Organisms for the Control of Pathogens in Protected Crops

Cultural Practices for Sustainable Agriculture

Serie **Agricultura** [**10**)

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Cultural Practices for Sustainable Agriculture

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ORGANISMS FOR THE CONTROL OF PATHOGENS IN PROTECTED CROPS. CULTURAL PRACTICES FOR SUSTAINABLE AGRICULTURE

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Index

Introductory preface of coordinators

"Preface" and "prologue" are equivalent linguistic terms. Both terms refer to the opposite ends of the text body in any work and are used to announce that the work has been finished or to give guidance. This introduction was a common procedure in Greek and Latin works of drama, and is indeed still relevant to classic drama of more recent times. It was recited in front of the public to inform them about the working of the plot that was going to be played out, to excuse the poet any censures addressed to him and to ask for indulgence, or for any other requisite purpose. It is not possible to find a better declaration outside of the term's own definition.

Why did we choose a book and not another communication media? Borges provided this answer which is well adapted to our intellectual background: Amongst all the instruments man has, the most amazing one is, without doubt, the book. The rest are just extensions of your body. The microscope, the telescope are extensions of the eyes; the telephone is an extension of the voice, then, we have the plough and the sword that are extensions of the arm. But the book is just something else: it is an extension of the memory and the imagination (sic).

Fundación Cajamar was the bank entity that ordered the coordination of this work, the contents and its authors. This banking entity is special as, since its beginning, it has been one of the driving forces propelling horticultural development in Almeria. This fact was sufficient to accept the task and the responsibility that it implied.

The rest has been possible thanks to the good will of the authors that has led to this work seeing the light in a very short time. The kindness they have showed us has been the real engine. Once again, thanks friends.

Why those contents?

A prior terminological explanation must be made that applies to the work. It is about the concept of crop **pests**. The Vegetable Health Law (L*ey de Sanidad Vegetal*, 43/2002 of the 20th of November) uses the term to designate pests as diseases of the crops. This fact breaks with a provision of science that, for more than a century and a half, has regulated vegetable health. On one side, the Vegetal Pathology or Phytopatology treating the study of diseases, and, on the other, Agricultural Entomology that includes the pests. For this reason, the use of the word “pest” can be misleading, although the law mentioned above specifies that this definition also includes diseases. One of the Royal Decrees that has developed the law (R.D. 58/2005 of 21/01/05) uses, in order to designate the causes of pests and diseases, the harmful **organism** couple that is defined in the following manner: any species, race or vegetable biotype, animal or pathogen agent that is harmful for vegetables or vegetable products. The content of what is called Vegetable Protection is an example of the collective terming of pests, diseases, weeds or adventitious buds and control procedures. Some dilettantes and professionals prefer, instead, to use the term Vegetable Health, as ‘protection’ and ‘fight’ have got connotations that are never defined but which could tarnish the archangelic character of human spirit. These explanations allow us to introduce the approach this work was written with. This essay mainly talks about the pest and disease management of protected horticultural crops. It is focused on the concept of the **pathosystem**, understood as a subsystem of an ecosystem (the agrosystem) defined by the parasitism phenomenon. As such, weeds could play a role, like a reservoir of plants of parasites, as they compete with but do not parasitize crops. This concept is important because it considers agrarian activity as a **system**, where each part meets a function and confers some properties to the whole. Alteration of one of the parts can unbalance the system in one or another sense. In fact, this perspective of agrarian system is the one that best defines the concept of **integrated pest and disease management** that could replace the more simplified concept of **control**.

This work was born as the result of a demand existing in most developed societies that food healthiness is guaranteed as well as conservation of the earth’s environments and atmosphere. A lot of written opinions have been given in response to these needs. Toxicity of pesticides and their residues, and the appearance of parasite resistance have sparked specific and informative coverage throughout the media and influenced on public opinion, and it is easy to understand why. Since the publication of “Silent Spring” by Rachel Karson, a large number of scientific studies have proved the empiric observations and intuitions of this American author; including: “Our stolen future” , where, from biphenyls to DDT, aspects have been shown that were never suspected a few years ago: their role as a hormone disruptor being amongst other aspects of its toxicology.

This work the reader has in their hands has been written in a short period of time, due to the fact that the European Union is now working towards the reduction of phytosanitaries. This reduction reflects a recent model, on a worldwide level, working on the process of methyl bromide removal, with the aim of its removal from agricultural use by 2015. This model has an important environmental foundation, in respect to the depletion of the ozone layer of the earth stratosphere and the consequential damages for human health and the environment. Some of the chapters in this book exist because of the “global project” to remove the fumigant gas, and some authors are active players in that process.

There are two case models concerning the reduction of phytosanitaries in intensive crops on the Mediterranean coastline in Spain. One model corresponds to pepper crops in the Field of Cartagena (Autonomous Community of Murcia), and the other is located in Almería, in its western and eastern areas: in just one crop season the yield raised from 800 ha in integrated management to more than 11000 ha. How did this change take place at such a fast speed? It is especially amazing that this fact has taken place in a sector as conservative as agriculture. Undoubtedly, there was the need to change the market requirements taking advantage of the circumstances that should have never taken place, especially because they could have been avoided. How could the market provide itself with so many million auxiliary insects and mites in such a short period of time? How was it possible to transfer technology so that the applications or “releases” were efficient?

In Spain it is normal to employ useful insects in citric crops. This tradition has been spread for over a century. It results paradigmatic the maintenance of the insectarium of the old *Estación Fitopatológica de Levante*, that was located in Burjasot (Valencia) and later moved to the premises of the Vegetal Protection Service in Silla (Valencia). In these premises, every year, two consumers of cochineal in orange trees (*Novius cardinalis* and *Cryptolaemus montrouizieri*) are produced. For the past twenty-five years, research about useful insects and their application within the integrated pest management has been locally developed for intensive horticultural crops at the Department of Vegetal Production of the Andalusian Regional Government in Almería, the current IFAPA, located in La Mojonera (Almería) and in the old *Estación Sericícola* located in La Alberca (Murcia). We cannot forget in, this imperfect inventory, the work of the private sectors from Almería and Murcia that have provided great results too. All this knowledge has made possible the provision of a satisfactory answer to the fast change concerning pest control.

Some of the authors who have written the chapters in this book are engines of those researches and its diffusion. This is more evidence of how important research and innovation is in the development and adaptation to changes in intensive horticulture. Maybe this "success" has had an impact in the authorization to create technological base businesses, boosted by different state administrations, to encourage a common approach between public research and business activity.

The control of plant diseases did not have nearly so drastic a change as the integrated pest management. Regardless, some of the most efficient results have been collected in the chapters of this book. These results are the consequence of the need to look for alternatives to methyl bromide as a fumigant of agricultural soils in the control of soil diseases. In these cases it is called **biodisinfection**. Biodisinfection techniques are referred to as "biofumigation" and "biosolarization". Contents of this work cover the control of edaphic fungi, nematodes, bacteria and viruses through the application of these procedures that accumulate the experience of reiterative trials that have taken place for more than ten years, which is a significant guarantee about its utility. Its disinfectant use is extended with a clear improvement of physical and chemical properties of soils, increasing its fertility and postponing its degradation. Some authors admit, within the biodisinfection term, which is a vaguely defined term at present, the introduction of antagonist microorganisms. In the texts of this work, some references shall be found concerning its efficiency. Antagonist microorganisms that are recommended for the management of aerial spread diseases are covered with a critical point of view.

Grafting use is complementary to these techniques of management of soil diseases, whose efficiency has been checked in thousands of hectares, every year, for the past twenty-five years.

Finally, we thought it was convenient to introduce a present-day topic. We are referring to biopesticides of vegetal origin. Some of them are not new, like the ones derived from nicotine, pyrethrum and rotenone amongst others. Maybe this resurgence is founded in neem oils and in "new agricultures" that adopt different denominations but that are concurrent in their contents: ecological, organic, biological, etc. The truth is that extracts of garlic, nettle, cinnamon, etc. are being introduced the market with a high level of acceptance and without a clear and fair registration rule and, above all, without the sufficient supported information for users.

We are sure that contents of this work will be increased and practically amended as soon as it comes to light. We are also sure that a large number of proposals, made by the experts that drafted its content, will remain in time. We would be happy if the reader finds its contents as useful as we found them.

We finish this preface in the same way we started it. We want to say to the critics that we apologize for any involuntary defects and ask readers to be indulgent with all the authors and their coordinators. These authors enjoyed a total freedom to express, in the way they wanted, the contents of their respective chapters.

Chapter 1

Integrated Production in Andalusia: Protected Horticultural Crops

Vicente Aparicio[1a], María Paz Rodríguez[2], Francisco Javier Cabrera[1], Martín M. Acebedo[1], Ana Belén García[2], María Encarnación Trujillo[2], Carmen M. Méndez[3]

1. Introduction

In this chapter, it is aimed to state, specifically and practically, the concept of Integrated Production (IP), as well as its origin, development, need to be implemented and future perspectives, referred always to the *production stage*. Although the contents of this article make reference to the protected horticultural crops from the coast of Almería, it can be also applied to other areas with similar agro climatic conditions. In Andalusia, the area used for protected horticultural crops of Integrated Production (IP), in the season 2008/09 amounts to 11331 ha in Almería, 383 ha in Granada, and 30 ha in Huelva.

Figure 1. Official logo of integrated production of Andalucia

Starting from the concept of IP as "Farming system to obtain vegetables which uses at maximum the *natural resources and production mechanisms* and secures a long-term *sustainable agriculture*, introducing biological and chemical control methods and other techniques that combine the *society needs*, *environment protection* and *farming productivity*, as well as the activities carried out for handling, packing, processing and labelling of products".

[1] Regional Ministry of Agriculture and Fish. Provincial Government Office of Almería.
[1a] Head of the Vegetal Health Department.
[2] Regional Ministry of Agriculture and Fish. Provincial Government Office of Almería. State company of Agrarian and Fish Development (DAP).
[3] Regional Ministry of Agriculture and Fish. Provincial Government Office of Almería. State company of Agrarian Transformation. (TRAGSATEC).

It aims to implement a production and marketing system that, observing the principles and objectives stated in the IP definition, obtains "high quality" vegetable products, and therefore, in addition to meet the quality requirements, the adequate use of techniques that have been contrasted as respectful with human health and environment must be justified. All the actions that can be done within the IP framework are stated within the corresponding specific IP Regulations. The actions carried out in accordance with the production stage regulations must be recognised by authorised certifying entities which shall certify the production as IP. If, in addition to production, the handling and presentation is made in accordance with the IP regulations for the handling centres, the final product shall be identified with the IP logotype.

The production factors considered with a higher impact on the aims proposed by the IP are *fertilizers* and *phytosanitary control methods*. The fertilisation that influences especially on production costs and on the possible pollution of the environment (soil, waters, etc.), is regulated in the "General Requirements" of the specific IP Regulations. Due to its possible harmful or unwanted side effects, the aspects related with phytosanitary control are, without doubt, the main worry in order to reach the goals set by the IP. The problems that horticultural intensive production has had in recent years (phytosanitary products residues, resistance of some harmful organisms to phytosanitary products etc.) have been a consequence of the pest control systems were based, mainly and exclusively, on the use of phytosanitary products.

With a clear forward-looking approach, the need to look for and implement other alternative control systems was already proposed during the years 1980-1985. It was the beginning of a series of studies and trials favoured by the Regional Ministry of Agriculture and Fish of Andalusia Regional Government in collaboration with other administration units specialised in vegetal health, as well as with phytosanitary and biological control organisms companies, valuing particularly the contribution of some agricultural associations, technicians and growers that have assumed with full confidence the IP system. In this manner, the foundations of IP were built, and later it was regulated with specific regulations for each one of the eight priority protected horticultural crops in Almería: aubergine, courgette, green bean, melon, cucumber, pepper, watermelon and tomato.

In accordance with the previous paragraphs, the Regional Ministry of Agriculture and Fish, developing practically the functions that it has entrusted such as *regulating, controlling and promoting* the agrarian activities and, particularly, the topics related with Vegetal Health, included IP as the fourth programme of the Andalusian Plan of Vegetable Health, together with the Phytosanitary Inspections, the Rapid Alert System and Phytosanitary Information (RAIF), the Campaigns and Phytosanitary Researches, and the Vegetal Health Laboratories; setting their goals in accordance with the Vegetal Health Act:

- Prevention and fight against pests.
- Control of phytosanitary defence mechanisms.
- Secure the fulfilment of legislation about Vegetal Health, through inspections and adequate penalising mechanisms, if appropriate.

Since then, the development of the IP programme has been a priority for the Regional Ministry of Agriculture and Fish, which regulated through specific regulations whether the technical aspects as well as the economic aids (technical support and innovative means of phytosanitary control), promoting the creation of Associations for Integrated Treatments in Agriculture (AITRAs), and teaching IP techniques to farmers.

Furthermore, Collaboration Agreements were entered into with agrarian entities to implement IP techniques, through trials and experiences, financing specially the hiring of technicians.

Following with the promotion of IP, the following support lines are currently in force:

- Techniques: Incorporation into the specific Regulations of studies, experiences and other innovations as a result of the collaboration with IFAPA, production and commercialisation companies of (Biological Control Organisms, (BCOs), phytosanitary companies, technical services of the agrarian entities of the sector, University of Almería, etc.
- Economic aids: Based on the direct support for the promotion of Integrated Production Associations (APIs), and aids aimed to control vector virus insects in horticultural crops, in accordance with the National Programme.

- Diffusion and training: Through talks, technical meetings, conferences, specialised courses for IP, publications, website, poster, trade fairs, audiovisual material, etc.

Currently, the IP is found in a standardization or effective convergence process with other certifications (Naturane, AENOR, GloblalGap), which shall permit technical advances, simplification and unification of audits to verify and certify the Regulations.

2. Specific crop conditioning factors and phytosanitary problems

The statements stated below are referred to protected horticultural crops that are developed under non hermetic closures conditions, which keep a very variable area, with average values which fluctuate between 0,5 and 1,5 ha, most of them covered with sand and with localised irrigation systems, structures with different systems and heights, and as insulation mean they use the combination of plastic and mesh to facilitate the side and/or zenithal ventilation.

Some specific factors of this crop system which are going to have a great influence on the presence and development of the different pests, and that are decisive to establish a proper and rational system of phytosanitary control are:

- Favour climatic conditions for the development of crops, and, therefore, of pests.
- Structures with non hermetic closures, which allow taking advantage of the favourable natural weather conditions with low cost.
- Inappropriate protection in the ventilation openings.
- Intensive nature of farms whether in time as well as space.
- Presence of spontaneous plants.

A direct consequence of the factors mentioned, is the presence of high pest populations. If in addition to this, the phytosanitary control system is based, mainly and almost exclusively, on treatments with phytosanitary products, the following problems can arise:

- Presence of phytosanitary products *residues* in the horticultural products for consumption. It is difficult to keep the productions without residues in the harvest, or not to exceed the maximum residue levels (MRLs) established, when it is tried to control pests that appear severity and close to harvest-time, due to there are only few active substances that can be used with the required effectiveness and the specific authorisation.
- Appearance of pest *resistances* to phytosanitary products, as a consequence of repeated applications with products of the same or very similar chemical family. This problem is worsened with the reduction of authorised active substances.
- Harmful effect for handlers' health. A higher and better protection is demanded as well as formulations and handling and application techniques, which guarantee the minimum exposure of the operator to phytosanitary products.
- Risks of environmental or ecotoxicological damages (flora and natural fauna, land, surface and underground waters, etc.).
- High economic costs due to excessive or unjustified applications.

3. Evolution of phytosanitary control

The evolution of phytosanitary control until reaching the current practical application of IP as a more advanced control measure, but always in constant evolution, can be broken down in the following stages:

- **First Stage**: Phytosanitary control based on *rudimentary cultivation measures and chemical control*. These cultivation measures referred to the use of several mechanical means as protection of agricultural holdings against the presence of pests. A simple structure with plastic and non adequate mesh as efficient barrier to limit the entry of pests, elimination of pest insects by mechanical means, etc.

  The *chemical control* is the direct and basic element of fight. The possible side harmful effects derived from its use are not taken into account. Mainly, immediate effectiveness is looked for pest control and its polyvalent effect. Many applications are carried out without assessing the real need to do them. It is kept thanks to the few requirements of the markets.

- **Second Stage**: *Preventive and cultivation measures* based on experiences with the purpose of avoiding or delaying the presence of pests. The closures are maximized so that they combine the ventilation with the limitation to pest entry. Planting densities are adjusted to facilitate ventilation with the purpose of avoiding favourable conditions for fungal or bacterial diseases. Fertirrigation is optimized. Seeds and seedlings are required to meet a minimum of health guarantee. Traps begin to be used to catch pest insects and weeds begin to be removed.

  The *chemical control* is applied in a *more rational manner*, with higher requirements for the phytosanitary products of possible use, applicable also with reference to the number of treatments and the application techniques used. The profile of the phytosanitary products must respond to: high effectiveness, expressed authorisation for crops, higher specificity, shorter safety period, good toxicological and ecotoxicological behaviour.

- **Third Stage**: It begins with the publication of the specific IP Regulation that lays down, expressly and based on studies and trials, the most advanced aspects with respect to:

a) *Preventive and cultivation measures:*

- Health guarantee of vegetal material: seeds and seedlings.
- Cleaning and health before the planting of structure and soil.
- Closures or barriers that avoid or delay to the maximum the entry of pests, keeping ventilation: mesh and double door with intermediate space.
- Distribution at the beginning of the crop of traps to catch pest insects (pheromones, chromotropic and light).
- Absence of spontaneous vegetal species. The proper handling of some of them is being studied as BCOs reservoirs.
- Balanced fertirrigation.

b) *Rational control with phytosanitary products:*

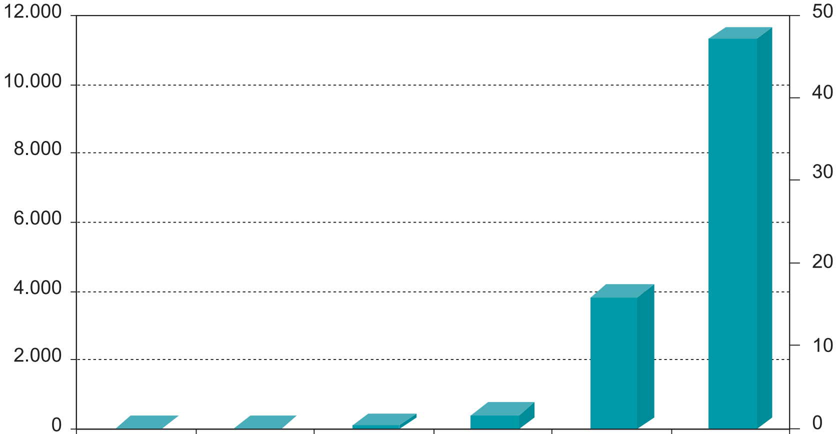
- Official Register to use in the crop where the characteristics and conditions of use are laid down (dose, formulas, safety periods, number of applications per crop cycle, toxicology, ecotoxicology, application techniques, etc.)
- Respect to pollinizers.
- Compatibility with BCOs.
- Effectiveness and use strategy. The applications shall be determined by the intervention criteria established in the Regulations and shall respond to the real need of carrying them out according to the level of pest population (according to observations and samplings), the biological conditions of the same, the crop phenology, the damages it causes in the specific crop, etc.

c) *Biological Control:*

- Control and regulation of pest populations through the BCOs action.
- Maintenance of natural populations of BCOs.
- Application of commercial formulas of BCOs, which appear in the register of Other Means of Phytosanitary Defence (OMPD), as complement of the autochthonous fauna.
- BCOs release and handling strategy according to their specific action, together with cultivation and preventive measures, and use of corrective measures when it is necessary, through phytosanitary treatments in early crop stages or to control other pests for which specific BCOs are not available.

To conclude, as a consequence of this evolution, that has favoured an increase in the effectiveness of pest control, together with the market demand of quality products, an exponential increase in the area of IP horticultural crops has been produced.

Figure 2. Surface protected in production integrated in Almeria



	2003/04	2004/05	2005/06	2006/07	2007/08	2008/09
has	21	25	128	431	3.838	11.331
(%)	0,1	0,1	0,5	1,6	14,5	42,8

4. Regulations

The ruling framework which regulates IP in Andalusia is the following one:

- Act 43/2002, of 20 November, of Vegetal Health.
- Royal Decree 1201/2002 of 20 November on integrated production of agricultural products.
- Decree 245/2003 of 2 September on integrated production and its indication in agrarian products and their derivatives.
- (Amendment). Decree 7/2008 of 15 January which amends Decree 245/2003, on integrated production and its indication in agrarian products and their derivatives.
- (Application). Order of 13 December 2004, which develops the Decree 245/2003.
- (Amendment). Order of 24 October 2005 which amends Order of 13 December 2004, which develops the Decree 245/2003.

- (Application). Order of 10 October 2007 which passes the specific Regulation on Integrated Production of Protected Horticultural Crops (tomato, pepper, aubergine, green bean, courgette, cucumber, melon and watermelon).
- (Correction of mistakes). Correction of mistakes of the Order 10 October 2007.

To access the regulations, go to the website of the Andalusian Regional Government[1].

5. Andalusian integrated production register

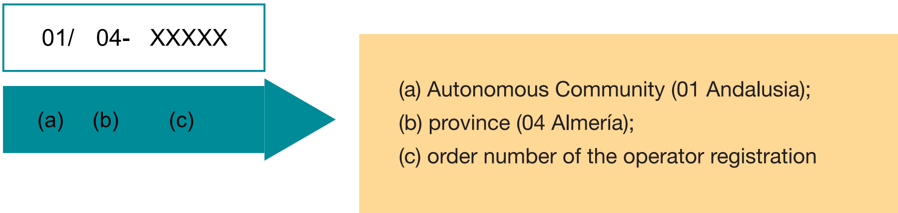
It is created and attached to the Regional Ministry of Agriculture and Fish of the Andalusian Regional Government, and all the identification data and activities carried out by persons or entities which operate Integrated Control or Production shall be registered in this Andalusian Integrated Production Register (RPIA). This register is organized in two sections: Integrated Control and Integrated Production.

The RPIA has administrative nature, is public and operates according to the coordination and communication principles with the State General Administration, where there is a General Register of IP.

The registration in the RPIA, which is a compulsory previous requirement to practice the activity as IP operator in Andalusia, is applied in the Provincial Government Office of Agriculture and Fish, where the agricultural holdings are located; if they are in different provinces, they shall be registered in the province which has the highest area. Each operator has assigned a registration card which shall include all his current information, and which responds to the following identification code, that must appear in all the documents related with the IP activity:

[1] Web of the Council of Agriculture and Fishing, of the Meeting of Andalusia. (\Agricultura\Producción Integrated) (http: // www.juntadeandalucia.es/agriculturaypesca/)

Figure 3. It registers registral of obliged use of the operators of PI



The RPIA is managed through the IT applications PRIN (administration) and PRIN-Mobile (operators). The registration and modification requests are carried out with the application PRIN-Mobile, available in the website of the Regional Ministry of Agriculture and Fish (1). Finally, the data shall be imported to the application PRIN in the Provincial Government Office.

It must be highlighted that only the operators that appear formally in the PRIN shall be considered as registered, and that they only shall be taken into account for the different actions to be carried out within the IP framework (grants, controls, etc...), the data referred to producers, parcel, technical services, etc… registered in such Register.

Given the topic we are dealing with, some aspects related with "*Operators who obtains Vegetable Products"* are going to be specified, who may appear as individual operators or Integrated Production Associations (APIs). The APIs must bring together at least five producers and an area of at least, the 25 % of the maximum established in the specific regulation of the corresponding product. If the cultivation area is located in a less-favoured area, according to the Council Regulation (EC) 1257/1999, the area shall be, at least, the 15 %.

The operators who obtain agrarian products must submit the registration forms in the RPIA, at least a month before the beginning of the production season, together with a favourable audit report carried out by an authorized certifying entity, and the compulsory documents. Once registered in the RPIA they must communicate through a reasonable request any modification related with its structure or its operation. However, due to the peculiarity of the protected horticultural crops in Andalusia and, particularly, in Almería, it is necessary to establish a period to submit registration and modification requests of RPIA (Order of 12 December 2007 of the General Direction of Agricultural and Livestock Production), which must be adapted to the productive cycles of the agricultural season that takes place in this intensive productive area. Therefore, there are two periods to submit applications to include new producers and / or parcels; from 1st November to 31st December for spring cycle and from 1st to 31st May for autumn cycle, always one month before planting the crop. Out of these periods, only modification applications may be submitted that laid down the discharge of farmers and /or parcels, without prejudice that the operators must communicate the Register, when these discharges are produced, any change that affects the legal personality, legal representative, composition of the technical service or certifying entity.

The operators are obliged to:

- Have a competent technical service with a composition according to the limits laid down in the specific product Regulation and the dispersion of agricultural holdings. The competent technical service is obliged to control the operator production process, being responsible for the fulfilment of the rules and specific Regulation of IP, as well as to fill in the agricultural holding notebook. All the members of the competent technical service have to take an IP training course before rendering the service.
- To have a holding notebook where the entire cultural practices are registered.
- To provide weekly the Provincial Government Office with the crop phytosanitary information, in accordance with the integrated control strategy laid down in the specific IP Regulations through the IT application TRIANA, to manage the agricultural holding notebook.
- To inform about its production programme to the certifying entity yearly.

The breeder operators from Almería registered in the RPIA, in the season 2008/09, are 77 (70 APIs and 7 individual operators). The number of producers that are applying IP is 3878 in 11965 protected Homogenous Crop Units (UHC). The parcels are identified through SIGPAC enclosures from Andalusia (Geographic Information System to identify Agricultural Parcels), where they must appear as greenhouse use "*IV*". The crop advising is carried out by 247 competent technicians.

6. Certifying entities

The certifying entities shall carry out the operation control in the different production stages, preparation, processing and commercialisation, if appropriate, following applicable control plans, supervising protocols and IP regulations, taking into account the process in which the person or operating entity participates, the IP involved and the distinctive guarantee sign that is going to be used. In case of "*Breeder operators*" they shall be in charge of controlling, verifying and certifying the correct fulfilment of the specific IP Regulations applicable by the operators registered in the RPIA. The certifying entity shall inform, the corresponding Provincial Government Office of the Regional Ministry of Agriculture and Fish about the non fulfilment detected and the corrective measures adopted by the affected persons or operating entities, as well as the claims made by them.

To carry out the controls established and the corresponding certifications, the certifying entities must be recognised according to the rules 45004 and EN 45011, respectively, have the authorisation of the Regional Ministry of Agriculture and Fish and be registered in the Register of Inspection and Certifying Entities of Agrifood and Fish Products of Andalucía. To know the certifying entities see the website of the Andalusian Regional Government (1).

7. Control and monitoring

Independently from the control carried out by the certifying entities to certify the IP, the Regional Government of Agriculture and Fish, establishes a Control and Monitoring Plan aimed at this quality rule. It is applied to all the registered operators in the RPIA for the obtaining or production stage of agricultural products, fulfilling the Order of 13 December 2004, which regulates IP.

Through this Plan, a series of inspections are carried out by technical staff of the Vegetal Health Departments of the Provincial Government Offices of Agriculture and Fish, to individual operators as well as APIs.

The procedure establishes annual inspections to at least the 30 % of the operators registered in the Register. Inspection visits are made in the highest activity period of the crop cycle, making the corresponding control reports. During the inspection, the responsible technician and the holder of the agricultural holding must be present. The control procedure follows the following order:

- Election of producers and their respective parcels.
- Identification of the technical staff responsible for the parcels to be controlled.
- Verification of the agricultural holding notebook.
- Verification of the coherence of the data of the IT application TRIANA with the prescriptions made by the technical services.
- Revision of the audit documents carried out to such operators by the Certifying Entity.
- Taking of samples.
- To make the Control Report.
- Application of the verification list with the sections:
    - Phytosanitary Applicator license.
    - Installations, equipment and staff.
    - Phytosanitary products warehouses.
    - Machinery and treatment equipments and fertirrigation.
    - Protection and measure equipments.
    - Staff.
    - Land, preparation and farm work.
    - Sowing and planting.
    - Pollination and setting.
    - Modifications and fertilization.
    - Irrigation.
    - Integrated Control.
    - Identification and traceability.
    - Residues management.
- Agricultural holding notebook.

The result shall be favourable if at least the 90 % of the aspects checked in the verification list are fulfilled. In the event of APIs, the result shall be favourable in all the producers who had been inspected. If the result of the inspection is unfavourable, a 10 days period shall be granted in order to it tries to rectify the irregularities detected. Once the period has elapsed, the submissions shall be studied and it shall be decided if the irregularities are solved. If the decision is refused, a report to propose the discharge from the registration in the RPIA shall be issued.

In addition to these controls, the proceedings resulting from support applications for the control of vector virus insects in horticultural crops and the promotion of APIs, shall be also subject to administrative and on land controls. In the first case, all the entities will be subject to be monitored, while in the second case a sampling shall be carried out as a result of; (i) the selection through some risk criteria, or (ii) the detection of any incident after have been subject to the administrative controls, remaining always a 25 % of the proceedings subject to be elected randomly. For this reason, once all the support applications have been submitted, the General Direction of Agricultural and Livestock Production shall assess and make an analysis of all the applications in accordance with the above mentioned criteria.

As a consequence of all this control and monitoring process, technical-practical information is obtained which is very important to improve and update the specific IP Regulations.

8. Subsidies

8.1. Aids for the promotion of the Integrated Production Associations (APIs)

The Autonomous Region of Andalusia has been one of the pioneering Autonomous regions in the implementation of the IP system, regulated since 1995 by the Decree 215/1995, of 19 September, by which also the mark IP of Andalusia was created. A year later, the Order of 8 November 1996, laid down the first regulations to enter into collaboration agreements with the agrarian entities; for the development of Integrated Production programs, repealed by the Order of 28 March 2003, which laid down the rules for the granting of aids to improve vegetal health through the signing of collaboration agreements for the development of Integrated Production programs.

At the same time that this aids program aimed to implement new production methods, the publication of the Royal Decree 1201/2002 of the Ministry of Agriculture, Fish and Food, and particularly, article 8.6 laid down the possibility that the APIs received also aids set by regulations, such aspect was laid down in the Decree 245/2003 of the Regional Ministry of Agriculture and Fish, which regulated the IP and its indication in agrarian products and its processed products; and the Order of 13 December 2004, which developed the Decree mentioned before.

Consequently, within this framework, the first Order of the Regional Ministry of Agriculture and Fish, of 12 January 2006, was published, which laid down the regulations to grant aids for the promotion of Integrated Production, through the encouragement of APIs. For the purposes of this Regulation, the following expenses were eligible: the competent technical service expenses, the agronomic analysis expenses, as well as the expenses derived from the control and inspection carried out by the control and certifying entities, with maximum eligible amounts of 2000€ monthly for the technical director and 1700€ for the field assistant technicians; 2500€ for analysis expenses; and 4000€ in the event of chargeable expenses for the certification of productions.

The procedure to grant the aids was carried out accordance with competitive concurrence, setting a series of valuable criteria, although all the applying entities were beneficiary without budgetary limit of the expenses chargeable to the submitted plans. This same circumstance has been repeated year after year until today.

The inclusion of these aids in the Measure 115 of the Rural Development Program of Andalusia for the period 2007-2013, meant that the European Union began to finance jointly such aids through the European Agricultural Fund for Rural Development (EAFRD). These new facts, the need to adapt some aspects in the management of the aid proceedings, and the incorporation of new crops promoted the modification of the first regulating Order by the Order of 23 May 2007. Among other changes, this new Order meant the reduction of the maximum expenses chargeable to the agronomic analysis made until 1000€ by unit or fraction of the calculation coefficient, as well as the expenses derived from the control and inspection which decreased to 3000€. The aids for the remuneration of the technical services also suffered changes, in this time referred to the percentage of aid to be received, which became decreasing (-5 % per year passed), with an initial aid of 60 %. The aids followed being compatible with others for the same purpose, although, the total amount of the aid could amount to the 60 %, a 10 % more than the previous call.

Other remarkable change was the inclusion of article 6 “bis” which laid down the responsible declaration about the internal commitment of each of the producers, with the applying entity for aid, to assume the obligations derived from the fulfilment of the system during the five-year period of the program.

The change of this aids line to the Measure 132 of the Axis 1 of the Rural Development Program, framework 2007-2013, implied again modifications in the regulations of the season 2008/09, laid down in the Order of 16 May 2008, which amended those of 12 January 2006. Among the most remarkable changes, there was the eligible percentage that stopped being decreasing across time, applying the fix rate of 55 %, referred to the expenses incurred by the technical services. On the other hand, a maximum eligible amount of 3000€ was fixed per agricultural holding, in accordance with the Council Regulation (EC) No 1698/2005 of 20 September 2005. In order to the producers can access to this aid, each of the APIs must define and fix an individual participation fee, according to a clear, objective and single criterion for all the producers joined to the Association, that must be specified in the budgetary report of the program. This fee must be paid before the end of the season object of the aid.

The last modification of this brief historic review, which affects fully the horticultural protected crops, has been laid down in the Resolution of 3 December of 2008, of the General Direction of Agricultural and Livestock Production, by which the Annex 1 of the Order of 12 January 2006 is modified, and in which the eligible period has been extended from 10 to 12 months for the APIs aids.

The evolution of these aids is showed in Table 1 and Table 2.

Table 1. Evolution of the aids to Collaboration Agreements for the development of IP programs, Almería

Period or Season	1994/95-2000/01	2001/02	2002/03	2003/04	2004/05	2005/06
No. of beneficiary entities	25 (1)	5	5	5	5	5
Final amount of the subsidy (€)	799.913 (1)	180.304	156.263	150.000	167.285	161.484

(1) Total amount of the period.

Table 2. Evolution of the aids for the promotion of APIs, Almería

Season	2006/07	2007/08	2008/09	2009/10	2004/05	2005/06
No. of beneficiary entities	8	45	61	54	5	5
Final amount of the subsidy (€)	79.607	1.486.768	1.881.212	2.157.451 (1)	167.285	161.484

(1) Requested amount.

8.2. Aids within the framework of the National Program for Controlling Vector Virus Insects in horticultural crops

The Royal Decree 1938/2004, by which the National Program for controlling vector virus insects in horticultural crops, describes as public utility the prevention and fight against such pests and defines, in article 5, the compulsory measures to prevent the development of its populations.

Those affected by the compulsory nature of fight against pests shall be benefited from the technical assistance and the economic aids that, if appropriate, shall be determined in the corresponding regulation.

The implementation of the aids laid down for the period 2007-2011, are under decisions of the European Commission referred to the State aids and shall be financed with funds of the Andalusia Autonomous Region and with funds of the State General Administration.

The Autonomous Region of Andalusia calls these aids since the season 2005/06 (Table 3), according to the competitive concurrence system.

Table 3. Aids and crop area in biological control within the framework of the national program for controlling vector virus insects of horticultural crops in the province of Almería

Season		2005/06		2006/07		2007/08		2008/09		2009/10	
Regulation		Order 18/01/2005		Order 13/03/2006		Order 25/05/2007		Order 09/05/2008		Resolution 09/06/2009	
Eligible concepts [(1)]		a) y f)		a) y f)		a)		a)		a)	
Maximum eligible amount		100 %		50 % of the justified expenses							
Maximum expenses limited by modules per crop (€/ha)		No		No		No		Yes		Yes	
Beneficiaries		APIs, ATRIAs and entities with agreements		APIs, ATRIAs and legal persons		APIs, ATRIAs and S. Coop. and SAT		APIs, ATRIAs and APEs		APIs, ATRIAs and APEs	
Crop area (Has) and (%) respect to the total provincial	Aubergine			30	2 %	403	27 %	615	38 %		
	Courgette	2	0 %	36	1 %	312	7 %	775	17 %		
	Green bean			7	0 %	131	7 %	112	9 %		
	Melon					1.666	33 %	3.376	68 %		
	Cucumber			18	0 %	532	13 %	1.192	26 %		
	Pepper	51	1 %	331	4 %	4.020	49 %	5.825	83 %		
	Watermelon					991	21 %	1.920	40 %		
	Tomato	76	1 %	334	3 %	1.184	12 %	2.161	21 %		
	Total	129	0 %	757	2 %	9.238	23 %	15.975	41 %		

[(1)] RD 1938/2004. Article 5. a) Promotion of biological fight through the development of autochthonous auxiliary insects and the introduction of auxiliary insects reproduced in insectaries and f) Any other measure different from the conventional chemical treatments, which is justified technical or scientifically as necessary for preventing the development of populations of these pests, including the physical barriers in the infrastructure of greenhouses.

The aids are aimed to defray the implementation costs of biological control, and they cannot exceed the 50 % of the justified expenses from 2006/07. From the season 2008/2009, the maximum expenditure per crop and area unit has been limited with modules.

The BCOs used for biological control, object of the aid, are autochthonous or exotic, and they may be produced and commercialized with different formulas and formats (Rodríguez Rodríguez, 2004; Navarro *et al*., 2004). The act 43/2002 of Vegetal Health, regulates its commercialization and use, as well as its registration developed by the Order APA/1470/2007, by which the communication of commercialization of different phytosanitary defence means (OMPD) is regulated, among which the BCOs are found. Now there are more than 200 commercial formulas of BCOs[2].

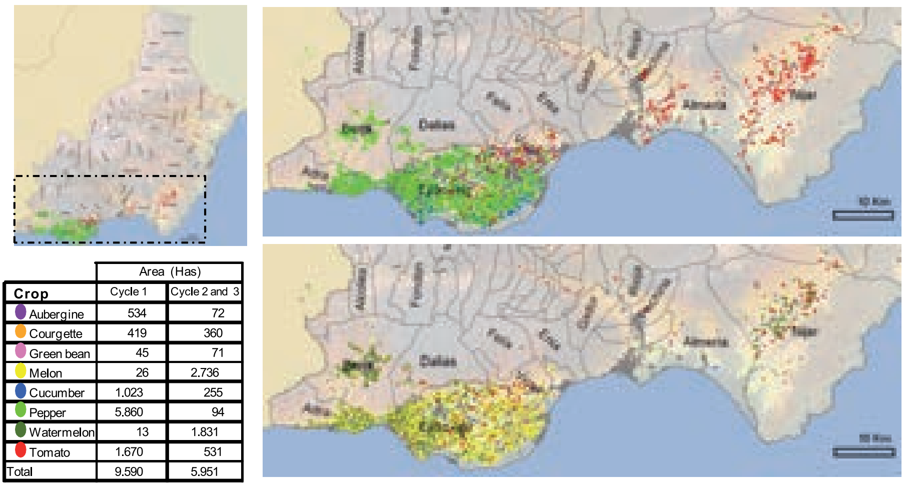
The area of biological control in Almería is distributed into all the crop areas. The current implementation degree is high, mainly in pepper, with more than the 80 % of the total provincial area within the program. The evolution of these aids is showed in Table 4.

Table 4. Evolution of the aids for controlling vector virus insects, Almería

Season	2005/06	2006/07	2007/08	2008/09	2004/05	2005/06
No of beneficiary entities	8	30	56	68	5	5
Final amount of the subsidy (€)	198.372	655.742	10.719.010	16.404.097 (1)	167.285	161.484

(1) Reserved initial amount.

Figure 3. Distribution of the crop area in biological control per cycle. Season 2008/09. Aids for the control of vector virus insects in Almería



Crop	Area (Has) Cycle 1	Cycle 2 and 3
Aubergine	534	72
Courgette	419	360
Green bean	45	71
Melon	26	2.736
Cucumber	1.023	255
Pepper	5.860	94
Watermelon	13	1.831
Tomato	1.670	531
Total	9.590	5.951

[2] Web of the Department of Environment, Rural Way and Sailor Phytosanitary (MARM), Record of Other Means of Phytosanitary Defense and of BCOs and Producer's Record. (Http: // www.mapa.es/es/agricultura/pags/fitos/registro/menu.asp)

9. Phytosanitary balance under integrated production in almería, season 2008/09

It can be affirmed that the IP system with the inclusion of biological control has meant a revolution in the phytosanitary control. The high effectiveness achieved, the guarantee of absence of residues and the respect to people health and environment, justified the actual acceptance and the continued growth experimented in the IP application.

The surveillance and the control of the phytosanitary conditions of crops, as well as the health controls of some vegetables or vegetal products, coming from Andalusian territory, are done through the Alert and Phytosanitary Information Network (RAIF), which includes: agro-climatic data and phytosanitary data. The reports made (agro-climatic data, phytosanitary applications, BCOs releases and levels of presence of pests of phytosanitary interest, and phenology) are published weekly in the web[3].

The crop season includes several cycles, beginning in June and ending in May of the next year, for the eight protected horticultural crops.

The reference *agro-climatic data* are obtained from the stations AL001 (San Isidro-Níjar), RIAL01 (La Mojonera) and RIAL02 (Almería), belonging to two automatic weather stations (EMAS): RIA (Agro-climatic Information Network of Andalusia) and RAIF.

These are interesting data due to the influence they have on the development of crops, pests and the BCOs.

During the season 2008/09, winter weather has been very extreme, with continuing frosts, abundant rainfall (above 40 mm) and predominance of windstorms coming form North and Northwest that have reached maximum speeds higher than 90 km h^{-1}. Summer has been very mild, with a registration of little extreme temperatures and abundance of local hydrometeors (morning mists), which has caused that relative humidity values near 100 % are registered continuously.

The technicians of the different APIs are the main source of *phytosanitary information*, who incorporate data into the system, through the IT application TRIANA, (Table 5).

[3] Web de la Red de Alerta de Información Fitosanitaria (RAIF). (http://www.juntadeandalucia.es/agriculturaypesca/raif/)

Table 5. Area of phytosanitary information per crop for the RAIF (2008/09)

Crop	Aubergine	Courgette	Green bean	Melon	Cucumber	Pepper	Watermelon	Tomato	Total
Has	163	69	19	149	178	351	99	174	1.039

It is shown the development of the most significant pests and the BCOs in the different crops, based on the control strategies laid down in the specific IP Regulation. In addition to the active substances of higher importance, applied in the phytosanitary treatments and the releases of BCOs made, to control the indicated pest (Figure 4 to Figure 11).

Figure 4. Evolution of whitefly, blackfly populations and BCOs (a). Treatments (b) and releases (c). Aubergine, season 2008/09. Almería

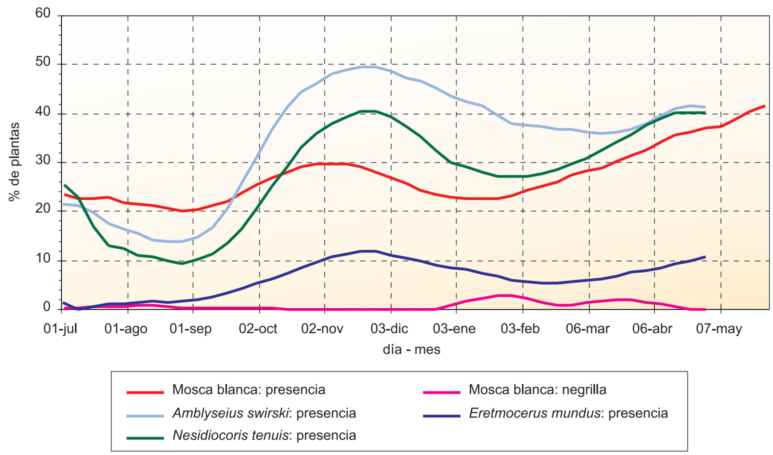


Figure 5. Evolution of whitefly population, most important viruses, and BCOs (a). Treatments (b) and releases (c). Courgette, season, 2008/2009. Almería

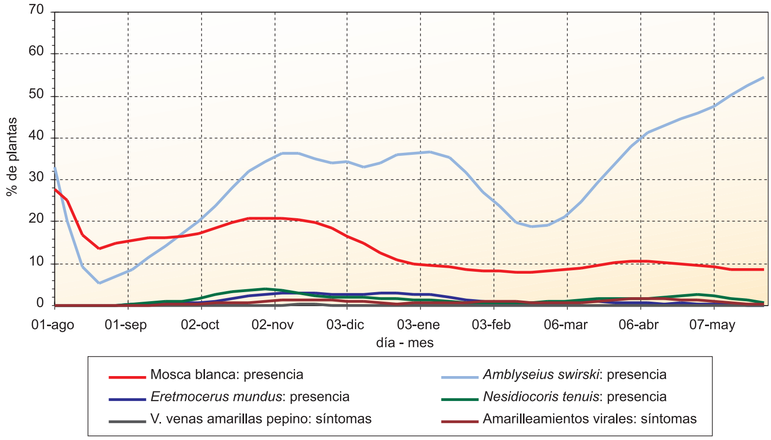


Figure 6. Evolution of whitefly population in plants, bean yellow disorder virus, and BCOs (a). Treatments (b) and releases (c). Green bean, season 2008/09. Almería

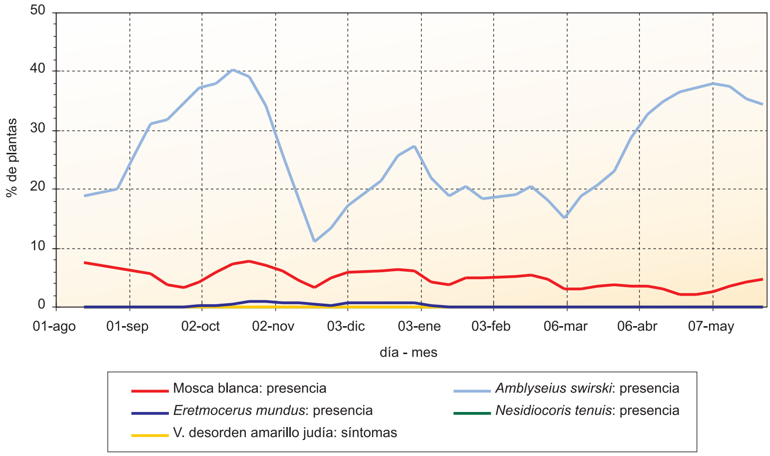


Figure 7. Evolution of whitefly population in plants, most important viruses and BCOs (a). Treatments (b) and releases (c). Melon, season 2008/09. Almería

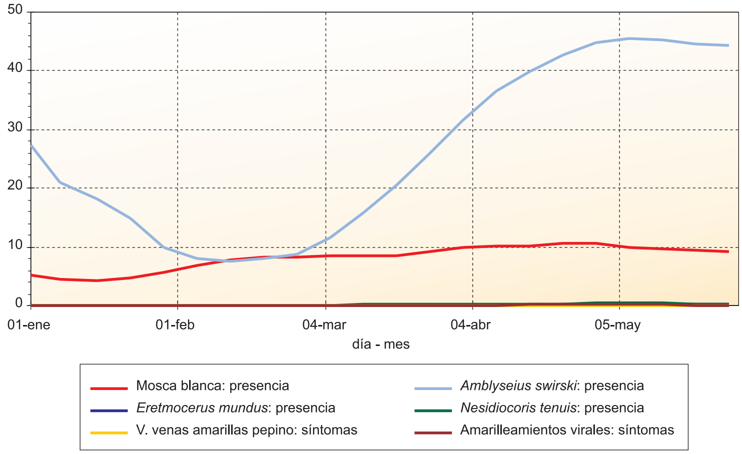


Figure 8. Evolution of whitefly population in plants, most important viruses and BCOs (a). Treatments (b) and releases (c). Cucumber, season 2008/09. Almería

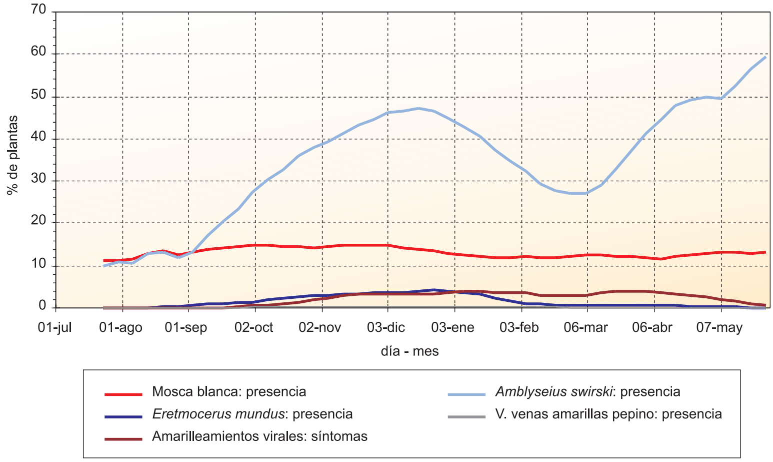


Figure 9. Evolution of thrips population, tomato spotted wilt virus, BCOs and damages to fruits (a). Treatments (b) and releases (c). Pepper, season 2008/09. Almería.

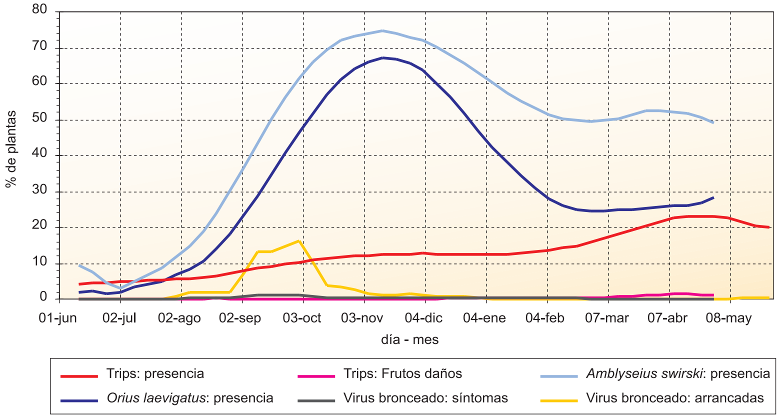


Figure 10. Evolution of whitefly population in plants, most important viruses and BCOs (a). Treatments (b) and releases (c). Watermelon, season 2008/09. Almería

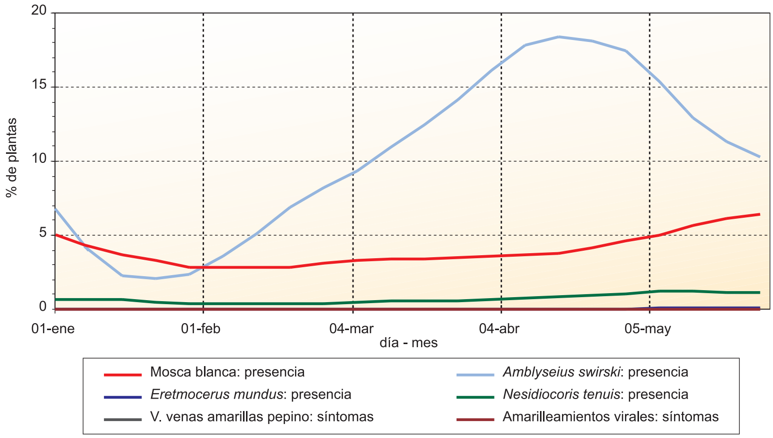
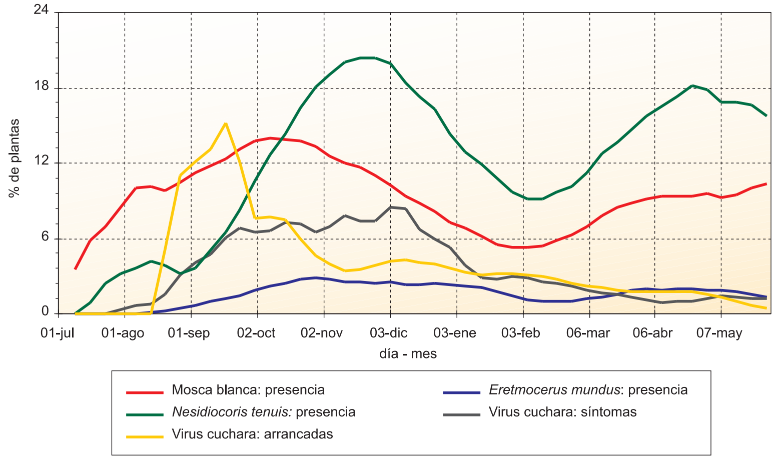


Figure 11. Evolution of the whitefly population, tomato yellow leaf curl virus (TYLCV), and BCOs (a). Treatments (b) and releases (c). Tomato, season 2008/09. Almería



To conclude it must be said that pest control has been effective in accordance with the IP strategies (use of BCOs, preventive measures, and phytosanitary products compatible with BCOs). Furthermore, it has been achieved:

- Adequate implementation of IP techniques in horticultural crops in Almería.
- Considerable increase of IP area.
- Obtaining food products free of residues.
- Reducing the use of phytosanitary products.
- Improving quantity and quality of productions.
- Optimum conditions to keep pollinizers.
- Higher confidence of the different agrarian sectors in the system.
- Increasing the incorporation of technicians.
- Decreasing the bad practices and improper use of phytosanitary products.

The emergence of new pests, autochthonous (*Nezara viridula*, *Lygus sp*.,...) or strange (*Tuta absoluta*, ...), that have not been a phytosanitary problem up to now, obliges to carry out a continuing revision of control strategies in IP. Therefore, IP cannot be considered as a static system.

10. Terms for the updating of the specific ip regulations: perspectives

The Order of 10 October 2007, which regulates IP for the eight horticultural crops is divided into two parts: the "*General Requirements*" to be applied to all the crops, and the Annexes 2 to 9 that laid down the "*Specific Requirements*" for each crop.

The higher limitation of use of active substances, higher requirements for their application and the need for they be compatible with BCOs (Biological Control Organisms) has caused the need for revising the specific IP Regulations. The experience accumulated in the last season has permitted its updating, introducing a set of changes directed to facilitate the application of Integrated Production. The updating of the specific IP Regulation shall come into effect through the pertaining Resolution which shall allow its immediate application.

The part of "general Requirements" is being studied for its later updating and standardization, with other Regulations and Quality Protocols. The main aspects included in such updating are highlighted below, and they are specified in the part of "Specific Requirements" for the different crops:

- Updating of the active substances, according to the updated register of the Ministry of Environment and Rural and Marine Affairs (MARM) (3), whether of the compatible phytosanitary products as those included in the respective Annexes.
- The strategies of the control methods are aimed at the priority use of BCOs. The list of BCOs has been updated, new commercial products are included and the application doses are eliminated.
- The use of OMPD different from the BCOs is included, to complement the action of these. Its use shall be determined by a favourable resolution, of registration in the register, issued by the

Ministry of Environment and Rural and Marine Affairs. (3). The competent technician shall be the responsible for guaranteeing the compatibility of these means with the BCOs used in the crop.

- Reservoir plants of BCOs may be used (with Phytosanitary passport or certificate of origin and health) to facilitate their introduction, reproduction and later incorporation to the crop. The competent technician shall give details in the agricultural holding notebook of the followed strategy (no. of plants, species, placing, and the control exercised).

Information is shown, to be considered, for a correct strategy to control pests in a representative crop for each of the cycles: pepper and watermelon (Table 6 and Table 7).

Table 6. BCOs used in the strategy of pest control in pepper crops (Pe) and watermelon (Wa)

Pest	Broad mite		Red spider mite		Whitefly		Caterpillar		Aphid		Thrips	
Crop BCO	Pe	Wa	Pe	Wa	Pe	Wa	Pe	Wa	Pe	Wa	Pe	Wa
Adalia bipunctata									2	2		
Amblyseius andersoni			2	2								
Amblyseius californicus	2		1	1								
Amblyseius cucumeris	2										2	
Amblyseius swirskii	2		2	2	1	1					1	1
Aphidius colemani									1	1		
Aphidoletes aphidimyza									1	2		
Banker-plant									1	1		
Chrysoperla carnea									2	2		
Encarsia formosa					1							
Eretmocerus eremicus					1	2						
Eretmocerus mundus					1	1						
Feltiella acarisuga			2									
Lysiphlebus testaceipes									1	1		
Macrolophus caliginosus					1							
Nabis pseudoferus ibericus							2					
Nesidiocoris tenuis			2	2	1	1						2
Orius laevigatus											1	
Phytoseiulus persimilis			1	1								

1: BCOs of priority use that exercise an effective control on the pest.
2: BCOs of secondary use that exercise a partial control on the pest, being necessary to complete its effect with other BCOs or with phytosanitary products and crop measures.

Table 7. Active substances used in the strategy of pest control in pepper crops (Pe) and watermelon (Wa)

Pest	Broad mite		Red spider mite		Whitefly		Nematode		Caterpillar		Aphid		Thrips	
Crop BCO	**Pe**	**Wa**	**Pe**	**Wa**	**Pe**	**Wa**	**Pe**	**Wa**	**Pe**	**Wa**	**Pe**	**Wa**	**Pe**	**Wa**
Abamectin	1		1	1										
Summer oil	1		1		1						1		1	
acrinatrin			2										2	2
Alpha cypermetrin						2			2	2	2	2		
azadirachtin				1	1				1		1	1	1	1
Sprinkling sulphur	1		1			1								
Wetable sulphur	1		1											
Bacillus thuringensis aizawai									1	1				
Bacillus thuringensis kurstaki									1	1				
Beauveria bassiana					1	1							1	
betacyfluthrin									2					
bifenthrin			2		2	2			2	2	2	2		
buprofezin					1	1								
Piperonyl butoxide + pyrethrins				1	1	1			1	1	1	1	1	1
cypermethrin									2	2	2	2		
cyromazine				1										
deltamethrin									2	2	2	2	2	
ethoprofhos							1							
fenamiphos							1	1						
fenbutatin			1											
flufenoxuron			1						1	1				
indoxacarb									1					
lambda cyhalotrin					2	2			2	2	2	2		
lufenuron									1	1			1	1
Chlorpyrifos methyl									2					
methiocarb													2	
methoxyfenozide									1					
Oxamyl	1		1	1	1	1	1	1			1	1	1	1
pymetrozin					1	1					1	1		
pyridaben			1		1	1								
pyrimicarb											1	1		
piriproxifen					1									
spinosad									1				1	
tebufenozide									1					
tebufenocida + bacillus									1					
teflubenzuron					1				1					
thiacloprid					1	1					1	1		
thiamethoxam				1								1		
Verticillium lecanii					1									
zeta-cypermetrin				2	2				2	2	2	2		

1: Active substances of possible use due to their compatibility with the BCOs or by their known effect on the same.
2: Active substances incompatible with BCOs, they shall be only used with technical justification and in the event of the control methods indicated for each pest are not effective.

The application of any practice or action which is not laid down in the Regulations, due to circumstances that may occur derived from climatic or other type of situations, have to be authorised by the Provincial Government Office of Agriculture and Fish with a previous technical justification.

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Chapter 2

Organic production regulation. Recommendations for pest and disease management in vegetables in greenhouse

*Luis Guerrero Alarcón**

1. Introduction

The organic cultivation of vegetables in greenhouse for fresh consumption is a practice laid down in the **Council Regulation** (EC) 834/2007, of 28 June 2007, on organic production and labelling of organic products and repealing Regulation (ECC) 2092/91, and in the Commission Regulation (EC) 889/2008, of 5 September 2008, on organic production, labelling and control, because both of them, in its article 1 refers to "live or unprocessed agricultural products". As all the European Community Regulations, they shall be applied directly to all the member states and any transitional national rules are required, and both entered into force on 1 January 2009.

Many **cultivation techniques** in greenhouses in Almería are perfectly assumed by organic agriculture (OA), as *retranqueo*[1] that, keeping the soil covered with sand, permits the supply of organic matter and little soluble fertilizers at the bottom, or the whitewashing of covers which facilitates temperature control without energy consumption, or the use of drip irrigation and its contribution to water saving, or the biological fight, that avoids, to a large extent, phytosanitary treatments. However, the hydroponic cultivation would be incompatible with OA.

To know the **current** situation of OA it is enough to begin saying that on 31 December 2008, 1317751 ha of OA were registered throughout the country. From this total number of hectares, 754067 ha were registered in Andalusia, and only 4003 ha are vegetables and tubers. In Almería there are 1320 ha of vegetables in OA, most of them -about 700 ha- in greenhouse, to a large extent located in the east area of Almería. The total value of organic production in Andalusia in 2005 amounted to 150

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[1] Set of operations intended for leaving the soil again as it was when it was covered with sand the first time.

millions Euros, while sales reached to 100 millions (250 millions nationwide). The Spanish organic consumption does not exceed the 1 % of the total food consumption, while in some countries where we export our products, the consumption already stands between the 5 and 10 %. All these data show a still incipient sector, but with a basis solid enough to accept these future challenges so important as the FAO announcement of 2002, which said that "*organic agriculture could become a realistic alternative to traditional agriculture over the next 30 years*" (Report: World agriculture: towards 2015-2030).

2. Health in organic agriculture

The two (EC) Regulations that regulate OA which refer to health lay down:

The **(EC)R 834/2007**, when it refers to the specific principles applicable to farming, it sets out that at the beginning the maintenance of plant health shall be tried by preventive measures, such as the choice of appropriate species and varieties resistant to pest and diseases, appropriate crop rotations, mechanical and physical methods and the protection of natural enemies and pests, (article 5 (f)), and also, in the plant production rules, it says that, the prevention of damage caused by pests, diseases and weeds shall rely primarily on the protection by natural enemies, the choice of species and varieties, crop rotation, cultivation techniques and thermal processes (article 12, (1.g)).

Furthermore, the applicable **(EC) Regulation R 889/2008**, in article 5, pest, disease and weed management, sets out in section 1 that where plants cannot be adequately protected from pests and diseases by measures provided for in Article 12 Regulation (EC) No 834/2007, only products referred to in Annex II of this Regulation may be used in organic production. Also, those operators shall keep documentary evidence of the need to use the product. And in section 2 it states that for products used in traps and dispensers, except pheromone dispensers, the traps and/or dispensers, shall prevent the substances from being released into the environment and prevent contact between the substances and the crops being cultivated. The traps shall be collected after use and disposed off safely.

In a crop as intensive as vegetables in greenhouse, to tackle plant health with the tranquillity of knowing how to respond in case of necessity, a phytosanitary **strategy** must be decided, that shall be the most diverse and complete compendium of pest and disease control methods, which are laid down in this document. First of all, sampling and monitoring techniques must be applied, so that the development and intensity of pests and diseases can be known.

Samplings are proposed when assuming that it is not possible to have a comprehensive knowledge of all the populations in each parcel, therefore, an "enough" number of plants is chosen at random, but not a very high number because it would mean the impossibility of sampling in practice. From the observation of the different vegetative organs and the assessment of various phytosanitary problems and other anomalies, decisions are taken about the actions to be carried out and the effectiveness of those that have been already implemented.

To **monitor** a few chromotropic, reticulated and sticky traps are placed in the sides, corridors, doors and windows and placed among the crop randomly; those of yellow colour shall be used for monitoring whitefly, leaf miner, winged aphids and thrips, and those of blue colour are practically specific for thrips. Unfortunately, also auxiliary ones shall be caught, but at least, we will obtain information about their presence. In order to detect the sexual activity of lepidopteran males, delta, moth or water traps are placed with impregnated pheromones on several types of dispensers or diffusers, such as rubber, polythene, etc. If it is possible, a light trap is placed outside the greenhouse. And as advances are produced about the knowledge of trap plants, also they may be used with this purpose.

A self-diagnosis can be tried with the help of the website of the Andalusian Regional Government, although for an accurate diagnosis of pests and diseases, samples must be taken to the official Vegetal Health Laboratory, or to a private one.

All the commercial products that are going to be used must be registered in one of the two **registers** of the Ministry of Agriculture: the Register of Phytosanitary Products and the Register of Other Means of Phytosanitary Defence, which includes, among others, the Biological Control Organisms. It is not necessary the certification of the agricultural inputs as available in OA, because it is enough if they are laid down in the Annex II of the (EC) Regulation No. 889/2008.

3. Preventive, cultural and physical measures

This group of measures is so important that if they were implemented it shall be enough for a correct health, together with the first good decisions about the type of installation and greenhouse construction. The main measures are detailed below.

- Actions carried out by **public intervention**. Some examples of implementing legal or administrative regulations aimed to dissuade, decrease or avoid the presence of phytoparasites in the field or their effects, are the Andalusian Order for the control of viral diseases in horticultural crops, the national programme for the control of vector virus insects in horticultural crops, the obligatory nature of the Phytosanitary Passport for seedlings, quarantines as that applied to the export of tomato to USA, the local plans of rural hygiene, aids for the fight against parasites of new introduction, for the enhancement of structures, for biological fight, etc. In accordance with the first results of the studies that are being done, the called “biological stoppage” shall consist of leaving mandatorily at least a 10-day period since the crop is pulled up until the new plantation, in order to decrease until acceptable levels the presence of thrips, and a 15-day period for whitefly.
- Protection of irrigation water **covering the pool** to avoid water pollution with fungi by air, and if the cover is opaque, the lack of light shall avoid the alga spread.
- The **design and construction** of the greenhouse must consider aspects such as giving priority to the easy control of ventilation, shading, lighting, to reduce problems related with temperature and humidity excess.
- To make difficult the **entry** of insects in the greenhouse, this shall have double door and chamber entrance where a fan directed to the exit shall be installed and it will begin to operate automatically when the exterior door is open. Also, it is important to fill in the possible plastic holes.
- To plant at **low density** in order to enhance lighting and increase aeration. The more light the less risk for the plants to grow poorly looking for light and to lay down, as well as the appearance of “blotchy” in fruits. The higher aeration the less proliferation of fungi, as *Botrytis* o mildew, and bacteria.

- The use of windbreak **hedges** and other barriers against pest invasion. For example, lines of trees or shrubs outside the greenhouse, and they can have a direct action against pests, and, at the same time, to favour the proliferation of beneficial animals because they shelter auxiliary insects and insectivore birds, that increase biodiversity, as we know, in simplified crop systems increase instability. Placing them at 2 or 3 metres distance to avoid shades, these fresh hedges cushion cold and hot air before arriving the greenhouse, decreasing wind strength until 80 % at a distance from the windbreak among 10 and 20 times its height, and also they have an important function of making the landscape beautiful. Other examples, the tough false yellowhead (*Dittrichia viscosa*) is a sticky autochthonous plant that has been used for catching any kind of insects, and lately, as reservoir plant of auxiliary myrids, the lantana or Spanish flag (*Lanthana camara)* for installation of Orius, in the rosebay (*Nerium oleander)* aphids (*Aphis* nerii) are installed that soon shall be parasitized, *etc*.
- Other solution against wind and pest invasion is the placing of agrotextile **meshes** of different thickness, preferably more closely-woven of 10*20 threads per cm^2. This is the most common practice, and partly it is also compatible with the use of fresh hedges.
- To decrease the transmission risk of fungal, bacterial and virus diseases, bleach diluted by 10 % shall be used for **disinfecting** pruning tools and placing the phytosanitary entrance of the greenhouse.
- Planting **resistant or tolerant species and varieties** to some phytosanitary problems. Experience shall give us this information, or even better, the commercial firm that provides us with the vegetal material. The decision adopted shall be combined with rotations, associations, *etc*., because in monocrops, more aggressive breeds of the pathogen can appear which overcome resistance. Some plants have resistance to several parasites and others to only one; others keep resistance during the whole vegetative period or only during some specific stages of their life. Horticultural varieties have been mentioned that lose their resistance to nematodes due to the high temperatures in soil.

- To use **patterns** resistant to soil diseases difficult to avoid, for example, it is very used the watermelon grafting on pumpkin rootstock to *Fusarium sp.* problems, and, although it is less developed commercially, also tomato plants grafted on tomato rootstock and melon plants grafted on pumpkin rootstock are used.
- In a situation of **balanced nutrition**, carried out mainly with organic fertilizers, strong plants shall be produced, with less content of water, and more content of polyphenols, lycopene and proteins difficult to digest by the pathogen microorganisms and insects. Also, when soil is too much enriched, plants can grow excessively and finally they shall be infected by bacteria such as *Pseudomonas corrugata* causing medullar necrosis in tomato. A balanced nutrition of potassium contributes to predispose plants against fungal and bacterial infections. On the other hand, a low relation C/N is adverse for the presence of nematodes, but a high content of nitrogen favours the aphid attack, and such aphid uses it to produce its own proteins.
- To carry out a **rational farming work**, when land requires farming works, avoiding in any case the formation of impermeable soil that, at the same time would favour root asphyxia. When working the land, some eggs, larvae and insect chrysalides shall die because they will be buried or exposed to sun and air on the surface.
- In **plant associations** the repellent or attractant effects of some vegetal species are used for some pests and, so that they protect other plants. Examples: **basil** repels whitefly and thrips in pepper plantations, in addition to it has a toxic oil for insects, in general; **garlic** and **onion** are repellent of several pests; **aubergine** plant attracts great amounts of whitefly and it is very appetizing for red spider mite which allow us to reproduce on it the predators *Macrolophus*, *Nesidiocoris* and *Phythoseiulus*; planting small amounts of **barley** plants (called commercially as *banker*) we can release barley aphids that they do not attack horticultural plants, and with these aphids we can reproduce *Aphidius* and *Aphidoletes* that later they will parasitize the aphid of the crop to be protected. *Nesidiocoris* is reproduced on **false yellowhead** and *Orius* on **apple mint**. Other reservoir plants are being studied, such as **tobacco**, **pumpkin** or **geranium**, to check their effectiveness as sheltering or hosting plants for the reproduction of auxiliary insects within the greenhouse. A good example of bait plant:

planting 1 line of sweet corn among 20 tomato plants against *Heliothis*, after that, **sweet corn** is cut and removed when caterpillars are on it; meanwhile the presence of **lemon geranium** in the plantation repels whitefly, and the **dandelion** repels nematodes. ***Tagetes minuta*** eliminates from soil wireworms, and other species ***Tagetes pátula*** (nemanon) eliminates nematodes. **Mint, nettle and southernwood** repel aphids and …so on and so forth. But we do not get tired of insisting on the need of carrying out trials at a smaller scale before generalising its use, among other things, to check its effectiveness under our conditions.

- With crop **rotations**, the main advantage is the temporal and space separation between plants and their pests and diseases, they are more effective when more specific they are and have less mobility. With this technique it is more difficult to fight against parasites that can come from other greenhouses. For example, if there are nematodes, the place affected shall be left without growing or resistant plants shall be planted on it, or melon crop shall be avoided if there is *Fusarium oxysporum melonis*, but if there is *Phytophthora*, the strategy is not so effective because it affects almost all the crops.
- Choice of the best **sowing date** for each crop in a specific place, avoiding, as far as possible, that the moment of highest aggressiveness of a pest or disease concurs simultaneously with the critical stage of highest sensitivity of the host plant. For example, the trend to plant autumn pepper earlier makes that *Spodoptera* attacks are more and more serious.
- The use of **healthy seeds and plants**, mainly free of virus and bacteria.
- The plastic whitewashing or the use of meshes to obtain **shadings** and in this manner, to avoid light and heat excesses, which can cause several physiopathies such as shrivelled fruits and burns in leaves of young plants.
- Provision of heating where it is really essential, or other form of **thermal protection** (thermal fleece, *etc.*), to reduce or avoid damages due to cold, when it is required to bring forward the production date.

- To irrigate in a **rational manner** and, if it is necessary, to install drainage systems, to make ridges, *etc.*, with the purpose of avoiding the formation of puddles, root asphyxia, hydric stress and proliferation of soil fungi.
- To proceed to the **mechanical or manual harvest** of pests and to remove the organs affected by diseases in order to decrease parasite pressure; but taking into account that when pulling leaves off, also natural enemies are removed. To take out the old crop before drying it up and to clean the greenhouse.
- After pruning, if big wounds have been made, to apply beeswax *mastic*, clay (bentonite) or other cicatrizants authorised in OA, to help **cicatrizing** and avoid the introduction of pathogens through the wounds. If there is already *Botrytis*, copper salt shall be mixed with the cicatrizant.
- Applying ash trail or throwing salt on the surface of the snail itinerary can avoid that they arrive to the plants.
- Bello defines **biofumigation** as the action of volatile substances produced in the biodegradation of organic matter for the control of the plant pathogens. It proposes the following method: a dose of organic matter (biofumigant) between 50 and 100 t/ha, distributed uniformly and incorporated immediately into the soil through passing a rotovator and smoothing down the surface. It is irrigated until achieving the soil saturation and it is covered with plastic to keep, at least during two weeks, the gases and other products resulting from the decomposition of organic matter, and that are: isothiocyanates, ammonium, nitrates, sulphydric, phenols, tannins, organic acids, *etc*. With this method we can act against nematodes, soil fungi (of neck and vascular ones), bacteria, *etc*. Biofumigation can be carried out with green organic matter, o with fresh manure or composted manure, and with a relation C/N between 8 and 20, and, at any time in the year because decomposition does not require too much high temperature (it is enough up to 30 ºC). Also, the composted manure shall provide the crop with the acquired resistance to nematodes and other parasites, because roots shall go through the manure layer.
- **Solarisation** consists of a soft pasteurization of soil, whose effect is produced by the application of heat and humidity for a long time, and it shall not be carried out systematically, only if it is

necessary. If we do not have full confidence in the origin of the organic matter, and it can have any kind of contamination as weeds seeds, bacteria, etc., it is advisable to carry out solarisation after *retranqueo*, and the biofumigation effect can be enhanced. With solarisation the population level of bacteria, pathogens causing vascular and neck diseases, nematodes, weeds and some stages of insects that spend a part of their cycle on the soil, such as thrips and tuta is reduced; it does not cause ecological gap and also it respects beneficial microorganisms, because they are thermophiles. Soil also is benefited from solarisation because of the enhancement of structure and the increase, at short term, of the capacity of cationic exchange.

The five steps to be followed for a correct preparation are:

1. Soil is prepared, if it is not covered with sand, as sowing was going to be done: it is crumbled, the existing plants are removed and it is smoothed down with a plough.
2. It is irrigated until field capacity to increase the thermal sensitivity of pathogen spores and seeds to enhance thermal conductivity. In case of localised irrigation with drippers from 3 to 4 L/h it is irrigated for 7 or 8 continued hours. After that, the irrigation branches must be collected because the heat is going to be produced under plastic would decompose them, and only in the event of soils that keep very little water it is justified to leave them in order to irrigate during solarisation.
3. When we can access to the parcel, the whole soil is covered with thin clear plastic layers (100-200 gauges) treated against ultraviolet rays. The edges are overlapped and fixed to soil with land or sand. This soil cover is removed before planting or sowing. Plastic can be used again if it has not suffered from breakages.
4. The covering period must not be lower than four weeks, and it must be extended to eight or more to fight against the pathogens located in the lower layers of the soil. The best time to apply this technique under our conditions is between the middle of June and the middle of September.
5. It must be taken into account that the roof plastic must be clean and not whitewashed with the purpose of collecting the highest possible sunshine. The sides as well as the roof must be closed

during the application period. As heat in the greenhouse interior is going to be very high, it is convenient to carry out solarisation the same year that it has been decided to change plastic.

- **Methods against superior animals**. Although it is very unlikely that wild boars, rabbits or other superior animals enter into the greenhouse, in case it happens it is convenient to use different dissuasive barriers, such as hedges, meshes and protective tubes, as well as traps and repellents (for example, for rats, moles and mice to place devices with rat poison glue with food attractant, traps, ultrasound devices, *etc.*). Against birds, the methods for frightening them away such as tying video tapes, hanging fishes in decomposition, hanging open containers with bleach, cannons, scarecrows, ultrasound devices, *etc*., are methods to be used while their effectiveness lasts, because birds get used to their presence soon.

- There are different types of **traps** against arthropods:

  - **Fly traps**: They are used against Mediterranean fruit fly (*Ceratitis capitata)*, a pest to be watched in tomato crop if they are going to be exported to USA. Fly traps shall be hung, they can be made of glass or plastic jars and bottles with holes made in their top third, with bait inside made of a part of vinegar or juices, other of sugar and five of water, or with pyrethroids (deltamethrin and lambdacihalothrine) at a dose of 50-100 cm^3/hl of water in addition to a food attractant that can be hydrolyzed protein, or sugar or molasses; although the most effective bait is crystalline biamonic phosphate by 2-4 % in water.
  - **Entomological glue**: it is spread on traps and devices strategically placed, according to the use we want to give it.
  - **Sticky chromotropic traps**: they are rectangular cards or tapes, with a sticky product. They are placed densely for massive catching, especially in the entrance area (doors, corridors, windows and other with openings), and also placed among the crop. They are blue for thrips and yellow for dipterans, winged aphids, whiteflies and thrips. A good strategy is to place them near soil, just when the previous crop is

pulled off and before planting the new one (they will catch mainly thrips), and after that, they must be raised or replaced if they are much damaged.

- **Lighting**: these traps catch mainly butterflies of nocturnal lepidopterans. They are usually place outside the greenhouse, and if they are placed inside, some measures should have to be taken in order to the adult forms cannot enter from the street and only the internal ones are caught.
- **Baits**: For caterpillars that are buried, baits with approximately 1 kg of bran + 10 g of sugar + 10 cm^3 of authorised insecticide in OA and water can be prepared until obtaining a doughy substance; a handful of this substance is applied at the foot of the plant at dusk in order not to get very dry. Against mole crickets and other soil insects, traps with natural cryolite can be used at a rate of 0.5 kg per 1 *kg* of bran, although, before applying it, the certifying body must be consulted to make sure of its authorisation. To attract “wireworms”, pieces of potato or carrot can be placed near the foot of the plants and buried, marking them with a stick and removing them when they have caught worms. Against slugs and snails there are granulated baits that are made of a methaldehyde formula, therefore, they must also incorporate a repellent of superior animals and be distributed out of the ecological parcel, or through traps; snails and slugs are also attracted by sawdust, bier and watered bread dough (that, on the other hand, they have less contraindications that methaldehyde), so that we can make any trap model to catch them, and after that collecting them. Against ants, a mixture of honey or sugar with authorised insecticide can be tested, as bait in a trap or, for example, soaked in a piece of foam rubber.
- **Pheromones** are sexual attractants; generally, they are aimed to males that are used as **fight method** and for monitoring. The fight methods can be based on two ways of action:

  a) to achieve **sexual disruption**, it is made with a massive release of pheromone dispensers to disrupt males when they try mating;

b) to make a **massive catching**: an enough number of traps with pheromones are placed in order to when the number of males decreases, also the population global fertility and the attack intensity is reduced because the total number of individuals decreases. The traps where pheromones are placed can be of delta type, with sticky card or a kind of moth **traps** with water or with an insecticide tablet (deltamethrin and lambdacihalothrine).

Currently, we can find synthesis pheromones for:

- **Lepidoptera**: *Agrotis segetum* (grey worm), *Heliothis (Helicoverpa) armigera*, *Plusia (Autographa) gamma, Spodoptera exigua*, *Tuta absoluta.*
- **Dipterans**: *Ceratitis capitata* (Mediterranean fruit fly).
- **Thrips**: *Frankliniella occidentalis.*
- **Sterile males**. Controlled releases of sterile males are made in citrus and fruit trees to fight against Mediterranean fruit fly. The pest danger is reduced when the percentage of fertile males decreases, due to the disruption and occupation of the territory.

4. Natural products

Natural products used for vegetal health in OA are organic or mineral products that strengthen resistance of plants to parasites (**plant strengtheners**) or eliminate them directly (**phytosanitaries**). In general, they are innocuous for superior animals and do not leave residues, but the **features** of each formula must be known to act consequently. Except for other instruction, treatments shall be applied avoiding the hottest and lightest moments, and being the product just prepared because they decompose very easily. They can be formulated for spraying or sprinkling, but, when applying them, products must reach the vegetal organs to be protected because they do not have systemic capacity, in other words: they act by contact, therefore the vegetal organs emerged after treatment and those to which treatment has not reached will not be protected. Many times, the adequate strategy shall be to locate the treatments in the first **focal points** of the pest or disease, and repeating it few days later. When it is necessary to carry out several generalised treatments, it is convenient to **alternate** products with different active principles to avoid the creation of resistances by parasites. Always the **safety period** that appears in the label must be observed before harvesting. Other important aspect is to know the effect of the product on **natural enemies**. As some products require to be used with a **pH** lower than water ph, to decrease it, vinegar and other authorised acids can be added to the phytosanitary mixture. In the surrounding area of the greenhouse, there will be a **specific place** fit to prepare the treatments in the tank, to wash it, to accumulate the empty containers, etc.

As an exception, and in order not to complicate very much the structure of this article, in this section we also include the few **synthesis** chemical products authorised in OA, with its corresponding limitations.

The traditional ways of obtaining the natural preparations are:

a) **Crushing** of raw material.

b) **Flower extract**: Flowers are moistened and crumbled, and after that, the paste is squeezed pressing it.

c) **Maceration**: Vegetal material must not ferment, it can be left in water 24 hours at the most, and after that, it must be filtered.

d) **Fermented slurry**: It is prepared in containers with the lid on, but stirring it every day in order to air is introduced into it. It is fermented for more than 14 days, until it stop giving off foam, at this moment, it can be already used. Slurry that has been fermented little time is used 4 or 5 days later.

e) **Infusion**: Herbs are put to soak in very hot water where they remain for 24 hours.

f) **Decoction**: Put the herbs into water for 24 hours, boil them on a low flame for 20 or 30 minutes and then, let them cool down.

Among the **natural preparations** used in OA, the following ones are found, grouped by their effect against diseases or pests, with some recommendations as guidance to obtain them if we want to prepare them at home.

Against diseases:

- **Horsetail**: It has a high content of silica, of a toxic saponin for fungi, equisetonin, of flavonoids and alkaloids (as nicotine that makes it to have some insecticide effect). It can be obtained by decoction of 1 *Kg* of fresh chopped horsetail, or 150 *g* in powder in 10 L of water. If it is mixed with some adherent its effectiveness enhances. When it is diluted in water at a rate of 1:5 is recommended against mildew, bacteria, rusts, etc.
- An extract of **pulp and citric seeds** that has been already formulated, with a high content of organic acids (mainly ascorbic), induces the synthesis of phytoalexins improving the natural defence of plants against bacteria, fungi and algae.
- Several proposals for the use of **garlic and onion**, they are used as antibiotic, fungiestatic and bacteriostatic due to the power of allyl polysulphide, and as insecticide and repellent thanks to alliin that is converted into allicine. An infusion of 700 g of cut bulbs in 10 L of water that can be used without dilution at a rate of 3 treatments at intervals of 3 days; or a slurry of 10 kg of onions or 1 kg of garlic in 100 L of water, using a dissolution of 10 % in classic spraying.
- The infusion of 50 g of **camomile** in 10 L of water protects against fungi, in general, and specially melon mildew.
- **Sulphur**. It is preventive and healing against oidium. It can be phytotoxic above 28 ºC. It must not be mixed with oils or to be applied after them. It can be used in spraying, as wettable dust, sprinkling and by sublimation. It is desirable to sprinkle during the highest humidity hours to facilitate its adhesiveness, and taking into account that it can damage the auxiliary fauna against pests.
- **Potassium permanganate**. Against oidium and bacteria, and it has also an algicide effect. It is applied spraying or brushing on the focus, without mixing with other products and leaving a period of more than 21 days from a treatment with oil. Although in Spain this product is registered for use in vegetables, the (EC) Regulation 889/2008 only authorises it for fruit trees, olive trees and vines.
- **Copper products**. As preventive treatment against mildews, *Botrytis*, bacteria, *etc*. They are formulated in the form of copper hydroxide, copper oxychloride, tribasic copper sulphate and

cuprous oxide; they are carried out by spraying, sprinkling or brushing if it is to spread paste. Do not mix with oils. The cupper accumulation on soil must be avoided due to its biocide power and blockage of other mineral elements (6 kg copper cannot be exceeded per ha per year).

- **Silica dust rocks** (quartz sand). Silicon dioxide acts as drying; therefore, it is used for fighting against fungi and bacteria.
- **Homeopathic and isopathic preparations**. They are obtained by crushing or incinerating the ill plants or the same parasites that cause the disease and its later dilution, because when they are diluted at infinitesimal doses induce the plant to a resistance reaction to adversity.
- **Dairy products**. Skimmed milk diluted between 10 and 50 % is used as disinfectant of hands and of pruning and grafting tools, because their proteins inhibit transmissible virus by contact (van der Berkmortel, 1977). A milky enzyme, lactoperoxidase, has anti-oidium capacity.
- **Beehive propolis**. It has a general antibiotic function, due to its high content of resins, waxes, flavonoids, organic acids, minerals and vitamins, and they are presented as aquous, hydroalcoholic and alcoholic solution.

Against pests:

- **Pyrethrum** (it comes from *Chrysanthemun/Tanacetum cinerariaefolium*). The pyrethrum is the oleoresin extract of dried chrysanthemun flowers and its active principles, the pyrethrins have effect on aphids, flies and a broad spectrum of pests. They act by contact, as they are lypophilic, penetrate quickly into insects and attack their nervous system. An infusion of 1-2 kg pyrethrum flowers can be prepared in 100 L of water. It is formulated in liquid and powder, and it contains usually the synergizer piperonyl butoxide, coformulating inhibitor of pyrethrins enzymatic degradation (in this case, before using consult the certifying body).
- **Quassia** (from *Quassia amara*). Against insects in general, as insecticide and repellent. It has a high content of active substances called quassinoids, which do not eliminate insects but stop their

development and cause rejection. Put 2-4 kg of quassia shaving to soak in 90 L of water for 24 hours, after that, remove the shaving and boil it in 10 L of water that will be then added to the 90 L.

- **Rotenone** (it can comes from roots of *Derris sp.,* de *Lonchocarpus sp.* or *Terphorosia sp*.). Against aphids, thrips, mites and broad spectrum. It acts by contact and ingestion on the nervous system and interferes the mitochondrial respiration of insects. It is very toxic for fish. A decoction of 1.5-2 kg of ground roots in 20 L of water can be made. If you are going to use a product that contains piperonyl butoxide as synergizer, consult the certifying body before applying the treatment.
- **Neem** (*Azadirachta indica*). It has a broad spectrum. It acts by contact and ingestion according to the different active principles it contains. Nimbin and salannin cause repellent and anti-alimentary effect on several insects and mites, azadirachtin affects insect physiology, mainly the larvae, inhibiting the growth and altering the metamorphosis when interferes with the steroid hormone ecdysone, on females, it acts reducing their fertility and causes egg sterility. It has also a certain upward and downward systemic effect on plants. Other acquired resistances have not been described. It is available in commercial formula, or preparing a maceration of 0.5 kg of ground seeds in 20 L of water.
- **Spinosad**. It is obtained from fermentation of actinomycete bacteria *Saccharoployspora spinosa*. It is composed of a mixture of spinosyns (A and D). It has neurotoxic effects against thrips and caterpillars. Be careful if hymenopteran parasitoids are released because it can be harmful for them.
- **Dust of silica rocks** (Quartz sand). It acts by dehydratation against sucking and defoliating insects, and against mites, and as snail and insect repellent when it is sprinkled at the foot of the plants.
- **Nettle**. A complete slurry with 1 kg of fresh nettle, or 250 g in powder of dried nettle, in 5 L of water that it is finally diluted at a ratio 1:10 to be used against aphids. Nettle maceration without diluting can be used to strengthen the plant indirectly against aphids.
- **Tobbaco**. The aqueous solutions of alkaloid nicotine are used as insecticide and acaricide. They can be obtained leaving to settle 1.5 kg of tobacco vein in 20 L of water for a day. Potassic

soap as wetting agent enhances its adherence. It acts, mainly, by inhalation, but also, by ingestion and contact because it is very volatile and penetrates the insect tegument. The applicator must maximize the security measures. The commercial formulas made of nicotine sulphate are not authorised in OA.

- **Garlic or onion**: See the section "Against diseases". Against red spider mite, caterpillars and aphids, and as gastropods repellent.
- General repellents of insects can be prepared with common **rue, tansy, wormwood or southernwood** through infusions of 0.5-1 kg of dried herb per 100 L of water, and with **eucalyptus** in maceration of 750 g of ground leaves in 20 L of water. At this point, I would like to suggest a big agreement of the whole sector to change the current trend consisting of researching about the biocide action of natural products, following the guidelines set out by the synthesis products, and to initiate lines of research about the **repellent** effect of some of these substances of natural origin.
- **Sulphur**. Preventively as general repellent, and as acaricide against red spider mite, broad mite and eriophids. It can be phytotoxic above 28 ºC. It must not be mixed with oils or to be applied after them. It can be used in spraying, as wettable dust, sprinkling and by sublimation. It is desirable to sprinkle during the highest humidity hours to facilitate its adhesiveness, and taking into account that it can damage the auxiliary fauna against pests.
- **Soft or potassium soap**. It has contact action because it softens the exoskeleton membranes that produce alterations of the cellular physiology; it also causes asphyxia. It is used against soft shell insects and mites, and it is also effective to clean molasses and to eliminate sooty mould fungus. We can find it in the market formulated from fatty acids of vegetal oils and potassium salts. A handmade formula: to prepare 5 kg of sieved ashes and ½ kg of soap in 10 L of water on to heat during 20 minutes; to use it, dissolve 1 L of that broth in 20 L of water.
- **Ferric triphosphate** applied on soil between the cultivated plants, against snails and slugs.
- **Gelatin**. 9 g of gelatine in powder are dissolved for each 1.5 L of warm water and it is sprayed on aphids and mites.

- **Vinegar**. Home remedy used normally by the farmers in spraying against aphid, dissolving it between a 10 and 20 % in water.
- **Crustaceans exoskeletons**. Actually, it is an organic provision for soil, but with a collateral effect against nematodes (thanks to chitin).
- **Paraffinic oil** of narrow range. Against thrips, miners, aphids, whiteflies and mites; it acts by asphyxia and disintegration of the chitin of the protective shield. It has to be applied in high volume and with high pressure, shaking the broth constantly. If a treatment with sulphur has been made previously do not apply paraffinic oil before a 30 day-period.
- **Vegetal oils**. Thyme, linseed, soya, mint, pine, caraway, neem, basil, etc. They are used as wetting agents, repellents and biocides of a wide range of pests.

5. Biologic fight

One of the basic principles of OA is that natural enemies of pests must be protected and promoted, that is, to implement biological fight. With this **biological fight**, the natural presence, mainly, or introduced of biological enemies is used to fight against pests. There are many types of introducing natural enemies, also known as auxiliary ones: through boxes, envelopes, cards, and even placing in our greenhouse vegetal organs of places where their presence have been detected (taking precautions of not introduce also a pest or disease that we did not have). We must work towards the achievement of acclimatizing the exotic auxiliary ones or, at least, their stay while the crop lasts, for example, with the management of reservoir plants, or if chemical products have to be used that are innocuous for auxiliary fauna, because, before a treatment, natural enemies die usually more easily than pests because they are more contaminated with pesticides due to they have more mobility and a higher exposure area, to they are of small size and also they have enzymatic systems less powerful. Other remarkable characteristic of the biological control organisms is that they are totally harmless for the farmer and the consumer. The enemies of phytoparasites are divided by their way of action, into predators and parasites. **Predators** are usually superior animals, mites or insects that act devouring their preys; and **parasites** are insects, nematodes, fungi, bacteria or viruses that act at internal pest level, insects (parasitoids) feeding on them and the rest (pathogens) making them ill and causing their death. The formulas made of fungi, bacteria and virus must be applied at dust, with wetting agent and without mixing with copper fungicides.

The main agents of biological and microbiological fight (fungi, bacteria and virus) used are described below:

- **Superior animals**. As in OA the interspecific relations and the maintenance of a live ecosystem are of paramount importance, all the individuals that can help to keep pests at bearable levels have to be protected. Among the insectivore birds we can highlight thrush, hoopoe, blackbird, *etc.*, as diurnal birds; swallow and swift as twilight birds; and little owl as devouring higher insects and rodents, as night bird. Other interesting superior animals as bats, hedgehogs, toads and snakes are protected by Law, therefore, any action that attempt on their life may be reported before SEPRONA and the Environment Provincial Office.
- **Predators**:

***Amblyseius (Neoseiulus) californicus**,* phytoseiid non exotic mite which acts on all the stages of red spider mite. Frequently, it appears in a natural way. Adults have a medium size of 0.3-0.5 mm and appearance of small spider, with pear shape and orangey-reddish colour, its legs are long and the back surface is reticulated. The speed of its biological development is higher than its prey's development, although its fertility is lower. It is found mainly on the leaf underside. It preys through small stylets which absorb the fluid content of its prey's bodies. It tolerates sudden temperature fluctuations and relative humidity, bearing relative humidity of 30-40 %, and temperature above 32 ºC.

***Amblyseius cucumeris**,* phytoseiid non exotic mite that feeds mainly on hatched eggs and thrips larvae of first stage. It can appear spontaneously together with thrips colonies. Adults have elongated bodies, almost piriformis, and with two side hollows in the central part. Their size is 0.3-0.5 mm. They are almost transparent and with long legs that allow them to move quickly. Optimum conditions for their development: temperature around 18-20 ºC, and relative humidity above 50 %. High temperatures and low relative humidity limit their activity.

Amblyseius swirski, phytoseiid non exotic mite, it is very similar to *A. cucumeris,* which has been introduced recently. It is very effective against thrips and whitefly; a certain residual population can survive even if the absence of preys.

Aphidoletes aphidimyza, non exotic dipteran, it is an aphid predator. Larva is about 2.8 mm long, is elongated and transparent orange with two whitish stripes in its body sides. Optimum temperature and relative humidity for the development of *Aphidoletes* is 23 ºC and 80-90 %, respectively. When the predatory larva finds the aphid, it injects a poison into the aphid to paralyse it, sucking later the liquid juice of the prey. It can be endured in long cycle crops.

Coccinella septempunctata and ***Adalia bipunctata***, are the seven spotted and two spotted ladybirds respectively, non exotic, predators, the larvae as well as the adults, of many species of aphids in all their development phases.

Coenosia attenuata, known as killer fly, it is autochthonous and it is an ally to fight against whitefly, it catches adults even in the air.

Cryptolaemus montrouzieri is the most used natural enemy against cotton mealybug. This is a exotic species. The adult forms, that look like ladybirds, as the larvae, which are covered with a waxy coating which make them similar to their preys, are predators of all the stages of mealybugs. The optimum relative humidity is between 70-80 %, and the search behaviour stops above 33 ºC and below 16 ºC.

Crhysoperla carnea*.* Common green lacewings are general predators (or little specific). *C. carnea* is not exotic and is a voracious predator of many species of aphids. Eggs isolated or in small groups, are found attached to leaves by a long filament at one end. Larvae are about 8 *mm* long; they have hairs in the back of the body, a pair of longitudinal dark stripes, together with several parallel transversal stripes. Adult common green lacewings are pale green with two pair of long transparent green membranous wings and abundant veins. Its predatory activity keeps with a temperature range between 12 and 35 ºC, and the interval of 60-90 % of relative humidity.

Feltiella acarisuga*.* Larvae of this non exotic dipteran are predators of all the stages of red spider mite. They are orangey-yellow and about 1.7-1.9 mm long. Pupae are formed within an off-white cocoon in the underside of the leaf attached to a nerve. Adults are pink-brown and have long legs, they can detect in the air the red spider mite focuses and to lay their eggs there. Its optimum development is with temperatures between 15-25 ºC and relative humidity between 60-90 %.

Macrolophus caliginosus, is a bug belonging to the family of *Miridae,* non exotic, whose adult and nymph forms prey on all the stages of whitefly, *Bemisia tabaci* as well as *Trialeurodes vaporariorum*, although it can be also feed on larvae and adults of thrips, mites, aphids and lepidopteran eggs. Eggs are embedded by females in the vegetal tissue. Nymphs are yellowish-green and their eyes are red. Adults have a greenish-yellow body, with spots or dark parts on the head, the antennae, the back and the leg ends.

Nabis pseudoferus ibericus, autochthonous bug that feeds only on insects. It is effective against aphids, whiteflies and lepidopterans.

Nesidiocoris tenuis, polyphagous bug belonging to the *Miridae* family, is similar to *Macrolophus*, it is not exotic. It is effective against thrips, whitefly, red spider mite and small aphids. A high population of *Nesidiocoris,* coinciding with a scarce presence of preys and long winters can also produce damages on crops.

Orius laevigatus, also known as “flower bug”. It is not exotic, it shows great ability to move and in adult stage can fly. The adult forms as well as larvae and nymphs act on thrips larvae as well as on adults. In the absence of preys they can feed on pollen. Larvae and nymphs are yellowish with visible red eyes. Adults are 1,4-2,4 mm long, they have a brown body, with a long mouth and they can move. Its optimum life conditions are between 20 and 30 ºC of temperature, minimum day length of 10-11 hours, and relative humidity above 50 %.

Phythoseiulus persimili*s,* it is not exotic, it appears spontaneously and is an exclusive predator of spiders of genus *Tetranychus*, to which it preys on all their stages. Adult has a bigger size than spider and great mobility, with pear shape, bright red colour and long legs. It is more effective with temperatures between 15 and 25 ºC and relative humidity between 60 and 90 % (it shows little tolerance to high temperatures and low relative humidity).

- **Parasitoids**:

Aphidius colemani is a non exotic wasp that parasitizes aphids. Adult is dark colour, thin, with long antennae and a remarkable wing venation. It is more effective with temperatures between 20-30 ºC. Female can detect an aphid colony at long distance, when it finds the colony it palpates the aphids with its antennae and lay an egg on it, the egg hatches and the resulting larva begins to feed within the aphid, which is taking gradually a golden colour ("mummy"). The new adult of parasitized aphid emerges through a round hole. Other hymenopterans parasitize also *Aphidius* (hyperparasitism) larvae, producing in this case an exit hole with its serrated edge.

Diglyphus isaea, this ectoparasite is the main enemy of leafminer larvae. It is not exotic. When a mini-wasp finds a leafminer larva, it paralyses it and stops feeding, then lays egg next to it within the gallery, so that the new born *D. isaea* larva feeds on leafminer larva, consuming it totally, black excrements can be observed through the leaf. The wasp emerges outside perforating the gallery by the upper leaf epidermis. Also, the predatory action of the adult female has been mentioned, that "bites" the leafminer larvae sucking their juice. Adult has short and articulated antennae, is dark colour with metallic highlights and is about 1.5 mm long. The development limits are between 6 and 25 ºC and low lighting affects negatively the parasite.

Encarsia formosa, is a exotic wasp parasitoid of whiteflies in greenhouses. When the *Trialeurodes* pupa is parasitized it turns black; when the *Bemisia* pupa is parasitized turns transparent brown. Adults chew a round exit hole before emerging. Adults measure about 0.6 mm and are black. Its suitable temperature is 24 ºC (between 15 and 30 ºC), requires medium lighting and relative humidity between 50 and 80 %.

Eretmocerus mundus, it is not exotic and is a natural enemy of whitefly larvae *Bemisia tabaci*. The larva of first stage of parasitoid is introduced within the whitefly larva. By transparency, the *Eretmocerus* larva can be seen when it is developed of yellow-golden colour, and later, the dark eyes and wing rudiments of adults. When adult emerges, the parasitized larva shows a round hole, while normal shedding of flies causes a T-shape hole. A small amount of *Bemisia* larvae die by feeding stings of adult *Eretmocerus*, which is a wasp 1 mm long, of yellow-brown colour, with three typical red points in triangle shape on the head, and dark green eyes.

Trichogramma evanescens, small exotic wasp that parasitizes lepidopteran eggs.

- **Preparations with nematodes**:

Steinernema feltiae, against larvae of soil flies (*Sciaridae*) and other insects that spend their life cycle on soil (thrips, *etc.*), and against nematodes. The product is applied with high humidity in order to it activates quickly and penetrates in the larvae by their natural holes. Once the nematode reaches the intestines, perforates them and releases a toxic bacterium that kills the larva. *Steinernema* reproduces in inside the parasitized enemies.

Heterorhabditis bacterióphora, entomopathogenic nematode. It is formulated with a natural polymer from mollusc's chitin (called chitosan). Against whitefly, thrips, worms and soil insects.

- **Fungal preparations**.

Home preparations can be made with simple laboratory techniques or using commercial products made of:

Ampelomyces quisqualis, oidium development suppresor, which may have healing action.

Arthrobotrys botryspora* and *Paecilimyces lilacimus, against nematodes.

Beauveria bassiana, for whitefly. When applying the product, the fungus spores come into contact with whitefly and germinate on it; the fungus degrades the epidermis using enzymes, and invades the insect body that shall die 7-10 days later. It is not necessary a high relative humidity.

Lecanicillium muscarium, before ***Verticillium lecanii,*** to control whitefly and aphid. It has to be used at high humidity to be effective, because spores are sprayed that have to be developed, because hyphae parasitize the insect body and destroy it in 7-8 days.

Gen*.* ***Trichoderma,*** against soil fungi and others, resulting also a good stimulator of root growth.

- **Bacterial preparations.**

From non genetically modified bacteria.

The most used is ***Bacillus thuringiensis,*** whose active matter is composed of bacteria spores and a protein crystal (endotoxin), being the crystal the main active principle; it is effective against young caterpillars of lepidopterans and little effective on miner larvae in horticultural crops (var. *aizawai* and var. *kurstaki*), and potato beetle (var. *tenebrionis*); it acts by ingestion, paralysing the intestine, and caterpillars stop feeding.

The ***Streptomyces griseoviridis*** preparations are used against soil fungi, and other fungi transmitted by seeds.

Others: there are also other bacterial preparations formulated with stimulators for control of algae, soil fungi, oidium, mildew, botrytis.

- **Viral preparations.**

Those belonging to the family ***Baculovirus*** are interesting in agriculture; the nuclear polyhedrosis virus and the granulosis virus, against young larvae of lepidopterans. They act by ingestion, larvae stop feeding and die. Commercial products are used or they are prepared taking infected larvae, they are macerated in clean water in a sterile container, and they are applied or kept in a hermetically sealed container, preserving them frozen or in a cool and dark place. Against bacteria also culture of bacteriophage virus of rhizosphere can be prepared, although with laboratory methods more sophisticated that require a specific preparation.

6. Control of adventitious plants

Weeds are not crop enemies in the narrow sense, in fact, if they are managed well, become interesting allies, because they are CO_2 drain, keeping carbon in their structure for photosynthesis, they are a sign of the presence of auxiliary enemies against pests and, cut in the surface, provide humus to soil when they decompose. When we do not want their presence, the best way is to act **preventively**, for example, avoiding bad composted manure that contains seeds.

- The **paddling** with non contaminated vegetal rests, as straw, fibber coconut sheets, jute sheets, *etc.*, with polyethylene plastics, even with the disadvantage that they do not allow soil to breathe, or with agrotextiles as polypropylene mesh, or sand, or cardboard, *etc.*, they are also a possibility to fight against weeds, but they cannot be improvised, they will have to be programmed in advance.

- **Mechanical farm works** as mowing or with hoes, or works with tractor equipped with different implements such as rotary tillers, cultivator and rotary brushes, with brushcutter or mower, shall be chosen according to the general strategy of greenhouse management.
- Also **thermal treatments** can be made through solarisation during summer, or with propane or butane burners.
- Other technique used is the **soil management** through the called false sowings. Land is prepared as is going to be sown, but sowing is not made, with the purpose of inducing the emergence of the first weeds, that we will destroy later not to compete with the first crop stages; this technique can involve the change of the habitual date of sowing.
- In some ecological crop systems, **livestock** is introduced to feed on weeds, at the same time they leave a small manuring, but also an unavoidable resowing of weeds.
- **Natural herbicides** begin to be used, that they would be formulated on fungal and bacteria preparations and inhibitory allelopathic substances. Allelopathy is originated from the chemical compounds released by plants that affect other plants. The release mechanisms of allelopathic agents are: by volatization, leaching, exudation and vegetal residue decomposition. As a starting point, we can consider, for example, the well-known deterioration effects as those produced by dissolution of walnut extract on tomato plant, the eucalyptus extracts on many plants, the presence of *Amarantus* and *Chenopodium* on green bean, of pumpkin on several arvensis, etc.

In OA the use of **chemical synthesis herbicides** is not allowed.

7. Specific actions

Although there are much work to be developed by scientists and experimenters of OA, we dare to propose as general **phytosanitary strategy** the biological fight in the fight against pests, the management of relative humidity and the temperature against fungi and bacteria, and the use of varieties resistant to virus. And only if necessary, the products of Annex II shall be used. Pesticides and phytosanitary products of (EC) Regulation 889/2008, which it will oblige us to programme again the releases of auxiliary insects, because these products are not fully innocuous for them.

Below, we have detailed some of the specific actions to be made according to the phytoparasites and the most foreseeable accidents under our conditions, if they mean a real danger for our crop. These actions have been grouped according to if they are made before planting, when planting is being made or with the crop already planted, and in each case, a brief description of the damages caused by the different pathogens is made and some action guidelines are given.

Before planting:

- If the presence of **soil fungi** can be foreseen, *Pythium sp., Phytophthora sp.* and *Rhizoctonia sp*., causing neck and root diseases, or vascular diseases caused by *Fusarium sp.* or by *Verticillium sp.*, that provoke green wilting of a part or the entire plant; or microscopic **nematodes** (*Meloidogyne sp.*) that once they have been introduced in the roots cause some knots or "root galls" that do not permit the plant development; and against **adventitious weeds** and **some bacteria**, in any of these cases, if there are precedents of previous attacks, a solarisation as a preventive measure have to be done during summer.
- To pull up the **weeds** and sulphurise the post structure to clean possible **pest reservoirs**.
- If there are **saline** outcrops on the soil, wash it.

When planting is being made:

- If soil fungi causing **neck and root diseases** (genus *Pythium sp., Rhizoctonia sp., Phytophthora sp.*) are detected, that produce green wilting in transplanting, the pool and channels should be covered as preventive measure; and, if necessary, to proceed to spray the root ball, or, if it is possible, to immerse or irrigate the seedlings with a copper solution , and, as a last resort, taking into account that the accumulation of copper on soil is harmful, a “cacharreo” (application of the treatment directly on the plant neck) would be made on the plant already transplanted. The following summer, solarisation would be made. Other option is the introduction of Trichoderma as antagonistic fungus.

With crop already planted:

- **Aphids**. *Myzus persicae,* of green colour, and *Aphis gossypii*, of dark colour, transmit virus, deform and dry the tender organs, and produce oily honeydew on which sooty mould grows. This pest is specially dangerous in cucurbits. Treatments can be made with rotenone, potassium soap or paraffinic oil. Biological fight with *Aphidius sp.,* placing first a “banker” and, if it is necessary, to do releases after that.
- **Caterpillars**. *Spodoptera* littoralis, caterpillar is brown and it has little incidence as pest, however, *Spodoptera exigua*, whose caterpillar is green, bites leaves respecting at the beginning the epidermis, also the skin of watermelon fruits and the terminal buds of peppers that come to close them, and then they devour the entire leaves. *Helicoverpa (Heliothis) armigera* feeds on leaves and penetrates into fruits. *Plusia (Autographa) gamma* or “camel” feeds mainly on leaves. The tomato moth (*Tuta absoluta*) attacks the fruit, in which it penetrates, as well as the leaves, where it makes galleries from which it feeds on mesophyll respecting the epidermis, and the stems to which it perforates. Treatments made with *Bacillus thuringiensis* or with a baculovirus or spinosad are recommended in the first larvae stages. Works are being carried out to introduce *Nabis* and with *Trichogramma,* with good expectations at short term.
- **Whiteflies**. *Bemisia tabaci*, adults have the wings stuck to the body with tile-shape, they transmit the tomato yellow leaf curl and the yellow stunting disorder virus in cucurbits and green beans; *rialeurodes vaporariorum*, the adults have the wings parallel to body in “delta” shape, it transmits the yellow virus in melon. The adults as well as larvae and the honeydew they produce are found in the underside of the leaves that are gradually filled up with the “sooty” fungus, even they become yellow and dry up.

As preventive method, yellow cards are placed in a massive way. In the treatments the following substances can be used: pellitory, potassium soap or paraffinic oil. The biological fight shall be made through *Eretmocerus mundus, Amblyseius swirski,* and preparations made of the fungus *Beauveria bassiana.*

- **Thrips**. *Frankliniella occidentalis* transmits the spotted wilt virus in tomato and pepper and, furthermore, it causes silver damages in leaves and fruits that are necrotised later. As preventive method, several blue cards are placed. If it is necessary to treat it, it will be made with: azadirachtin, spinosad or paraffinic oil; the addition of sugar as synergizer enhances the treatment effectiveness. The biological fight is made with Orius *laevigatus* (little effective in tomato), *Amblyseius cucumeris, Amblyseius swirski* and *Nesidiocoris* mainly.
- **Leafminer**. *Liriomyza trifolii.* The presence of adults causes slight damages in the upper side of the leaves due to whitish dotted caused by feeding and laying wounds, or attempted laying. Galleries in the leaves are produced by larvae feeding. Yellow sticky cards are placed to catch adults. Treatments can be applied with paraffinic oil, rotenone, pellitory or azadirachtin; or to carry out biological fight with *Diglyphus isaea*.
- **Red spider mite**. *Tetranychus urticae.* It is detected by the presence of red mites with two dark side spots, and the symptom, in all the vegetal organs, is bleaching and yellowing, spider's web is observed in more intense attacks. To control it, early treatments shall be made to the focus with wettable sulphur or sprinkling sulphur, but taking into account that it can damage the auxiliary ones, or with paraffinic oil. Biological fight with *Phythoseiulus persimilis, Feltiella acarisuga o Amblyseius californicus.*
- **Broad mite**. *Polyphagotarsonemus latus*. Tarsonemid microscopic mite that produces deformations, nerves curling and, sometimes, defoliation, mainly in pepper. To control it, treat early the focuses with sulphur or paraffinic oil. For its biological control, release *Amblyseius californicus* or *Amblyseius swirsk*.
- **Tomato russet mite**. *Aculops lycopersici*. It is an eriophyidae mite which sucks the sap and causes bronzing or rusting in stem and leaves from the basal part in upward way. Leaves are dried

up. This process is accelerated under high temperature and low humidity conditions. Its spread must be avoided with cultural practices and tools. The focus must be treated with sulphur from the first symptoms.

- When we consider the presence of **ants** harmful for plants, pots or beehives, several strategies can be tried: treat with rotenone the anthill, to place traps with entomological glue or grease when ants pass, to sprinkle repellents made of silica or sulphur, to place baits with authorised insecticide in OA and honey or sugar or ground grains that the ants will carry to the anthill feeding on them, or to spray with wormwood or tansy as repellents.
- When we stop using synthesis phytosanitary products, we must know in order to prevent them that, some insects that were not considered as **pests** any more, arise again, as the southern green stink bug (*Nezara viridula*), the European mole cricket (*Gryllotalpa gryllotalpa*), the citrus mealybug (*Planococcus citri)*, the wireworm *(Agriotes sp.)*, the red bug or San Antón *(Oxycaremus lavaterae)*, the beetle *Gonocephalum rusticum*, etc. Generally, the preventive measures shall decrease the presence of these pests.
- **Powdery Mildew** of Solanaceae *(Leveillula taurica)* and of cucurbits *(Podosphera fusca).* The optimum conditions for its development are relative humidity 70 %, 10 ºC<Tª<35 ºC. At the beginning there appear whitish felts in the upper and underside of the leaves, or yellowish spots on the upper leaf surface of solanaceae, which evolve to necrotic spots. Preventive measures: to use resistant varieties and to remove the basal leaves with pathogen presence to diminish the amount of inoculum. From the beginning, the attack shall be treated with sulphur. Some experiments are being testing to fight against them, with algae and fungi preparations, enzyme formulas that increase the resistance mechanism of plants, etc., but we do not have still conclusive data with respect to effectiveness and safety.
- **Late blight of solanaceae** *(Phytophthora infestans)*. At the beginning, oily spots are observed on leaves, then they are necrosed, and rhomboid shapes appear in the leaflet tips from the central nerve, or necrotic spots in stem or in fruit peduncles.

- **Cucurbits mildew (downy mildew)** *(Pseudoperonospora cubensis)*, the oily spots become soon polygonal respecting the veins, necrosing the upper side and appearing a greyish felt in the underside of the leaf. The optimum conditions are: relative humidity 90 %, and temperature between 10 and 25 ºC. To control it, it is necessary to enhance ventilation in the greenhouse. Treatments against both mildews shall be made with copper products or silica dust. The formulas with enzymes are still in development stage.
- **Gray mould**. *Botrytis cinerea* needs optimum conditions to develop, 95 % of relative humidity, and temperature between 17 and 23 ºC. Gray mycelium of fungus appears in wound areas (due to removing of stems or other reasons) and sensitive organs; in tomato fruits, the called “ghost spot” is shown, which consists of small rings with external white crown and dark centre. Once fungus has appeared, the area affected by mould has to be cleaned and after that, apply a copper paste with a brush, or carry out a general treatment with copper products or silica dust, favouring always the greenhouse ventilation to lower relative humidity. Preparations with enzymes are still in development phase.
- **Bacteria**: *Erwinia sp.* causes watery rot in stems and fruits which gives off bad smell and survives in soil, water, vegetal rests, *etc*. *Pseudomonas sp.* produces necrotic spots and is transmitted by seeds and vegetal rests. *Clavibacter sp.* produces a general aspect of “burnt” on the plant, it is spread by seeds, vegetal rests, *etc*. They are developed in high humidity periods and temperature between 20 and 25 ºC. To control them, copper products must be applied preventively, without forgetting to increase ventilation in greenhouse and act about the rests of previous crops (remove them, solarise, *etc.*).
- **Virus**: Although there is a spread trend to think that plants grown organically tolerate better the presence of pathogen virus than those grown conventionally, however, this theory has not been shown scientifically and in view of the uncertainty of a virosis, and due to it is impossible to keep the presence of vector organisms to zero, it is recommended to grow resistant plants and maximize the preventive measures.

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9. Annex II of R (EC) 889/2008

Pesticides - plant protection pruducts referred to in Article 5(1)

Notes:

A: Authorised under Regulation (EEC) 2092/91, and carried over by Article 16(3) (c) of Regulation (EC) 834/2007.

B: Authorised under Regulation (EEC) 2092/91.

1. Substances of crop or animal origin

Authorisation	Name	Description, compositional requrement and conditions for use
A	Azadirachtin extracted from Azadirachta indica (Neem tree)	Insecticide
A	Beeswax	Pruning agent
A	Gelatine	Insecticide
A	Hydrolysed proteins	Attractant, only authorized applications in combination with other appropriate products of this list
A	Lecithin	Fungicide
A	Plants oils (e.g. mint oil, pine oil, caraway oil)	insecticide, acaricide, fungicide and sprout inhibitor
A	Pyrethrins extracted from chrysanthemum cinerariaefolium	Insecticide
A	Quassia extracted from quassia amara	Insecticide, repellent
A	Rotetone extracted from Derris spp. and Lonchocarpus spp. and Terphrosia spp.	Insecticide

2. Micro-organisms used for biological pest and disease control

Authorisation	Name	Description, compositional requrement and conditions for use
A	Micro-organisms (bacteria, viruses and fungi)	

3. Substances produced by micro-organisms

Authorisation	Name	Description, compositional requrement and conditions for use
A	Spinosad	Insecticide Only where measures are taken to minimize the risk to key parasitoids and to minimize the risk of development of resistance

4. Substances to be used in traps and/or dispensers

Authorisation	Name	Description, compositional requrement and conditions for use
A	Diammonium phosphate	Attractant, only in traps
A	Pheromones	Attractant, sexual behaviour disrupter, only in traps and dispensers
A	Pyrethroids (only deltametnrin or lambdacyhalothrin)	Insecticide, only in traps with specific attractants, only against Bactrocera oleae and ceratitis capitata Wied

5. Preparations to be surface -spread between cultivated plants

Authorisation	Name	Description, compositional requrement and conditions for use
A	Ferric phosphate (iron (III) orthophosphate)	Molluscicide

6. Other substances from traditional use in organic farming

Authorisation	Name	Description, compositional requrement and conditions for use
A	Copper in the form of copper hydroxide copper oxychloride, (tribasic) copper sulphate, coprous oxide, copper octanoate	Fungicide. Up to 6 kg. copper per ha year For perennial crops, members states may, by derogation from the previous paragraph, provide that the 6 Kg copper limit can be exceded in a given year provided that the average quantity actually used over a 5 year period consisting of that year and of the four preceding years does not exceed 6 kg
A	Ethylene	Degreening bananas, kiwis and kakis; Degreening of citrus fruit only as part of a strategy for the prevention of fruit fly damage in citrus, flower induction of pineapple, sprouting inhibition in potatoes and onions
A	Fatty acid potassium salt (soft soap)	Insecticide
A	potassium aluminium (aluminium sulphate) (kalinite)	Prevention of ripening of bananas
A	Lime sulphur (calcium polysulphide)	Fungicide, insecticide, acaricidi
A	Paraffin oil	insecticide, acaricidi
A	Minerals oils	insecticide, Fungicide Only in fruits trees vines, olive trees and tropical crops (e.g. bananas)
A	potassium permanganate	Fungicide, bactericide Only in fruits trees, olive trees and vines
A	Quartz sand	Repellent
A	Sulphur	Fungicide, acaricidi, Repellent

7. Other substances

Authorisation	Name	Description, compositional requrement and conditions for use
A	Calcium hydroxide	Fingicide Only in fruits trees including nurseries, to control *nectria galligena*
A	potassium bicarbonate	Fungicide

Chapter 3

Biopesticides obtained from plants, another result from coevolution. Current situation and usefulness

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1. Introduction

Synthetic pesticides are still the most used solution for plants parasite control (insects, mites, mammals, fungi, bacteria, parasitic plants, etc.) and their competitors (weeds). This fact is even more common in protected intensive horticultural crops, where certain habits have been established between farmers and technicians generating procedures that are very difficult to change. However, the growing social concern about the effects that pesticides may have in the environment and food safety has lead in the implementation of new management systems designed to deal with plant parasites. A proof of this concern on a planetary scale has been the agreement reached to remove the methyl bromide in agriculture procedures (soil disinfection, barns, quarantine uses, etc.). In 2005, the first phase of this agreement was completed in developed countries and it will be fully complete in 2015 when this ban will be applied worldwide. This system, whose origin is the Protocol of Montreal, is acting as a model for the decrease of the number of synthetic pesticides used in the European Union.

Not more than five years ago, it seemed that transgenic plants obtained by genetic engineering would replace phytosanitaries in crop protection. However, consumers and farmers´ distrust in respect to the application of these varieties has caused, at least in the meantime, the use of chemical pesticides of synthesis. Besides, in cultivation under plastic, the use of these pesticides has been limited by the employment of beneficial insects, that has been developed rapidly thanks to research and experimentation attempted in the last 30 years. Other substitution

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options are microbial insecticides (*Bacillus thuringiensis,* entomopathogen baculovirus), pheromones (sexual behaviour alteration, capture and elimination) and plant derived phytosanitaries.

Plant derived pesticides have been known and used for more than a century. This has been the case with nicotine, pyrethrum and rotenone among others, although its use has been on the decline since the 50s of last century; improved effectiveness, extended action, persistence and ease of use of synthesis phytosanitaries led to their generalization, which is still in force. The change experienced in society, based on the current concern about the respect for the environment and food safety has caused again the interest for the discovery and use of natural agents concerning crop protection. The fact that those natural agents are obtained from plants or other live organisms (bacteria, fungi, etc.) does not mean that they are innocuous for consumers´ health. At this point, we should remember the famous phrase of Paracelso, a Swiss doctor and chemist from the 16th Century: only the dose makes the poison ("solo *quantitas venenum fecit*"). As classical examples, the foxglove or the curare can be illustrative examples. To mention a recent example, the research that showed how the incidence of Parkinson's disease increased in rats when those animals had been chronically exposed to rotenone.

During the last few years, many researches have been carried out focused on characterizing hundreds of plants extracts and their secondary compounds and the possible active matters. Nevertheless, the number of plant based insecticides used nowadays is very small. Maybe this fact has its origin in a substantial difference with respect to synthesis phytosanitaries: biopesticides do not have acute toxic effects on insects. This is a transcendental fact that clearly has an impact on the use.

Plants have got their own chemical defence strategy which is 300 million years old and has allowed them to survive until now. These substances allow cohabitation (attractant substances) or defence relations (repellent or toxic substances). This system could explain why it is so uncommon that toxins from plants cause acute effects (which is the case of pyrethrum I), as they normally cause sub lethal effects. The effects among the insects are growth inhibition, development of larvae, behaviour alteration (antifeedants, pheromones that prevent the laying, repellent agents, etc.). In other words, the phytopesticides can mean alternative methods of pests and diseases, as action mechanisms are different to the pesticides of chemical synthesis which, in general, only affect the nervous system of insects.

However, the weak presence of pesticides of vegetable origin in the market is not only due to the fact that they do not cause acute toxic effects in their targets organisms. Other production aspects must be taken into account before launching a substance to the market. For instance; 1) The natural resource, the raw material, shall have a continuous availability; 2) The active matter preparation must be obtained at reasonable cost and must have a uniform and constant quality; 3) technology protection (patent that grants exclusivity) and 4) standardization in every country. It is worth, if we consider how important it is, taking into account these premises as, currently, substances are being offered to control the pests and diseases that have got legal registries for other uses, especially nutritional and strengthening uses for the plants, but not as phytosanitaries. Nevertheless, it is convenient to point out that no patent is needed for domestic or local use of pesticides of vegetable origin whose production does not pursue lucrative interests.

Ten years ago, the global market of pesticides was valued at fifteen thousand millions US dollars. It is estimated that nowadays biopesticides are just around 1 % of that amount, that is to say, 150 million US dollars. Pellitory dominates this market, monopolizing 70 % of sales. After years of promises, insecticides based on neem oil are starting to be a threat for preponderance of pyrethrins. According to some authorized opinions, the market based on essential oils has just started. It seems that the market for vegetable origin pesticides could increase by 10 or 15 %, while the market for synthetic phytosanitaries has started its decline. In the USA and European Union, this trend is causing, among others, the removal of organophosphorated phytosanitaries and carbamates. There is no doubt that this freed space is a great opportunity for phytopesticides. Also, developed countries, which are the main pesticide users, prefer natural products instead of synthetic products. This is, in fact, the growing trend of Organic Agriculture. In spite of all of this, it seems clear that plant pesticides shall not totally replace, in the short-term, chemical synthetic products. It is required that raw materials are available to obtain phytopesticides, which will take some time.

2. Biopesticides. Definitions

From an etymological point of view, a biopesticide is any pesticide of biological origin. That is to say, live organisms or natural origin substances synthesized by them. Generically speaking, it would comprise to any product for crop protection that has not been chemically obtained.

Some authors stated that the term is restricted to the biological agents used for pest management: Beneficial arthropods, fungi, virus (baculovirus, granulovirus) and bacteria (*Bacillus thuringiensis* and its varieties). In fact, this definition does not take into account products derived from live organisms' metabolism and, therefore, excludes, for example, semiochemical compounds like pheromones or allelochemical molecules. It also seems appropriate to include biological synthesis molecules, calling the phytochemical molecules with phytosanitary nature as plant origin biopesticides.

Bibliography generated by this topic is very wide and some authors think that knowledge provided can constitute a doctrinal body very difficult to refute. This is what we think happens in essays, almost of an encyclopaedic nature, published under the coordination of REGNAULT-ROGER, PHILOGÈNE and VINCENT (2003, 2004 y 2005), that have been a basis for a large part of this chapter. Thus, concerning innovative aspects about relations between pest insects and their host, aspects relating to behaviour of insects have been shown with respect to chemical emissions of plants disclosing aspects that are only known in part or not known at all. Plant emissions that behave as herbicides have been analysed in more depth and their nature has been studied. If we consider this, we must say that it was worth that all this knowledge must be reviewed. Also, we cannot deny how useful this knowledge is in terms of crop protection. Unfortunately, this research has not had until now a parallel development in terms of management of diseases produced by fungi, bacteria, virus, viroids, mycoplasma, etc. Its development has been wider for phytoparasite nematodes.

Biopesticides usefulness, which is another way of defining them, can be seen by applying food quality criteria summarized under a rule of 4 elements: satisfaction, service, health and safety, which are opposed to ecological disorders produced by abusive and indiscriminate use of synthetic phytosanitaries, that could be summarized under another rule of 4 elements: breakup (food chain), continuance (persistence), resurgence (that appear again) and resistance (from parasites to pesticides).

Requirements for biopesticides are high: selectivity and absence of toxicity for non-target species; biodegradability to non toxic molecules and absence of resistant phenomena from the target pest. If we remember what happened with the synthetic pesticides, this will cause biopesticides to combine with other management strategies. For instance: a) increase of genetic resistance of plants to parasites, b) preparation of cultivation techniques, c) reduction of biotic potential of parasite through sterile insect techniques, use of development regulators, d) use of beneficial arthropods, e) physical techniques of protection, f) eco-chemical management: Sexual disruption, "attract and kill" techniques (for example, association of pheromones with insecticides).

All these requirements for biopesticides should be completed with a level of scientific accuracy equivalent to the one required for synthetic pesticides. An example could be the neem. These aspects are complex and very expensive, but they apply to a big number of cases. At least this can be deduced from the inventory carried out in 1998, where it is shown that more than 2000 vegetable species have got insecticide properties.

2.1. Historical biopesticides

Their use was motivated by the need of controlling pests and they also provided a good availability, as it is the case of arsenic and its derivative substances, or animal oil or petroleum-derivative products. They were not only used during the 19th and 20th centuries, but they are still used today, although their use is not as important as in those centuries (table 1).

Table 1 shows three of the historical biopesticides. The oldest one formed by nicotine and analogue substances has not been included. Their use goes back to the 60s, when aqueous extracts from tobacco (*Nicotiana tabacum*) and other species of the same type were used for the control of sucking and chewing insects. Nicotine, along with its derivative substances (where we can highlight the anabasine), are very toxic alkaloids for insects that increase their activity when they stabilize themselves as salts (sulphate, oleate, stearate). This substance is very toxic and causes, in human beings, breathing muscle paralysis with a dose of 50 to 60 ppm.

Table 1. Some characteristics of the main insecticides of vegetable origin available in USA

Characteristics	Pyrethrin or pellitory	Rotenone	Neem	Essential oils
Country of origin	Kenya, Australia	Southeast Asia, Venezuela	India	Worldwide
Active matters	Pyrethrins (esters)	Rotenoids (isoflavonoids)	Azadirachtin (limonoids)	Monoterpenes, simple phenols
Statutory situation	Approved	Approved, although it could be withdrawn	Approved	Exempt
Use in biological agriculture	Approved	Approved	Approved	Approved
Action against target pests	Contact “Knock-down”	Cytotoxin ingestion	Antifeedant ingestion	Contact “Knock-down”
Persistence	Very limited	Limited	Limited	Limited
Toxicity for mammals	Minimum	Moderately toxic, but very toxic for people	Non toxic	Non toxic
Plants from where they are extracted	Chrysantemun, cinerariaefolium	Lonchocarpus nicou*	Azadirachia indica	Thymus vulgaris**

* Derris elliptica, Tephrosia virginiana and others.
** Mentha puleginum, Eugenia caryophyllus.
Source: Adapted from ISMAN, 2003.

These bioinsecticides served the industry (and this perspective should be kept) as a base to synthesize copying from natural substances. Thus, in 1984 the first pyrethroid of synthesis was obtained from allethrin, which is unstable to light with its eight isomeres. Halogenation with bromine atoms in cypermethrin molecule gave rise to deltamethrin, with a strong insecticide activity and stability against light action. This work seems important to solve the problems that these plants extracts present. Another example of that is the oil (or better to say, the oils) of neem. Known and adored in India for more than 4000 years for its usefulness to control pests of stored products, it has also been used as a spermicide to decrease semen viability in man. Its insecticide activity is mainly due to a limonoid compound known as azadirachtin, while efficiency of salamines, nimbins and analogue substances is less in this case. Every molecule has got a different activity against insects. While salamines and nimbins are antifeedant, azadirachtins (more than a dozen of analogue substances have been identified) are growth inhibitors. In spite of all studies carried out and its proven insecticide effectiveness, the neem could not, until now, replace insecticides of synthesis due problems to apply them at large scale: small persistency due to its photolability (20 h of average life on the leave surface), degradation hydrolysis in water at 37 °C and pH8 (20 h are enough to degrade 50 %). This example and the difficulty to implement the proper formulations means a considerable limitation and alerts us about existing difficulties for an efficient use of biopesticides, in spite of its unquestionable properties against pests.

3. Current state of knowledge about new biopesticides

When plants are the source of active matter, adjectives like 'new' or 'innovative' in pest and disease control research can be doubtful yet tempting. Let's use as an example BALACHOWSKY's quote (1951) on insect control in barns using the insecticidal properties of aromatic plants, with the recipe that the priest of St. Sulpicio Parish in Paris gave to his parishioners in 1760: "take some wormwood, two handfuls of savin juniper and a similar amount of tansy; take some small basil, big sage and small sage and some leaves of parsley, a handful of each and two handfuls of green leek. Shred thoroughly and put into a big caldron". This empirical practice shows that farmers would choose plants with properties that avoided the development of insects. The 'secrets' of such plants have been revealed in a more systematic and experimental manner. To do so and to reveal such properties, a basic method has been set, from which many techniques derive. This has enabled REGNAULT-ROGER (2004) to reveal the insecticidal activity of some essential oils and their main compounds (monoterpenes) against the bean weevil (*Acanthoscelides obtectus*). Table 2 shows the activities of the oils and monoterpenes against the above mentioned weevil. This weevil especially affects beans, although it can feed on chickpeas and broad beans, among others. Results in Table 2 show the importance of ethnobotanical surveys. In this regard, St. Sulpicio Parish priest's recipe is quite eloquent. Precisely one of the pillars of Organic Agriculture rests on bringing back the rural knowledge on crops. This knowledge should not have been underestimated for indoor crops.

Table 2. Activities of the essentials oils and monoterpenes, as well as the vegetal species, on bean weevil (*Acanthoscelides obtectus SAY*)

	Inhalation activity on adults		
	More active (CL50<10 mg•dm^{-3})	**Intermediate (11-99 mg•dm^{-3})**	**Less active (≥ 100 mg•dm^{-3})**
Essential oils	*Thymus serpyllum*	*Laurus nobilis*	*Myristica fragans*
	Thymus vulgaris	*Verbena officinalis*	*Petroselium sativum*
	Origanum majorana	*Mentha piperita*	*Apium graveolens*
	Origanum vulgare	*Anethum graveolens*	
	Cinnamorum verum	*Eucalyptus globulus*	
	Rosmarinus officinalis	*Citrus limon*	
	Ocimun basilicum		
	Salvia officinalis		
	Satureja hortensis		
	Coriandrum sativum		
	Cuminum cyminum		
Monoterpenes	**More active (5 mg•dm^{-3})**	**Intermediate (5<CL50<20 mg•dm^{-3})**	**Less active (CL50 >20 mg•dm^{-3})**
	Carvacrol, linalol, eugenol, thymol, terpineol	Cuminaldehyde, p-cymen, anethole, cinnamaldehyde	Estragol, borneol, a-pinene
	Reproduction inhibition		
	Lay	**Larva penetration**	**Emerging**
Essential oils	*Eucalyptus globulus*	*Thymus vulgaris*	*Thymus serpyllum* (1)
	Salvia officinalis	*Origanum vulgare*	*Thymus vulgaris* (1)
	Apium graveolens	*Eucalyptus globulus*	*Origanum vulgare*
	Verbena officinalis	*Laurus nobilis*	*Eucalyptus globulus*
			Lavandula angustifolia
			Laurus nobilis
			Salvia officinalis
			Coriandrum sativum
			Cinnamorum verum (1)
			Rosmarinus officinalis (1)
			Ocimum basilicum (1)
			Petroselinum sativum (1)
Monoterpenes	Linalol, thymol, carvacrol	Linalol (1), thymol (1), eugenol, anethol	Carvaclol (1), linalol (1), eugenol (1), thymol (1), terpineol (1)

(1) Complete inhibition.

It is important to point out that the toxicity works in different ways: by inhalation against the adults (in the case of *A. obtectus*, it affects males more commonly than females), ovicidal and larvicidal activity and anti-nutritional toxicity for larvae in cotyledon tissue. Equally important for application purposes is to determine the most sensible target species and to choose the form of application (spraying or seed impregnation).

But monoterpenes are not the only compounds found in essential oils showing insecticidal activity. Polyphenols, acid phenols and flavonoids are equally active. Rosmarinic acid and luteolin-7-O-glucoside are among the most abundant. The activity of these polyphenols focuses on the alteration of the insect's motor functions, accompanied in some instances by a 'knock-down' effect (momentarily loss of its mobility).

So the essential oils from plants and their aromatic molecules show a dual activity:

a) Against adults, through instant toxicity by inhalation (monoterpenes) and, on the other hand, an activity that focuses on the insect's mobility (polyphenols).

b) Against the reproduction rate, by altering fertility and showing ovicidal and larvicidal properties.

This research model enhances the reputation of aromatic plants found in the Mediterranean Basin. The seasonally high luminosity and temperatures of this climate seem to demand from the plants some adaptation efforts that translate into a significant evolutionary molecular richness. This richness can be noted on its multiple uses, such as a condiment, in industries such as medicine, perfume, cosmetics, pharmacy, herbalists, and as an aroma in the food and agriculture sector, and probably the list could go on.

But if the diversity of vegetal species of plants is important in the Mediterranean Basin, it is even more important in tropical regions, where 65 % of total diversity of flora is concentrated, which can mean that many molecules could be extracted from these plants for phytosanitary purposes.

3.1. *Meliaceae* Family

Known for being the source of neem from India and that of tusedamine, a limonoid marketed in China as an insecticide.

The Meliaceae family comprises a group of more than 14 genera of neotropical plants. Table 3 sums up family species with reported insecticidal activity.

Table 3. Species of Meliaceae family that present an insecticide activity

Vegetal species	Insects on which the trial has been tested	Type of activity	Type of preparation	Nº of identified molecules
Swietenia humilis	*Ostrinia nubilalis*	Growth regulator	Bark extracts	7
S. macrophyla	*Spodoptera frugiperda*	Antifeedant	Bark extracts	3
S. aubrevillana	*Spodoptera frugiperda*	Antifeedant	Bark extracts	3
Cedrela odorata		Ingestion toxicity	Bark extracts	2
C. salvadorensis		Ingestion toxicity	Bark extracts	2
Guarea grandifolia			Bark extracts	6
Trichilia martiana		Growth regulator	Bark extracts	3
T. hirta	*Spodoptera litura*	Growth regulator	Bark extracts	2
T. americana	*Spodoptera litura*	Growth regulator	Bark extracts	2
T. trifolia	*Sitophilus zeamais*	Antifeedant	Bark extracts	3
Aglaya spectabilis	*Spodoptera littoralis*	Ingestion Toxicity		11
A. dookoo (Lanisium domesticum)	*Sitophilus zeamais*	Antifeedant	Bark extracts	3

Most of the extracts from these plants show antifeedant and growth-reducing activity, but they are not toxic. The chemical nature of their active molecules against insects classifies them as limonoids, and more rarely as terpenoids.

3.2. *Piperaceae* Family

The pantropical family Piperaceae has been used traditionally as a source for insecticides, spices (white and black pepper) and medicines. Unlike the Meliaceaa, the compounds showing insecticidal activity, demonstrated as acute toxicity and ‘knock-down’ properties, are piperamides and, to a lesser extent, lignans and benzoic acids.

Apart from the insecticidal role of the piperamides, the family is known for its production of synergists. When a product with pesticidal activity is combined with a non-toxic product (synergist) and the effect is higher than expected, we are talking about a synergist association in the strictest sense of the term. The clearest example occurs when mixing pellitory and piperonyl butoxide, where the latter allows the pellitory to act with the same efficiency with minor doses to achieve its known toxicity. However, when two toxic products are mixed and the resulting toxicity is higher than the mere arithmetic combination of each product's toxicity, the term 'potentiation' is used. The mechanism of piperonyl butoxide focuses on inhibiting the detoxification enzymes, so these cannot neutralize the toxic substances, boosting the toxic effect and delaying the appearance of resistance in the target insects.

Lignans are substances very commonly found in plants, and Piperaceae are especially known for producing some of them (Table 4). Again the relationship between plant and parasite is striking. To defend themselves from insects, plants have developed toxic molecules at a high energetic cost, and these are accompanied by others which are able to emphasize the toxic effects of the former without, apparently, increasing the cost used to produce the toxic molecules.

Table 4. Lignans present in different plants that have insecticide and /or synergic action

Family	Genus and plant species	Lignan	Activity
Asteraceae	Piper longum	sesamine	S
	Piper cubeba	cubeb	S
	Piper aductum	dilapiol	S/I
	Artemisia absynthium	sesartamin	S
	Chrysamthemun sp	sesamine	S
	Erigeron sp	dilapiol	S/I
Umbeliferae	Atriscus sp	podophyllotoxin	S
	Pasticana sativa	myristicin	S

Source: Adapted from PHYLOGÉNE, 2004.

3.3. *Liliaceae* and *Brassicaceae* (Cruciferae) Families. A thoroughly studied model

We have thought that these two families, so common in our diets, deserve special treatment. So far, only the insecticidal effects of the biopesticides have been mentioned, but the absence of fungicidal, bactericidal and nematicidal activity is striking. Simply, we have found only very general references. These aspects may have not been investigated, but it is very likely that they exist, as a consequence of the coevolution of such molecules with anti-microbiotic activity. The protective role played by the polyphenols in the disease process is very suggestive. The phenolic compounds show inhibition in the activity of the hydrolitic enzymes (pectinases, cellulase and proteases).

The Liliaceae (*Allium*) and Brassicaceae (Cruciferae) families are an exception. Because of their use as biological soil disinfectants, some aspects of their activity as fungicides and nematicides are known. Sulfur compounds of the genus (*Allium)* and some Cruciferae are characterised as insecticides, acaricides, nematicides, herbicides, fungicides and bactericides.

Sulfur compounds from plants can be split into two main categories: non-protein amino acids (and their derivatives) and glucosinolates (and their derivatives). Table 5 sums up sulfur compounds originated from plants, *Allium* and species from the Brassicaceae family.

Table 5. Sulphur compounds and plants from which they have been obtained

Name of molecule	Species, Genus and/or Family of plants and fungi
Alkenyl cysteine sulphoxide Disulphide Thiosulfinate Thiosulfonate Trisulphur	*Allium porrum*, *A. cepa, A. sativum, Brodiaea uniflora, Tulipa edulis, Tulbagia violacea, Asparagus* sp., *Asparagus officinalis, Adenocalymna alliaceum* (Bignoniaceae), *Albizzia lophanta* (Mimosaceae), *Ferula* sp. (Umbeliferae), *Azadiracha indica* (Meliaceae), *Nuphar luteum* (Nynphaeaceae), *Losianthus* sp. (Runiaceae), Bignoniaceae Fungi: *Marasmius* sp., *Lentinus erodes* Seaweed
Glucosinolate Alkil isiothiocyanate	Cruciferae,Capparidaceae, Resedaceae, Moringaceae, Tropeolaceae, Solvadoraceae, Limnantaceae, Caricaceae, Euphobiarceae, Filocaceae, Plantaginaceae Fungi: *Agaricus bisporum*

Since glucosinolates are the most commonly studied sulfur compounds in Cruciferae, we know other plant families can produce them. More than 100 glucosinolates have been identified, which have been classified into three main categories: alkyl glucosinolates, alkenyl glucosinolates and indole glucosinolates, although apparently they are not the only ones. Volatile sulfur molecules are produced from glucosinolates, during the process of decomposition of plant tissue, through the intervention of the myrosinase enzyme, leading to thiocyanates, nitriles and isothiocyanates being the most abundant substances. In the case of *Allium* and fungi, volatile sulfur molecules are obtained from sulfur amino acids (cysteine, cystine and methionine), stored in the cell cytoplasm in the shape of dipeptides and released by the action of enzyme y-glutamyl peptidase.

3.3.1. Phytosanitary potential of *Allium* and Cruciferae

3.3.1.1. Insecticidal activity of *Allium*

We need to distinguish the effects originated from plant extracts from those originated from volatile sulfur compounds. For the extracts, a large group of them has been summed up offering a glimpse of their activity. This activity reveals itself both as a toxicity (mortality similar to that of synthesis insecticides), and as an effect on the behaviour and physiology of development. For such purpose, see Table 6. We have to keep in mind that the referred insects are those on which tests have been carried out, which does not allow generalization, but leads to carrying out observations on other species, especially those that are relevant to protected intensive crops.

The effect of the association of crops using *Allium* as pest control plants has been studied as well. Association of onion with carrot; onion, garlic and potato; beetroot and onion, and aromatic plants with onion and garlic. The results were contradictory and, therefore, did not allow a specific recommendation. Nevertheless, the insecticidal effects of the *Allium* extracts seem real and we should develop a technology to improve their performance.

Table 6. Insecticide activity of *Allium* extracts

Type of preparation	Target insect	Effect on the insect
Onion extract	*Pieris brassicae, P. napi*	Lay inhibition
	Delia radicum	Repellent
	Myzus persicae	Antifeedant
Garlic extract	*Myzus persicae*	Antifeedant
	Epilachna variventris	Antifeedant, development alterations
	Cocopsylla pyricola	Reduce the level of lay
	Spodoptera litura	Juvenomimetic activity
	Spodoptera litura	Ovicide action
	Diadromus pulchellus (parasitoid)	Larvae mortality
	Sitobion avenae	toxicity
	Rhopalosiphum padi	
	Schistocerca gregaria	
	Leptinotarsa decemlineata	
	Pieris brassicae	
	Phthorimaea operculella	
	Culex y Aedes	
	Musca domestica	Anticholinoesterasic activity
	Trogoderma gramarium	
	Bemisia argentifoli	Toxicity in eggs, larvae and nymphs
	Tribolium castaneum	
	Sitophilus zeamays	
	Dysdercus koenigii	Ovicide action
	Eearias vitella	
	Helicoverpa armigera	
Leek extract	*Drosophila melanogaster*	Toxicity

The effect of the pure productsl of the sulfur compounds from *Allium* was valuated. The observed insecticidal activity is of two kinds: they act both on the insect's physiology and on its locomotive behaviour. The most common compounds are:

- DSM2: dimethyl disulfide, widespread in, apart from *Allium*, Leguminous plants and Cruciferae.
- DSP2: diallyl disulfide. Apart from *Allium*, present in Bignoniaceae
- TiA2: diallyl thiosulphinate or allicin
- TiM2: dimethyl thiosulphinate

Table 7 shows doses and insect species that have been tested on.

The volatile compounds of *Allium* may have negative effects on some entomophagous insects. Thus, when swallowed by *Romalea guttata* feeding on wild onion (*Allium canadense*), disulfides (DS) are repellent for two species of predatory ants. Equally, 5-Methyl-L-cysteine sulfoxide (PCSO), present in leek (*Allium porrum*) repels some ant species of genus *Formica*, which are predators of the leek moth (*Acrolepiopsis assectella*). These disulfides are toxic against hymenopteran parasitoids, such as *Dinarmus basilis* y *Diadromus pulchllus*. In the latter, the repellent effect has been observed in leek moth faeces (*Acrolepiopsis assectella*).

Table 7. Lethal concentrations 50 (CL50) obtained after 24 hours of exposure, expressed in mg•L^{-1} of air, for five sulphur compounds of *Allium*

Species	Phase	DSM2	DSP2	DSA2	TiM2	TiA2
Acanthoscelides obtectus	Ad	1,8	75	24	-	-
Acrolepipsis assectella	Ad	0,4	23	3,5	-	-
	L5	3,8	> 150	50	-	-
Bruchus atrolineatus	Ad	0,2	2,0	0,6	0,15	0,18
	L4	2,8	12	-	-	-
Callosobruchus maculatus	Ad	1,1	2,6	0,5	0,25	0,16
	L4	2,0	-	-	-	-
	Egg	0,2	-	-	-	-
Diadromus pulchellus	Ad	0,5	20	4,5	-	-
Dinarmus basalis	Ad	0,3	-	0,4	-	-
	L4	1,6	-	-	-	-
Drosophila melanogaster	Ad	0,2	2,5	0,7	-	-
Ephestia kuehniella	Ad	0,2	-	0,02	0,04	0,02
Plodia interpunctella	Ad		-	-	0,02	-
Oryzaephilus surinamensis	Ad	0,8	5,5	3,5	-	-
Sitophyllus oryzae	Ad	1,2	-	-	0,02	-
Tineola bisselliella	Ad	0,9	1,3	0,02	-	-

Ad= adult; L= larva; Egg.
Source: REGNAULT-ROGER, *et al*., 2004.

3.3.1.2. Insecticidal potential of Cruciferae. Compounds derivated from glucosinolates (GLU)

Glucosinolates are sulfur compounds, whose best known sources are plants from family Cruciferae. Other close families that also produce them are *Capparidaceae, Resedaceae and Moringaceae*, for instance. Also, the common mushroom (*Agaricus bisporum*). As mentioned before, more than 100 GLU have been identified so far. Several GLU can coexist within the same species, its concentration varying according to the plant's organ and age.

Besides GLU, sulfur phytoalexins can be found in Cruciferae, which belong to the defense induced by several kinds of stress on the plants.

It has been observed that these plants have some attracting effects on insects; which could enhance parasitoid and predator development of pest insects.

Activity of GLU on insects is similar to that developed by *Allium*: repulsion to adults, lack of larvary appetite and toxicity.

Among GLU, the methyl isothiocyanate is one of the most studied (MITC) that shows a toxic and repellent activity but, at the same time, it can attract insects that parasitize cruciferae. For the first case, relating to insects in barns, especially weevils and moths, they have shown an active behaviour against adults and larvae. Concerning the attraction power, traps have been successfully used based on allyl isothiocyanate for fleas (*Phyllotreta sp*), cabbage maggot (*Delia radicum*), cabbage seedpod weevil (*Ceuthorrynchus assimilis*), seedstem weevil (*Ceuthorrynchus napi*). This attraction power takes also place in generalist parasitoids (*Diaretiella rapae* and *Meteorus leviventris*) of aphids and with Lepidoptera Noctuidae.

3.3.1.3. Activities against other plant parasites

a) Acaricidal Effects

Little information has been generated on this topic. It has been observed that garlic extracts act as repellent for *Tetranychus urticae*, one of the “red spider mite” found in many crops. Also, it has been observed that a similar repellent effect of the abovementioned extracts occurs on *Varroa jacobsoni*, a parasite that attacks honeybees.

b) Nematicidal Effects

Allium fistulosum and *Allium grayi* extracts have proven to be very active against *Meloidogyne incognita* eggs and juveniles. A strong nematicidal activity has been revealed for asparagusic acid. The isothiocyanates have been shown to have a nematicidal activity on *Meloidogyne*, *Heterodera shachatii* and *Pratylenchus*. In contrast, methyl isothiocyanate used as a soil disinfectant, generated from precursors such as dazomet or metam sodium, does not show sufficient control in intensive crops.

Broad information can be found on compounds extracted from plants (other than *Allium* and Cruciferous) that have an active effect against nematodes, especially on eggs and juvenile stages. The main types studied, *Meloidogyne* is highlighted. Among the families that these active principles belong to are: carboxylic acids, lipid compounds, glucosides, amino acids and proteins, phenolic and aromatic compounds and heterocycles with oxygen, sulphur or nitrogen, alkaloids, terpenoids, etc. (table 8).

Table 8. Molecules of vegetable origin identified with nematicide effect

Active parts of the plant	Nematode assessed and cycle phase	Identified molecule
Peanut roots	*Pratylenchus coffeae* (juv)	Di-n-butylsuccinate
Tigernut oils	*Pratylenchus spp.* (juv)	Oleic, linoleic, palmitic and stearic acids
Macuna aterrina stems and roots	*Meloidogyne incognita, Heterodera glicines* (juv)	Allantoin, sytosterol, stigmasterol L-Dopa
Root exudates, asparagus stems and leaves	*Heterodera glicines, H. rostochiensis, Meloidogyne hapla, Pratylenchus penetrans* (ovi, lar and adul)	Asparagusic acid
Root exudates of canavalia (*Canavalia ensiformis*)	*Meloidogyne incognita, Naccobus aberrans* (nema)	Concavaline A (lectin)
Ricin mash	*Meloidogyne spp. Tylenchulus semipenetrans* (juv)	Ricin (lectin)
Soya roots	*Meloidogyne incognita, M. javanica* (nema)	Glyceollin (phytoalexin)
Common bean and Lima bean roots	*Pratylenchus scribeni, P. penetrans* (nema)	Cumestrol, psoralidin, phaseolin (phytoalexins)
Rue plant	*Meloidogyne spp.,Xiphinema index* (lar and adul)	Anthranilic acid Limonene, pinene, cineole (monoterpene)
Root exudates of marigold	Different nematodes phytoparasites (lar and adul)	Bithienyl derivatives and α-terthienyl
Root exudates of safflower	*Meloidogyne incognita* (juv)	Serpentine
Crotalaria spectábilis plants	*Meloidogyne incognita* (nema)	Pyrrolizidine
Tobacco leaves	*Meloidogyne incognita* (juv)	Nicotine
Sophora flavescens roots	*Bursaphelenchus xylophilus* (lar, adul)	Cytisine and anagyrine derivatives
Leaves and flowers of *Artemisia milagirica*	*Meloidogyne incognita, Rotylenchulus reniformis* (juv)	Santonin (terpenic lactone)
Seeds and root exudates of cinnamomum or neem	*Different nematodes phytoparasites* (ovi, lar, adul)	Nimbidine, thionemone, azadirachtin (triterpenes limonoids)
Essential oils of basil and geranium	*Meloidogyne spp., Herodera spp., Anguina tritici, Tylenchulus semipenetrans, Rotylenchus reniformis* (juv)	Citral, citronellol, geraniol
Root exudates of cucumber	*Meloidogyne spp.*(rep)	Curcubitacin (triterpene)
Essential oils of *Inula helenium*	*Meloidogyne incognita* (juv)	Helenia
Potato roots (cv. Tempo)	*Dytilenchus dipsaci, D. destructor* (nema)	Risitin (sesquiterpenoid, phytoalexin)

(juv)=larvicide; (ovi)=ovicide; (adul)=adulticide; (nema)=nematostatic; (rep)=repellen.
Source: adapted from DIJAN-CAPORALINO *et al*., 2004.

c) Fungicidal and bactericidal effects

It seems that these aspects have not been studied on derivatives of *Allium* and Cruciferous as thoroughly as on nematodes control. And above all, research focused on the evaluation of plants or plant extracts with fungicide or bactericide effect is scarce.

The toxic effects of *Allium* on pathogenic fungi for humans is well-known. With regards to plant pathogens, the activity of thiols and sulfides on *Botrytis allii* has been reported. Also the activity of methyl disulfide on *Aphanomyces eutiches*, causing root rot of peas and other leguminous plants. GRAINGE and AHHED (1988) mention in their general review the different fungi sensitive to *Allium*: *Alternaria tennuis, Aspergillus niger, Fusarium oxysporum, F. poae, Verticillium albo-atrum*, on which onion and garlic act upon. Meanwhile *Phytophthora infestans*, which causes downy mildew of tomato and potato is sensitive to garlic chives (*Allium tuberosum*).

The soil biodisinfection (biofumigation, biosolarization, etc.) has shown how Cruciferous decrease the number of pathogen populations found in plant roots (*Phytophthora capsici, Fusarium oxysporum* f. sp. *dianthi*, among others). It seems that the isothiocyanates are effective, to the point that improvement programmes have been established, aimed at obtaining varieties of mustard seed containing a greater quantity of isothiocyanates in the roots, in order to enhance the management of *Helminthosporium solani* and *Verticillium dahliae* in potato crops.

As in the case of fungi, the bactericidal effect of *Allium* on human pathogenic bacteria has been known for many years. However, little is known about their activity on plant diseases. It is known that the bacteria *Erwinia carotovora* and *Agrobacterium tumefaciens* are sensitive to different species of *Allium*. The same would apply to Cruciferous.

d) Herbicidal effects

Albeit the fact that research carried out for weed control is not as exhaustive as the one described for insects, few aspects are known on the herbicidal effect of *Allium*. Thus, the volatile compounds of garlic and radish, the disulfides cause the breaking of dormancy of gladiolus, peony and the ornamental plum trees. Glucosinolates and their derivatives from cruciferous show a clear herbicidal activity.

3.4. Herbicides derived from plants. Allelopathic substances

The effect of crop residues when soil biofumigation is applied is, in many cases, herbicidal. It has been known since ancient times that asparagus should not be replanted on the same soil due to the negative effect that it represents for the survival of the new crop. It was equally known about the toxicity of lettuce crop when the soil had been previously planted with asparagus. The fall of peanut crop yields when it was preceded by sorghum has been attributed to the sorgoleone originated when crop residues decompose, and this substance inhibits the development of numerous adventitious roots. This type of interaction is different from parasitism and symbiosis, but so is the competition (the resource is limited). The relation has been defined with the name of allelopathy. Almost all of the molecules characterized as allelopathic agents are secondary metabolites of plants. These molecules can be grouped into three broad categories: phenolic compounds, terpenes and alkaloids. All plant organs contain compounds that can be allelopathic and released into the environment in different ways: volatilisation, root exudation, leaching and decomposition of plant residues. The release seems to be closely linked to the environment so that the emission of volatile toxic substances is more common in arid and semiarid regions. Thus, *Salvia* type plants produce volatile compounds such as camphor, 1,8 cineole and α pinene and β-pinene, having an effect on the growth of neighbouring plants. Table 9 shows some examples of the activities of allelopathic molecules.

Table 9. Herbicidal activity of allelopathic molecules

Compounds	Activity	Target plant
Coumarins	Root inhibition	Radish
Flavonoids	Growth delay	Lucerne
P-hydroxybenzoic acid	Germination inhibition	Thyme
Isoflavons	Germination inhibition	Onion

Source: adapted from CHIAPUSIO *et al*., 2004.

If there is an example of widely studied allelopathic substances then it would be, without doubt, hydroxamic acids.

3.4.1. The allelopathic potential of hydroxamic acids

Both DIBOA (2,4-dihydroxy-1, 4 benzoxazin-3-one) and DIMBOA (2,4-dihydroxy-7-methoxy-1, 4 benzoxazin-3-one) have been subject to special and on-going research and are mainly produced in rye, maize and wheat. They are to be found in any part of the plant, except in the seeds and are released mainly by root exudates. On the soil they undergo a chemical and microbial degradation that convert a DIBOA into BOA and DIMBOA into MBOA.

Hydroxamic acids have toxic effects on various pests found on cereals. The studies developed for the control of Asian and European corn borer (*Ostrinia furnacalis* and *O. nubilalis*) are paradigmatic, especially those related to genetic improvement aimed at developing varieties that synthesize a higher quantity of DIBOA and DIMBOA. Hydroxamic acids have also been associated with the control of wheat mycosis caused by *Septoria tritici* and their inhibitory effect has been evaluated on bacteria such as *Erwinia spp, Agrobacterium tumefaciens, Staphylococcus aureus, Escherichia coli* and the yeast *Candida albicans*.

Furthermore, the chemical industry has shown interest in the herbicidal activity of DIBOA and DIMBOA. Laboratory tests have shown that the growth of roots and coleoptiles of numerous plant species is inhibited by them. The inhibition has also been evaluated in field tests. Thus, rye residues containing DIBOA and BOA reduced weeds by 93 %, proving that DIBOA is particularly active for monocotyledons and dicotyledons for BOA. Also, it seems that hydroxamic acids could reduce the toxicity of some herbicides such as atrazine, which converts into hydroxyatrazine in the presence of DIMBOA.

The chemical industry has changed the molecular structure of these hydroxamic acids in order to increase their phytotoxicity, as in the case of 1-8 cineole, marketed as an herbicide. Similarly, the genetic improvement of rice has focused on obtaining varieties with a greater allelopathic power against weeds. Therefore, cultivating rice Dular inhibited the development of 80 % of *Cyperus* plants.

3.5. Other plant sources of active compounds for insect control

3.5.1. *Phytoecdysteroids*

In 1966, phytoecdysteroids were discovered by chance among plant secondary substances. Their role in the plant world is unknown, but they are very similar to those substances generated by insects in order to shed their skin. The substances are known in the insect world as juvenile hormones and molting hormones. Both belong to the group of ecdysteroids, which are derived from sterols produced by plants and insects extract them from these. They transform them into cholesterol and then convert them into molting hormones. This has led to further research and assume that plant steroids could have a synergistic or antagonistic effect against the insect's own hormone.

Currently there are more than 200 different substances obtained from different plant species. The bracken (*Polypodium vulgare*) is one of those which contain more hydroxyecdysone. 3000 species of plants have been studied and 5 to 6 % of them contain ecdysteroids in significant quantities. Table 10 shows the activity of the ecdysteroids on insect development.

Table 10. Minimum concentrations of ecdysteroids (ppm) required to affect insect development

Species	Ecdysteroid	Concentration (ppm)	Source	Effect
Aedes aegypti	Ponasterone A	10.000	Environment	Development inhibition
Tribolium confusum	Ecdysone	5.000	Food	Development inhibition
	Ponasterone A	500	Food	Development inhibition
Musca domestica	Ponasterone A	150	Food	Reduction of pupa formation and adult emerging
	Cyasterone	150	Food	
Bombyx mori	Ponasterone A	150	Food	Supernumerary molts and death
		> 1,5	Food	Nymphosis acceleration
Drosophila melanogaster	Ecdysone	20	Food	Premature formation of pupae

Source: extracted from MARION-POLL *et al*., 2004.

These brief observations show that phytoecdysteroids play a role in the plant's defence against arthropods.

Equally, phytophagous insects have a wide range of possible responses to these compounds: some are resistant against ingested ecdysteroids and are quickly detoxified and others are semi-tolerant or sensitive and often have developed sensory receptors that allow them to detect these substances and avoid those plants that contain them. It could be reasonably stated that all this behaviour is the result of coevolution.

Would it be possible to use phytoecdysteroids for crop protection? It could be a possibility. To this aim its mild toxic action must be taken into consideration as well as the role of molecules as antifeedants, which would make them useful when combined or integrated in other control techniques.

3.5.2. *Idioblastic oleaginous cells*

Readers may be asking themselves the following: what are the idioblasts? They are plant cells that secrete oils that are visible with the naked eye because of their different morphology and size when compared to the rest of the cells. Its contents are terpenes, fats and flavonoid aglycones. These oils have fungicidal properties and thus the persin seems to be responsible for slowing down the advance of hyphae of *Colletotrichum gloeosporioides* in the pericarp of unripe avocado. It has been suggested that the idioblasts are the precursors of the glandular trichomes, more complex structures that have been referenced for years for their activity against phytophagous insects.

A much-studied model is the avocado *(Persea americana*). Idioblastic cells are found in fruits (ripe and young), leaves, roots, pedicels and peduncles. They are also common in other species of the genus and other members of the family Lauraceae. Its idioblasts contain, among other compounds, alkaloids, sesquiterpene hydroperoxides and other terpenes.

Furans have been evaluated for their activity against the bacteria *Bacillus subtilis* and *Staphylococcus aureus* and found to inhibit their growth. Triolein has an insecticidal effect at high concentrations. It also seems that there is a synergising effect between triolein and furans. Persin is the third molecule obtained from avocado in which an insecticidal activity has been detected by inhibiting the growth of larvae of silkworm (*Bombyx mori*), in addition to the fungicidal activity pointed out above.

The insecticidal activity of furans on *Spodoptera exigua*, Lepidoptera Noctuidae very harmful and polyphagous to various horticultural crops has been more widely and in detail evaluated. The toxic action is exerted on larvae at different stages of development, inhibiting growth and the weight of the non-imaginal forms. Sublethal doses dissuade larvae from taking food. The inhibition of larval growth is produced by persin under similar test conditions to those used for furans. All these previous observations are necessary in order to obtain new categories of insecticides, but the fact seems promising.

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Chapter 4

Greenhouse technology and biological control

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1. Introduction

With the final purpose of obtaining productions with a certified and recognizable quality, the advances in the production systems under greenhouse, as in other agricultural systems, must be aimed to consolidate a rational productive model which curbs the use of resources and respects the environment and that includes health guarantees for consumers and producers.

In the last years, a higher and higher concern has been highlighted for the consumption of high quality products and safe for food, produced under environmental sustainability criteria. The limited and rational use of phytosanitary products in the control of pests and diseases has an outstanding role to satisfy this aim. As a response of quality and food safety demand, the vegetable producers of Almería are carrying out in the last years, successfully, a change of strategy in the control of pests and diseases, promoting and giving priority to the biological control over the chemical control, supported by the results of an important and intense research and innovation work developed in the last decades by researchers and technicians of public entities and companies. This work, that has allowed the development of essential tools (knowledge, techniques, auxiliary fauna, reservoir plants, …) necessary for producers and technicians to implement the biological control of pests and diseases in greenhouses from Almería, must follow growing to achieve that this strategic change can be solid and sustainable throughout the time, more and more effective and efficient and can solve skilfully and quickly the new problems to which it will have to face inevitably in the future.

* Experimental Station of the Fundación Cajamar.

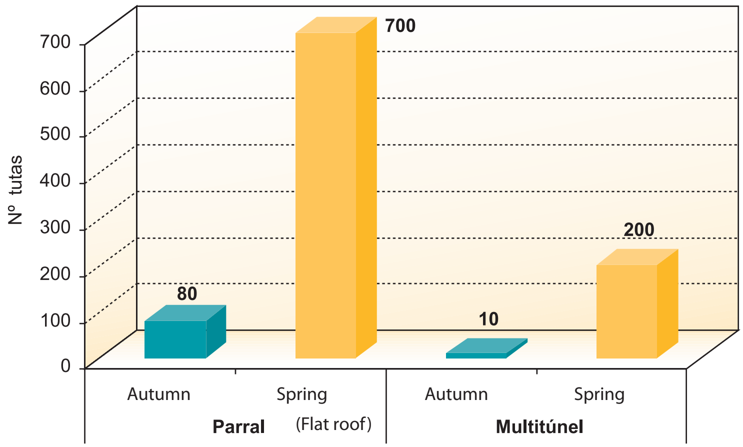
In order to study the variables that affect the incidence of pests and diseases, it is necessary to consider the greenhouse as a system where the crop, the populations of the different pest species that affect it and the auxiliary fauna linked to them are included. We must also take into account that different typical factors of this system interact in the ecosystem balance such as the closing structure (hermeticity, characteristics of materials), the equipment installed in the same (heating, cooling, CO_2) or the management carried out in such system. In short, the technology used in the greenhouse and its management affects in a decisive manner each and every one of the live elements of the greenhouse: plants, pests and auxiliary fauna, and shall determine the intensity of the phytosanitary problems or the success to solve them for each greenhouse.

The assessment of the interactions of the mentioned factors with the behaviour of pest populations and auxiliary fauna must provide useful information to guarantee the success in the incorporation of biological fight in pest control. In this sense, in the Experimental Station of Fundación Cajamar, different aspects have been assessed with respect to structures, the closing systems (plastics and meshes), the climate control equipments or crop techniques that interact with the complex plant-pest-natural enemy, and therefore, they influence on the effectiveness of biological control.

2. Greenhouse structures and photoselective or anti-pest plastic materials

The greenhouse confines the space where crops are developed and establishes the most important physical barrier to avoid the entry of pest insects or virus vectors that can cause economic damages to production. A hermetical closing, where the connection points between the inside and outside of the greenhouse through which pest insects can reach the crops (windows, doors, holes) are properly protected, is one of the first measures to observe in the integrated control of pests. Figure 1 based on the observations made in the Experimental Station of the Fundación Cajamar, shows that the hermetic degree of the structure affects the incidence of *Tuta absoluta*, one of the most recent and devastating pests for greenhouse agriculture in Almería.

Figure 1. Captures of *Tuta absoluta* in different greenhouse structures



The use of plastics known as anti-pest (photoselective), that block a part of the UV radiation (Salmerón *et al.*, 2001) and eliminate the wavelength corresponding to the most visible colour for insects, makes difficult the development of pest insects (Salmerón *et al.*, 2001; Antignus *et al.*, 2001; Lapidot, *et al.*, 2002), or of virus transmitted by insects that are sensitive to the decrease or lack of ultraviolet radiation (Gonzalez *et al.*, 2003; Monci *et al.*, 2003; Rapisarda, *et al.*, 2006). However, it can also have a negative effect on the activity of pollinisers that need the ultraviolet radiation spectrum (Bertholf, 1931; Weiss, 1943; Hollingsworth *et al.*, 1970; Varela, 1974; Brown *et al.*, 1998; Chittka and Thomson, 2001), limiting their sight (Cabello *et al.*, 2005 a, 2006; Soler *et al.*, 2005), so that the ultraviolet light conditions can change the pollinisers perception, bee (*Apis mellifera*) and bumblebee (*Bombus terrestris*), on the different colours of flowers, increasing their difficulty to locate flowers among the crop (Cabello *et al.*, 2005b; 2006). However, this negative effect can be lessened by the response capacity of pollinisers, so bumblebees have an excellent and fast learning capacity and they can manage to adapt themselves to the absence of ultraviolet light (Dyer y Chittka, 2004).

The limitation of ultraviolet light reduces, decreases, and even avoids the growth and sporulation of pathogen fungi such as *Botrytis cinerea* (Jarvis, 1997; Díaz *et al.*, 2001).

To assess the influence of filters for ultraviolet radiation additive to plastic materials, on the presence of *Bemisia tabaci* and *Frankliniella occidentalis*, as well as on the activity of natural pollinisers (*Bombus terrestris* and *Apis mellifera*), different studies have been done in the Experimental Station of the Fundación Cajamar since 2005 comparing closing systems with different levels of ultraviolet radiation absorption (1 %, 10 %, 23 %, 55 % y 65 %, respectively) in tomato, melon and mini-watermelon crops.

With relation to pest insects (Fig. 2), the results obtained show that anti-pest plastics absorb the ultraviolet radiation that comes to the greenhouse, and limit the insect's mobility, and consequently, reproduction, so that this is an important tool for the control of whitefly and thrips in greenhouse, because the trials made recorded reductions by 65 % of *Bemisia tabaci* as well as *Frankliniella occidentalis* under anti-pest plastic with relation to the control test (Pérez *et al.*, 2009).

With respect to pollinisers (Fig. 3 and 4), the experimental results show that there is an specific interaction between the anti-pest plastics and the pollinating species, so that the bumblebee activity (*Bombus terrestris*) is not affected by the use of anti-pest plastics, and it does not affect the crop yield, while the bees activity (*Apis mellifera*) is affected (López *et al.*, 2006; Pérez *et al.*, 2007), registering a reduction by 46 % in the number of bees that enter into and come out the beehive, which caused maximum reductions of production until 34 % (Pérez *et al.*, 2009).

Figure 2. Evolution of the accumulated number of Bemisia tabaci (a) and Frankliniella occidentalis (b), in chromotropic cards, under plastic with a transmissivity of 1 % (Antipest) and 55 % of UV radiation (Control test)

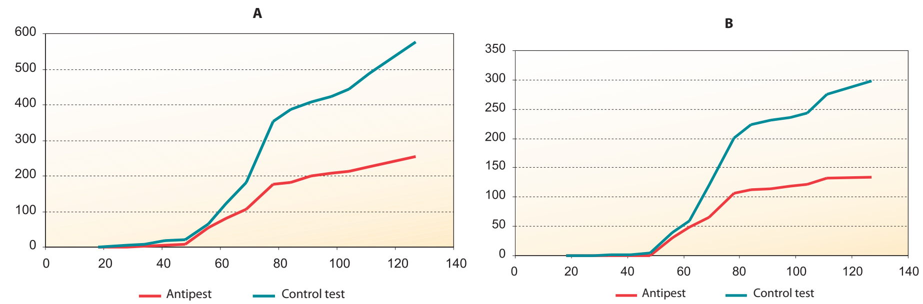
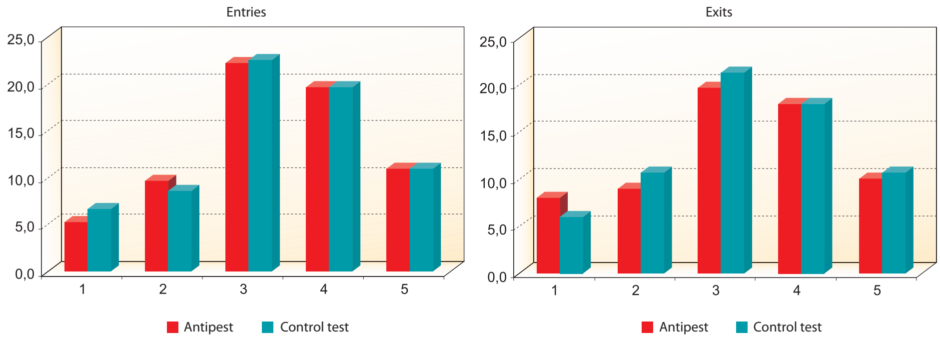


Figure 3. Following of the bumblebee activity (*Bombus terrestris*) under plastics with a transmissivity of 1 % (Antipests) and 55 % of UV radiation (Control test)

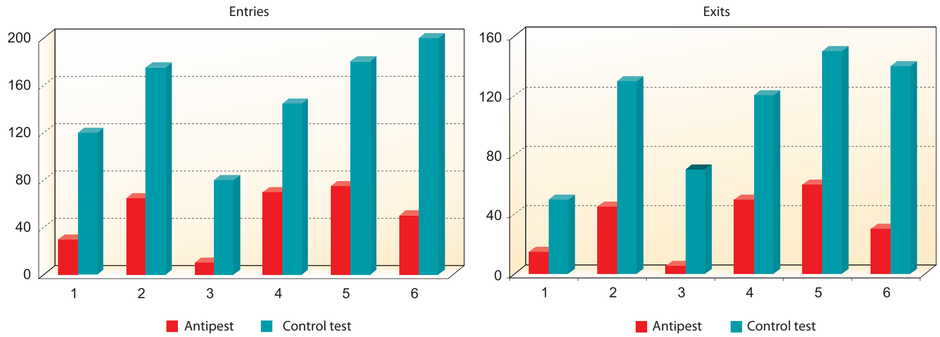
Mini-watermelon crop



Entries: Average number of bumblebees that enter into the beehives during 15 minutes of activity. Exits: Average number of bumblebees that come out the beehives during 15 minutes of activity.

Figure 4. Following of the bee activity (*Apis mellifera*) under plastics with a transmissivity of 1 % (Antipests) and 55 % of UV radiation (Control test)

Melon crop



Entries: Total number of bees that enter into the beehives during 10 minutes of activity. Exits: Total number of bees that come out the beehives during 10 minutes of activity

The results obtained show that the anti-pest plastics reduce the incidence of whitefly and thrips significantly, and do not affect the implementation of biological control and, in addition to this, the bumblebee (*Bombus terrestris*) was not affected by the use of anti-pest plastic, while *Apis mellifera* was affected. Therefore, in the crops where bees are not used as pollinating insects, such as tomato, pepper, cucumber, aubergine, green bean and even courgette, the incorporation of anti-pest plastics can allow carrying out a better control of the main horticultural pests and improve the results obtained by the biological control.

Photo 1. Bee flight (*Apis mellifera*) above melon flower

Photo 2. Bee (*Apis mellifera*) on a watermelon flower

Photo 3. Bumblebee (*Bombus terrestris*) on a tomato flower

Photo 4. Bumblebee (*Bombus terrestris*) on a watermelon flower

2.1. Anti-insect meshes

Pests, in general and specially, those acting as vector virus such as *Bemisia tabaci* and *Frankliniella occidentalis*, vector virus of tomato yellow leaf curl (TYLCV) or the tomato spotted wilt virus (TSWV), respectively, have turned into the problem of great economic repercussion within protected horticulture. For this reason, the use of meshes in the windows as physical barriers to reduce the entry of insects is an essential preventive measure in intensive production systems to reduce the phytosanitary applications and improve the successful possibilities of biological control.

Currently, the catalogues of anti-insect meshes available for farmers are very wide (meshes of different thread densities, different colours, photoselective meshes, etc.), but the need of combining the mesh effectiveness in the insect exclusion with an adequate air permeability which allows keeping proper ventilation conditions, makes difficult the selection process of a mesh.

During the last years, several studies have been carried out to characterize geometrically the commercial meshes, just as to estimate its porosity (Ross y Gill, 1994; Bell y Baker, 1997; Teitel, 2001; Bartzanas *et al.*, 2002, Cabrera *et al.*, 2002; Valera *et al.*, 2003, Cabrera *et al.*, 2006), with the purpose of determining their effectiveness as physical barrier before the entry of insects as well as the effect on natural ventilation within greenhouses.

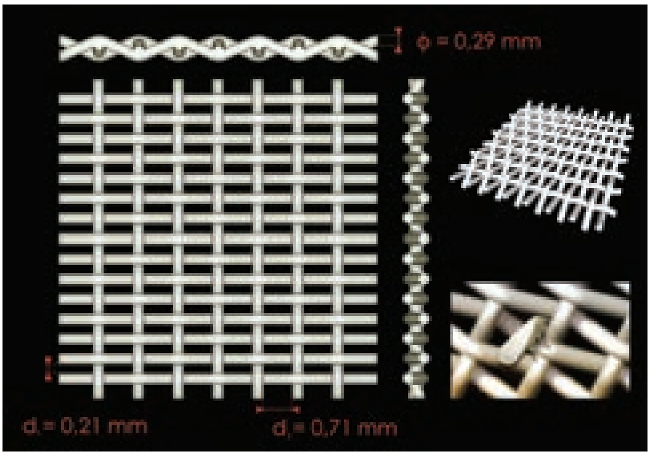


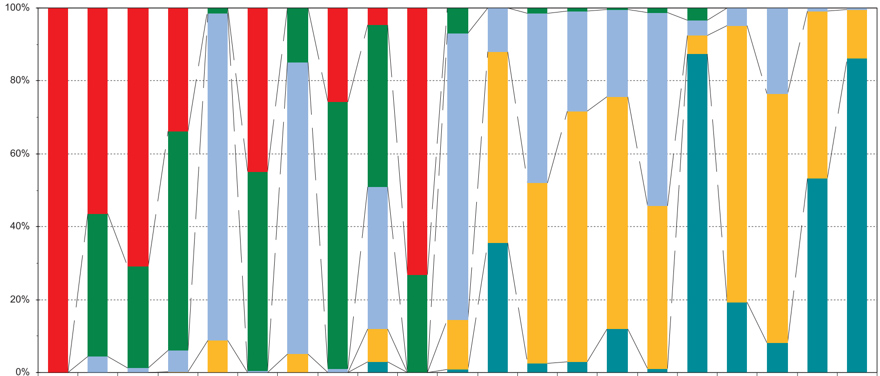
Photo 5. Digital image of an anti-insect mesh

Many of these works show that the properties of the meshes are not always well defined in the commercial offers. In the Experimental Station of the Fundación Cajamar, since 2002, several works have been carried out about description of meshes which evaluate the physical characteristics of the same and their uniformity, their effectiveness as barrier against pest insects and the effect on the ventilation rate (Fig. 5). These works have shown the lack of uniformity in the hole size and its geometry, that the mesh properties are not well defined and that it is necessary to improve the commercial identification of the meshes and to specify their characteristics. A correct definition of a mesh must include information (or allowing its estimation) about the following aspects:

- Average hole size and percentage of insect exclusion.
- Thread diameter or both diameters if it is oval, expressed in millimetres.
- Number of threads per square centimetre, describing firstly the number of threads in warp and secondly the number of threads in weft (example: 20x10 threads cm^{-2}).
- Resistance to airflow (or porosity): relation between the hole area and the total area.
- Homogeneity
- Optical properties: spectral transmissivity and spectral reflection or absorption
- Mechanic properties (Resistance to UV radiation)

Effectiveness of anti-insect meshes is due to they act as physical barriers (Bethke *et al.*, 1994; Baker and Shearin, 1994; Bell, 1997, Bell and Weatherley, 1999; Antignus, 1999; Teitel *et al.*, 2000; Critten and Bailey, 2002; Díaz Pérez *et al.*, 2003; Hanafí *et al.*, 2003; Teitel 2006), or as light filters (Bethke *et al.*, 1994; Antignus *et al.*, 2001; Teitel, 2001; Klose y Tantau, 2004) resulting in a negative effect on the crop.

Figure 5. Study of the exclusion percentage of insects for 21 commercial meshes



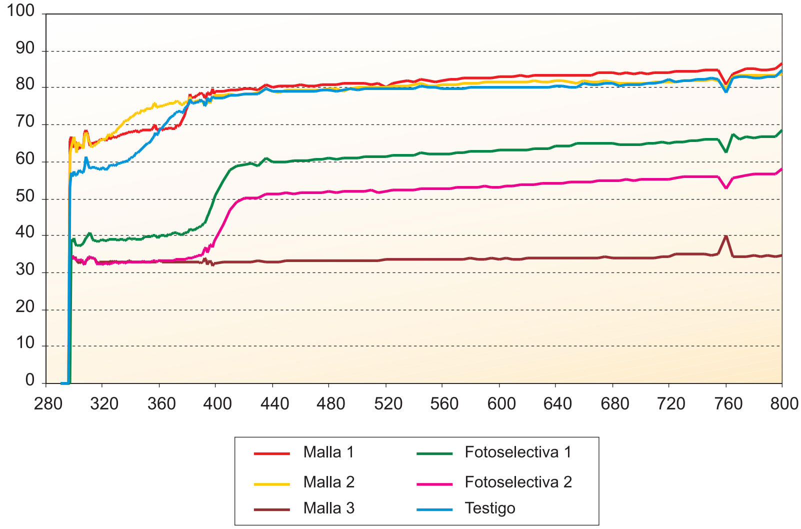
NXxNY (hilos cm^{-1})	4,6x2,8	15,3x8,7	15,0x9,2	15,3x8,6	20,5x8,7	15,6x9,5	20,7x9,2	16,1x10,2	18,4x9,6	14,9x10,4	19,8x10,2	27,9x13,4	20,2x9,3	20,7x10,6	19,3x11,1	19,8x10,6	30,1x21,4	22,6x10,7	20,8x10,3	22,3x10,8	22,5x11,0
G (mm)	(0,36)	(0,32)	(0,32)	(0,33)	(0,26)	(0,33)	(0,27)	(0,32)	(0,29)	(0,33)	(0,28)	(0,20)	(0,30)	(0,29)	(0,30)	(0,31)	(0,19)	(0,28)	(0,30)	(0,29)	(0,30)
0,32<Ø mm	1	0,563988	0,707692	0,338392	0	0,449184	0	0,256881	0,046626	0,731852	0	0	0	0	0	0	0	0	0	0	0
0,24<Ø=0,32 mm	0	0,391369	0,280769	0,60091	0,01372	0,545797	0,149062	0,732798	0,444172	0,268148	0,069259	0,000506	0,015448	0,009242	0,004263	0,013055	0,033166	0	0	0	0
0,19<Ø=0,24 mm	0	0,044643	0,011538	0,059181	0,897866	0,005019	0,800592	0,010321	0,388957	0	0,786148	0,119494	0,465499	0,273567	0,238704	0,529156	0,041651	0,048137	0,234445	0,008371	0,00326
0,15<Ø=0,19 mm	0	0	0	0,001517	0,088415	0	0,049358	0	0,090798	0	0,136087	0,524557	0,494336	0,68854	0,636829	0,447346	0,051678	0,759317	0,6844	0,458143	0,134474
Ø=0,15 mm	0	0	0	0	0	0	0,000987	0	0,029448	0	0,008505	0,355443	0,024717	0,028651	0,120205	0,010444	0,873506	0,192547	0,081154	0,533486	0,862266
	M20	M03	M01	M05	M11	M02	M14	M04	M12	M08	M07	M17	M10	M21	M06	M09	M18	M19	M13	M16	M15

Ø=0,15 mm 0,15<Ø=0,19 mm 0,19<Ø=0,24 mm 0,24<Ø=0,32 mm 0,32<Ø mm

In the Experimental Station of the Fundación Cajamar the spectrum of different commercial meshes was analysed (Fig. 6), highlighting as some of them (mesh 3) exert a strong light reduction, transmitting equally the entire spectrum (around 34 %). Photoselective meshes reduce significantly the transmission of ultraviolet radiation (a 36 and 33 % with respect to the 60 % that the rest of meshes transmit: control test, mesh 1 and mesh 2). In the visible spectrum, the average transmissivity of non photoselective meshes (control test, mesh 1, mesh 2 and mesh 3) is higher than 80 % compared with 61 % of phoselective mesh 1 or the 50 % of photoselective mesh 2 (Gazquez *et al.*, 2009).

The effectiveness depends mainly, as physical barrier, on the hole size, defined by the thickness and the number of threads, and the size and /or morphology of the pest insect. The size (thoracic and abdomen width) of the main pest insects in the greenhouses of Almería is shown in Table 1.

Figure 6. Spectrum of different commercial meshes



Mesh1: Mesh 28*13 threads cm-2 of Macrotex; Mesh 2: Mesh 30*21 threads cm-2 Econet-t of Ludvig Svensson; Mesh 3: Mesh 20*10 threads cm-2 of black colour; Photoselective 1: Mesh 21*9 threads cm-2 BioNet of Klayman Meteor L.T.D.; Photoselective 2: Mesh 21*11 threads cm-2 OptiNet of Polysack.

Table 1. Dimensions of the common pest insects present in the greenhouses of Almería

PEST INSECT	THORAX WIDTH (microns)	ABDOMEN WIDTH (microns)
Liriomyza trifolii	640	850
Trialeurodes vaporariorum	288-400	708
Bemisia tabaci	239-320	565
Frankliniella occidentalis	192	265

Photo 6. *Bemisia tabaci*

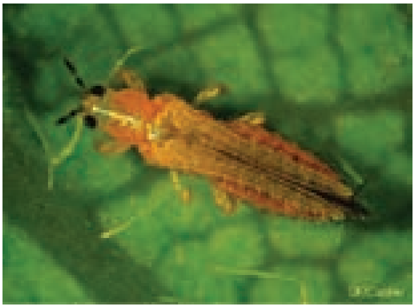


Photo 7. *Frankliniella occidentalis*

Photo 8. *Tuta absoluta*

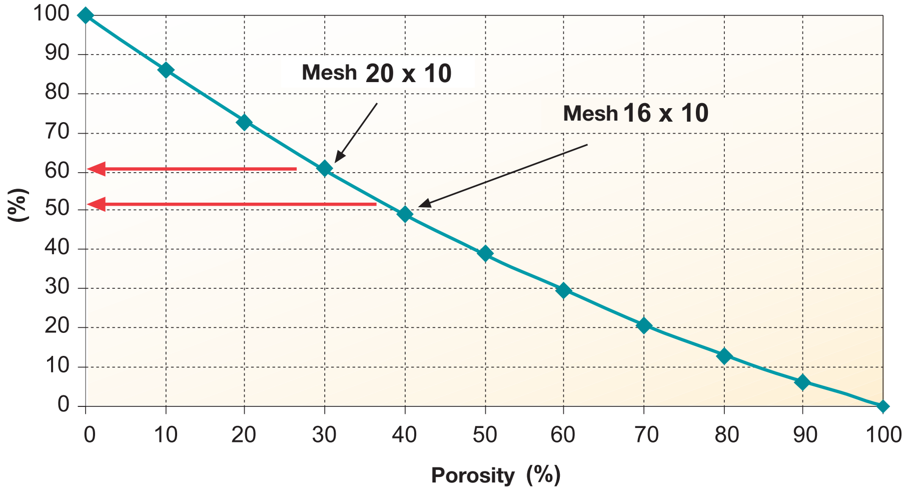
However, as it is shown in figure 7, the meshes in the windows cause a reduction of the air renewal (Muñoz *et al.*, 1999; Fatnassi *et al.*, 2002, 2006; Kittas *et al.*, 2002; Pérez-Parra *et al.*, 2003; Molina-Aiz *et al.*, 2004, 2005) (higher temperatures, more humidity, less CO_2) that must be offset either through the increase of the ventilation area (Pérez-Parra *et al.*, 2004), or to obtain a higher porosity with a higher insect exclusion, that it to say, a higher effectiveness, it is necessary a lower thread diameter (Cabrera *et al.*, 2006; Gázquez *et al.*, 2009).

Taking into account aspects such as the three-dimensional nature of the meshes, the thoracic diameter of insects and an small thread diameter (0,19 mm) Cabrera et al (2006) defined the most effective type of mesh for the exclusion of the most important two pests in agriculture under plastic (*Bemisia tabaci* and *Frankliniella occidentalis*). (Table 2)

In the Experimental Station of the Fundación Cajamar, meshes as cover material of greenhouse with different properties physical as well as optical have been compared during the spring and summer time for three consecutive years (2005, 2006 y 2007).

The influence of meshes of different thread density and photoselectivity used as covering material has been analysed on the productive response of a cluster tomato crop in summer season and with integrated control, as well as the incidence of *Bemisia tabaci*, *Frankliniella occidentalis*, Tomato Yellow Leaf Curl Virus (TYLCV) and Tomato Spotted Wilt Virus (TSWV).

Figure 7. Reduction of ventilation rate (%) according to mesh porosity (Pérez-Parra *et al*., 2004)



Source: Pérez Parra *et al.,* 2004.

Table 2. Most effective meshes for the exclusion of the two most important pests in agriculture under plastic (*Bemisia tabaci and Frankliniella occidentalis*)

Pest insect/three dimensional hole diameter (mm)	$Ø_{3D}$ (mm)	Porosity (m2 m-2) (a)	Ventilation reduction (%)	Type of mesh (cm threads^{-2})
Bemisia tabaci (*$Ø_{3D}$: 0,24*)	0,24	0,42	33	24 x 12
Trialeurodes vaporariorum	0,19	0,36	41	28 x 14

Source: Cabrera *et al*., 2006.

Photo 9. Different types of commercial meshes

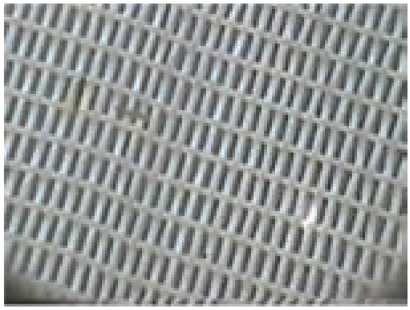


Photo 10. Mesh 28 x 14 threads/cm^{-2}

The results obtained (Fig. 8 and 9) show that the use of photoselective meshes or with a higher density than standard meshes of 20x10 threads cm^{-2} reduce significantly the levels of *Bemisia tabaci* and of TYLCV (Gazquez *et al.*, 2007). Likewise, the use of photoselective meshes does not reduce significantly the incidence of *Frankliniella occidentalis*, but this effect has been observed when the reduction of radiation under the mesh is important (black mesh). However, the strong reduction of the radiation transmitted by some meshes (black or photoselective) has very negative effects on the final yield, with yield reductions until 50 % in tomato crop (Gázquez *et al.*, 2009).

Figure 8. Evolution of the day accumulated incidence (DAI) of *Frankliniella occidentalis* in plant, under different commercial meshes

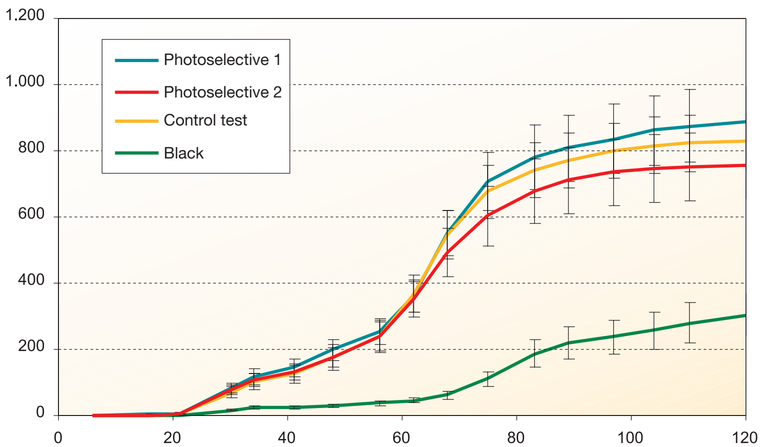
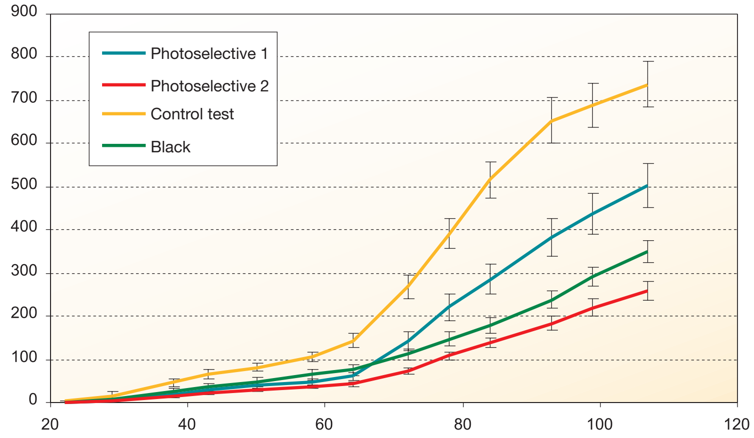


Figure 9. Evolution of the day accumulated incidence (DAI) of *Bemisia tabaci* in plant, under different commercial meshes



2.2. Cooling techniques and pest control

The need to satisfy the market demands for a continuing provision of products during the whole year, which must be stable in amount and quality, is obliging to extend the crop cycle until summer time, when inside the greenhouse, temperatures far above the optimum (T>35 ºC) and very low humidities are reached, with high deficits of vapour pressure (DPV>3 kPa). Therefore, it is necessary to dispose of some cooling systems within the greenhouse in order to keep more favourable conditions for plants which allow obtaining adequate harvests with respect to quantity and quality for a longer time.

The adequate control of environmental temperature in greenhouse is an essential factor to obtain a homogeneous and quality yield during a big productive cycle, because they intervene decisively on many physiological processes of (Gonzalez-Real and Baille, 2006). If we consider the habitual crop cycles in Almería, it is necessary to reduce air temperatures from early spring to late autumn (Kittas *et al.*, 1996; Montero *et al.*, 1998). The most practical and economic strategy, and for this reason, the most used to lower the temperature of greenhouse during day, is the combination of natural ventilation in addition to whitening of the cover (Meca *et al.*, 2007).

The incorporation of anti-insect meshes in the greenhouse windows to protect the crops from pests and diseases is a generalised practice that has been adopted in horticulture from Mediterranean Southeast. Muñoz *et al.* (1998), Pérez-Parra (2002) have quantified considerable reductions of the ventilation rate within the greenhouse which varies from a 35 until 60 % for meshes known as anti-aphid and anti-thrips, commonly used in the greenhouses. Therefore, these physical barriers favour that natural ventilation is not enough to reach a thermal and hygrometric system acceptable for the development of horticultural crops as well as for natural enemies that are introduced in the same.

The main problem of most of the multi-span flat greenhouses is the lack of adequate natural ventilation. This causes excessive temperatures inside the greenhouse and problems derived from these thermal excesses, especially if the levels of relative humidity are low: hydric stress, problems of fruit setting, fruit physiopathies ("blossom end rot", "blotching" (irregular ripening), epidermis cracking, etc.). But poor natural ventilation also has harmful effects on cold times, because these coincide with the full development of crops in Almería, whose transpiration causes the appearance of high levels of relative humidity. These levels, together with the night cooling of the cover, shall cause condensation on the interior face of plastic. Such condensation produces dripping on the crop which favours the development of fungal and bacteria diseases of the aerial part (Hand, 1984; Mistriotis *et al.*, 1997; Papadakis *et al.*, 2000). Furthermore, the condensation layer reduces the light transmission inside the greenhouse (until a 40 % at midday hours, Jaffrin and Makhlouf, 1990) limiting the light interception by the crop, and in short, the yield. Furthermore, nutritional disorders associated with the high levels of relative humidity can appear. Finally, and not less important, a bad air renewal rate can cause a drastic fall of the CO_2 levels inside the greenhouse as it is fixed by the crop as a consequence of its photosynthetic activity. With poor ventilation conditions, the CO_2 concentrations have been measured showing reductions by 25 % with respect to outside concentration with winds higher than 5 m s^{-1}, (Lorenzo, 1990); and until a 44 % with winds inferior to 1,5 m s^{-1}, which result limiting for the greenhouse productivity.

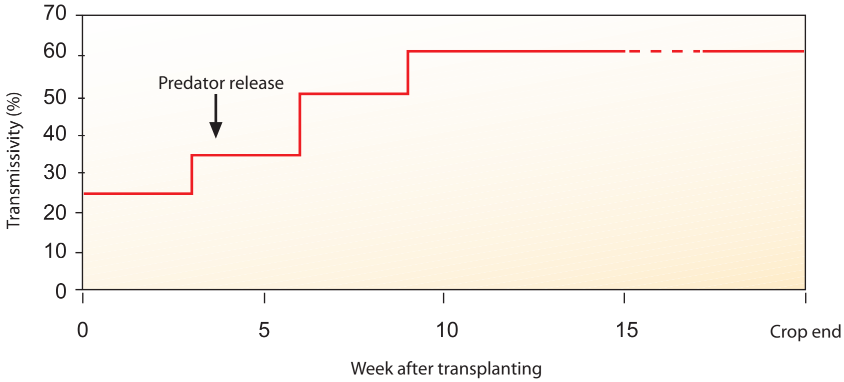
The convenience of ventilating adequately the greenhouse to avoid undesirable phytopatological problems and to provide the introduction and establishment of beneficial auxiliary fauna, specially when the installation of anti-insect mesh is unavoidable, makes necessary to enhance the design of the actual ventilation systems, increasing the ventilation area, adding more efficient windows (hopper instead of rolling) or combining side and zenithal ventilation (Pérez-Parra *et al.*, 2004)

In any case, the shading consisting of cover whitening presents a series of problems as the lime permanence during cloudy days and the lack of homogeneity when applying it (Montero *et al.*, 1998). Improvements such as the establishment and progressive wash of whitening (Fig. 10), will help the development of the crop as well as the appropriate evolution of natural enemies populations.

When the combination of natural ventilation and whitening in the higher thermal load periods are not enough to avoid high temperatures, the incorporation of other cooling systems, as forced ventilation, evaporative cooling through nebulisation, can be alternatives to take into account, due to their high efficiency. But the incorporation of technology to control excessive temperatures have effects, that must be studied, on the incidence of pests and its natural enemies to correct undesired effects.

In the Experimental Station of the Fundación Cajamar a research programme about cooling systems in greenhouses is being carried out, and different systems have been assessed: natural ventilation, forced ventilation, nebulisation, at high and low pressure, whitening at different concentrations and combination of these techniques (Aroca, 2003; Maillo, 2005; Sáez, 2005; Rodríguez, 2006; Parra, 2007; González, 2008;

Figure 10. Advisable guideline of whitening management of the greenhouse cover in pepper



Meca, 2008; Gázquez and col., 2006; 2007 and 2009; Meca and col., 2006 and 2007; Pérez-Parra and col., 2005) and the effects of such cooling techniques on the incidence of pests and diseases have been assessed.

The results (Fig. 11 and 12) show that the use of forced ventilation increased significantly the populations of *Bemisia tabaci* and *Frankliniella occidentalis* with respect to whitening and nebulisation, due to the higher penetration of the same through the mesh of the entry windows because of the fall of pressure induced by the extractor-fans. With respect to virus and diseases, there was also a higher incidence of TSWV in the treatment of forced ventilation and of *Botrytis cinerea* in the nebulisation (Gázquez *et al.*, 2007).

Photo 11. Greenhouse with natural ventilation

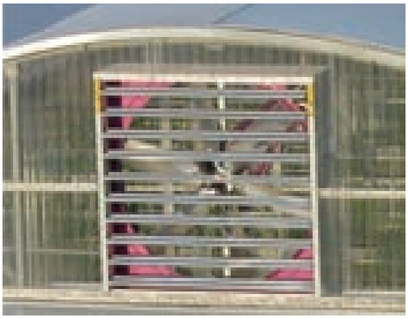


Photo 12. Forced ventilation

Photo 13. High pressure nebulisation nozzle

Photo 14. Low pressure nebulisation nozzle

Figure 11. Number of accumulated whiteflies per day (MDA) for a pepper crop under three cooling systems (T1: Forced V., T2: Nebulisation and T3: Whitening)

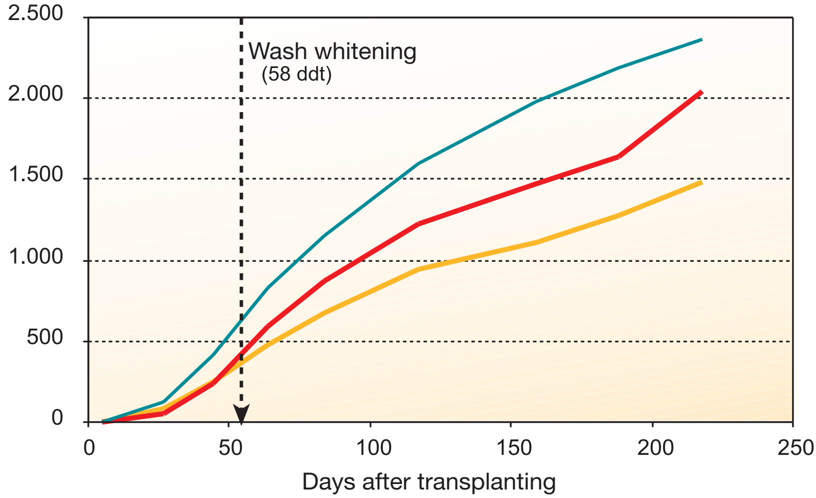
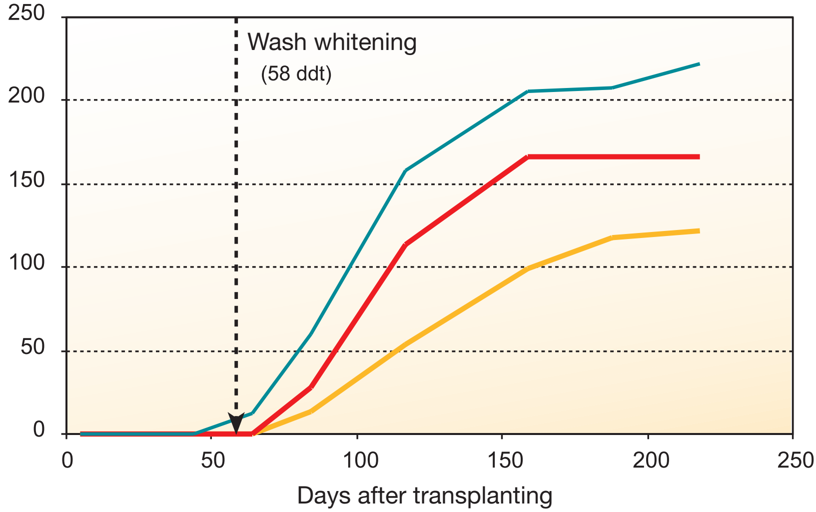


Figure 12. Number of accumulated whiteflies per day (MDA) for a pepper crop under three cooling systems (T1: Forced V., T2: Nebulisation and T3: Whitening)



In subsequent trials the influence of two cooling strategies (whitening at standard dose vs. nebulisation) was assessed on the pest incidence (*B. tabaci* and *F. occidentalis*) and TSWV in a California pepper crop in greenhouse. The nebulisation treatment, reduced the humidity deficit and hydric stress during the first weeks, but favoured the development and reproduction of thrips, keeping higher levels with respect to whitening treatment (Gázquez *et al.*, 2007). The differences found in the presence of thrips were moved to a higher incidence of TSWV in nebulisation treatment, with a percentage of plants affected of 72 % at the end of the crop, being December the month when more ill plants were found (more than 30 % of the total); in the whitening treatment, infected plants did not reach to 5 % (Fig. 13) (Gázquez *et al.*, 2009).

The results showed that the strategy of nebulisation combined with cover whitening (whitening dose: 12.5 kg of calcium carbonate (blanco España) per 100 L of water) increases significantly the populations of *Frankliniella occidentalis* with respect to standard whitening (25 kg of blanco de España per 100 L of water) (Fig. 14 and 15). With respect to the virus transmitted by thrips, there was also a higher incidence of TSWV under such treatment, and however, differences were not observed in the incidence of *Bemisia tabaci* (Gázquez *et al.*, 2009).

Figure 13. Evolution of the virus percentage TSWV, in a pepper crop under two cooling strategies: cover whitening with standard dose and nebulisation more cover whitening with reduced dose (50 %) (T:1 Whitening, T2: Nebulisation + Whitening, T:1 Nebulisation + accumulated whitening and T3: Accumulated whitening)

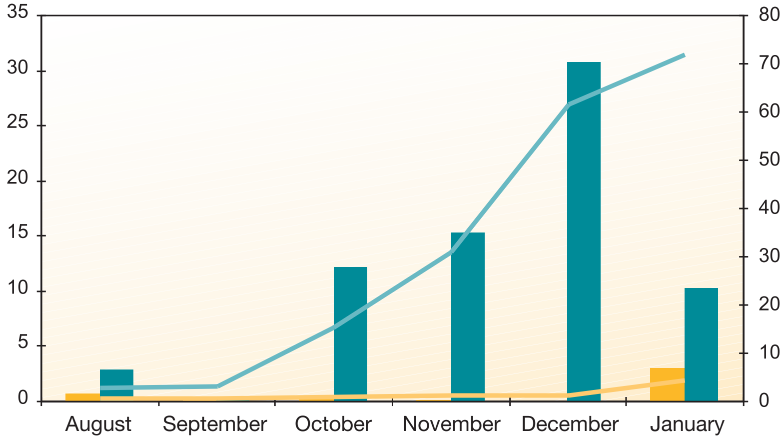


Figure 14. Evolution of Accumulated Day Whitefly (ADW) in plant, for a pepper crop under two cooling strategies: cover whitening with standard dose and nebulisation more cover whitening with reduced dose (50 %)

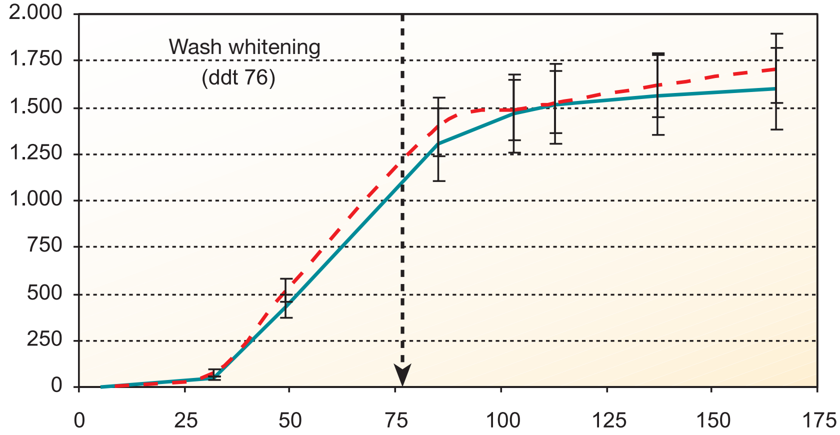
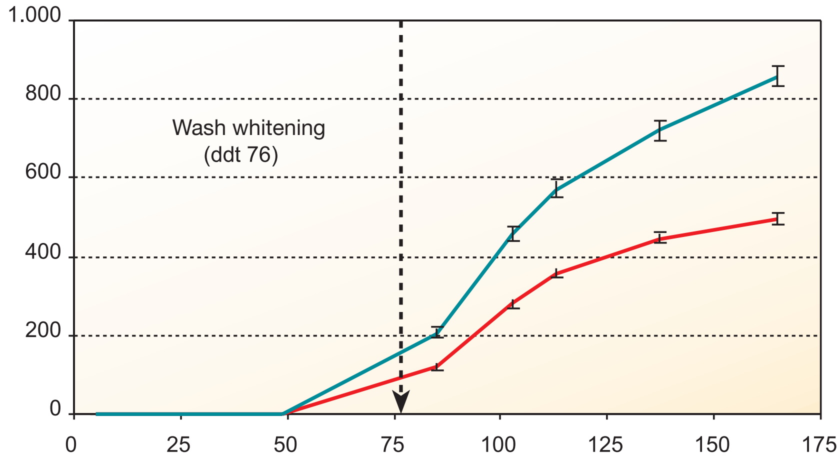


Figure 15. Evolution of Accumulated Day Thrips rate (ADT) in plant, for a pepper crop under two cooling strategies: cover whitening with standard dose and nebulisation more cover whitening with reduced dose (50 %)



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Chapter 5

Sulphur sublimators and shelter plants

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1. Introduction

In all integrated pest management programmes (IPM or ICM) it is necessary to integrate all compatible techniques between each other (cultural measures, crop operations, etc.) that permit the reduction of pathogen levels (pests and diseases). In recent years there have been evaluations of new crop techniques that interact with the complex pest-natural enemy, therefore, they influence on the effectiveness of biological control.

An example of how some cultural practices can affect the biological complex plant-pest-auxiliary fauna can be seen in the way of applying sulphur in the greenhouse or the way of introducing natural enemies into greenhouses, etc.

The crop is an essential element of all pest control strategies. Its phytosanitary state is going to depend on the cultural practices which it is subject to (irrigation, fertilization, stem removal, pruning, etc) and, at the same time, these will influence decisively on the management of the auxiliary fauna population introduced into the greenhouse. For this reason, it is necessary to synchronize the programming of the cultural measurements (pruning, stem removal...) with the releases of auxiliary fauna to stop these tasks interfering during the installation process of the natural enemies. This way, the cultural pruning tasks should be carried out only when it is possible before each release of natural enemies and should not be repeated within two weeks after the release.

In the selection of vegetable material, the characteristics that favour the introduction of natural enemies must be considered. For example, many species, mainly mites and predator bedbugs depend on flower pollen for their reproduction. Therefore, it has to be tried that the flowering

* Experimental Station of the Fundación Cajamar.

period is kept stable during the full crop cycle, which can imply a change to the way of pruning the plants, changes in the fertilization process to produce more vegetative plants and also in the method of harvesting, staggering the harvests.

The management of the releases of natural enemies can condition the success of its establishment. The releases should be done preferably at the end of the day, or if it is not possible early in the morning, to avoid they suffer from stress due to the big change from recommended conservation temperatures to greenhouse conditions in the middle of the day (generally high temperatures and very low relative humidity).

2. Use of sulphur sublimators

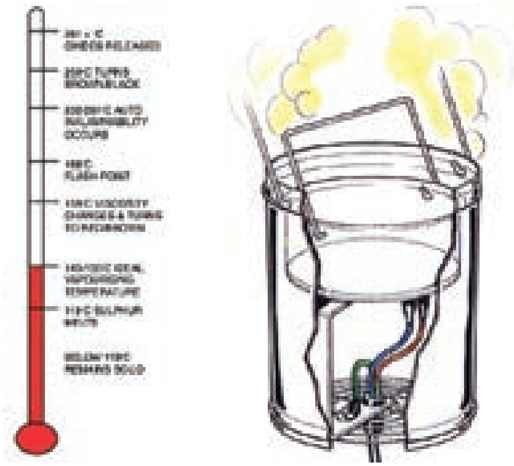
The drastic reduction of the amount of phytosanitarian treatments for diseases like powdery mildew (*Leveillula taurica)*, derived from the implementation of biological controls in crops like pepper, has turned this disease into the main fungal problem of this crop. In the greenhouses of Almería, sulphur, for its anti-mildew and acaricide properties, is one of most used products, being able to be applied in different ways: i) sprinkling (powdered sulphur), ii) foliar (liquid or wettable sulphur) and iii) sublimed (powdered sulphur). Currently, the use of sulphur sublimators to prevent the appearance of mildew is a very common technique in Holland and in the fields of Cartagena, in pepper and rose crops. This technique is not very common in Almeria, although it is expected to experience a great expansion once its effectiveness as well as its influence on natural enemies is known. In fact, the Specific Regulations of Integrated Production of Protected Horticultural Crops of the Andalusian Regional Government (2007) recommends the use of sublimators as a preventive measure for the control of *Leveillula taurica*.

The term sublimation comes from Latin *sublimare* and it is the process which consists of the state change of the matter from solid to a gaseous state without ever being liquid. Sulphur in a vapor state penetrates the cells of the mildew as a result of the solubility in lipids in cell walls of the fungus (García, 1997) In the interior of the cell the sulphur is reduced to hydrogen sulphide interfering with various metabolic processes by blocking cell breathing and inhibiting the synthesis of nucleic acids and proteins. These processes happen during the eight hours following treatment, with a maximum activity around the third hour.

Photo 1. View of a sulphur sublimator

Photo 2. View of the sulphur tablets used in the sublimators

Figure 1. Diagram of a sublimator and temperature scale and processes occurred during sublimation



Source: Brinkman, Holland.

A sublimator (or sulphur evaporation) consists of a box of stainless steel that contains an evaporation plate of aluminium where the solid sulphur is applied, under which there is an electric resistance. The temperature control is a very important factor in order to avoid unwanted generation of sulphur oxides ($SO_{2,}$ SO_4, etc.), so it should be between 145 ºC and 155 ºC. The electrical consumption of a sublimator is normally 100 W.

Recommendations for the use of sublimators:

- Use sulphur tablets with >99,0 % richness, micronized sulphur at 98,5 % can also be used but it has a higher proportion of impurities.
- Place them 30 cm over the plants and continue elevating them as the crop develops.
- Install a sublimator each 250-300 m^2.
- Watch the working order and remove the residues of the pans frequently.
- Do not overload sublimators, because they can spill once liquefied.
- Start with 2-3 hours of operation at the beginning of the crop and progressively increase until a maximum of 6-8 hours, depending on the density of sublimators, climatic conditions, sensibility of the cultivars to pests and diseases, greenhouse structure, crop cycle, etc.
- If they are working for more than 4 hours, establish various periods of 2-3 hours, with rest periods of 0,5 hours.
- Stop the sublimators 1-2 hours before entering the greenhouses and ventilate the greenhouse early in the morning or leave a safety period of 4 hours if it is not ventilated.
- Place some device/protector that prevents the sublimed sulphur to concentrate on the plastic that is immediately over the sublimator.

There are trials about the incidence of mildew in pepper in which its presence was detected in the greenhouse where the sulphur was applied on the leaves (Fig. 2), increasing gradually from the middle of December and reaching 50 % of the plants of said greenhouse in February (Gázquez y col., 2009).

Generally, the three ways of applying sulphur must not affect the implementation of biological control, however, a reduction in the populations of *Amblyseius swirskii* has been observed in the treatments of powdered sulphur compared with foliar sulphur (Fig. 3).

Figure 2. Evolution of oidium incidence in a pepper crop with application of foliar sulphur

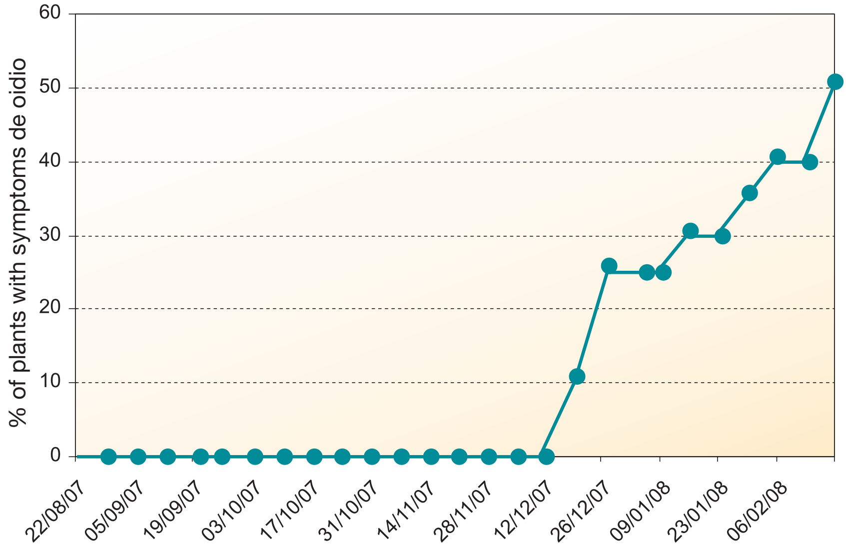
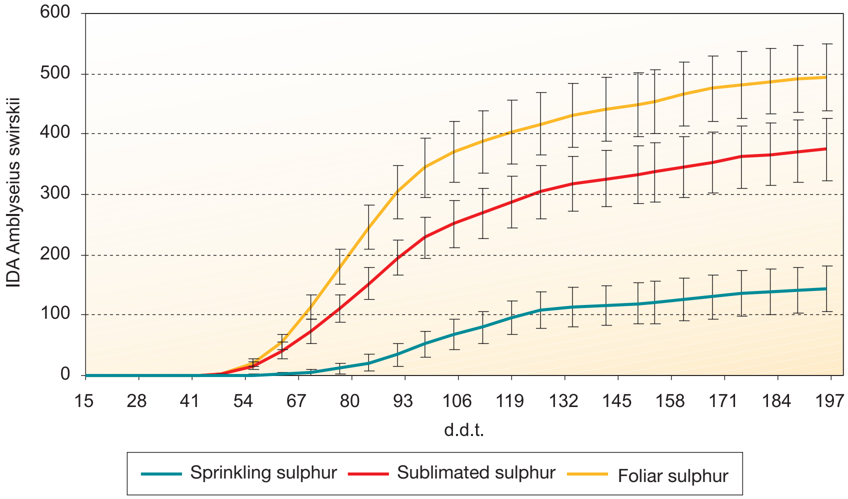


Figure 3. Evolution of oidium incidence in a pepper crop with application of foliar sulphur



Another advantage of sublimation against other ways of applying sulphur is that the plants show more vegetative development, and their aspect, especially leaves and fruits, is much better due to the absence of visual deposits of sulphur derived from the phytosanitarian applications.

Photo 3. Aspect of the plants and fruits with applications of sulphur powder

Photo 4. Aspect of the plants and fruits with applications with sublimed sulphur

Photo 5. Aspect of plants and fruits with applications of foliar sulphur

Common practice in the area of Almería, as well as in other pepper crops areas under greenhouse, is to carry out sprinkling before the release of natural enemies followed by applications of foliar sulphur. In recent trials of Gazquez and col. (2009) they compared this strategy with the application of two doses of different sublimation (a maximum 5 hours against a maximum of 8 hours), highlighting that the application of sublimed sulphur at a high dose was the most effective method to control mildew (Fig. 4).

Figure 4. Evolution of the plant percentage with oidium symptoms in a pepper crop under three different strategies of sulphur application

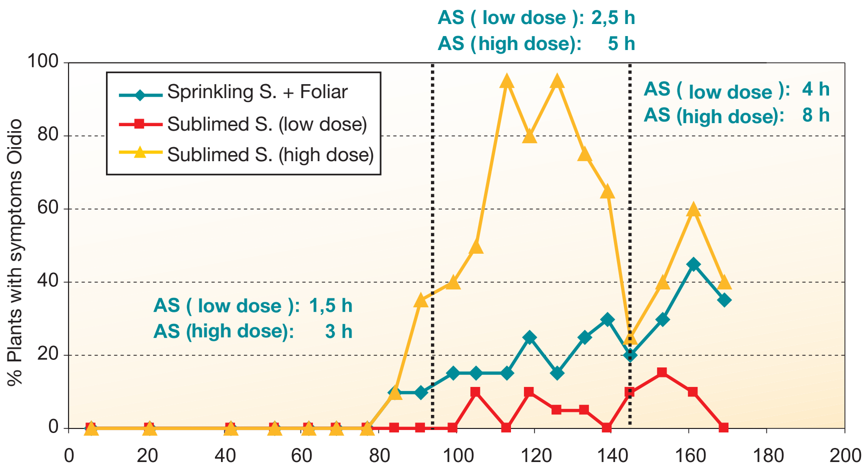


Photo 6. View of pepper plants with oidium problems under an application strategy of sublimed sulphur at low dose (2,5 hours during November and December)

Photo 7. View of pepper plants under an application strategy of sublimed sulphur at high dose (5 hours during November and December)

Experiments with sublimators have also been made in tomato crops in spring cycles where similar results to the ones from pepper crops have been obtained, that is, their good effectiveness to prevent mildew. Also, it did not affect the implementation of biologic control in the case of the tomato, at the establishment of *Nesidiocoris tenuis* (Fig. 5), nor interfering with the pollinisers (*Bombus terrestris*). However, it did not exert a good control over tomato russet mite (*Aculops lycopersici*), being even less effective the treatment with foliar sulphur.

Figure 5. Evolution of the accumulated day incidence of *Nesidiocoris tenuis* in a tomato crop strategies of sulphur application

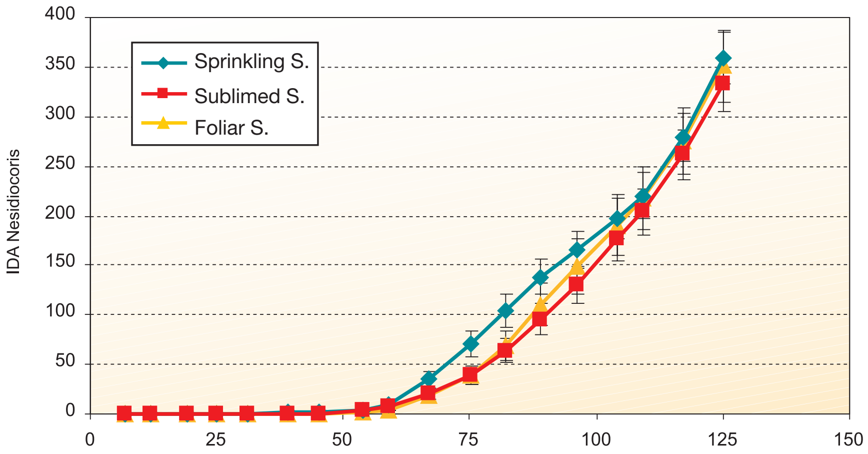


Photo 8. View of a tomato crop with foliar sulphur applications affected by Aculops lycopersici (tomato russet mite)

The service of prevention of Coexphal made a technical report about the inhalation risks of sulphur dioxide by the workers. In several trials with sublimators, the maximum levels of concentration of sulphur dioxide inside the greenhouse have been measured (1,2mg/m^3 of sulphur dioxide) and found to be much lower than the maximum accepted (5 mg/m^3). Therefore, toxic levels for people at risk of inhalation of sulphur dioxide in trials with sublimed sulphur have not been found. However, it is recommended to stop the sulphur sublimators approximately 1-2 hours before entering the greenhouses and ventilating the greenhouse at least one hour before entering to work.

We can affirm, in view of the results obtained in different field trials, that the use of sulphur sublimators to prevent the appearance of mildew (*Leveillula taurica*) is a very effective technique, though it is necessary to manage and optimize the working time of the sublimators depending on the design of the installation (number of sublimators/ha), the climate conditions, the sensibility of varieties to mildew, greenhouse structures and the release of natural enemies.

However, they have the problem that, in areas close to the sublimators, the permitted concentration levels of sulphur on plastic can be exceeded. Specifically, the sulphur concentration on the plastic of a greenhouse, in which the sublimators worked for a maximum of 8 hours a day, was measured, and in just 6 months the level was 628 ppm of sulphur. Just above the sublimator, however, a concentration on the plastic of 1684 ppm was reached, a value very close to the maximum limit (2000 ppm) fixed by the *Comité Español de Plasticos para la Agricultura* (CEPLA) (Spanish Committee of Plastics for Agriculture) for plastic of three campaigns. This can involve a risk of premature degradation of covering plastic, for which it is recommended to place some type of protector/ diffuser that mitigates this effect (Photo 9).

Photo 9. Sulphur sublimator with a device to protect the plastic above it

3. Shelter plants

The plants have a very important role in the conservation of the auxiliary insects. Not only do they find shelter or prey and alternative hosts, especially when there is a lack of such in the crops, but also many require food in the form of nectar (floral or extra-floral), pollen, seeds or plant juices. This need and the benefit of the vegetable food are well documented for many parasitoids. But maybe it has been undervalued, in the case of predators, which also depend on these vegetable resources. For example, in Sirphids, the larvae are predators, while adults need the nectar and the pollen. Various phytoseiid mites can use the pollen in the place of the prey. In diverse families of Heteroptera, both stages are omnivorous and the mixed diet of plant and prey favours the biological effectiveness in comparison with the purely carnivore diets. As a whole, this confers an advantage as opposed to other entomophagous, being able to subsist and settle in the crop when there are few pests.

The reservoir plants provide habitat, food and alternative hosts for the parasitoids and predators with which they permit an effective and efficient control of the pests in greenhouse horticultural crops. This is a preventive strategy that permits to maintain and multiply the population of natural enemies within the greenhouse, independently of the presence or not of a pest. Shelter plants consist of vegetables taxonomically very different to the crop, where natural enemy populations are bred, that is to say they cannot develop themselves in the crop or they serve as support and favour the increase of the population.

The use of the reservoir plants to help the installation of natural enemies is very little developed. Currently, in Almeria, in view of the massive increase of crop area under integrated control, it is necessary to implement this technique to optimize the installation of natural enemies.

The heteropterus *Nesidiocoris tenuis* is often used in biological control programmes in tomato crops, mainly in the control of whitefly (*Bemisia tabaci*) but as it is a polyphagous insect that feeds itself on diverse pests (thrips,...) and pollen, sap,..., it can withstand bigger populations before the excess of population causes damage to the crop. The installation process of *Nesidiocoris tenuis* is very slow and the use of reservoir plants can help.

There is a need to develop methodologies to use and maintain the natural enemies in the crop and close to it. Shelter plants can improve the biological control. The introduction of shelter plants in the crop is a key factor, and the understanding of the behaviour, movement and feeding of natural enemies is essential to obtain the desired benefits. In particular, a methodology designed to the conservation of the individuals to support an approach of integrated pest control should be developed, based on the long-term establishment of natural enemies in the environment. For example, in the case of the *Nesidiocoris tenuis*, the insect can be fed at the beginning to help the installation with eggs of *Ephestia kuehniella* Zeller (Lepidopterous: Pyralidae) (Gerling and col., 2001)

The study and assessment of those vegetable species that can act like a shelter for natural enemies could result in great interest to improve the techniques of biological pest control, as the system, if effective, could lead to a significant reduction in the application costs, due to the replacement of inundative releases for inoculative ones. Also, the presence of certain natural enemy populations established in the greenhouse, previous to the entry of pest populations, can provide an improvement in the control techniques of said populations, minimizing initial damages to the crop.

The abundance of predators in a determined host is not the only condition required for this plant to be considered “useful” in relation to the colonization of the crop. The shelters should not only assure the conservation of the entomophagous, but should also be the source of the predator and “release” it in the opportune moment. Diverse factors can determine its retention and/or dispersion from the plant, as well as its subsequent establishment in the crop: the phenological state of the plant, an excessive presence of preferred preys, a lower preference relative to the crop, an adaptation of the plant that delays the acceptance of the new host, etc.

The studies carried out by Arnó and col., (2000) show that the use of tobacco plants helps the early establishment of the Miridae in early tomato crops within greenhouse. However, to apply successfully this technique, the following aspects have to be considered:

- Check its suitability as a reservoir plant or source of food for the natural enemies, that is to say, its effectiveness in the conservation of auxiliary insects, and to guarantee the colonization of the crop.
- Determine the risk of these plants in acting as an inoculation for diseases, mostly viruses, either they can be reservoir or important hosts of its vectors.

- Verify that these reservoir plants are not too good Miridae hosts as the predator might prefer to stay on the plant, rather than colonise the crop. The shelters not only should assure the conservation of the entomophagous, but also should be the source of the predator and “release” it at the opportune moment. Diverse factors can condition its retention and/or dispersion from the plant, as well as its subsequent establishment in the crop: the phenological state of the plant, an excessive presence of preferred prey, a lower preference relative to the crop, an adaptation to the plant that delays the acceptance of the new host, etc.
- The reservoir plants should be uniformly distributed throughout the entire greenhouse to guarantee a homogeneous colonization.
- Optimize their management: the moment of the introduction, number of plants per area (ha), crop techniques, etc.

Currently, an industrial research project called CENIT - MEDIODIA is being developed (www.cenitmediodia.com) - an acronym of *“Multiplication of Efforts for the Development, Innovation, Optimization and Design of Advanced Greenhouses”*. The main objective of the project is to carry out a research basis of strategic character in the field of agriculture under plastic, for the obtaining of new multi-discipline knowledge that permits the development of a new concept of advanced greenhouse: highly automated, energy and water efficient consumption; diversified crops and profitable in any period of the year in different Spanish climates through integrated production; renewable energy and water supply; optimisation of the management of products and the value of waste.

Within this project there is a sub-project led by Agrobio S.L. in collaboration with the IFAPA-Centro La Mojonera, denominated “*Selection of shelter plants for the conservation and increasing of natural enemies in horticultural crops*”. The objective is to select autochthonous vegetable species as shelter for predators (mirids and Anthocoridae) and develop the implementation and use in the horticultural crops of Spanish south east. With its introduction in greenhouses it is intended to favour the inoculation and early dispersion of beneficial organisms, improving current biological pest control programmes. The Experimental Station of the Fundación Cajamar is collaborating in the assessment of the dispersion of *Orius laevigatus* and *Nesidiocoris tenuis* from the hosting plants to the horticultural species and the handling of the shelter plants: number of plants and placing of them inside the greenhouse.

3.1. Use of the shelter plants for the installation of aphidius

The great development capacity of the aphids makes the dispersion of the pest throughout the crop very quick if it is not controlled at an early stage. For this reason, traditionally it has been advised to keep a close monitoring of the pest, as well as the carry out preventive releases of natural enemies, that have the problem of their high costs (Vila, 2008).

The best example of the application of shelter plants is the "Banker-plant". These supply the aphid parasitoids with alternative hosts and permit the effective control of the aphid pests in the horticultural crops in greenhouses. This is a preventive strategy that allows the maintenance and multiplication of the population of natural enemies within the greenhouse, as the *Aphidius colemani*, independently, or not, of the presence of the pest.

Photo 10. Cereal shelter plant or *Banker-plant*

Photo 11. Detail of the aphid mummies parasitized by *Aphidius colemani*

Shelter plants consist of crops like wheat or barley type cereals, where aphid populations specific for these plants are bred, meaning that they cannot develop in the crop, as for example *Ropalosipum padi* (specific cereal aphid). This replacement organism serves as a host for parasitoids such as *A. colemani,* which permits the carrying out of an efficient preventive control of the aphids with low doses of natural enemy releases and with reasonable costs. (Vila, 2008).

The results of several trials have allowed a fine-tuning of the use protocols of shelter plants for the introduction and breeding of *A. colemani* in the horticultural crops of Spanish south-eastern greenhouses. With just 3 plants it was observed that it is possible to produce, in spring, 1 individual of *A. colemani* per m^2 per week. *Aphidius colemani* moves very well around the whole greenhouse and has a high capacity for searching out aphids, which allows the placement of shelter plants in any zone of the greenhouse (E. Vila, personal communication).

Currently shelter plants are being used successfully in the greenhouses of Almeria and in the Field of Cartagena. It is recommended to introduce between 4 and 6 shelter plants per hectare, depending on the crop. One week after the introduction, a release of *Aphidius colemani* should be made at 500 individuals per each 4 shelter plants. If the parasitoid develops well, more introductions are not required. The shelter plant can be replaced each 3 or 4 weeks for a new one if the cereal aphids (*Ropalosiphum padi*) run out.The management of these shelter plants is simple as it does not require detailed counts. Simply, it has to be verified that there are cereal aphids at the time of the release of the parasitoid and observe that there are mummies in the plant 2 weeks after the releases, watching for the possible development of hyper-parasitism, in this case, it is convenient to carry out releases of predators. However, the irrigation has to be managed correctly and they have to be placed in a place with good light, as well as avoiding the arrival of ants (Vila, 2008). Currently it is necessary to replace these plants several times throughout the crop cycle. For this reason, it is convenient to select other more resistant species and of more bearing, which would allow the increased availability of parasitoids.

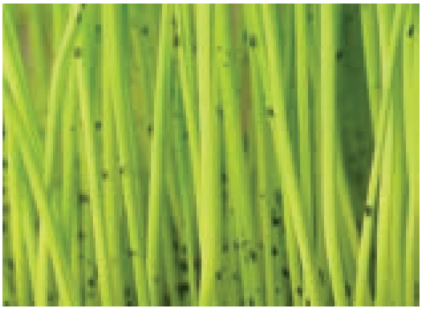


Photo 12. Detail of *Rophalosipum padi* populations, which is the substitute host used for breeding *Aphidius colemani* within the crop

Photo 13. Detail of the *Rophalosipum padi* populations (specific cereal aphid)

3.2. Use of the shelter plants for the installation of anthocoridae (orius)

Currently it is necessary to wait for the pepper crop to be flowering before carrying out the introduction of *Orius laevigatus*, so that in absence of thrips population this predator can feed on pollen and establish itself conveniently. The use of shelter plants can solve this problem and with it we can improve and, more importantly, to bring forward the establishment of the Orius.

The apple mint (*Mentha suaveolens*), is a plant that can be found naturally in the west of Almería and is a good shelter during the months of summer for *Orius*, and many farmers harvest them in order to introduce them inside the greenhouse, either extracting the insects present on this plant or cutting the inflorescences and depositing them over the pepper crop. It has also been verified that *M. suaveolens* does not present any risk of hosting the main viruses that affect peppers.

Photo 14. View of the apple mint plants (*Menta suaveolens*)

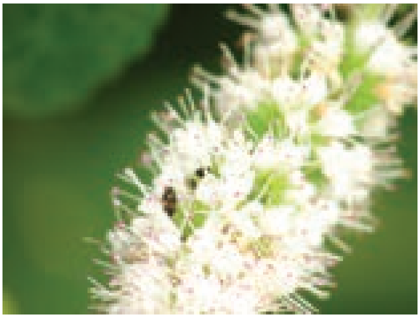


Photo 15. Detail of the apple mint inflorescence, *Orius* shelter

In the Experimental Station of the Fundación Cajamar they have carried out experiments using apple mint (*Mentha suaveolens*) as a shelter plant of *Orius,* being a good host of the same during the flowering stage. However, some problems have been presented. This plant acts as an attractant for thrips, red spider and especially whitefly. The presence of other pests has also been identified, for example *Nezara viridula*. So, special attention has to be paid to the healthy state of these plants as we can introduce other pests into our crops.

It flowers only during the summer period (May to October).

Photo 16. Detail of apple mint plants as whitefly attractant (*Bemisia tabaci*)

Photo 17. *Nezara viridula* and the damages that causes on pepper fruits

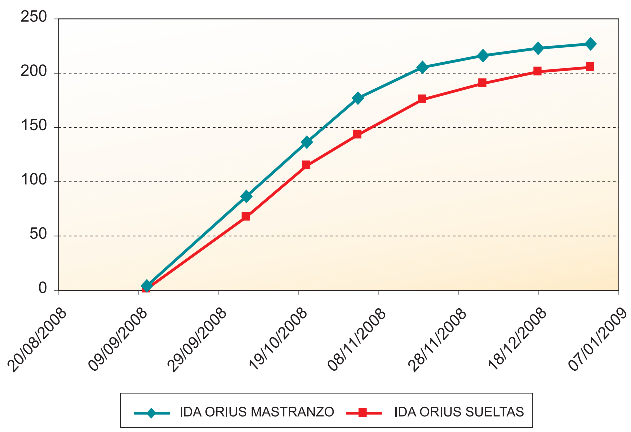
Inside the framework of the Cenit-Mediodia project trials have been carried out in the field of the Experimental Station of the Fundación Cajamar to evaluate the dispersion of the predators from the shelter plants and their establishment in the crop with very successful results. Specifically, "shelter plants" that can favour the early installation of mirids and Anthocoridae in tomatoes and peppers have been assessed.

Photo 18. View of the apple mint (*Menta suaveolens*) in a pepper crop

Shelter plants of *Dittrichia viscosa* (false yellowhead) for *Nesidiocoris tenuis* were assessed, a key predator in the biological control of whitefly in tomato crop, and *Mentha suaveolens* (apple mint) for installing *O. laevigatus*, a predator of great importance in the control of thrips in pepper crops.

The results obtained confirmed that *Mentha suaveolens* (apple mint) is a good host of *Orius laevigatus*, that are well dispersed from these plants to the crop, and also allowing the early installation of the predator in the pepper crop (Fig. 6).

Figure 6. Evolution of accumulated day incidence of *Orius laevigatus* in a pepper crop under two introducing strategies of *Orius*: Apple mint plants vs. releases



3.3. Use of the shelter plants for the installation of mirids (nesidiocoris)

The biology, behaviour and effectiveness of *E. mundus* is well documented (Stansly andcol. 2004, 2005; Urbaneja and Stansly 2004), and releases of this parasitoid are made for the control of *B. tabaci* in greenhouse crops in this region (Urbaneja and col. 2003).

On the other hand, there is very little information and the *N. tenuis* potential for the control of this whitefly has been slightly researched (Calvo and col., 2008). It is often quoted for its poliphagous character (Goula, 1985; Urbaneja and col., 2005) or its zoophytophagous behaviour (Dolling, 1991). *N. tenuis* has been considered a good agent of biological control as it preys on whitefly, thrip, leafminer, mites and lepidopterans in greenhouses (Arzone and col., 1990; Calvo and Urbaneja, 2003; Carnero and col., 2000; Marcos and Rejesus, 1992; Solsoloy and col., 1994; Torreno, 1994; Trottin-Caudal and Millot, 1997; Vacante and Benuzzi, 2002; Vacante and Grazia, 1994).

The heteropterus *Nesidiocoris tenuis* is often used in biological control programmes in tomato crops, mainly in the control of whitefly (*Bemisia tabaci*) as it is a polyphagous insect that feeds itself on diverse pests (thrips,..) and pollen, sap, etc. The installation process of *Nesidiocoris tenuis* is very slow and the use of shelter plants can help it.

The management of predator mirids in the protected crops of the Mediterranean is based mainly on the natural colonization of the greenhouses from the surrounding vegetation. Alomar and col. (1994) identified various non-cultivated species as important sources of *D. maman* and *M. caliginosus* and it has been demonstrated that the quantity of coloniser individuals is influenced by the abundance and closeness of these plants to the tomato fields, though the crops can also be colonised from relatively distant sources (Alomar, 2003). More recently, (Gabarra and col. (2004), have reached a similar conclusion in a study about the role of the vegetation around the greenhouses in their colonization by the mirids.

The establishment of these mirids in the crop is the biggest limitation as the capacity of these mirids to control pests is closely linked to its good establishment (Trottin-Caudal and Millot, 1994). And even getting to establish itself in the crop, its dispersion and the control of the pest are generally too slow if the introduction doses are not very high and to increase said doses is not economically feasible.

Photo 19. *Nesidiocoris* in a tomato plant

Photo 20. *Nesidiocoris* in geranium

Photo 21. Placing of geraniums in the greenhouse

Photo 22. View of the geranium inflorescence

A deeper knowledge of its feeding habits, the relations that they establish with their hosts, of the factors that intervene in its dispersion behaviour and the improvement of diverse aspects of the commercial breeding, as the substratum of lay and the use of artificial or semi-artificial diets can have a bearing on its effectiveness for the control of pests.

It is also known that the mixed diets (plant and prey) in comparison with purely carnivorous or phytophagous diets improve the development, survival, fertility and longevity rate.

During the spring season in 2007 in the Experimental Station of the Fundación Cajamar a trial was carried out of *Nesidiocoris tenuis* establishment using the geranium as a shelter plant (*Pelargonium spp.* in tomato crop (Gazquez and col., 2007).

Figure 7 shows the evolution of the populations of Nesidiocoris, thrips and whitefly in a spring tomato crop using geranium plants as a shelter for Nesidiocoris. We can observe how the population of Nesidiocoris took nearly 100 days to present adequate establishment levels,

Figure 7. Evolution of Nesidiocoris, thrips and whitefly populations in spring tomato crop using geranium plants as Nesidiocoris shelter

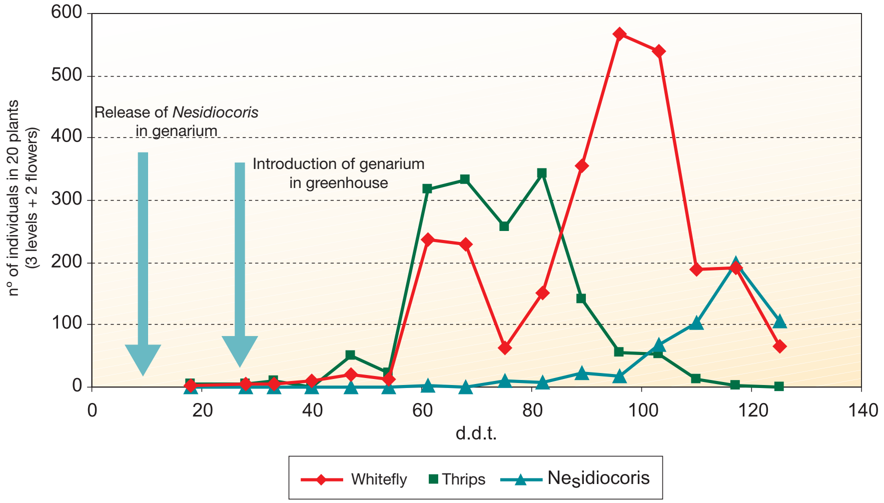


Photo 23. Introduction of *Dittrichia viscosa* plants (false yellowhead) in a greenhouse with tomato crop

Photo 24. View of the *Dittrichia viscosa* (false yellowhead) plants used as Nesidiocoris shelter

achieving an adequate control of fly and thrip populations from that moment. Therefore, the use of geranium as a shelter plant, in spring, helped the establishment of Nesidiocoris but in an excessively slow manner (Gazquez and col., 2007). A later trial in the autumn-winter season was carried out and a good establishment of the insect in the shelter plant was not produced, which means that it is necessary to study in depth the use of shelter plants exploring the possibility of using other species.

Figure 8. Evolution of the number of Nesidiocoris per tomato plant (area with false yellowhead vs. without false yellowhead)

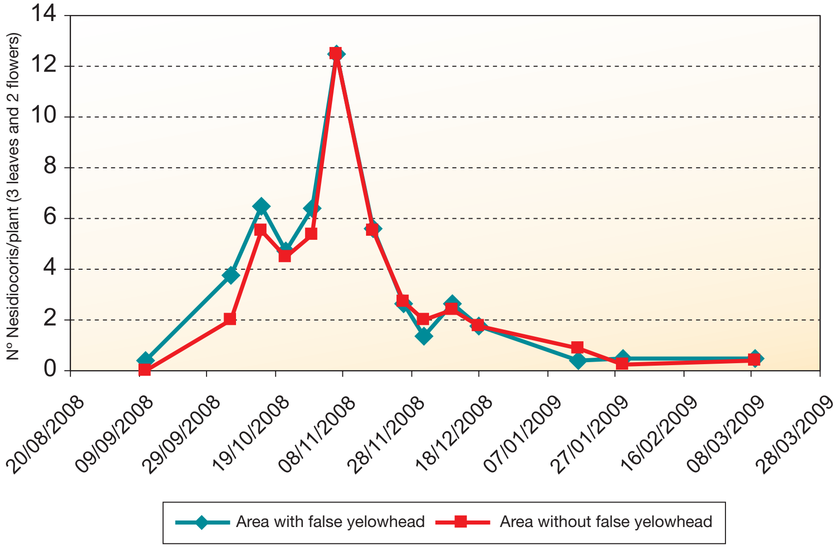


Figure 9. Evolution of the number of Bemisia per tomato plant (area with false yellowhead vs. area without false yellowhead)

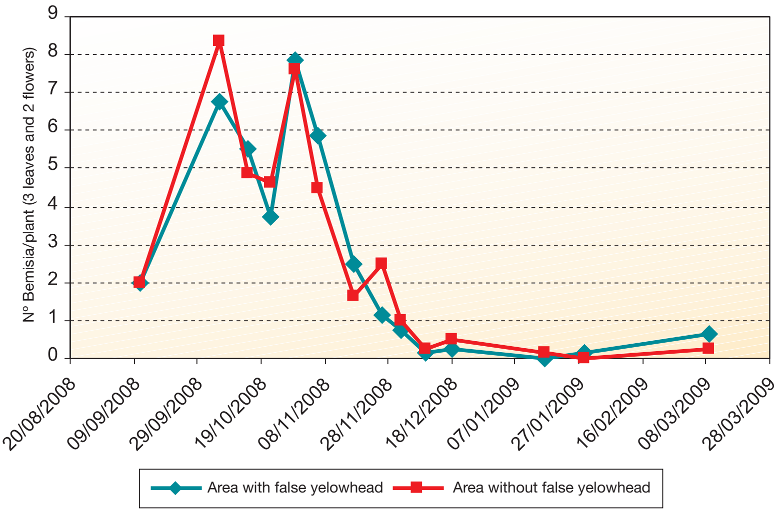


Photo 25. False yellowhead plant within the greenhouse

In the Experimental Station of the Fundación Cajamar they have assessed shelter plants of *Dittrichia viscosa* (false yellowhead) to install *Nesidiocoris tenuis* in tomato crops within the Cenit-Mediodia project framework. Figures 8 and 9 show the evolution of the populations of *Nesidiocoris tenuis* and *Bemisia tabaci*, respectively, in the trial greenhouse, checking how Nesidiocoris tenuis is able to complete its biological cycle over *Dittrichia viscosa,* presenting a great dispersion from the false yellowhead plant to the crop to all the greenhouse zones, doing a good control of *Bemisia tabaci*.

Photo 26. Nesidiocoris feeding on Ephestia eggs

In line with this, another experiment was carried out in a tomato crop in autumn in which a greenhouse (900 m^2), where the standard protocol of biological control in tomatoes in the area was followed, and releases of doses of 1 individual/m^2 were made, was compared with another greenhouse (900 m^2) where no releases of Nesidiocoris were made but 4 false yellowhead plant pots were introduced (previously inoculated 6 weeks before with 5 couples of *Nesidiocoris tenuis* that were fed on *Ephestia sp* eggs until observing the second generation of adults). Figures 10 and 11 show the evolution of the populations of *Nesidiocoris tenuis* and *Bemisia tabaci*, respectively, in these two greenhouses, showing as the greenhouse where shelter plants of false yellowhead have been used, *Nesidiocoris tenuis* has been established before than in the greenhouse where the release of Nesidiocoris was made and has exerted a better control of *Bemisia tabaci*. However, it is necessary to be prudent in regards to these initial results and to be aware that it is necessary to continue carrying out studies which do not allow this technique to be optimized to be able to add it to our release of natural enemy protocols with the maximum guarantee.

Figure 10. Evolution of the number of Nesidiocoris per tomato plant (area with false yellowhead vs. without false yellowhead)

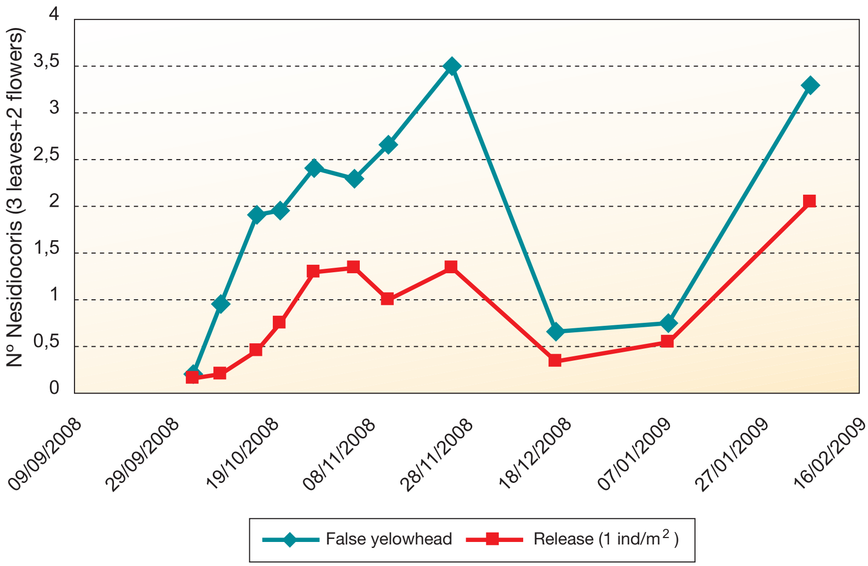
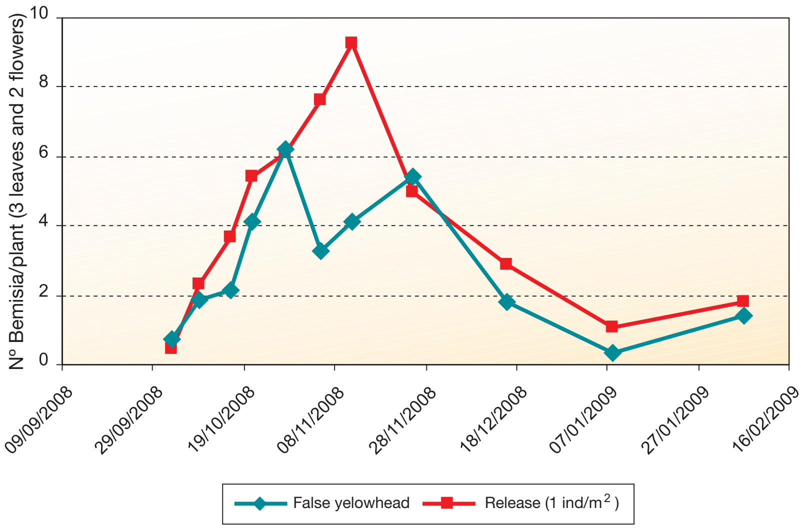


Figure 11. Evolution of the number of Bemisia individuals per tomato plant (area with false yellowhead vs. without false yellowhead)



4. Trap plants and vegetal barriers

Trap plants are those plants which are placed in the greenhouse with the objective of attracting the pest and exerting biological control over said pest. The trap plant has to be more attractive for the pest than the crop and has to offer good installation possibilities and the maintenance of natural enemy populations. Some trap plants are already being used in small scale crops. The clearest example is the use of tobacco plants as "trap plants" in seedbeds with *Macrolophus caliginosus* established over its leaves, which act as a trap for whitefly.

The use of barrier crops as a control method of non-persistent virus can be an efficient strategy. There are experiments in the outdoor that have demonstrated that the use of sorghum as a crop has reduced the virus levels of CMV and PVY in peppers. These barrier crops do not act like a physical barrier that reduces the levels of pests, but they reduce the rate of infection of some viruses by inoculating the vector of the virus in the barrier crop before reaching our crops.

Photo 27. View of the high concentration of greenhouses, where there is hardly physical space to place shelter plants of natural enemies

Photo 28. Detail of the vegetal barriers that could be placed outside the greenhouses

The horticultural crops in the province of Almeria form a vast and dense extension of greenhouses practically uninterrupted by other types of vegetation. This density makes the crops highly vulnerable to the phytopathogens that affect the production most and to the viruses that are transmitted by insects. When the insects come out a greenhouse with a virus, they practically do not have any other option than to enter another nearby crop, infecting it consequently with the virus they are carrying.

An interesting environmental measure to slow the dispersion of phytopathogens is the creation of vegetal barriers between the horticultural crops, with the objective of attracting and controlling in a natural way the pests proceeding from the greenhouses. The effect of vegetal barriers is described as effective in relation to many monocultures as different as fruit trees (apple and pear orchards), cereal or lettuce fields and outdoor horticultural crops (Albajes and Alomar, 1999; Alomar, 2003; Vila, 2004). For these crops, plants have to be selected as:

- Attractive for pests.
- Good shelters or food sources for the natural enemies.
- It is also very important to determine the role that they can play as a source of a disease inoculation - mainly virus - that usually affect the crops of the area and do not take unnecessary risks, either being important shelters or hosts for their vectors.

Photo 29. View of the spontaneous vegetation that can act as shelter of natural enemies

Photo 30. Detail of the catching operation of natural enemies in spontaneous vegetation

The polycultures (associated or mixed crops, intercropping, in strips, by relieving) and the rotations have shown their capacity to favour the presence of different entomophagous. A known example is the alternate cut of different strips in a lucerne field that allows the retention of predators; the harvest of the whole field allows them to be released towards the adjacent crops (eg: sweetcorn or cotton). Since some years ago, in England perennial grasses are sown in ridges inside cereal fields («beetle banks») to offer shelter and prey for the diverse Carabidae during the winter. The use of shelter plants allows the maintenance of a localized breeding in the greenhouse before the appearance of the pest and even before the installation of the crop. The development of soil conservation systems through reduced farming techniques and the use of living vegetable covers (spontaneous or sown) in the perennial crop streets (fruit, vine, olive grove) can also be used to create shelters of entomophagous, especially when specific areas are left without cutting.

The adventitious flora of the crops requires a special consideration. The results of the different prospecting in the crops show clearly that many natural enemies of their pests are found in plants traditionally considered as «adventitious weed».

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Chapter 6

Lepidopteran management

Luis Miguel Torres-Vila[1]

1. Introduction

Many lepidopteran species, butterflies and moths, can cause different kinds of damages in greenhouse crops. However, fortunately, only a reduced number of these cause economic losses important enough to require direct control actions. Here we will emphasize on the species that are currently more relevant and the techniques that can be used in integrated control, with special stress on biological control.

2. Lepidopteran pest species[2]

The main biological information provided for the species of the families Noctuidae and Pyralidae has been mainly gathered from Cayrol (1972), Guennelon (1972) and CABI (2000). Literature about Gelechiidae family is provided in the corresponding section.

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[2] The pictures shown in this chapter have been made by P. del Estal, A. Lacasa, P. Bielza, P. Bueno and L.M. Torres-Vila

2.1. *Helicoverpa armigera* Hb.

Noctuidae

Common name: heliothis, tomato fruitworm

Description

Eggs are yellowish, sub-spherical (0.5-0.6 mm) and with longitudinal striations. Larvae can become up to 40-45 mm long in the last instar and are green, ornamented with longitudinal lines and brown tubers. Pupae (20-25 mm) are dark brown. Adults (35-45 mm of wingspan) show a marked sexual dimorphism, having greenish-gray forewings (males) or light brown (females) with a spot in the middle and a darker distal band.

Biology and behavior

H. armigera develops usually three annual generations in the south Iberian Peninsula with flights in May-June, August and September-October. Oviposition lasts from 1-3 weeks, and a female can lay more than 3000 eggs (> 400 in 24 hours). Eggs are laid singly, especially on the underside of the leaves close to the flowers. The flowering period exerts a strong attraction on the ovipositing female. Hatching occurs 3-4 days after egg laying and, at the beginning, larvae feed on biting superficially leaves and stems. When larvae reach the third instar, they bear the fruits (tomatoes) or the floral buds (carnation, cotton) to which they are associated definitively, although they can change several times of fruitful organ. When completing the development, the larvae go down to the soil and bury themselves to pupate, although it has been described that they can pupate also inside the tomato fruits, preparing them properly (Torres-Vila *et al*., 1996). In about 1 to 3 weeks, depending on temperature, the adult emerges. In the third generation, diapause is induced in larvae due to the photoperiod and temperature, which shall be expressed in the pupa. The insect overwinters in this stage, completing the annual cycle. The fast larval development and the staggered emergence of first flight adults cause often an overlapping between generations, and all the evolutional stages of the insect can be observed simultaneously. This situation is even more complicated the years when immigrations are produced, because *H. armigera* shows, in addition to a great mobility in adult stage, a marked migratory potential (Torres-Vila *et al*., 2002a, 2005, Torres-Vila 2003).

Photo 1. Adult and caterpillars of *Helicoverpa armigera* Hb.

Damages

H. armigera causes important damages in several greenhouse crops (horticultural and ornamental), being especially serious in those whose flowers or fruits are marketable such as carnations and tomatoes. Damages are shown at two levels: reducing the yield and decreasing quality. The bites and borings produced by larvae cause the fall of flowers and young fruits. Often, the most developed fruits do not fall from the plant, but they are damaged and unusable. The injuries favor the colonization of different saprophyte microorganisms which cause rottenness that, together with insect remains (exuviae and frass), provoke an important reduction of health and commercial quality. This aspect is especially severe in *primor* crops or presentations of fruits *in cluster* because small aesthetic damages are not tolerated. The predilection of this species for marketable vegetal organs rich in nitrogen (sprouts, flowers and fruits), high polypaghy, geographic extension, dispersal power, migratory potential, facultative diapause, high fecundity, and tendency to develop insecticide resistance are the main factors that contribute to its major pest status (Fitt 1989, Zalucki 1991, CABI 2000, Torres-Vila *et al*., 2002a,b, Torres-Vila 2005).

Photo 2. Damages of *Helicoverpa armigera* Hb.

2.2. *Spodoptera exigua* Hb.

Noctuidae

Common name: beet armyworm

Description

Eggs are brown-yellowish (0.4 mm) and striated. Larvae show very different colors, greenish to brown-grayish, with longitudinal brown stripes bordered by yellowish lines on the sides and dorsum. Larvae can reach 30-40 mm long in the last instar. When they are disturbed, they protect themselves rolling up as a ring-shaped pastry, from which one of its Spanish common names derives. Pupae (20-25 mm) are brown and are located in the soil forming an earthy cell joined with silk. In pepper crops, pupae are very often found inside the fruits. Adults (25-30 mm of wingspan), without a clear sexual dimorphism, have mottled brownish forewings, with two characteristic orange spots.

Photo 3. Adult of *Spodoptera* exigua Hb.

Photo 4. Caterpillars of *Spodoptera exigua* Hb.

Biology and behavior

Usually it presents 2-3 annual generations, but in the protected crops from Almería it can have even six. The female oviposits mainly on the underside of the most mature leaves, medium and basal ones, forming throughout the oviposition period 2-3 egg clusters (ooplates) of 50-250 eggs each one (the last clusters are often smaller) protected with white-grayish abdominal scales. After hatching, the larvae remain grouped, keeping this gregarious habit until the third instar when they disperse and then cause the greatest damage. *S. exigua* shows a considerable migratory potential (Torres-Vila, 2003, Torres-Vila *et al*., 2005).

Damages

Unlike *H. armigera*, whose more severe damages are caused in the fruitful organs, *S. exigua* is a species mostly defoliator, although it can also damage young fruits. In ornamental crops, the most severe damages are caused in shouts and flowers. In the first instars, the larvae feed on foliar epidermis respecting the venation. The most developed larvae consume the entire leaf thickness, causing important foliar losses and decreases of the photosynthetic capacity of the plant. The larval injuries make easier the entry of saprophyte fungi and bacteria and the development of rottenness. *S. exigua* is a polyphagous pest that can attack tens of cultivated species and, among the most attacked crops under greenhouse, we find pepper, tomato, watermelon and melon.

Photo 5. Damages of *Spodoptera exigua* Hb.

2.3. *Chrysodeixis chalcites* Esper

Noctuidae

Common name: tomato looper moth

Description

Eggs are whitish, sub-spherical (0.5 mm) and with longitudinal striations. Larvae are 35-40 mm long in the last instar, they are bright green with a longitudinal white-yellowish stripe in each side of the body and finer dorsal white lines, spotted with ocelli. The larvae only have three pair of prolegs, therefore they move around arching the body as the geometrids, from which the common name is derived. Their morphology is characteristic, the body being gradually enlarged from the head to the back part. Freshly formed pupae (20 mm) are greenish and then turn dark brown. Adults (35-40 mm of wingspan) have dark brown forewings spotted of light brown and violet color, with two characteristic pearl-white spots. At rest, they show a prominent thoracic crest of modified scales.

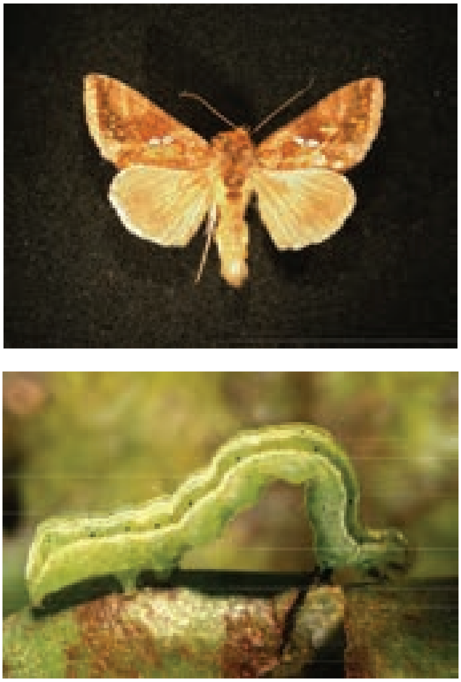

Photo 6. Adult and caterpillar of *Chrysodeixis chalcites* Esper.

Biology and behavior

This species develops two or three annual generations, with flights between June and November. Females lay the eggs singly or in small groups mainly on the leaves, being the fecundity about 500 eggs. The larvae are primarily phyllophagous and complete their development in 2-3 weeks. The pupae are located in the aerial part of the crop, protected by a light silken cocoon. In greenhouse, it can appear in larval stage during the whole winter. The adults of *C. chalcites* also show migratory habits.

Damages

This is a polyphagous species that develops on many horticultural and ornamental crops, including tomato, pepper, green bean, courgette, cucumber, aubergine, melon, watermelon, rose and gerbera. When the larvae are small, their damages are limited to small bites in the foliar parenchyma, especially on the underside of the leaves. In the last stages, they consume the entire foliar thickness, damage being especially severe in recent plantations. The damages in fruits are usually of scarce consideration.

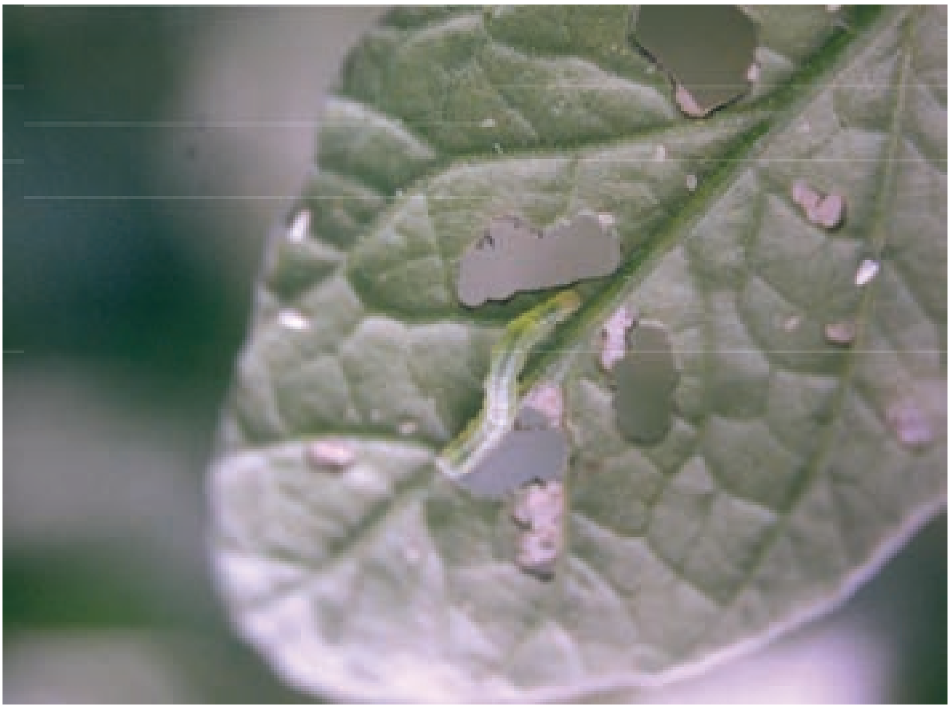

Photo 7. Damages of *Chrysodeixis chalcites* Esper.

2.4. *Autographa gamma* L.

Noctuidae

Common name: plusia, silver Y moth

Description

Eggs are very similar to those of *C. chalcites* but a little bigger. They are whitish, sub-spherical (0.7 mm) and with longitudinal striations. Larvae can reach up 40 mm long in the last instar and their morphology is very similar to *C. chalcites*, with a fusiform body and movement arching the body because they have only three pairs of prolegs. The color varies from intense green to bluish green bordered on either side by a clear white line and dorsal thinner white lines. Pupae (20-25 mm long) are also greenish when just formed and then turn dark brown. Adults (35-40 mm of wingspan) have brown-grayish forewings, darker in some areas, on which a reniform pearl-white spot stands out which reminds of the Greek letter *gamma*, from which the species takes the name. At rest they show a thoracic crest of modified scales.

Photo 8. Adult of *Autographa gamma* L.

Biology and behavior

Just like *C. chalcites*, it develops two or three annual generations, but adults of *A. gamma* may be found the whole year. Females can lay up to 2000 eggs, singly or in small groups, arranged on leaves and sprouts. The larvae consume mainly leaves and complete their development in 3-4 weeks, pupating on the leaves and stems inside a light silken cocoon. It is characteristic the high daylight activity of adults, in addition to the twilight and night activity which is usual in the species of this family. The species shows an extreme migratory potential that in some occasions has caused some social alarm (Torres-Vila 2003, Torres-Vila *et al*., 2005).

Photo 9. Caterpillars and damages of *Autographa gamma* L.

Damages

A. gamma is a polyphagous species that develops on many horticultural and ornamental crops, practically the same that those mentioned for C*. chalcites*, the damages due to defoliations also being very similar. Occasionally, it can also damage the tomato fruits.

2.5. *Ostrinia nubilalis* Hb.

Pyralidae

Common name: European corn borer.

Description

Eggs are lenticular (1 mm in diameter) and they are grouped in clusters, slightly overlapped each other. They are off-white when just laid and then turn yellowish. The larvae may grow up to 20-25 mm long in their last instar and they vary of color, from off-white to grayish or light brown and even pinkish, with dark head and prothoracic plate. The pupa (20 mm) is dark brown, and it is smaller and sharper in males. The adults (25-30 mm of wingspan) show a light sexual dimorphism, being females, in general, of light brown color and males of a darker color. The spread wings on the imago body at rest remind of an arrow tip which ends forming a peak in the maxillary palpi, very prominent in prognata position.

Photo 10. Caterpillar of *Ostrinia nubilalis* Hb.

Biology and behavior

This is a very polyphagous species, and it has been described on many crops and weeds. It presents several pheromone races worldwide, characterized by its pheromonal blend (E, Z or its hybrid E/Z), the trait being regulated genetically and with polymorphic variation, which is important when synthetic sexual pheromones are used in the management of the species. In Spain, it develops usually two annual generations with flights in May-June and July-August. The adult life span is 10-15 days. The female can lay up almost 1000 eggs (an average of 500-600) in groups of 5-50 units (an average of 20-30) forming ooplates or egg clusters. In pepper crops, eggs are usually laid directly on the fruits. Hatching occurs within 4-10 days and larvae feed on making tunnels in the stems, or bearing the fruits and staying inside them when they develop on pepper. When reaching the last larval instar (15-30 days according to temperature) larvae pupate in the vegetal remains and, in the case of pepper, also inside the fruits. The hibernation takes place with the diapause induced in last-instar larvae. When diapause is broken after overwintering, larvae spin a light cocoon and pupate, and adults emerge usually 7-12 days after.

Damages

O. nubilalis is a key pest of corn and sorghum, but many years ago it was also mentioned, sometimes anecdotally, on some crops that can be produced under greenhouse, such as pepper, escarole, gladiolus, chrysanthemum and dahlia. In the last years, and due to unknown causes, its incidence in pepper has worsened greatly, even in outdoors crops, and it has become a primary pest to be controlled in this crop. The larvae bore into the fruits, and as they are hollow, the symptoms of their activity are not shown until damages are very advanced. Sometimes larvae move from a fruit to another dispersing not only saprophyte microorganisms which cause rottenness, but also pathogens as the bacteria *Erwinia carotovora* Jones. The larval damages of *O. nubilalis* in pepper crops usually get worse in later crop cycles.

2.6. *Tuta absoluta* Meyrick

Gelechiidae

Common name: tomato moth

Description

Eggs are creamy or yellowish with a pseudo-cylindrical shape (0.35 x 0.20 mm). Larvae are clear brown in the first instars and then turn greenish or brown-pinkish, reaching a length of almost 10 mm at the end of their development. The pupa (10 mm) is brown-greenish at the beginning and it gets darker in the course of time. Adults (10 mm long and 15 mm of wingspan) have brown-grayish forewings with chestnut-brown patterns and three black points in the middle line (Monserrat Delgado 2009, EPPO 2009a).

Biology and behavior

T. absoluta shows a high biotic potential as it can complete more than 10 generations per year (approximately one generation per month) and each female can lay more than 250 eggs. Female oviposits in the tenderest parts of the plant, mainly in the sprouts and the underside of the leaves. Eggs hatch in 4-5 days and the larvae develop quickly making mines and tunnels in leaves and fruits. When completing larval development larvae pupate in the same plant or in the soil. Hibernation may take place practically in any stage, but when food is available and temperature is not a limiting factor, the development is extended to the whole year without diapause (Monserrat Delgado 2009, EPPO 2009a).

Photo 11. Adult, caterpillars and pupa of *Tuta absoluta* Meyrick

Damages

The first outbreaks in Spain of this oligophagous pest of South American origin were detected in Castellón in 2007 and in Almería in 2008, being tomato the most attacked crop by far. The larvae can affect the crop from the seedling stage, and they cause damages mainly in the leaves and sprouts, although they also affect the fruits. Occasionally, tunnels can appear in stems, especially in the base of foliar and peduncular insertions. In the leaves, the larvae consume the thickness of the mesophyll, respecting only the foliar epidermis, which provides the insect some protection from the outside environment. These damages have a wide tunnel shape and an undifferentiated path which will necrotize, and they are very different from the elongated and winding mines of *Liriomyza* spp. In the foliar tunnels observed against the light, the larva and its droppings can be clearly observed. In the fruits, the damages are shown as borings and more or less superficial tunnels. The damages can be located in any part of the fruit, but often the borings start from a sheltered area for the larvae, as for example the contact area with leaves and other fruits or under the sepals of the calyx. The larvae can damage the fruits from very early phenological stages, even in fruit setting. The injuries in young fruits, even though they are small and cicatrize later, can result in undesirable deformities during their subsequent vegetative growth.

Photo 12. Damages of *Tuta absoluta* Meyrick

The larval damages in leaves and fruits can certainly decrease yield, but the most serious problem derives from fruit quality loss. Tomato fruits which are going to be sold in the fresh market greatly reduce their value due to the aesthetic damages that, as in the case of *H. armigera*, can be even worsened due to insect remains and the presence of saprophyte bacteria and fungi. In addition to this, we must take into account the important problem, limiting in some markets, derived from the marketing and exportation of fresh fruit, because *T. absoluta* is currently listed as a quarantine pest (EPPO 2009a) and therefore, it is subject to a phytosanitary and legislative specific framework in each geographical area (cf. Chapter 14).

2.7. *Keiferia lycopersicella* Walsingham

Gelechiidae

Common name: tomato pinworm

Description

Eggs are pale yellow when laid, but turn orange before hatching. The larvae can reach 10 mm long, and they are greenish gray with pinkish spots in the dorsal part of the segments at maturity. Therefore, macroscopically, they are very similar to the *T. absoluta* larvae. Pupation is made in a silken cocoon on the plant leaves although it can also occur in the soil, in this case the pupa being also protected with sand grains. The adult is between 5 and 7 mm long, of grayish color with darker spots (Geraud-Pouey and Pérez 1994). The study of genitalia is essential to avoid identification mistakes between the gelechiids *K. lycopersicella* and *T. absoluta*.

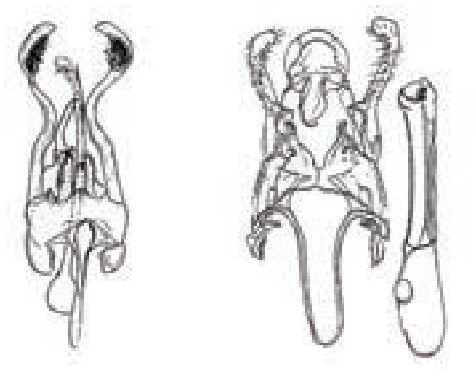

Figure 1. Genitalia of *Keiferia lycopersicella* Walsingham (left) after Zimmerman (1978) and of *Tuta absoluta* Meyrick (with the aedeagus extracted) after Povolny (1975)

Biology and behavior

Females lay eggs singly or in small groups of 2-3 eggs on the plant foliage. Larvae are leafminers at least during the two first larval instars. When larvae increase their size, they can leave the mine and feed externally on the leaves. Throughout the crop cycle and as population increases, fruit damages also increase (Zimmerman 1978). In the origin area of *K. lycopersicella* between 7 and 8 generations may occur per year, the different development stages often being overlapped with one another.

Damages

The species is native from America and was described on specimens from the Caribbean island of Saint Croix. Currently, it is distributed by the north of Brazil, Hawaii, Cuba, Haiti, Bahamas, Colombia, Venezuela, Guiana, Mexico and the south of the United States. *K. lycopersicella* was reported for the first time in Europe from Italy at the end of the year 2008 (Sanino and Espinosa 2009). Therefore, this moth is a potential danger to tomato crops in the rest of the continent, especially in the Mediterranean Basin. This species feed on plants from the Solanaceae family, so that in addition to tomato we can find potato and aubergine between their most important hosts. The larvae of *K. lycopersicella*, as the larvae of *T. absoluta*, are mainly leafminers, making translucent mines in the leaves and, to a lesser extent, tunnels in the fruits, especially those in contact with the attacked leaves. The most important damages derive generally from the attack to fruits.

2.8. Other Noctuidae

In this section we are going to mention only other species of noctuid lepidopterans that can also cause damages in several horticultural and ornamental greenhouse crops. The bad so-called *gray worms* include several species of the genus *Agrotis* (*A. segetum* Den. and Schiff. and *A. ipsilon* Hufnagel, among others) but also representatives of other genera such as *Noctua pronuba* L. and *Peridroma saucia* Hb. All these larvae are hidden during the day buried in the soil, and at night they can cause an important damage to roots, plant neck and low stems, especially in nursery seedlings and recently-transplanted plants.

Other species as the tomato moth (*Lacanobia oleracea* L.), the cabbage moth (*Mamestra brassicae* L.), the semi-looper (*Trichoplusia orichalcea* Fabricius) and specially the Egyptian cotton leafworm (*Spodoptera littoralis* Boisduval) can cause important defoliations in addition to contaminate the fruits with frass.

Photo 13. Entomophagous predators of lepidopteran eggs and caterpillars: adult (a) and larva (b) of *Chrysopa* sp., adult (c) and larva (d) of *Orius* sp., adults of *Podisus maculiventris* Say attacking a *S. exigua caterpillar* (e), and adult of *Nesidiocoris tenuis Reuter* (f)

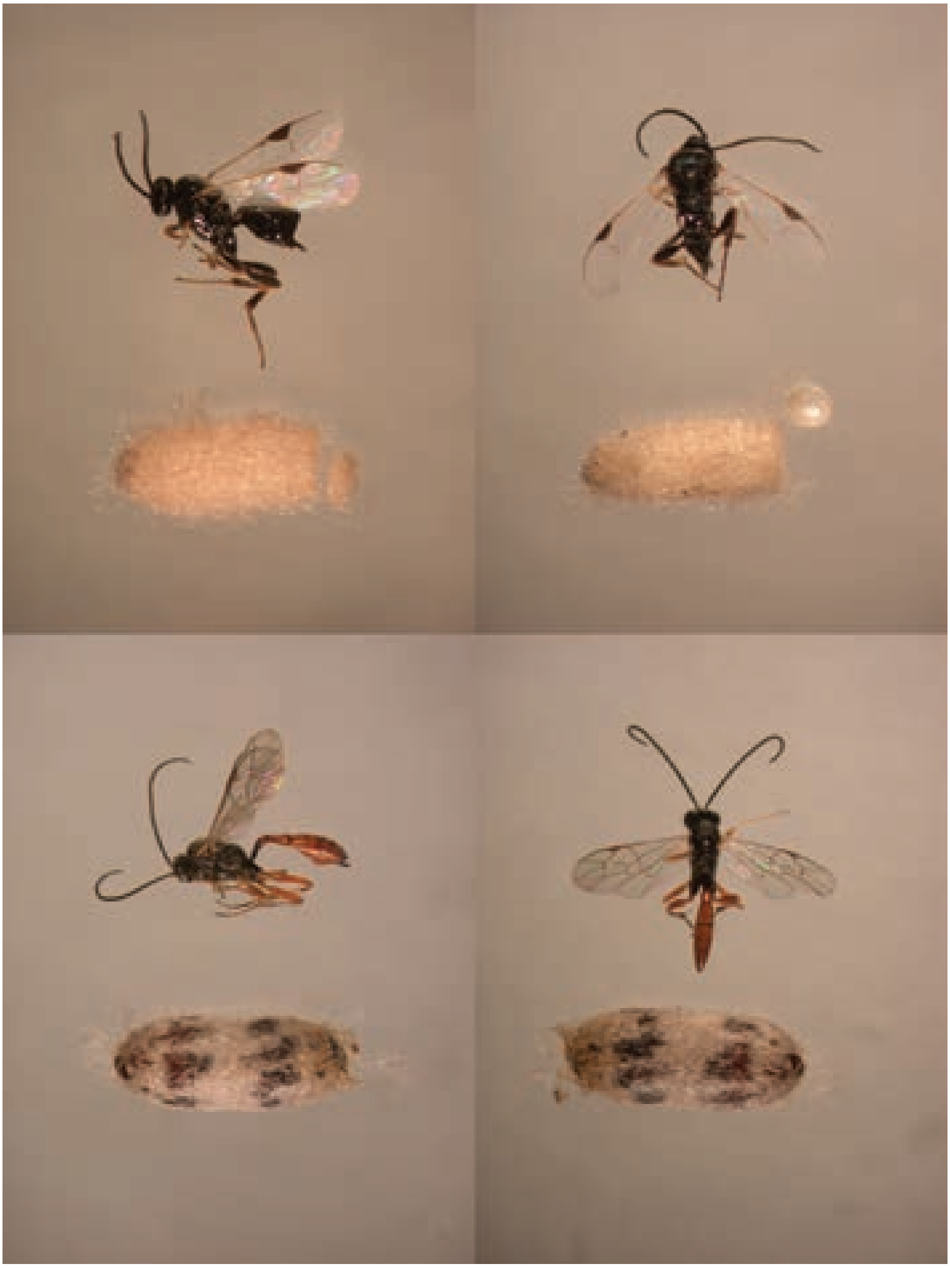

Photo 14. Entomophagous parasitoids of lepidopteran caterpillars: adults (lateral and dorsal positions) and cocoons of Cotesia kazak Telenga (above) and Hyposoter didymator Thunberg (below)

Photo 15. *S. exigua* caterpillar died by SeNPV infection

3. Integrated control of lepidopteran pests

3.1. Biotechnical methods: sexual pheromones

The sexual pheromones of insects are exocrine hormones or semiochemicals that act from a distance allowing the encounter of both sexes. In the lepidopterans, these substances are species-specific isomeric blends of long-chain hydrocarbons produced by female in most cases. There are two distinct ways of using synthetic sexual pheromones in an integrated protection system depending on the intended purpose: control (mass trapping and mating disruption) or monitoring.Mass trapping

The purpose of mass trapping with synthetic female sex pheromones is to attract, capture and eliminate the males from the area to be protected. Mating is avoided and consequently females cannot lay viable eggs so that there is neither offspring nor crop damage. The trap types commonly used for this purpose are the funnel traps for medium-sized lepidopteran species and the sticky delta traps for smaller species. Mass trapping has the advantages of being environmentally friendly and very species-specific, but method also has constraints in species highly polygynous (in which males mate with numerous females), with high dispersal potential and/or with high adult population densities (Torres-Vila 2007). The high population density that characterizes the species studied here is thereby a problem as mass trapping is more likely to succeed with pests that, despite causing much damage at the larval stage, have relatively low adult population levels (Howse *et al*., 1998). Mass trapping has been recommended to control some of the species discussed herein, including *C. chalcites* and *A gamma*, maintaining 8-10 traps/ha during the whole crop cycle, though effectiveness must be tested in each field situation. The oil-water traps seem to be effective with *T. absoluta*, for initial infestations or low populations, at a density of 20-40 traps/ha.

Mating disruption

As in mass trapping, the purpose of this method is to avoid matings, the offspring and damages, but in this case causing the air to become permeated with the synthetic sexual pheromone in the crop area to be protected. The aim is to eliminate the males' capability to recognize the chemical trail of calling females, known as the pheromone plume. The diffusion of the pheromone is carried out using special devices that may vary in shape and size, called dispensers. Currently, there is technology available to produce dispensers that can last all season without the need to be replaced, which has a positive impact on cost. The mating disruption method is also environmentally friendly (if managed correctly it does not generate waste) and highly specific (Torres-Vila 2007). The greenhouse structure greatly prevents the pheromone cloud being swept away by the wind and avoids the consequent efficiency loss. The dispersive capacity of adults, particularly high among noctuids, favors invasion from the outside of the greenhouse (especially in the case of interior lighting at night) and this may be avoided by using an appropriate mesh over the ventilation windows, which may be the same as those used against thrips or whiteflies, thus achieving dual protection. Technical advice and on-going (and adequate) surveillance is essential to achieve good efficiency with this method. It is worth remembering that mating disruption in a greenhouse must not only provide significant results from a biological perspective but must also be valid from an agronomic standpoint. This is a necessary, but not sufficient condition to be able to move from a research and development phase to a commercial phase in a given pest species.

Monitoring

The purpose of the monitoring process is to estimate the flight curve reflecting the population dynamics of adults (males if female sex pheromones are used) in order to optimize the timing of phytosanitary interventions employing insecticidal, biological or other control methods. The abovementioned funnel or delta traps are also used for monitoring, in which at regular intervals (1-3 times per week) the number of males captured is counted. A standard design for monitoring could be established with a density of at least 1-2 traps/ha, increasing the number in smaller greenhouses. Population monitoring with sexual pheromone traps is a well-established and widely used method among farmers, ATRIAs and ADVs in Spain. The main advantages are that it is an ecological method (does not generate waste), highly specific and low cost. Several companies provide commercial dispensers with the synthetic sexual pheromones of the lepidopteran species mentioned in this paper.

3.2. Insecticidal control

In recent years, new types of synthetic insecticides presenting an innovative mode of action have been added to the classic groups (carbamates, organophosphates, pyrethroids). They are characterized by a low environmental impact and by its low or no effect on auxiliary fauna and therefore, they are a valuable tool in integrated control programmes if necessary. It is worth mentioning as active ingredients the examples of flufenoxuron and teflubenzuron (benzoylureas inhibiting chitin synthesis), indoxacarb (oxadiazine that acts by blocking the sodium channel), especially recommended for the control of *T. absoluta*, or tebufenozide (non-steroidal ecdysoid mimetic of the molting hormone).

There are also included the so-called *natural products* that incorporate other novel active ingredients, often of plant origin (cf. Chapter 3) or microbial origin. They are difficult to classify, as they are not synthetic products and cannot be classified as chemical insecticides *sensu stricto*, but either as bioinsecticides as their mode of action is not the direct result of a pathology. Among the most commonly used for the control of lepidopteran and other insect pests, it is worth mentioning azadirachtin (tetraterpenoid of neem tree seeds, *Azadirachta indica* A. Juss., which acts as a growth regulator (IGR) affecting both the metabolism of the ecdysone and juvenile hormone, but with an uncertain mode of action) or spinosad (a spinosyn antagonist of the nicotinic receptor of the acetylcholine obtained from the soil actinomycete *Saccharopolyspora spinosa* Mertz and Yao), also particularly effective against *T. absoluta*. The biological insecticides or bioinsecticides are included in the following section on biological control due to their own nature and mode of action.

For the proper use of both these and conventional active ingredients, and especially to avoid or minimize the occurrence of insecticide resistance, we must scrupulously follow good general practices with phytosanitary products and particularly the recommendations for insecticide resistance management (IRAC 2009). Insecticide resistance is an underlying problem with the use of pesticides, which is exacerbated in species with several generations per year, especially given that the supply of authorized active ingredients has declined dramatically in recent years. Insecticide resistance to several chemical families, especially pyrethroids, has been experimentally shown in Spain in the case of *H. armigera* (Torres-Vila *et al*., 2002a,b, Torres-Vila 2005). Chemical control is still necessary today against lepidopteran pests in protected crops in most situations, although it is also implicitly recognized that it is essential to promote other complementary control methods.

3.3. Biological control

Biological control techniques include the use of natural enemies (entomophagous and entomopathogens) and bioinsecticides (commercial preparations based on microorganisms and entomopathogenic nematodes). Tables 1, 2 and 3 show the current most important biological control agents (or whose potential use is promising), selected for their applied interest from the extensive list of species preying on, parasitizing or causing diseases in the lepidopteran species studied here (CABI 2000, Cherry *et al*., 2001, Jacas and Urbaneja 2008, EPPO 2009b, Liñán 2009, Urbaneja *et al*., 2009, NHM 2009).

Entomophagous insect species (Table 1) capable of preying on lepidopterans in its early stages (eggs and larvae) are mainly bugs (Hemiptera: Miridae, Nabidae and Pentatomidae), orius (Hemiptera: Anthocoridae), lacewings (Neuroptera: Chrysopidae) and to a lesser extent, ladybugs (Coleoptera: Coccinellidae). However, given that orius, lacewings and ladybugs are specialized mainly in the predation on aphids, whiteflies or thrips, in most situations they only offer a complementary control on lepidopterans. In any case, since the management of lepidopteran pests in greenhouse must be further integrated into the programmes implemented against other insect pests, especially whiteflies and thrips, the right choice of the predator species in each case is essential to optimize the biological control.

Table 1. Entomophagous predators used in the biological control of lepidopteran pests in greenhouse crops

Biological control agent		Pest	
Order / Family / Species[a]	**Status[b]**	**Species[c]**	**Stage[d]**
Hem.: Anthocoridae			
Orius sp.	+++	*H. armigera, S. exigua, S. littoralis, C. chalcites, A. gamma, Agrotis sp., O. nubilalis*	E C
Hem.: Miridae			
Dicyphus tamaninii Wagner	+	*H. armigera*	E C
Macrolophus caliginosus Wagner	+++	*H. armigera*	E C
Macrolophus pygmaeus Rambur	++	*H. armigera, T. absoluta*	E C
Nesidiocoris tenuis Reuter	+++	*T. absoluta*	E C
Hem.: Nabidae			
Nabis pseudoferus Remane	+++	*H. armigera, S. exigua, C. chalcites, A. gamma, Agrotis sp., T. absoluta*	E C
Hem.: Pentatomidae			
Podisus maculiventris Say	+++	*H. armigera, C. chalcites*	E C
Podisus nigrispinus Dallas	+	*C. chalcites. T. absoluta*	E C
Neur.: Chrysopidae			
Chrysopa sp.	+	*H. armigera, S. exigua, C. chalcites, A. gamma*	E C
Chrysoperla carnea Stephens	+++	*H. armigera, S. exigua, C. chalcites, A. gamma, Agrotis* sp., *M. brassicae*	E C

[a] Only the species with a higher applied interest, current or potential, are shown.
[b] Status: research: +, development: ++, or commercial: +++. The assignment to each class is sometimes intuitive.
[c] Species where the trophic relation has been positively shown. This non exhaustive list is subject to be extended due to the generalist character of predators.
[d] Development stage susceptible to be preyed on, E: Egg, C: Caterpillar.

The most important entomophagous parasitoid species (Table 2) from the viewpoint of applied biological control of lepidopterans include small wasps parasitizing eggs (Hymenoptera: Trichogrammatidae) and larvae (Hymenoptera: Braconidae and Ichneumonidae). It is worth mentioning for its effectiveness *C. oculator* y and specially *Trichogramma* spp., without forgetting the native and foreign species of *Cotesia* (Torres-Vila *et al*., 2000, Urbaneja *et al*., 2002, Cabello *et al*., 2005). The management of entomophagous may be tackled in various ways but, in the case of greenhouse crops, augmentative strategy (massive and directed releases of the entomophagous species) is almost essential. A very important practical aspect to consider is the toxicity and side effects of insecticides for entomophagous species (cf. Chapter 13), particularly when the biological and chemical control come together into an integrated control strategy. In these cases, it is necessary to use active ingredients that have been experimentally proven to have either no effect or a residual effect for auxiliaries. What is more, the production of lineages of entomophagous resistant to certain active ingredients by artificial selection has been taken into consideration. A strict control of genetic quality must be imposed upon those companies that supply beneficial arthropods that are mass-produced in a laboratory. The objective is to minimize inbreeding and genetic drift due to uncontrolled artificial selection pressures, guaranteeing maximum biological effectiveness of entomophagous in the greenhouse.

Table 2. Entomophagous parasitoids used in the biological control of lepidopteran pests in greenhouse crops

Biological control agent		Pest	
Order / Family / Species[a]	Status[b]	Species[c]	Stage[d]
Hym.: Braconidae			
Chelonus oculator F.	+++	*S. exigua, S. littoralis*	E
Cotesia kazak Telenga	++	*H. armigera, S. exigua,*	
		C. chalcites, A. gamma	C
Cotesia marginiventris Cresson	+++	*H. armigera, S. exigua,*	
		S. littoralis, C. chalcites,	
		A. ipsilon	C
Hym.: Eulophidae			
Eulophus pennicornis Nees	+	*A. gamma, M. brassicae,*	C
		L. oleracea	
Necremnus artynes Walker	+	*T. absoluta*	C
Hym.: Ichneumonidae			
Hyposoter didymator Thunberg	+	*H. armigera, S. exigua,*	C
		S. littoralis, C. chalcites,	
		A. gamma	
Meteorus pulchricornis Wesmael	+	*H. armigera, S. exigua,*	C
		S. littoralis, C. chalcites,	
		A. gamma	
Hym.: Scelionidae			
Telenomus ullyetti Nixon	+	*H. armigera*	E
Hym.: Trichogrammatidae			
Trichogramma achaeae Nagaraja & Nagarkatti	+++	*H. armigera, Spodoptera* sp., *C. chalcites, T. absoluta*	E
Trichogramma brassicae Bezdenko	+++	*H. armigera, C. chalcites,*	E
		A. gamma, Agrotis sp.,	
		M. brassicae, O. nubilalis	
Trichogramma evanescens Westwood	+++	*H. armigera, S. littoralis,*	E
		C. chalcites, A. gamma,	
		A. segetum, A. ipsilon.,	
		M. brassicae, O. nubilalis	
Trichogramma pretiosum Riley	++	*H. armigera, S. exigua,*	E
		Agrotis sp., *O. nubilalis,*	
		T. absoluta	
Dipt.: Tachinidae			
Lydella thompsoni Herting	+	*O. nubilalis*	C

[a] Only the species with a higher applied interest, current or potential, are shown.
[b] Status: research: +, development: ++, or commercial: +++. The assignment to each class is sometimes intuitive.
[c] Species where the trophic relation has been positively shown.
[d] Development stage susceptible to be parasitized, E: Egg, C: Caterpillar.

The biological insecticides or bioinsecticides (Table 3) include species of entomopathogenic microorganisms of very diverse taxonomic position (viruses, bacteria and protozoa-microsporidia) as well as fungi and nematodes. These species may be highly specialized as viruses or show a less specific host range such as bacteria and fungi. In any case the objective, once the bioinsecticide has been applied, is to start a virulent infectious process and to reach as quickly as possible the epizootic level necessary to achieve an effective control of the pest. In addition to the direct mortality thus obtained, it is necessary to consider the sub-lethal effects of bioinsecticides (Vargas Osuna 2001). For example, reduced mobility and decreased feeding of diseased larvae result in smaller adult size which may have a very negative impact on the biotic potential of the pest and therefore be favorable for crop protection in the short or medium term. Amongst the most developed biological insecticides, the one that has been widely used for decades is the bioinsecticide of bacterial origin *Bacillus thuringiensis* (Balsamo) Vuillemin, whose subspecies (serovars) *arzawai* and *kurstaki* include several strains with high pathogenic activity on lepidopteran larvae, especially effective in the early instars. This gram+ bacteria is characterized by its proteinic parasporal crystalline inclusion with insecticidal properties. *Cry* genes codifying for the protein have been incorporated using molecular biology from *B. thuringiensis* into the genome of some crops that thus express the insecticidal properties of the bacteria, the so-called GMOs.

Table 3. Entomopathogens (bioinsecticides) used in the biological control of lepidopteran pests in greenhouse crops

Biological control agent	Pest		
Taxa / Family / Species[a]	Status[b]	Species[c]	Stage[d]
Virus: *Baculoviridae*			
AsGV (Granulovirus of *A. segetum*)	+++	*A. segetum*	C
AsGV (Granulovirus of *A. segetum*)	+++	*A. segetum*	C
AsNPV (Nucleopolyhedrovirus of *A. segetum*)	+	*A. segetum*	C
CcNPV (Nucleopolyhedrovirus of *C. chalcites*)	+	*C. chalcites*	C
HaNPV (Nucleopolyhedrovirus of *H. armigera*)	+++	*H. armigera*	C
MbNPV (Nucleopolyhedrovirus of *M. brassicae*)	+++	*M. brassicae*	C
SeNPV (Nucleopolyhedrovirus of *S. exigua*)	+++	*S. exigua*	C
SlNPV (Nucleopolyhedrovirus of *S. littoralis*)	+++	*S. littoralis*	C
TaGV (Granulovirus of *T. absoluta*)	+	*T. absoluta*	C
Bacteria: *Bacillaceae*			
Bacillus thuringiensis Berliner	+++	*H. armigera, S. exigua, C. chalcites, A. gamma, A. segetum., A. ipsilon, M. brassicae, O. nubilalis*	C
Microsporidia: Nosematidae			
Nosema pyrausta Paillot	++	*O. nubilalis*	E C P A
Fungi: *Clavicipitaceae*			
Beauveria bassiana (Balsamo) Vuillemin	+++	*H. armigera, S. exigua, C. chalcites, A. gamma, A. segetum, M. brassicae, O. nubilalis*	C
Nematoda: *Steinernematidae*			
Steinernema carpocapsae Weiser	+++	*S. exigua, S. littoralis, Agrotis sp., T. absoluta*	C

[a] Only the species with a higher applied interest, current or potential, are shown.
[b] Status: research: +, development: ++, or commercial: +++. The assignment to each class is sometimes intuitive.
[c] Species where the pathogenic relation has positively shown.
[d] Development stage susceptible to be infested, E: Egg, C: Caterpillar, P: Pupa, A: Adult.

3.4. Cultural methods and prophylactic measures

The reasoned management of crop operations with potential phytopathological implications, the use of prophylactic measures and ultimately the good agricultural practices, are fundamental to any horticultural crop and especially in greenhouse crops. The use of certified plant material, adventitious vegetation control, appropriate rotations of susceptible and non-susceptible crops, proper removal of crop residues, the use and maintenance of adequate mesh fencing in relation to the size and behavior of each pest ... are among the actions that should not be obviated in any case.

3.5. Integrated Control

The definition of integrated control (or Integrated Pest Management, IPM) is as complex as its concept. The FAO defines it as *a pest management system that in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains the pest populations at levels below those causing economic injury*. The definition embraces all methods described so far and implies the concepts of monitoring and damage thresholds.

Whichever is the action plan against a pest, it is imperative its monitoring in order to have periodical and updated information (preferably in real time) on their development stage and, optimally, on its abundance at every crop cycle phase, to infer potential damage before it occurs and take action if necessary. Catches in pheromone traps or the mathematical modeling of insect life cycle allow us to optimize the dates of intervention against the pest. This is crucial because the integrated control of lepidopterans currently demands the reduction of insecticide treatments, conventional or not, to a minimum together with a gradual increase of biological control. Modeling often comes up against insurmountable biological or ethological obstacles for the appropriate mathematical validation of the models. Pheromone traps can also present collateral problems as sometimes the flight curve does not truly reflect the population dynamics of the pest in the crop (Torres-Vila 2007), and, above all, there is no reliable, solid and widespread correlation between the adult population of a given species (estimated by catches in traps) and larval offspring or their damage in the next generation. Maini and Burgio (1993) provide a good example of the inconsistency of the catch-damage correlation in the case of *O. nubilalis* on greenhouse peppers. Thus, trap monitoring only provides a qualitative rather than quantitative treatment threshold as would be optimal, i.e. catches indicate when intervention should be implemented but not if it is necessary. The so-called *negative forecast* is a semi-quantitative approach to the problem that can be very useful for an acceptable prognosis in certain cases (Torres-Vila, 2007).

The counting of eggs, larvae or damages per crop unit, provides more valuable information, albeit more costly, since it allows the application of quantitative damage thresholds, which are essential in decision-making about phytosanitary interventions of any kind, including non-intervention. Unfortunately, the experimental determination and development of damage quantitative thresholds with lepidopterans have not yet been widely developed in Spain, being practically reduced to the case of *H. armigera* in processing tomato (Torres-Vila *et al*., 2003a,b). In the case of greenhouse crops, lepidopterans are special candidates for the appraisal and implementation of damage quantitative thresholds, particularly if attack focuses on the parts of the crop that are not marketable.

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Chapter 7

Whiteflies Management

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1. Introduction

"Whiteflies" is the common name of an insect group (Hemiptera: Aleyrodidae) which has around 1556 described species (Martin & Mound, 2007), although only about thirty species have been mentioned in Spain which are included in the table 1 (Martin *et al*., 2000). The origin of this group of insects is very varied, as its current spread. But, in general, these are organisms from hot climates: more than 724 species have been described in tropical areas, and only 420 species in warm areas (Bink-Moenen & Mound, 1990).

In general, there are two types of damage caused by whiteflies: direct and indirect. The first is caused by the insects feeding on the plant, adults as well as nymphal stages (immature), causing the sap-sucking that leads to weakening and reduction of plant yield, and also inducing very different physiological disorders on plants. The indirect damages are referred to as all the problems derived from the production of honeydew by the insect immatures, and especially, the capacity of the adults of some species to transmit very different vegetal viruses, that can lead to the continuity of the crop being put at risk in a specific area.

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Table 1. Whitefly species present in Spain

Subfamily *Aleurodicinae*	
Aleurodicus dispersus Russell, 1965	
Lecanoideus floccissimus Martin et al., 1997	
Paraleyrodes minei Iaccarino, 1990	
Subfamily *Aleyrodinae*	
Acaudaleyrodes rachipora (Singh, 1931)	*Aleurolobus olivinus* (Silvestri, 1911)
Aleurothrixus floccosus (Maskell, 1895)	*Aleurotrachelus atratus* Hempel, 1922
Aleurotrachelus rhamnicola (Goux,1940)	*Aleurotuba jelinekii* (Frauenfeld, 1867)
Aleurotulus nephrolepidis (Quaintance, 1900)	*Aleuroviggianus* adrianae Iaccarino, 1982
Aleuroviggianus polymorphus Bink-Moenen, 1992	*Aleyrodes elevatus* Silvestri, 1934
Aleyrodes proletella (Linnaeus, 1758)	*Aleyrodes singularis* Danzig, 1964
Asterobemisia carpini (Koch, 1857)	*Asterobemisia paveli* (Zahradnik, 1961)
Bemisia afer (Priesner & Hosny, 1934) sens lat.	*Bemisia medinae* Gómez-Menor, 1954
Bemisia tabaci (Gennadius, 1889)	*Bemisia spiraeoides* Mound & Halsey, 1978
Dialeurodes citri (Ashmed, 1885)	*Dialeurodes setiger* (Goux, 1939)
Parabemisia myricae (Kuwana, 1927)	*Pealius quercus* (Signoret, 1868)
Simplaleurodes hemisphaerica Goux, 1945	*Siphoninus phillyreae* (Haliday, 1835)
Tetralicia ericae Harrison, 1917	*Tetralicia iberiaca* Bink-Moenen, 1989
Trialeurodes ericae Bink-Moenen, 1976	*Trialeurodes ricini* (Misra, 1924)
Trialeurodes vaporariorum (Westwood, 1856)	

2. Pest-species of horticultural crops in spain

Although all the whitefly species are phytophagous, that is to say, they feed on vegetables, only a few of them can be considered as significant pests of agricultural crops, taking into account that there are more than one thousand five hundred species described.

In the scope of this book, that it to say, horticultural plants grown under greenhouse, we can mention two species of whiteflies that have represented and nowadays represent a serious problem in vegetables, especially in crops under greenhouse: *Bemisia tabaci* (Gennadius, 1889) and *Trialeurodes vaporariorum* (Westwood, 1856).

It is worth mentioning also the economic importance of *Aleurothrixus floccosus* (Maskell), *Paraleyrodes minei* Iaccarino, *Dialeurodes citri* (Ashmead) and *Parabemisia myricae* (Kuwana) in citrus trees (Soto *et al*., 2001), and of *Aleurodicus dispersus* Russell and *Aleurodicus floccissimus* (Martin *et al*.) in ornamentals and sub-tropical crops from the Canary Islands (Hernández-Suárez *et al*., 1997). Other species that has increased its incidence in outdoors horticultural crops in the last few years, like Cruciferae, is *Aleyrodes proletella* (L.), the cabbage whitefly (Castañe *et al*., 2008).

T. vaporariorum, known with the common name of greenhouse whitefly, has been the species of whitefly that caused major problems, since being considered as a greenhouse pest in the seventies (Llorens & Garrido, 1992) until the eighties and nineties. At that time, a very effective control of its populations was achieved, and furthermore, its importance in crops under greenhouse was substituted for *B. tabaci*, known as the cotton or tobacco whitefly; largely due to the great capacity to generate populations resistant to phytosanitaries by *B. tabaci* and the absence of an effective method to control it, as well as its great importance as a vector of very severe plant viruses. In the nineties, the presence of 3 different biotypes of *B. tabaci* (biotypes called B, Q and S), was detected in Spain, each of them with its biological peculiarities and, therefore, with its economic importance on the crops (Guirao *et al*., 1997; Banks *et al*., 1999; Moya *et al*., 2001; Beitia *et al*., 2001; Baraja *et al*., 2002).

Currently, both species are considered as harmful for horticultural crops in our whole country, although we can say that there is a predominance of *T. vaporariorum* in warmer geographical areas (as in Catalonia), in contrast with a predominance of *B. tabaci* in hotter areas (such as Andalusia and Canary Islands). Both species are effective vectors of vegetal viruses in different horticultural plants (table 2) (Amari *et al.*, 2008; Berdiales *et al*., 1999; Célix *et al*., 1996; Font *et al*., 2003,2004; García-Andrés *et al*., 2006; Jordá, 2004; Lozano *et al*., 2004, 2009; Monci *et al*., 2002; Navas-Castillo *et al*., 1997, 1999, 2000; Sanchez-Campos *et al*., 1999; Segundo *et al*., 2004).

Table 2. Vegetal virus transmitted by the two species of whitefly in horticultural crops in Spain

Vegetables Viruses	Initials	Vector
Beet pseudo-yellows virus	BPYV	Tv
Cucumber vein yellowing virus	CVYV	Bt
Cucurbit yellow stunting disorder virus	CYSDV	Bt
Sweet potato chlorotic stunt virus	SPCSV	Bt
Tomato chlorosis virus	ToCV	Bt y Tv
Tomato infectious chlorosis virus	TICV	Tv
Bean yellow disorder virus	BnYDV	Bt
Tomato yellow leaf curl virus, Tomato yellow leaf curl Sardinia virus, Tomato yellow leaf curl Málaga virus, Tomato yellow leaf curl Axarquía virus	TYLCV TYLCVSarV TYLCMalV TYLCAxV	Bt
Tomato torrado virus	ToTV	Bt y Tv
Sweet potato leaf curl virus, Sweet potato leaf curl Spain virus, Sweet potato leaf curl Canary virus, Sweet potato leaf curl Lanzarote virus	SPLCV SPLCESV SPLCCaV SPLCLaV	Bt

Bt: *Bemisia tabaci*.
Tv: *Trialeurodes vaporariorum*.

3. General characteristics of whiteflies

B. tabaci as well as *T. vaporariorum* are polyphagous species, they develop on several crops and also on spontaneous herbaceous plants, which facilitates the maintenance of their populations throughout the year. Both sexes are present, with haploid adult males and diploid females; reproduction is carried out by arrhenotokous parthenogenesis, so that fertilized eggs give rise to females and unfertilized eggs give rise to males.

The biological cycle of both species is similar and it can be summarized as follows:

Initially **the egg** is whitish and then turns caramel (*B. tabaci*) or blackish (*T. vaporariorum*) with the embryonic development. It has an oval, reniform or elongated shape and is usually held on to the vegetal support (generally on to the underside of the leaves) by a pedicel of variable size.

When eggs hatch, a **nymphal instar**, which is mobile, appears first. It moves on the leave (mainly on the underside) until it fixes itself to the plant, through its mouth stylet, and remains there until the adult emerges. After this nymphal instar, there are three more that are identified by the "molting" of the old cuticle and the subsequent increase of size. All the nymphal instars are very similar amongst themselves, the differences between them are their size, their appearance, and in some cases, the different wax secretions, as well as the presence of silks or setae. The first nymphal instars have a flat and translucent aspect, then they turn more opaque and develop the wax secretions and the typical setae of each species. At the end of the 4^{th} nymphal instar, the nymph stops feeding and the typical compound eyes of the imago begin to be appreciated clearly (by transparency), then, also the wings can be seen, and ultimately, the transformation into an adult is produced within the "pupal casing". This process, when any cuticle molting is produced, is improperly referred to as "pupa" (the insect state that is found on the leaf) by some authors.

It is relatively easy to carry out an identification of both species, from the adult stage as well as from the fourth nymphal instar:

At first sight, the adults of both species are very similar, but *B tabaci* has a slightly yellower colour and a smaller size. Both species have roof shaped wings on the back, leaving head and thorax in the open; but *B. tabaci* has "small roof" shaped wings forming an angle of 45 degrees with respect to the leaf surface, while *T. vaporariorum* has triangle shaped wings (Photos 1 and 2).

Photo 1. *T. vaporariorum* eggs

Photo 2. *B. tabaci* eggs

Another very noticeable difference is referred to its eyes. The whitefly adults have compound eyes formed by two groups of "omattidia" (visual units or simple eyes). In the case of *B. tabaci*, both groups of omattidia are joined, while in *T. vaporariorum* they are totally separate (in an upper and a lower groups) (Figure 1) and (Photo 3).

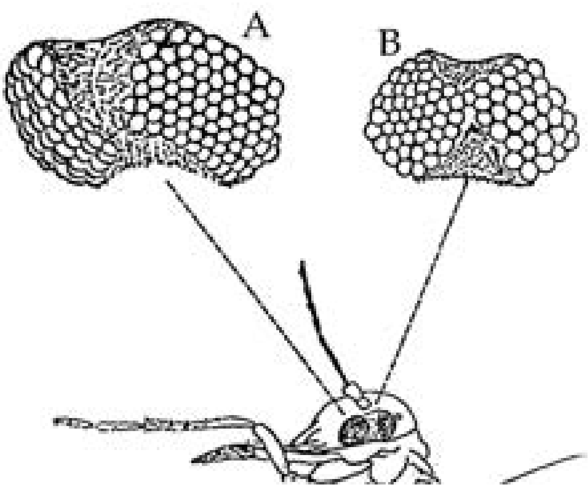

Figure 1. Differences between the ommatidia groups in *B. tabaci* (right) and *T. vaporariorum* (left)

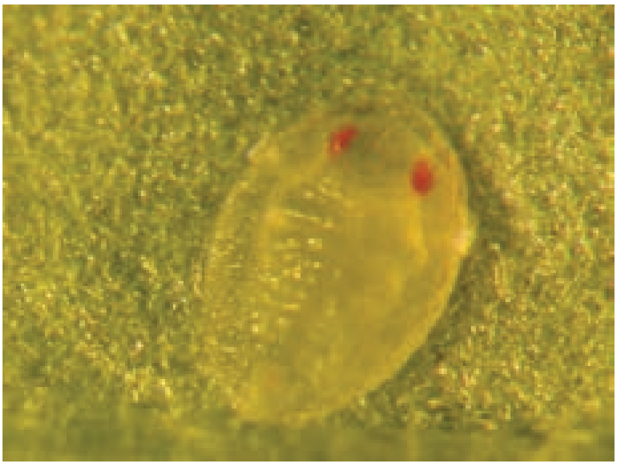

Photo 3. Fourth instar nymph of *B. tabaci*

From the 4th nymphal instar, it is also relatively easy to distinguish both species. In *B. tabaci* the back surface is convex and the outline is irregular. However, *T. vaporariorum* has a totally flat back surface and high respect to the vegetal substrate, and also it is surrounded by long transparent silks in its whole perimeter (Photo 4 and 5).

As it has been mentioned before, eggs from both species can be also distinguished, when just laid, they are yellowish white but the eggs of *T. vaporariorum* turn blackish when mature, while the eggs of *B. tabaci* turn caramel colour (Photo 6 and 7).

Photo 4. Fourth instar nymph of *T. vaporariorum*

Photo 5. *B. tabaci* adult

Photo 6. *T. vaporariorum* adult

Photo 7. Joined ommatidia groups in *B. tabaci*

4. Natural enemies of both species

As with many other phytophagous organisms, these two species of whiteflies in Spain have a great number of natural enemies (or beneficials) which are developed at the expense of them; amongst them we can find parasitoids, predators and also entomopathogens. These natural enemies can be autochthonous species of the Mediterranean Basin or exotic species introduced and adapted to our geographical area, which carry out their control activity of whitefly populations as usual.

Only some of these natural enemies have been considered as effective agents of biological control and are commercialized by specialised companies and distributed to be used in greenhouses, at the right moment and in the appropriate way. The trouble is that not all the species of beneficials are capable of inducing a significant mortality in the whitefly populations, which means they are not capable of controlling these pests under the economic threshold of damage to crop, and sometimes, they can even go unnoticed.

The species of natural enemies to which we will give more attention in this publication are those available commercially; however, and since the presence of other beneficials associated with populations of these two species of whiteflies is often observed, we have deemed convenient to mention these non-commercial natural enemies for general knowledge.

4.1. Parasitoids

The parasitoids of whiteflies belong to the order Hymenoptera (that is to say, they are "tiny wasps") which are included within the superfamilies Chalcidoidea (families *Aphelinidae, Eulophidae, Pteromalidae, Encyrtidae and Signiphoridae*) and Platygastroidea (family *Platygastridae*) (Polaszek, 1997). Particularly, within the family *Aphelinidae* there are a great number of parasitoids of *B. tabaci* and *T. vaporariorum*, being the genera *Encarsia* Föster and *Eretmocerus Howard,* those which have a higher number of species related with both whiteflies.

In table 3 we can find all the parasitoid species mentioned in Spain regarding the two species of whitefly *B. tabaci* and *T. vaporariorum* (Hernández-Suárez, 1999; Castañé *et al*., 2009; Natural History Museum, 2009).

The parasitoids are organisms that, in general, present a great specificity, that is to say, they have a reduced range of host species on which they can develop, and in some occasions, they are limited to only one species.

Table 3. Main species of parasitoids mentioned in Spain on *B. tabaci* and *T. vaporariorum*

	White fly guest	
Species of Parasitoide	***Bemisia tabaci***	***Trialeurodes vaporariorum***
Aphelinidae		
Encarsia acaudaleyrodis Hayat	+	+
Encarsia azimi Hayat	+	+
Encarsia formosa Gahan	+	+
Encarsia hispida De Santis	+	+
Encarsia inaron (E. partenopea)	+	+
Encarsia lutea (Masi)	+	+
Encarsia melanostoma Polaszek & Hernández	+	+
Encarsia mineoi Viggiani	+	+
Encarsia noahi Polaszek & Hernández	+	+
Encarsia pergandiella Howard	+	+
Encarsia sophia (Girault & Dodd)	+	+
Encarsia protransvena Viggiani	+	
Encarsia tricolor Förster	+	+
Eretmocerus mundus Mercet	+	
Eretmocerus eremicus Zolnerowich & Rose	+	+
Platygastridae		
Amitus fuscipennis MacGown & Nebeker		+

Photo 8. *Encarsia formosa*, pupa

Photo 9. *E. formosa,* adult

Photo 10. *Encarsia hispida,* pupa

Photo 11. *E. hispida,* adult

Photo 12. *Encarsia lutea,* pupa

Photo 13. *E. lutea,* adult

Photo 14. *Encarsia noabi,* pupa

Photo 15. *E. noabi,* adult

Photo 16. *Encarsia pergandiella,* pupa

Photo 17. *E. pergandiella,* adult

Photo 18. *Encarsia sophia*, pupa

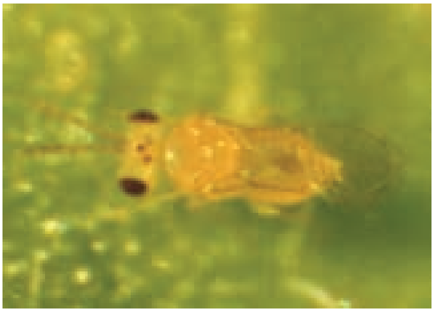

Photo 19. *E. sophia*, adult

Photo 20. *Encarsia tricolor*, pupa

Photo 21. *E. tricolor*, adult

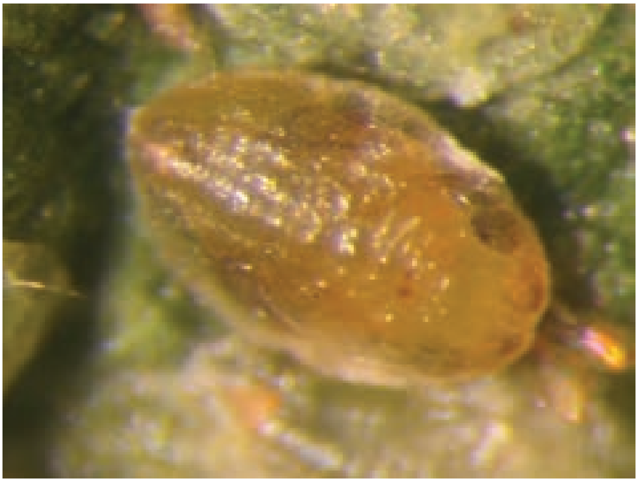

Photo 22. *E. eremicus*, pupa

Photo 23. *Eretmocerus eremicus*, adult

Photo 24. *Eretmocerus mundus*, pupa

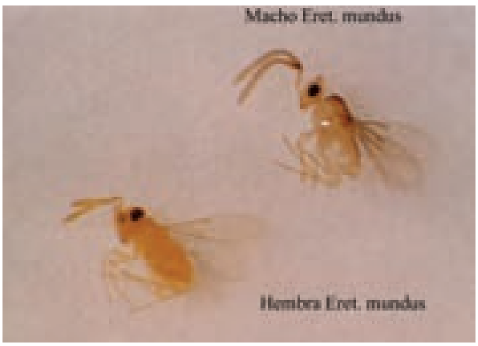

Photo 25. *E. mundus*, adult

4.2. Predators

Amongst the predators of whiteflies we can find different groups of insects, such as anthocorids and mirids (Hemiptera), coccinelids (Coleoptera), chrysopids (Neuroptera) and drosophilids, syrphids and muscids (Diptera), as well as *Phytoseiidae* and *Stigmaeidae* mites (Acarina).

Predators are organisms that show a great polyphagy habitually, that is to say, they have a broad spectrum of prey species, on which they can feed the adults as well as the immature stages.

Within the predator neuropterans of whiteflies, *B. tabaci* and *T. vaporariorum,* two species stand out in Spain: *Chrysoperla carnea* (Stephens) and *Conwentzia psociformis* (Curt.).

Within the dipterans, the main predatory species of aleyrodids are found within the *Drosophilidae* (*Acletoxenus formosus* (Loew)) and *Muscidae* (*Coenosia attenuata* Stein) families, although the action of other generalist dipterans that occasionally feed on these whiteflies, such as cecidomyiids and syrphids, is also mentioned.

Among the coleopterans, particularly in the *Coccinellidae* family, we can find some predatory species that have achieved the greatest successes in biological control (De Bach & Rosen, 1991). The *Coccinellidae* family is the most important in relation to the biological control of aleyrodids; on the two species of whitefly, *Clitostethus arcuatus* Rossi (Bt and Tv), *Delphastus catalinae* (Horn) (Bt and Tv), and other predatory coccinelids of scale pests such as *Cryptolaemus montrouzieri* (Mulsant) (Bt) and *Coccinella undecimpunctata* L. (Bt) which have been mentioned in Spain.

Although the Hemiptera order is composed mainly of phytophagous insects, the hemipterans include several families with predatory species, highlighting in particular *B. tabaci* and *T. vaporariorum*: the mirids *Macrolophus caliginosus* (Wagner), *Nesidiocoris tenuis* (Reuter), *Dicyphus tamaninii* Wagner and *D. errans* (Wolf). Also significant is the action of other hemipterans such as *Nabis pseudoferus ibericus* Remane (Nabidae) and *Orius* laevigatus (Fiebre) and *O. majusculus* (Reuter) (Anthocoridae).

Finally, the use of the predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae), of Mediterranean origin, should be highlighted in the biological control of *B. tabaci* in pepper and cucumber crops (Belda y Calvo, 2006).

Photo 26. *Acletoxenus formosus*, adult

Photo 27. *Amblyseius swirskii*, adults and eggs

Photo 28. *Delphastus catalinae*, adult

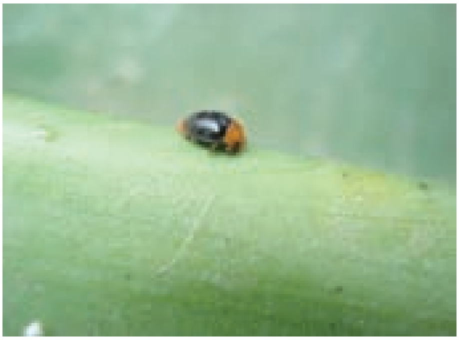

Photo 29. *Cryptolaemus montrouzieri*, adult

Photo 30. *Macrolophus caliginosus*, adult

Photo 31. *Nesidiocoris tenuis*, adult

4.3. Entomopathogens

Entomopathogens are organisms that cause diseases on insects, being the causal agent of very different viruses, bacteria, fungi, protozoa and nematodes. The entomopathogens have the problem that they do not look for their hosts actively, as parasitoids or predators do, therefore, their use in biological control is directed and limited to a mass production and application as a "biopesticide". Furthermore, their main limitation is their dependence on high humidity. On the other hand, however, they have the advantage of being compatible with the use of chemical treatments and they are very easy to manage (Fransen, 1990).

Among the entomopathogens that are capable of attacking whiteflies we can highlight the entomopathogenic fungi *Aschersonia aleyrodis* Webber, *Paecilomyces fumosoroseus* (Wize) Brown & Smith, *Beauveria bassiana* (Balsamo) y *Lecanicillium* (=*Verticillium*) *lecanii* (Zimm.) Viégas and *L. muscarium* (Petch.). These fungi germinate in the insect's cuticle, pierce it and then colonise the inside host (Fransen, 1990).

Also the nematodes *Steinernema feltiae* (Filipjev) (Nematoda: Steinernematidae) and *Heterorhabditis bacteriphora* Poinar have been assessed as possible biological control agents of *B. tabaci* (Cabello and Ruiz-Platt, 2007).

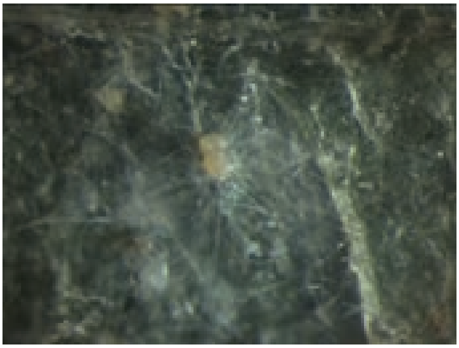

Photo 32. *Paecilomyces fumosoroseus*, on on whitefly eggs

Photo 33. *Lecanicillium lecanii*, on *T. vaporariorum* nymph

5. Biological control of whiteflies through natural enemies

As it has been mentioned before, to carry out the biological control of the two species of whitefly, through natural enemies, and considering the characteristics of the horticultural crops of greenhouses, which are temporary and favour the disappearance of the auxiliary organisms of the different pests, we must turn to the introduction of such beneficials from the mass breeding and commercialisation of them by companies devoted to it.

To reference the auxiliaries that are being used now in Spain against *B. tabaci* and *T. vaporariorum*, there is nothing better than turning to the information provided by the Spanish Ministry of Environment and Rural and Marine Affairs (MARM, 2009), as well as the Vademecum of Phytosanitary Products (De Liñán, 2009). In accordance with these two sources, nowadays, the natural enemies commercialized against the two species of whitefly are:

5.1. Amblyseius swirskii Athias-Henriot,1962

Phytoseiid mite, effective predator of eggs and young nymphs of whiteflies. Also it is known for its high activity on thrips larvae, *Frankliniella occidentales* (Pergande).

The geographical origin of this mite is the Eastern Mediterranean Sea (Greece, Israel, Turkey, …) which permits it to survive and act in high temperatures such as those reached inside greenhouses (it reduces its effectiveness from 40 ºC). It is effective in several horticultural crops, but it has been shown that it does not have any effect on tomato crops.

Adults are white-orangey, with a pear shaped body. Eggs are usually laid on the underside of leaves, close to nervations, as well as on flowers. Initially eggs are oval and white, and then turn caramel colour when hatching is approaching. It displays one larval stage and two nymphal stages (protonymph and deutonymph), which are differentiated by the number of legs (6 the larvae and 8 the nymphs).

Adults as well as nymphs are very mobile and they are capable of eating a great number of preys (from 15 to 20 whitefly eggs or larvae per day), which makes it an effective predator; in addition to its capacity to feed on pollen and nectar, which permits its establishment in the greenhouse in the absence of preys, once that flowering has begun, it becomes an effective biological control agent of whiteflies.

It must be also highlighted, as it has been mentioned before, that it is capable of establishing itself in crops even though humidity and temperature conditions are not favourable for other auxiliary species, which permits also that once it is established in the greenhouse, it is not necessary to introduce it again.

Method of use

It can be used as a preventive measure (in the absence of pests) and as a curative measure.

The introduction (with pollen) of 25-30 individuals/ m^2 is recommended as a **preventive** measure. If the minimum presence of whiteflies has already been detected, the introduction must be repeated 1 or 2 times leaving a 15-day interval.

The **curative** release requires the introduction of 50-100 individuals/m^2, releasing the highest dose in the areas of higher presence of the pest. And if it is necessary, this action must be repeated 15 days later.

The organisms provided by the different producing companies must be used within 24 hours after their receipt, and in any case, they must be kept at a temperature of 10-15 ºC until they are used.

At the moment, it is commercialized by two companies, Certis and Koppert. The first one sells it under the name of AMBSURE, in sprinkler packs of 1.000, 50.000 or 100.000 units (adults and nymphs). Koppert commercialises it in sachets with 250 individuals mixed with bran and in boxes of 100 and 500 sachets (SWIRSKII Mite Plus), and also in bottles of 500cc which contain 50.000 individuals (SWIRSKII Mite). Técnicas de Control Biológico (TCB) commercialises it with the trademark TCB-Swirskii. It sells it in cardboard containers of 25.000 individuals and sachets of 250 individuals in boxes of 500 sachets. MIP System Agro (BIOMIP); commercialises it with the trademarks SWIRSKII MIP 25 (cardboard containers with 25.000 units) and SWIRSKII MIP S in sachets.

5.2. Encarsia formosa (Gahan, 1924)

This is an aphelinid hymenopteran whose adults have an approximate size of 1 mm and they have a yellow abdomen and blackish thorax. Its populations are composed exclusively of females (98 % approximately) because the males of this species are very rare (they are brown-blackish and have a morphology similar to females).

E. formosa has a neartic origin (Polaszek *et al*., 1992), although it is currently spread worldwide, because it has been introduced in several countries as a biological control agent of *T. vaporariorum*.

It is a solitary endoparasitoid (it lays the eggs inside the whitefly nymphs) although superparasitism (more than one egg in the same nymph) can be observed when a population is abundant (Agekyan, 1982). Although it is known that it acts on *B. tabaci*, it is used mainly in the control of *T. vaporariorum*: when both species of whitefly are present, it shows a clear preference for the second one; it parasitizes all the nymphal stages of this whitefly, although it prefers the third and fourth instars, emerging only when the host reaches the fourth. *E. formosa* is capable of distinguishing between the pupae of *T. vaporariorum* parasitized and non-parasitized, avoiding oviposition in the first ones (van Lenteren *et al*., 1976).

There is a clear difference in the parasitism generated on *B. tabaci* and *T. vaporariorum*: in the first one, the development of the parasitoid turns the puparium caramel colour, while in the second case it turns black.

Its thermal range of effective action stands between 18 and 30 ºC approximately; it can hardly fly under 18 ºC and above the highest temperature an adult's longevity is reduced considerably. A female is capable of parasitizing between 80 and 100 specimens of whitefly in the course of its life.

Method of use

As it has been mentioned before, this species is highly recommended for the control of *T. vaporariorum*. This insect is distributed in puparial stage, inside the whitefly puparium, which are stuck to cardboard cards, each of them with 60-100 pupae/card according to each producing companyIt must be used with preventive introductions at the rate of 50 pupae/100 m^2, but when there is a low population of whiteflies, it must be introduced to the order of 1-3 pupae /m^2.

The companies that commercialise it in Spain are: Agrobío (ENCARSIA Formosa), Certis (ENSURE (f)) and Koppert (EN Strip). This last company also commercialises a mixing of *E. formosa* and *Eretmocerus eremicus*, with the trademark ENERMIX, and other mixing of *E. formosa* and *Eretmocerus mundus* with the trademark BETRIMIX.

After receiving them, the organisms can be kept between 1 and 2 days at a temperature of 8-10 ºC.

5.3. *Eretmocerus eremicus* Rose & Zolnerowich, 1997

It is an hymenopteran from the Aphelinidae family, parasitoid of different species of aleyrodids, but it is used in the biological control of *B. tabaci*. Also it was known (and commercialized) with the name of *Eretmocerus californicus*.

Adults are tiny wasps similar to those of *E. mundus*, endoparasitoids of aleyrodid nymphs, whose egg is laid under the nymph, between the nymph's body and the foliar area, and the first larval instar penetrates inside. The parasitized whitefly nymphs follow their development until the parasitoid completes its own development, and at that moment, the whitefly dies. Females prefer parasitizing the 2nd nymphal instar of whitefly. Also the predatory activity of adult females, which feed on whitefly nymphs that can entail a significant mortality in their populations, around 40 % of the general mortality due to the parasitoid, should be highlighted. Adults live an average of 8-10 days, at an average temperature of 25 ºC.

Method of use

This insect is spread as pupae, inside the whitefly puparia, which are placed stuck to cards, or within *blisters* or other containers. Usually, there is an amount of 50 to 250 pupae/distribution unit.

The recommended usage dose contains from 10-12 parasitized nymphs/m^2, carrying out 3 releases with a 2-week interval. It is advisable to carry out the release at a not very high room temperature, that is to say, early in the morning or at dusk. The release must be done soon after receiving them, but if that is not possible, the insects should be kept at a temperature between 8-10 ºC.

The companies that commercialise this auxiliary are: Biobest (ERETMOCERUS-SYSTEM), Certis (ERETSURE (e)) and Koppert (ERCAL).

Also, Koppert commercialises a mixing of *E. eremicus* and *E. mundus*, with the name of BEMIMIX.

5.4. *Eretmocerus mundus* (Mercet, 1931)

Like the previous species, this is an Aphelinidae hymenopteran, a parasitoid of several species of whiteflies, among them *B. tabaci*. As it is a species of Mediterranean origin, it is clearly adapted to the conditions of our horticultural crops in greenhouses.

It is a primary parasitoid and also an ecto-endo parasitoid, because as *E. eremicus* does, females lay the eggs outside of the whitefly nymph's body and the first larval instar of the parasitoid is introduced in the whitefly nymph's body.

Adults have an approximate general size of 1 mm. Females are lemon-yellow, except for the end of the ovipositor, and males are smaller and darker.

Eggs are oval and transparent when just laid, but turn brown later. After eggs hatch, three larval stages follow, the first one is transparent and pear shaped, similar to eggs; the second is oval and the third is almost transparent and shaped round. The prepupa is lightly yellow, and the pupa which is located inside the whitefly puparium with the ventral surface directed to the dorsal surface of the host, is dark yellow, turning brown as it is developed.

E. mundus distinguishes the host larvae that have been parasitized, only laying eggs in non-parasitized larvae. Although it parasitizes all the larval stages of *B. tabaci* it prefers the second and third instars (Urbaneja y Stansly, 2004).

Also, it can carry out a predatory action because it feeds on parasitoids through the wound in the vasiform orifice of the aleyrodid with its ovipositor (Geling ,1983).

The larva parasitized by *E. mundus* turns gold-yellow and has displaced mycetomas and more globose exuviae than the larva without being parasitized. Before the adult emerges, the dark eyes and the wing rudiments can be picked out through its transparency. When it is going to emerge from the puparium, the adult parasitoid makes a round hole in the cover of the puparium with its jaws, this aspect allowing the distinguishing of the nymph that has been parasitized, because in this last case, the exit of the whitefly adult from the puparium causes an inverted-T shaped opening.

Under the climatic conditions of southeast Spain and the Canary Islands, the activity of this parasitoid has been observed throughout the year. In general terms, the development from egg to adult, at 25 ºC, usually lasts between 18-20 days. The females usually live between 9-11 days and with an average lay of 30-50 eggs over the course of their lives.

Females are very long-lived during winter, and they are capable of remaining active during this season, which favours their spread on any vegetal host (Gerling, 1983).

Method of use

As it is effective against *B. tabaci*, it must be introduced in the crop after the presence of this whitefly has been identified. It has been shown that it can act with temperatures lower than 20 ºC and also higher than 30 ºC, although it is less effective.

It is distributed just like *E. eremicus*, in puparia of parasitized whitefly. A **preventive dose** of 1-2 puparia/m^2 can be used, compared with a normal **dose** of 2-3 puparia/m^2, and **curative dose** of 3-9 puparia/m^2. The releases must be carried out with mild temperatures, that is to say, early in the morning or at dusk, scattering the puparia contained in the containers between the leaves. The first nymphs of the parasitized whitefly may be observed two weeks later.

If they are not used directly after receipt, the insects can be stored for 1-2 days, in the dark and at a temperature of 8-10 ºC.

The insect is presented in different formats, according to the company that commercialises it, but two ways of sending it can be distinguished: separate parasitoid pupae, in containers or *blisters* with sawdust or other similar material; pupae stuck to cardboard cards.

The companies that commercialise it in Spain now are: BEMIPAR, Koppert; MUNDUS_SYSTEM, Biobest; ERMUNcontrol, Agrobío; MUNDUSCOLOR, Biocolor; MunduPAK3000, Bioplanet; ERETSURE (m), Certis and MUNDUS-BG, BGreen Biological System, TCB-MUNDUS, Técnicas de Control Biológico and MUNDUS MIP, Mip System Agro (BIOMIP).

5.5. *Macrolophus caliginosus* Wagner, 1951

It is an heteropteran from the Miridae family, predator of *B. tabaci* and also of *T. vaporariorum*. It is very voracious and capable of attacking whitefly in all its development stages, although it prefers eggs and nymphs. It looks for its prey actively and when it finds it, it sticks its stylet in and fully sucks out its insides.

Adults are between 3mm and 3.5mm long, are light green and have a thin appearance, red eyes, long and green antennae with a black base, and long legs which allow them move easily. Furthermore, this predator has the advantage that it can feed on other pest organisms in horticultural crops such as aphids, red spider mites, butterfly's eggs and caterpillars, miner larvae and thrips. Therefore, it can help to control these pests with high populations in the crop.

Adults live on plant sprouts, and lay the eggs on the leaves, preferably on the nerves and peduncle tissues. After eggs hatch, 5 nymphal instars follow before the adult's appearance. The female's longevity is 40 days, with a lay that ranges between the 100 and 250 eggs; depending on the environmental climatic conditions.

An adult of this mirid can eat more than 30 whitefly eggs per day. And it must be taken into account that under special conditions, of high population of mirids and low presence of prey, this insect can cause damages to crops, because it is also a vegetal feeder.

Method of use

It has been used for the control of both species of whitefly. In the particular case of tomato crops, the results obtained from *M. caliginosus* have been very positive. This predator is able to control the two species of whitefly that appear in tomatoes and even contribute to the control of other pests. However, it has a disadvantage, in that it has a very slow population development, and therefore, it must be introduced as soon as possible. In general, it is released some weeks around the whitefly foci so that *M. caliginosus* benefits from the presence of whitefly for its development and helps the parasitic wasps to control the first foci.

The insect is commercialized as adults and nymphs, in containers with vermiculite or other similar inert material. After the receipt of the insects, these can be kept for 1-2 days (if they are not used immediately), at a temperature between 10-12 ºC.

A normal usage dose consists of 0.5 individuals/m^2 and a curative dose, of 5 individuals/m^2. And always with 2 introductions, leaving a 2-week interval in between.

At present, the possibility of releasing this predator in the nursery is being considered, with the purpose of this being established when transplanting. Also the use of reservoir plants, the called “banker plants”, is subject to study, these plants permit their presence inside the greenhouse, feeding on other hosts, before the whitefly population appears (Urbaneja *et al*., 2002).

The commercialising companies of this insect in Spain are: Certis, MACSURE (c); Biocolor, CALIGICOLOR; Agrobío, MACROcontrol, and Koppert, MIRICAL and MIRICAL-N.

5.6. *Nesidiocoris tenuis* (Reuter, 1885)

It is also a heteropteran from the family Miridae, and comes from the Mediterranean Basin.

It is a predator of eggs and nymphs of the two species of whitefly, and also feeds on red spider mites, moth’s eggs, thrips, and even aphids and miner larvae.

Adults are 6 mm long, green and thin with long legs and antennae. They are placed mainly on stems and leaves. Eggs are translucent and females lay them on stems and leaves; in general, at 25 ºC, hatching occurs 6-8 days later, and then the insect goes through 5 nymphal instars. It is yellowish-green and placed mainly on the underside of the leaves, until adults emerge.

Adults and nymphs are active predators that look for their prey, to which they attach their mouth, sucking out the content of their bodies, so that they only leave the prey’s tegument on the plant.

They can consume between 40-50 eggs and 20-25 nymphs of whitefly, daily. Adults can also feed on plants, in the absence of prey; in fact, under some circumstances it is difficult to determine if it is an auxiliary or a pest for the crop (Sánchez et. al, 2009).

Method of use

This insect must be introduced early in the crop, because its population development is a little slow, especially under low temperatures.

In general, the recommended dose is 0.5-1.5 individuals/m^2, in weekly releases, up to the level of 2 individuals/m^2 if it is necessary.

After the receipt of these insects, they can be kept 1-2 days at 8-10 ºC.

In tomato crops, if there is a high population of bedbugs, and especially with a low prey/predator relation, damages can be produced in the plant apexes.

The following companies commercialise it in Spain: Agrobío, NESIDIOcontrol; Certis, NESISURE (t), Koppert, NESIBUG, Técnicas de Control Biológico, TCB-NESIDIOCORIS and Mip System Agro (Biomip), NESIDIOCORIS MIP.

5.7. *Beauveria bassiana* Bassi, 1835

B. bassiana is a parasitic fungus, whose conidia constitute the infectious unit. It has two stages on insects: one saprophytic and the other pathogenic. The pathogenesis stage is developed when the fungus comes into contact with the live tissue of the insect and humidity reaches 85 % within the microclimate.

The infectious process that leads to the insect's death goes through three stages: 1) germination of conidia and penetration of the hyphae in the insect's body, 2) invasion of the internal tissues of the insect, and 3) sporulation and the start of a new fungus cycle.

Usually, colonies grow slowly and are white although they can turn yellowish or pinkish in the course of time.

This fungus has a wide field of action; not only whiteflies, but also scale insects, aphids, thrips and other insects.

Method of use

This fungus is presented in three formats to be used against whiteflies: as a concentrated suspension of spores, as an oily dispersion of spores and as a spore concentrate in the form of a wettable powder.

Each of these formats is commercialized by a different company. The first one by Futrureco, with the name of BOTANIGARD SC. A different concentration of use is recommended depending on the crop to which it is going to be applied. It is recommended to apply with normal spraying, beginning the treatment when infestation has just started. The parasitized individuals die after 4-6 days. It is not advisable to mix it with fungicides. It must not be stored at temperatures higher than 30 ºC. At room temperature, in a cool and dry place, it can be kept for 2 years.

The second preparation is commercialized by Agrichem, with the name of NATURALES L. Independently from the crop, it is recommended to apply between 0.75 and 1 l/ha of preparation; as the application on leaf spraying at the beginning of the infestation and, if it is necessary, to repeat the treatment every 7 days. The parasitized individuals die after 7 days. This product must not be mixed with fungicides. And it must be stored at a temperature lower than 25 ºC and for a period lower than one year.

Finally, we can find the product commercialized by C.Q. Massó, with the name of BASSI WP. For this compound it is recommended a different dose depending on the crop to be treated, and it is also advisable to apply the product in normal spraying and at the beginning of the infestation. The parasitized individuals die after 4 and 6 days. It must not be mixed with fungicides and must not be stored at temperatures higher than 30 ºC. In a cool and dry place, at room temperature, the product can be kept for 2 years.

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Chapter 8

Thrips management

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1. General characteristics of the thrips that cause damage in protected crops

1.1. Morphology, biology and ecology

Thrips are grouped in the order Thysanoptera (insects with feathery wings). The thrips species considered as pests are between 1 and 2 mm long, have an elongated and cylindrical body, an inverted pyramid head shape, asymmetric, with the left mandible transformed into a solid stylet and the maxillae also transformed into stylets, semicircular shaped, forming a tube by coercion through which they inject the saliva and suck the content of plant cells at feeding sites. The antennae placed on the top of the head have from 6 to 9 condyles. The wings have a membranous part, with long cilia, placed on the back edge and, sometimes, also on the front edge. In the species of the suborder Tubulifera the last segment of the abdomen in adults is tubiforme, while in the suborder Terebrantia the edge of the abdomen is conical, sharper in females than in males. Terebrantia females have a falciform ovipositor, formed by two valves, articulated in the posterior edge of the eighth abdominal segment, and two more articulated in the ninth, which permits the insertion of eggs in vegetal tissues. The valves are curved towards the abdomen in the species of the family Thripidae, and opositively in the family Aeolothripidae, permitting the insertion of eggs in vegetal tissues in both cases. In the Tubulifera the genital opening is located between the ninth and tenth segments, and females lay the eggs on the host surface.

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The species of the suborder Terebrantia have five life stages: egg, two active larval stages, two inactive not-feeding stages (prepupa = prenymph and pupa = nymph) and the adult stage). Tubulifera have six stages, because there is an additional molting stage (between the two pupa instars (I and II)) than in the Terebrantia.

The two active larval stages have different sizes and feed in the same way as adults do. Once larvae have completed their development, they may pupate on the host plant or move to the ground where they establish in the litter at a few centimeters from the surface.

Most of the species are haploid (males) – diploid (females). Some of the species , [e.g. *Frankliniella occidentalis* (Pergande)] reproduce by arrhenotoky showing facultative parthenogenesis: unfertilized eggs produce males while fertilized females produce females. Other species (e.g. *Thrips Tabaci* Lindeman) reproduce by thelytokous parthenogenesis. Sometimes, the egg fertilization is a facultative action of females and can be influenced by the environmental conditions, the food availability and the host plant.

Under natural conditions, the relation between sexes in species with facultative arrhenotoky varies according to the season of the year, the host plant and the population densities. In the case of *F. occidentalis*, the proportion of males also shows seasonal changes, depending on temperature, because this influences longevity. In the mild winter of the Spanish Mediterranean coast, males are rare in *F. occidentalis* populations when temperatures are close to the lower thermal development threshold and the rate of population growth is low.

The duration of the life cycle, longevity, fertility and the reproductive potential of females are greatly influenced by the environmental conditions (mainly the temperature) and the amount and quality of food. These are the main factors driving populations in opportunistic species such as *F. occidentalis* or *T. tabaci*, which are hemodynamic in warm subtropical areas.

The species associated with protected crops are mainly phytophagous. Although, some namely phytophagous species like *F. occidentalis* may facultatively prey on Tetranychidae mites, and some predatory species (e.g. Aeolothrips spp.) may also feed on plant resources (e.g. pollen). Obligated predatory species,such us *Scolothrips longicornis* which feeds on Tetranychidae, are also common in protected crops.

The knowledge of the ecology and behaviour of the species is compulsory to define control strategies suitable for each area, situation, species and crop. In the case of species such as *F. occidentalis*, which pupate on the ground, the microhabitat conditions substrate may influence greatly the demographic population growth. In warm areas of the Mediterranean coast, some species, such as *F. occidentalis* or *T. tabaci,* are commonly found in protected and open field crops, where they reproduce or remain active during the whole year. On the contrary, in areas of the interior of the Iberian Peninsula with extreme climate these species are inactive during winter.

The thrips disperse by active flight of winged adults, usually over relatively short distances. However, long-distance movements occur, by passive translocation via wind, active flight or infested vegetal material. The adults of opportunistic species are markedly pollenophagous. Pollen is a highly nutritive food, which allows the reproduction and increase population growth. Many of these species, such as *F. occidentalis*, show an aggregative distribution pattern in flowers.

1.2. Type of damage

Direct damage

The thrips cause damage in two ways:

a) Feeding

The thrips have piercing and sucking mouthparts that injecting saliva on plant tissues and suck the content of the cells. The cells empty by feeding become whitish initially and then turn dark brown. Organs fed upon by thrips grow deformed.

Photos 1 to 4. Feeding damage of *Frankliniella occidentalis*: Necrotic silvering in: pepper leaf; silvering in pepper; pepper deformity; silvering in tomato

b) Oviposition

The incisions produced during oviposition by Terebrantia females (phytophagous and carnivorous) favor the infections and proliferation of fungi and bacteria causing the rot of tissues. This damage has more repercussions during the commercialization process of flowers or fruits. Sometimes, the substances surrounding thrips eggs are toxic for the plant and tissues react producing a whitish ring protuberance or hollow around, which reduces the flower or fruit market value at harvests.

Photo 5. Small spots surrounded by whitish rings in tomato due to damage caused by the lay of *Frankliniella occidentalis*

Indirect damage

The adults of some species such as *T. tabaci* or *F. occidentalis* are involved in the direct or indirect transmission of the virus.

a) Transmission of viruses via pollen

They can indirectly transmit some Ilarvirus such as Tobacco streak ilarvirus and Prunus necrotic ringspot ilarvirus. The adults may carry pollen contaminated with virus which attached to their bodies while visiting flowers. When thrips feed on infected pollen grain on healthy plants, the stylets may injure the underlying tissues and the inside of the pollen grain, together with the virus particles, may split over the wounded tissues producing the infection of the plant.

b) They transmit the virus of the genus Tospovirus directly

From the six thrips species known to transmit Tospovirus in nature, only *F. occidentalis* and *T. tabaci* are present in Spain. Three of the twelve described tospovirus species have been reported in Spain: the Tomato spotted wilt virus (TSWV), the Impatiens necrotic spot virus (INSV) and the Iris yellow spot virus (IYSV). All of these virus are transmitted in a persistent and circulatory way. Young first and second instar larvae may ingest the virus when feeding on infested tissues during a few minutes. As the insect develop the viral particles pass from the medium intestine to the general cavity, and from here to the salivary glands. From that moment on the thrips (in the larval or adult stage) is infectious. The average time required for inoculation is about 30 minutes of feeding, increasing the effectiveness of the infection as time increases. The circulation or latency period (since acquisition to the translocation of virions to the salivary glands) changes with temperature and with the virus, ranging from 4 to 18 days in *T. tabaci* and TSWV. Viruses are replicated inside the insect's body, and females, as well as males, remain infectious during their whole life, but the virus is not transmitted to their offspring.

The transmission of viral diseases is the main concern of thrips as potentially pest species. The binomial thrips-virus is a crop-limiting factor, with *F. occidentalis* and the Tomato spotted wilt virus (TSWV) as the most common example worldwide .

Photos 6 to 9. Damage of tomato spotted wilt virus. Reduction of development and bronzing; death of the affected plant; necrotic and circular spots in fruits; Circular discoloration in fruits

Photos 10 to 13. Damage of the spotted wilt virus in pepper: Circular spots in fruits; depressed spots in fruits; Necrotic spots in leaves of a resistant variety; Arabesques on leave of a non-sensitive variety

2. Most important pest-species

Many thrip species are associated with protected crops, although, only a minority of them cause the majority of damage. Until the introduction of *F. occidentalis* in Spain, *T tabaci* was the principal species causing damage in crops such as the carnation, the chrysanthemum or the gladiolus. Other species like *T .meridionalis* (in carnation), with *Frankliniella intonsa* (in rose) or *T. simplex* (in gladiolus) were occasional and less relevant. Shortly before the presence of *F. occidentalis* was detected in the protected crops in the Southeast of the Iberian Peninsula, small populations of *Frankliniella shultzei* were found in rose greenhouses, but never reaching pest status after 25 years. In some ornamental crops grown in pots in greenhouses with relative high humidity, populations of three species of the Panchaetothripine family (*Hercinothrips femoralis*, *H. bicinctus* and *Haeolothrips haemorrhoidalis*) have been found causing considerable damages to plants. In non-sprayed crops *Scolothrips longicornis*, a predator of tetranychid mites with interest from a biological control point of view because of its impact on red spider mite populations, may show up. Sometimes, other thrips species with no pest status appear in the flowers of vegetable or cut flower crops. These are pollenophagous species that do not multiply in the crop. Finally, we will refer to other species, not present in Spain but considered as pest in protected crops of other world areas, and therefore, they are monitored in imported vegetable, flower and ornamental plants.

2.1. *Frankliniella occidentalis* , the key pest

This is a very polyphagous and cosmopolitan species found in many agroecosystems. It is distributed in subtropical and mild climate areas where its impact, as a pest, is higher due to its great biotic potential and effectiveness in virus transmission, particularly, the tomato spotted wilt virus.

In coastal areas, it reproduces continuous throughout the year; the over-wintering adults and the wintering generations are dark brown, with the prothorax and the head paler than the abdomen. In spring and summer adults are paler than in winter, but the head and the prothorax continue to be clearer than the abdomen. Young individuals have dark spots on the tergites of the abdomen, getting darker segment as they mature. Males are clear or, at least, paler and smaller (0.8 to 0.9 mm) than females (1.2 to 1.6 mm).

Photo 14 and 15 . Adult and larva of *Frankliniella occidentalis*

Eggs are whitish. First and second instar larvae are whitish, turning yellowish as they develop. At the end of the larval stage they are from 1 to 1.1 mm. The nymphal instars are yellowish-white and remain immobile. The pronymph has the short antennae directed forward, while the nymph has the wings folded on the back of the body. In both instars incipient wings are present, although they are not functional. Legs in both stages are not articulated.

The activity and the reproduction of this species is continuous in areas with warm climate, although the development and population growth rate and decrease in winter. In summer, the high temperatures also limit the population growth. In the areas with mild climate and areas of the interior of the Iberian peninsula, several generations are completed yearly; the species over-winters as adutl and the population are mainly integrated by females, as they live longer than males.

The hatching of the eggs lasts approximately 13 days at 15 ºC, 3 at 25 ºC and 2.5 at 30 ºC. As soon as the larvae emerge they begin to feed on tissues or pollen, taking approximately 7 days at 15 ºC, from 2 to 3 days at 20 ºC, and from 1 to 2 days at 25 ºC and 30 ºC to complete the first stage. The second stage lasts approximately 12 days at 15 ºC, 9 days at 20 ºC, from 4 to 5 days at 25 ºC and from 2 to 3 at 30 ºC. At the end of the larval stage, larvae move to the ground where they pupate; pupation lasts approximately 10 days at 15 ºC, 7 days at 20 ºC, from 3 to 4 days at 25 ºC and from 2 to 3 days at 30 ºC. Females start laying eggs after a preoviposition period of 6 to 10 days at 15 ºC, from 2 to 3 days at 20 ºC, from 1 to 2 days at 2 5 ºC and at 30 ºC.

The minimum temperature for development is 10 ºC, with the first instar larvae being the most sensitive to temperature. The larval mortality is very high at 35 ºC, although nymphs can bear similar temperatures on ground. When temperature increases, the duration of development and longevity decreases. This species reproduce by arrhenotokous parthenogenesis, with unfertilized eggs producing males. Fertility increases with temperature, up to a limit (50 eggs/female at 15 ºC, 125 at 20 ºC, 135 at 25 ºC, 228 at 27.2 ºC, 40 at 30 ºC and 5 eggs/female at 35 ºC). The highest rates of population increase takes place at temperatures close to 25-27 ºC and relative humidity of 75 %. The longevity of the females varies with temperature (46 days at 15 ºC, 75 days at 20 ºC, 31 days at 25 ºC, 12 days at 30 ºC and 9-10 days at 35 ºC).

Photo 16 and 17 . Adults and larvae of *Frankliniella occidentalis* on pepper flowers. Nymphs on the soil

The nymphs (pupae) are sensitive to soil humidity, becoming dehydrated on totally dry grounds and dying from asphyxia when ground is flooded for more than 3 consecutive days.

This is a polyphagous species that multiplies in several cultivated and spontaneous plants, or simply feeds on them (adults feed mainly on flowers, being the pollen the most propitious food for larval development and the reproduction of females).

Adults are good fliers, they may move several meters by active flight and they may displace long distances by horizontal air currents. The colonization of protected crops normally occurs by plants infested in nurseries or by immigration from the neighboring vegetation, with the first specimens showing up at crop edges. When the host is appropriate and the environmental conditions are favorable (temperature close to 25 ºC and RH of 75 %), demographic outbreaks difficult to control are common in *F. occidentalis* populations. In southern Spain *F. occidentalis* is the only vector of the tomato spotted wilt virus. Considering all the species of this genus or other thrips genera implied in the transmission of this virus, *F. occidentalis* is the most effective virus vector, even in plants that are not good hosts for thrips, as it occurs in tomato plants where the biotic potential of the species is very low. The triggering of TSWV epidemics in protected crops depends on the environmental conditions and the type of virus. For example, the minimum time for the virus acquisition by the first instars or young second larvae, is estimated in about 30 minutes, but longer the longer period the higher the probability of acquiring the virus. Furthermore, the latency period of the virus inside the insect body (period of time from the acquisition of viral particles up to the point they arrive in the salivary glands) changes with temperature and the virus (Table 1).

If the latency period is lower than the period elapsed between the acquisition and the beginning of nymphosis, larvae can transmit the virus and infect new tissues of the same plant where it acquired the virus, or those of a new plant. However, if the latency period is higher than the length of the larval development, the adult is infectious, but not the larva. At temperatures higher than 20 ºC the length of the larval development (Table 2) is higher than the latency period, therefore, adults are the only one responsible for the transmission of the disease.

Table 1. Average period of latency (in hours) of the virus in the body of *Frankliniella occidentalis* at three temperatures, depending on the Tospovirus and the stage in which transmission is carried out (larva, adult) (according to WIJKAMP y PETERS, 1993)

Temperature (° C)	INSV		TSWV	
	Larvae	Adults	Larvae	Adults
20	*157*	*169*	*171*	*176*
24	*103*	*118*	*109*	*119*
27	*82*	*98*	*84*	*103*

To model the spread of epidemic, it is necessary to take into account that thrips spends the nymphosis period on the ground and that during this phase is viruliferous. This particular aspect must be also taken into account when establishing thrips control measures.

Table 2. Duration (in days) of the development stages of *Frankliniella occidentalis* at different constant temperatures in radish (ROBB, 1989)

	15 ºC	20 ºC	25 ºC	30 ºC	35 ºC
Egg incubation	10,1	6,6	3,2	2,5	2,4
Larval stage	17,1	12,3	6,2	3,4	3,9
Nymphal stages	12,2	7,1	3,6	3,2	2,7
Total development	**39,4**	**26,0**	**13,0**	**9,1**	**10,0**

Identification of the species

Some morphological characters permit identifying adults:

Antennae with 8 condyles, the first one lighter than the second. Anterior angles of the pronotum with a long seta and the posterior angles with a pair of setae. Clear forewings with 20 to 22 setae in the anterior venation. Long interocelar setae. Posterior edge of the eight abdominal tergite with a continuous comb of microsetae. Male abdominal sternites III to VII with elongated glandular areas in transverse position.

2.2. *Thrips tabaci*

Thrips tabaci is a polyphagous and cosmopolitan species, with a great ability to adapt to very different environments. The presence of two *T. tabaci* ecotypes with different modes of parthenogenetical reproduction, and different hosts and ability to transmit virus, is an indication of the broad ecological range of this thrips species. For example, the Spanish ecotypes do not seem to have the ability to transmit the tomato spotted wilt virus.

Adults from winter generations are brown, while in spring and summer they are completely pale or slightly brown. Wings are uniformly clear. Females are from 0.9 to 1 mm and males from 0.7 to 0.8 mm; males are always paler than females.

First eggs are translucent and turn white at hatching. Larvae of the two instars are whitish at the beginning, turning pale yellowish when they develop. At the end of their development they are between 0.7 to 0.8 mm. The nymphal instars (pupae) are whitish and develop on the plant;

prenymphs have antennae directed forward and nymphs have them folded on the back of the head and thorax. In both instars the insect do not feed and remains almost immobile because legs are not articulated.

In mild or cold regions it overwinters in the adult stage, but in warm regions it is active throughout the whole year.

Photo 18 and 19. Female and larva of *Thrips tabaci*

Females insert the eggs in the tissues of the flowers and the leaves. Egg hatching takes approximately 6 days at 25 ºC; young larvae look for protected places for feeding (inside the flowers, underside of the leaves, young sprouts, etc.). The first instar larval period lasts between 2 or 3 days at 25 ºC and the second one takes twice as long. When larvae complete development, they look for a protected place (flower organs, fruit calyx, close to the basal veins of leaves, etc.) to carry out nymphosis, which takes between 3 or 6 days at 25 ºC. The adults need other 2 or 3 additional days to mature.

Fertility varies with temperature, ranging between 20 and 120 eggs per female, distributed across a 20 day period (at medium or high temperatures) to 50 days (at lower temperatures). Reproduction is generally sexual or by thelytokous parthenogenesis. Arrhenotokous parthenogenesis is not to be excluded in warm regions or seasons, where a 10-50 % proportion of males is normally present.

Development occurs between 8 ºC and 38 ºC, with optimum thermal conditions between 25 and 30 ºC. In warm areas of the east coast of Spain, the activity does not stop, maintaining a constant level of multiplication in protected crops where several generations are completed. The longevity of males is approximately half of the females.

The Amaryllidaceae are the preferred hosts for this thrips species, but it can maintain a good reproduction rate in other hosts such as cucumber, peppers, carnations, etc. In addition to the cultivated plants, they can multiply on a great number of wild plant species.

The thelytokous ecotypes from eastern Europe do not seem capable to transmit the tomato spotted wilt virus, even though the arrhenotokous types transmit it; nevertheless, the effectiveness in transmission is lower than in *F. occidentalis*. The Mediterranean ecotypes do not transmit viral diseases that affect protected crops, although they are considered vectors of Iris yellow spot virus (IYSV) which affects garlic.

Identification of the species

The characters that permit identifying *T. tabaci* adults are:

Body uniformly clear or light brown. Antennae with 7 condyles, the first one clearer than the second. Posterior angles of the pronotum with a pair of long setae. Clear forewings with 3 or 5 setae in the distal part of the main vein. They do not have secondary setae in the abdominal sternites. Lateral edges of the tergite of the second abdominal segment with 3 setae. Posterior edge of the eighth abdominal tergite with a continuous comb of microsetae. Sternites III to V of the male abdomen with elongated glandular areas.

2.3. Other species

a) Other thrips species in protected crops

On rare occasions we have found specimens of other thrip species in protected vegetable crops, but not causing relevant damage. However, we have found, more frequently, cases of other species associated with flower crops, and in some cases, the damage has been significant. We are going to refer to these species.

Frankliniella shultzei

In cut flower crops in greenhouses, damage has been reported by pale specimens, which do not seem able of transmitting TSWV, contrary to dark specimens.

Adults are similar to *F. occidentalis* adults; the only difference is that the first ones are uniformly paler. The location of the interocelar setae between the posterior ocelli, the presence of campaniform sensilla in the metanotal plate, makes it different from the other *Frankliniella* species known to Spain. It has been found in rose but producing barely any damage; damage to gladiolus are more conspicuous.

Frankliniella intonsa

This autochthonous species has been observed to cause considerable damage to rose crops in greenhouses in the Mediterranean area. Adults are uniformly brown and 0.8 to 1 mm long. Populations are high between the middle of spring and early summer. The differences between this species and the other species of *Frankliniella* genus are: the uniform dark colour, non-prominent head, interocelar setae inside the ocelar triangle, striated metanotal plate and the lack of campaniform sensilla. We can find it often in rosaceae, legumes and cereal crops and in some spontaneous plants.

Thrips meridionalis

Until the arrival of *F. occidentalis*, *T meridionalis* was, together with *T. tabaci*, the most abundant species in greenhouse carnation and gladiolus from Southern Spain and responsible for significant damage. However, its seasonal character (from the middle of winter to the end of spring) limits the populations and damage to crops. Adults are black, wings having a pale base, which gives it a striped aspect. Within the genus, *Thrips*, it is included in the group of species with 8 antennae condyles. Adults are 1.4 to 1.8 mm long, and the larva, when it is fully developed, is approximately 1 mm long. The following characteristic differentiate *T. meridionalis* from other *Thrips* species: long setae in the posterior angles of the pronotum, three setae in the distal part of the anterior forewing vein, the abdominal sternites with non-aligned secondary setae and 8 condyles in the antennae. This is a polyphagous species which colonizes and multiplies in crops and wild plants.

Thrips simples

This is a species with a marked specificity to gladiolus, being one of the most significant pests to this crop in warm areas. It causes whitish spots on flowers and deformity, and inflorescence abortion. It is sheltered in the scaly leaves of corms; therefore it spreads easily, taking into account that adults are capable of bearing the low conservation temperatures of corms. Adults are dark and 1.2 to 1.5 mm long, with the base of forewings hyaline, giving the impression of a clearer stripe at the end of the thorax. The differences between this species and the other species of the genus are: the interocelar setae inside the ocelar triangle, the presence of 5-7 setae in the edge of the main forewing vein, the aligned secondary setae of the abdominal sternites, the 8 condyles of the antennae and the long setae in the posterior angle of the pronotum.

Hercinothrips femorali and H. bicinctus

This species causes damage on the leaves of several ornamental plants appreciated by their leaves. The brown spots originated at feeding sites are correspond to faeces, under which eggs are usually inserted. The antenna of the Adults end in sharp and long condyles; most of the surface of the body is reticulate and the abdomen is dumpy. The wings have two dark stripes and two light stripes in *H. femoralis*, and two dark stripes and three light stripes in *H. bicinctus*. Larvae are whitish and carry, as adults do, a ball of excrement at the tip of the abdomen, which protects them against predators. They are slow in their movements and nont good flyers. The nymphal stages develop in the same places as larvae do, being gregarious and lacking much mobility. The wing stripes permit differentiating the two species from other similar species in the genera.

Heliothrips haemorrhoidalis

This pest develops in ornamental crops in humid, mild or warm environments. Most of the body surface of adults is reticulate, being so more conspicuously in the head and thorax. They are black, with hyaline wings, dumpy abdomen and falciform forewings. Newborn larvae are whitish, becoming yellow-waxy at the end of their development. Larvae carry a ball of excrement on the long setae at the end of the abdomen, as well as adults do. Just emerged adults have a red abdomen (for this reason they are called *haemorrhoidalis*) and the rest of their body is black. Nymphs are not very mobile, remaining in the place where larvae complete development.

This species is classified within the Panchetothtipinae because of its reticulate body, falciform forewings and the pointed ending of antennae. It differentiates from *Hercinothrips* species by the uniform hyaline wings.

Photo 20 and 21. Female and larva of *Heliothrips haemorrhoidalis*

Scolothrips longicornis

It is a predator of Tetranychidae. Adults are clear brown, with striped wings (two dark stripes and three clear stripes). Larvae are whitish, turning to cinnamon as they develop due to the color of the mites they fed upon. The nymphal instars are whitish and remain in the place where larvae complete their development. Populations grow along the spring and the activity depends on the presence of prey because this is a specific predator of mites and obliged carnivore. In the greenhouses where the biological control of pests is carried out, it appears spontaneously at the end of spring, and it also collaborates with other predators (phytoseeid mites, cecidomyiids, etc.) to an effective control of red spider mites.

Photo 22 and 23. Adult and larva of *Scolothrips longicornis*, predator of red spider mites

Scolothrips longicornis can be differentiated from the other species by the 8-condyles antennae, striped wings, long setae in the posterior, anterior angles and lateral edges of the prothorax, and the body spotted with strong setae.

b) Species that may be introduced in Spain

Echinothrips americanus

This species of american origin was introduced to Central Europe. It affects mainly pepper and saintpaulia, and has been occasionally found in other ornamental plants. It is included within the Panchaetothripinae subfamily. The adults are black with (falciform) the base of the forewings hyaline, 8 condyles in the antennae, and head and thorax more reticulated than the rest of the body. Females are 1.5 to 1.7 mm; males are smaller and thinner than females. Young larvae are whitish, turning yellow-orangey as they develop. The nymphs are whitish, with a pair of long setae on each abdominal segment. At 20 ºC the larval development takes approximately 35 days, 15 days at 25 ºC and about 12 days at 30 ºC. This species attacks several ornamental plants and vegetable crops producing silvery spots on the upper side of leaves.

E. americanus can be differentiated from other native species of Panchaetothripinae by the dark wings with a clear stripe on the base, the reticulate body surface, long setae on the main vein of forewings that widen toward the end, the long setae of the abdominal tergites and the small circular glandular areas of the abdominal sternites in males.

Scirtothrips dorsalis

This polyphagous species has been intercepted by quarantine authorities in ornamental plants, and it may become a pest in horticultural crops such as peppers, tropical crops such as mangoes and citrus. It was originally distributed in Asian countries, but in the last few years, it has spread to African countries with warm or mild climate. Although it was initially implicated in the transmission of the tomato spotted wilt virus, it was later proved that there was a mistake in the identification of the specimen used in the essays, which were ultimately determined as *Frankliniella shultzei*. *Scirtothrips dorsalis* adults are clear, small, dumpy, with 8 condyles in the antennae, and a pair of long setae in the posterior angles of the pronotum; the wings are narrow, transparent, with two setae in the apical part of the posterior vein of forewings and dark plates in the centre of the abdominal sternites. Males, which were frequent in samples gathered at quarantine ports, are smaller and paler than females. This species is well adapted to live in greenhouse conditions. It produces deformations of fruits and leaves from feeding.

S. dorsalis can be differentiated from other species found in protected crops by its small size, light colour; head larger than longer, the dumpy aspect and the reticulate metanotum and other small details on males.

Thrips palmi

This species has been frequently found in imported ornamental plants, fruits, vegetable and cut flower at quarantine ports. This thrips species has spread worldwide from Asia. The eradication program implemented in the European countries where it has been detected gave good results and nowadays the species is in the quarantine list. This species transmits some species of Tospovirus as Watermelon silver mottle virus (WSMV) or Melon spotted wilt virus (MSWV), which affects cucurbits, therefore, its introduction in Spain would be particularly troublesome.

Adults are less than 1 mm long, pale, with 7 condyles in the antennae, being the first and second pale, with two pairs of long setae in the posterior angles of the pronotum, 2-4 setae in the apical part of the main vein of forewings and 4 setae in the lateral edge of the second abdominal tergite. The larvae are whitish, turning yellowish as they develop. The nymphs are whitish and develop in protected places near to the places where larvae developed. The optimum development occurs at 25 ºC and the lower thermal threshold at 12.6 ºC. A generation lasts approximately 80 days at 15 ºC and only 21 days at 30 ºC. Vegetables such as aubergine, watermelon, cucumber and the ornamental plants such as carnation, chrysanthemum and some ornamental plants grown in pots are good host for the species. The species feeds on plants producing silvering on the under side of leaves and the flowering organs, deformations of shoots and flower abortions. The transmission of viral diseases is produced persistently, and virus circulates and multiplies within the insect's body.

T. palmi can be differentiated from the other *Thrips* species associated with protected crops by the following features: adults are fully pale, 2-4 setae in the edge of the main vein of forewings, the interocelar setae located outside of the ocelar triangle, the first two basal condyles of the antennae are pale, the metanotal plate is striated with a pair of campaniform sensilla, 4 setae in the lateral edge of the second abdominal tergite, no secondary setae in the abdominal sternites and abdominal sternites III and IV of males with elongated glandular areas.

3. Control methods

3.1. Native and exotic natural enemies

In the natural systems a good number of predators, parasitoids, fungi and entomopathogen nematodes regulate thrips populations in a balanced and stable way. However, in protected crops the situation is different because they are vulnerable to crop practices, which makes it easy for the thrips to reach the pest level.

The use of natural enemies for the control of thrips in protected crops have increased greatly in the last 30 years, mainly due to the search of effective antagonists adapted to the conditions where they are going to be applied and easy to multiply under semi-artificial conditions. They have arisen due to the need to control, in a stable and lasting manner, the species that affect the protected crops and transmit viral diseases. Table 3 shows the most relevant natural enemies for the biological control of thrips in protected crops.

Table 3. Natural enemies of thrips and its possible use as biocontrol agents of pest-species

Natural enemy	Generalist Facultative Specific	Crops	Type of management	Availability	Pest
Parasitoids					
Ceranisus spp.	Specific (larvae)	Several	Inoculative release/ favouring installation	Possible commercial production	*F. occidentales* *T. tabaci* *T. meridionales* *H. haemorrhoidalis* *H. femoralis*
Tripobius spp	Specific (larvae)	Ornamental	favouring installation		*H. femoralis* *H. haemorrhoidalis*
Megaphragma spp	Specific (eggs)	Ornamental	Favouring installation		*H. haemorrhoidalis*
Thripinema ssp.	Specific (larvae and pupae)	Several	Favouring installation	NO	*F. occidentalis*
Entomopathogens					
Beauveria basiana	Generalist	Several	Massive release	Commercial production	*F. occidentales* *T. tabaci* *H. haemorrhoidalis* *H. femoralis*
Lecanicillium lecanii	Generalist	Several	Massive release	Commercial production	
Metarhizium anisopliae	Generalist	Several	Massive release	¿	*F. occidentales* *T. tabaci*
Paecilomyces spp.	Generalist	Several	Massive release		*F. occidentales* *T. tabaci*
Predators					
Mites					
Amblyseius spp	Generalist, Facultative	Several	Inoculative release	Commercial production	*F. occidentales* *T. tabaci* *H. haemorrhoidalis* *T. simplex*
Neoseilus spp	Generalist, Facultative	Several	Inoculative release	Commercial production	
Hipoaspis spp.	Specific	Several	Inoculative release	Commercial production	*F. occidentales* *T. tabaci*
Thysanopterans					
Aeolothrips spp.	Specific	Several	Favouring installation	NO	Several
Franklinothrips spp.	Specific	Several	Inoculative release	Possible	*H. haemorrhoidalis* *F. occidentales* *T. tabaci* *H. femoralis*
Heteropterans					
Orius spp.	Generalist	Several	Inoculative release	Commercial production	*F. occidentales* *T. tabaci* *T. meridionales*
Anthocoris spp.	Generalist	Several	Inoculative release	Commercial production	*F. occidentales* *T. tabaci*
Geocoris spp.	Generalist	Several	Favouring installation	NO	*F. occidentales*
Nabis spp	Generalist	Several	Inoculative release	Commercial production	*F. occidentales* *T. tabaci*
Macrolophus spp.	Generalist	Several	Inoculative release	Commercial production	*F. occidentales* *T. tabaci*
Dicyphus spp.	Generalist	Several	Favouring installation	Commercial production	*F. occidentales* *T. tabaci*
Dereaocoris spp.	Generalist	Several (outdoors)	Favouring installation	NO	*F. occidentales* *T. tabaci*
Nesidiocoris tenuis	Generalist	Tomato	Inoculative release	Commercial production	*F. occidentales*

3.1.1. Predators

The activity and effectiveness of predators in the control of thrips is determined by the vegetal hosts and the environmental conditions. This explains the good response of some predators in a crop (the case of *O. laevigatus* on *F. occidentalis* in pepper), but not in others close to it (the same pest and the same predator in tomato plants for example); this is because pepper is a good host for the pest and the predator, while tomato is not a good host for the pest nor predator, despite thrips being one of the major tomato pests because it transmits the tomato spotted wilt virus.

a) Anthocorids

Most of the thrips predators are generalists, therefore the abundance of alternative prey, preferred by the predator, determines its effectiveness in thrips control. Anthocorids have been shown to be very efficient in controlling natural populations of several thrips species. *Orius* species, with carnivorous feeding habits, provide acceptable levels of control in wild and cultivated plants, showing a preference for thrips versus other arthropods of similar size. The size of thrips and the plant organs colonized can be the reason for such preference. In coastal areas of the southeast peninsular several species coexist and their populations can be followed across time as it occurs with *O. laevigatus* and *O. albidipennis* in pepper crops in greenhouses and outdoors. The commercial production of these two species permits their use in thousands of hectares of pepper crops for the control of *F. occidentalis* and the tomato spotted wilt virus (TSWV). They are also effective for the control of western flower thrips and the onion thrips in pepper, strawberry, melon and aubergine crops and some ornamental crops and cut flowers.

In cold areas, *O. laevigatus* shows reproductive diapause induced by the short photoperiod, while this does not occur in areas with Mediterranean climate, which permits using this predator during winter periods in greenhouses in these areas, where there are a high risk of severe attacks of *F. occidentalis*.

In tomatoes, the abundance of trichomes with sticky substances makes the establishment and the predatory activity of *Orius* difficult, as in the case of thrips (*F. occidentalis and T. tabaci*). The predator small nymphs remain immobilized when the sticky substances of the trichomes are accumulated in the legs and they die by starvation, providing poor control of thrips and of viral diseases that *F. occidentalis* transmit. In rose crops, the release of *Orius spp.* has not been effective for the control of thrips (*F. occidentalis, F. intonsa)* due to the characteristics of the plant and the lack of pollen, which is used as substitute, and survival, food for predators.

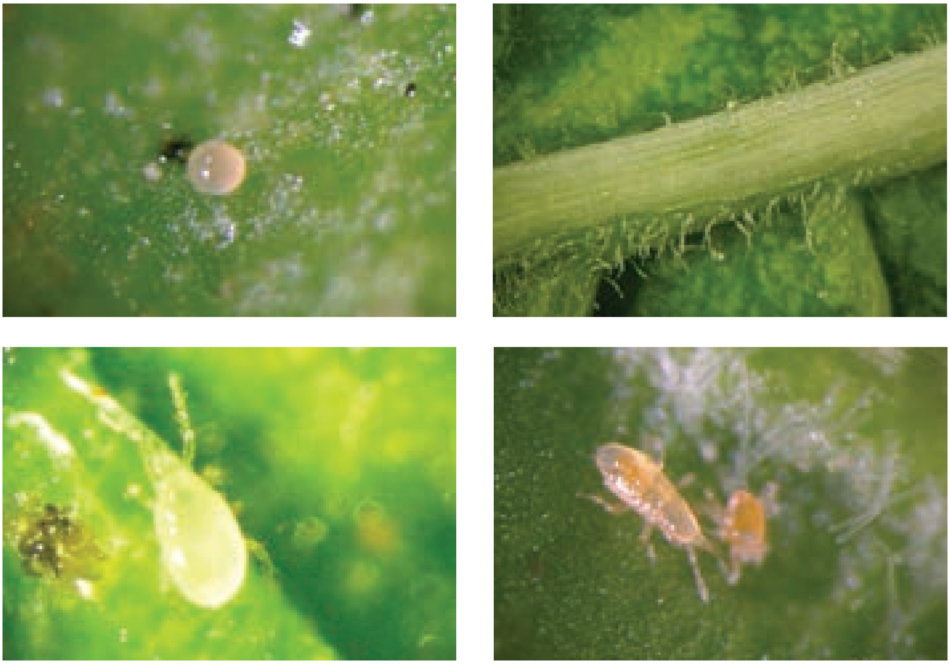
b) Mirids

Mirids are generalist predators feeding upon thrips and regulating thrips populations under natural conditions; however, they do not show any preference for this prey in crops and wild host plants, and their use against thrips gives poor results. In those crops such as tomatoes, on which many predators have difficulties establishing and surviving, mirids, such as *Macrolophus pygmaeus*, *Dyciphus* spp., or *Nesidiocoris tenuis*, reduce the populations of *F. occidentalis*. In some Mediterranean areas, *Dereacoris punctulatus* is frequently associated with thrips, such us *F. occidentalis* in the spontaneous vegetation, being of specific interest in the reduction of thrip populations in the agroecosystem.

In the spontaneous vegetation, Nabids (e.g. *Nabis pseudoferus*) have an important role in the control of thrips populations, although they prefer bigger size prey. *Geocoris* spp. (e.g. G. *atricolor, G. palles*) are less common in the natural systems and, therefore, their incidence on the thrips populations is protected crops is also lower.

c) Phytoseiids

The generalist predatory mites were studied and used as the first option for the control of thrips (*T. tabaci*) in protected crops (cucumber and tomato), before the introduction of *F. occidentalis* in Europe. *Neoseiulus* (*Amblyseius*) *cucumeris* and *N. barkeri* were the first produced commercially to be used effectively for the control of *T. tabaci* in greenhouse pepper crops, and later against *F. occidentalis*. In cucumber crops, the control of *F. occidentalis* and *T. tabaci* by *N. cucumeris* was insufficient, as it occurs in pepper greenhouses when there is TSWV infections. These mites consume young thrips larvae and are not very efficient against second instar larvae and adults. Other species of phytoseeids such as *Iphiseius degenerans* may prey upon *F. occidentalis* in pepper and cucumber crops, but their massive production is expensive.



Photos 24 to 27. Egg of *Neosiulus californicus*; egg of *Amblyseius swirskii*; adult of *Amblyseius swirskii*; nymph of *Orius laevigatus* preying on an adult of *N. californicus*

Other efficient predators of mites (*Neoseiulus californicus)* or whiteflies (*Amblyseius swirskii*), used for the control of those pests in pepper and cucumber protected crops, prey occasionally on thrips larvae; although those phytoseiids do not exert a good level of control on thrips, they certainly contribute to reduce thrips populations. In some crops, *Hypoaspis milles* has been reported as good predator of *F. occidentalis* larvae, although it is found mainly in the ground and prey preferably on sciarids and phorids larvae.

The combination of *N. cucumeris* and *O. laevigatus* has been reported as an effective alternative for the control of *F. occidentalis* in pepper protected crops in the Mediterranean and Central European areas. The phytoseiid exerts an acceptable level of thrips control at the beginning of the growing season, serving as food for *O. laevigatus* and facilitating the establishment of the anthocorid when the thrips densities are low.

3.1.2. Parasitoids

The parasitoids show a high degree of specificity and their efficacy is subject to the characteristics of the host plant and the species of thrips.

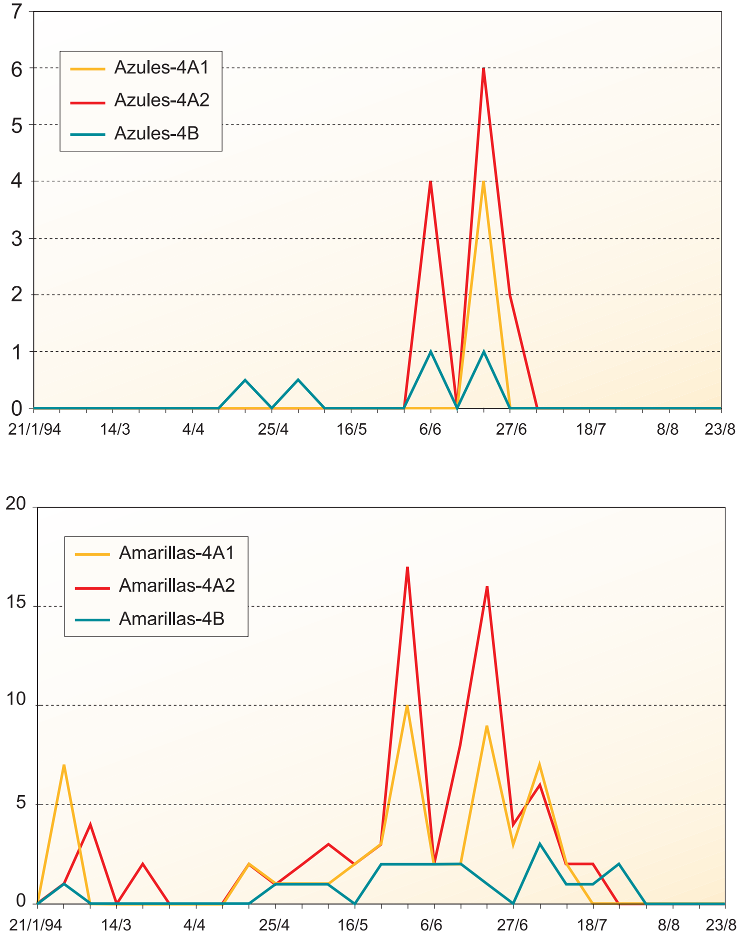
a) Parasitoids of thrips larvae

In some ornamental crops *Tripobius semilutens* parasitizes *H. haemorroidalis*, larvae, although the incidence on the population is not enough to obtain a good level of pest control.

The four species of *Ceranisus* (*C. menes, C. lepidotus, C. platinianus* and *C. ruselli*) reported to Europe, parasitize larvae of several species of the family Thripidae. They are common in some agrosystems where *F. occidentales* proliferates, but they are not very abundant (Figures 1 and 2), therefore they do not participate very much in the natural regulation of western flower thrip populations. The duration of the biological cycle is more than double the length of some species such as *F. occidentalis* or *T. tabaci*. The use of colored sticky cards is not recommended to enhance the activity of thrips parasitoids (Figures 1 and 2). The introduction in Europe of *C. americensis*, native to North America, as a possible bio-control agent of *F. occidentalis* was not at the level of the expectations, in spite being produced commercially.

It is necessary to take into account the possible intraguild predator/parasitoid interactions when establishing mixed strategies for the control of pests.

Figure 1 and 2. Evolution of the captures of Ceranisus menes in blue (1) or yellow (2) chromotropic traps in pepper greenhouses in the Field of Cartagena (Murcia). 4B: Chemical control against thrips and meshes in the side openings; 4A1: Chemical control against thrips without mesh in the opening; 4A2: Without specific treatments against thrips



b) Parasitoids of eggs

Several species of the genera *Megaphragma* and *Polynema* have been found parasitizing eggs of thrips species of the subfamily Panchaetothripinae, as well as species from other families such as *Thrips tabaci*, or *T .palmi*. These parasitoids are not very frequent and abundant, thus their incidence in the control of thrips pests is very low.

3.1.3. Entomopathogens

The activity and efficiency of the entomopathogens is influenced by environmental conditions. The favorable conditions for the entomopathogen fungi are also suitable for phytopathogen fungi of aerial development, which are controlled by chemical method incompatible with the entomopathogens. The use of these bio-control agents is affordable in crops that do not require fungicide treatments and crops in mild and humid areas; in any case, entomopathogenous are most effective against life stages of the thrips that take place on the ground.

Different *Bauveria bassiana*, *Lecanicillium lecanii, Metarhizium anisopliae or Paecilomyces fumosoroseus* isolates produce high mortalities of *F. occidentalis* and *T. tabaci* larvae under controlled conditions. The commercial formulations of *L. lecanii* and *B. bassiana* applied to plants and to the ground in greenhouse crops of the Spanish Mediterranean coast reduced the populations of *F. occidentalis* locally, but their effect persisted only for a short time.

Most of the fungi that have activity on thrips are compatible with the other natural enemies, and they can be used in integrated pest control strategies.

There are very limited references about thrips parasitism by nematodes. Only two species of *Thripinema* (*nicklewoodii* and *remiran*) have been reported as parasites of larvae of *F. occidentalis*, and both with low incidence rates. However, commercial preparations of *Steinernema spp.* and *Heterorhabditis spp.* have been tested for the control of the “western flower thrips” in protected crops with poor success.

3.2. Cultural methods

The management of thrips populations in greenhouse crops looks for the harmonic integration of all available pest control methods, after having evaluated their efficacy and compatibility.

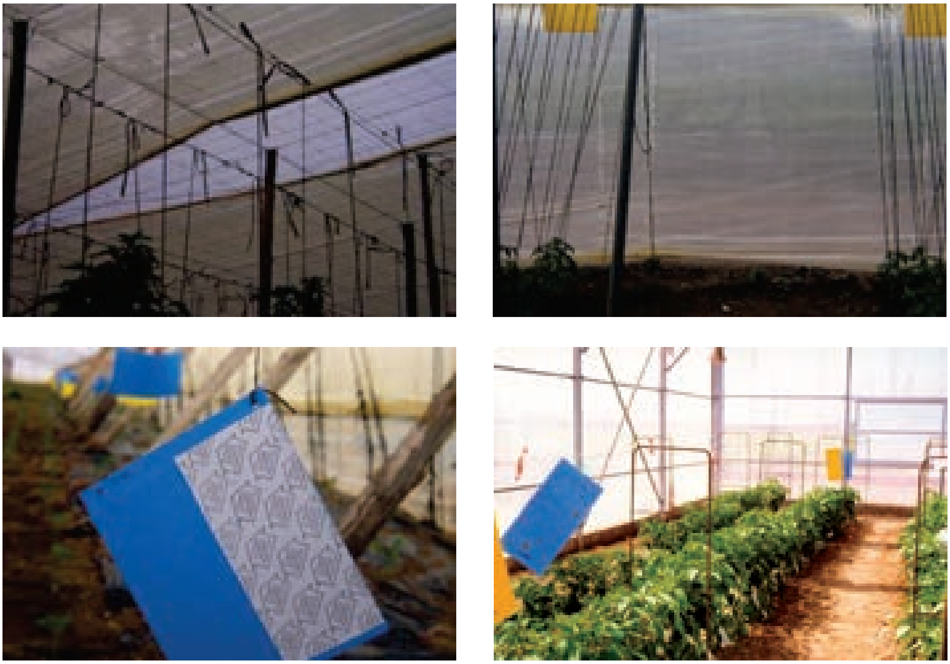
3.2.1. Preventive and cultural measures

a) Plantation material without thrips

Precautions are adopted to avoid the introduction of any kind of material infested with insects, not only in crops but also in production areas. The use of insect-free plant material, the installation of crops in non favorable seasons for the activity of insects, and the development of specific-species preventive hygienic measures, as well as the elimination of weeds or crops that serve as pest reservoir, are very useful in the control of polyphagous (e.g. *F. occidentalis, T. tabaci)* or specific (e.g. *H. femoralis*) thrips species, in protected crops of both vegetables and ornamental plants or cut flowers.

b) Exclusion or dissuasion barriers

Physical measures of exclusion (e.g. dense meshes) or dissuasion barriers (e.g. photoselective plastics or plastics with repellent) have been used to protect crops susceptible to *F. occidentalis* transmitted Tospovirus. At present, the installation of meshes in the ventilation openings and accesses to greenhouses is a common practice in high virus incidence areas. The use of 16 x 10 threads/centimeter in lateral openings and 6 x 10 threads/centimeter meshes in the zenith openings has been reported to produce delays in the immigration of *F. occidentalis* adults to pepper greenhouses of approximately a 4 to 6 weeks, both in crops initiated in winter and summer; similar delays were observed in the introduction of tomato spotted wilt virus and in the subsequent spread of the diseases.



Photos 28 to 31. Meshes in zenith and side ventilation and coloured traps situated in the possible places of entry of thrips; yellow and blue traps for the monitoring of populations

c) Wild plant management

The management of wild vegetation in the surroundings of greenhouses is utterly important for the control of thrips pest of protected crop; although the elimination of weeds has to be done in a selective way because, as we have already mentioned, many weed species serve not only as host for pests but also for natural enemies. Therefore, this issue has to be considered when defining strategies for the conservation of native natural enemies or the establishment of those released in the production system. That is, by favoring the spontaneous vegetation it may be possible to dispose of a population of natural enemies out of the greenhouses that may control the thrips population and reduce the immigration to crops. Nevertheless, a lot of spontaneous plants infested by Tomato spotted wilt virus (TSWV) in a symptomatic or asymptomatic way are also good host for thrips and sometimes for their natural enemies. Since the virus transmission is the most dangerous risk issue, weeds that are to be TSWV potential should be eliminated before setting up crops in order to reduce the initial inoculum of the virus, regardless of the number of thrips and natural enemies they host. It is estimated that between 4 and 6 weeks before setting up crops, depending on the temperatures, weeds should be eliminated from the surroundings of greenhouse.

The removal and elimination of plants infested with TSWV reduces considerably the incidence of the virus by decreasing the risk that thrips develop on plants infested with the virus. By removing infested plants, both the number of viruliferous thrips and the virus incidence decrease (Figures 3 and 4).

Figure 3. Evolution of the populations of F. occidentalis adults and larvae in a greenhouse where half of the plants infected by TSWV were pulled out

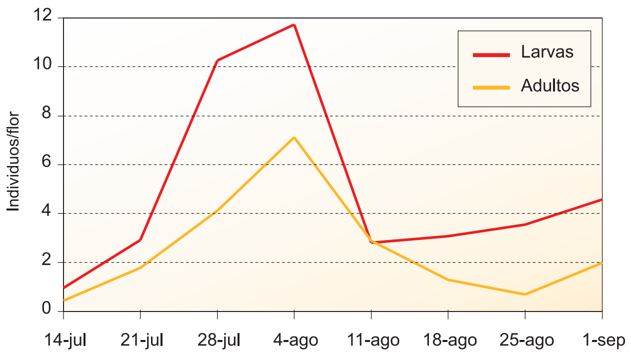
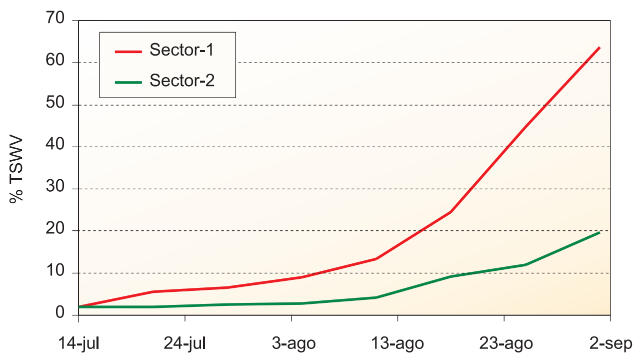


Figure 4. Evolution of the percentage of plants affected by TSWV in the half of the greenhouse where plants with symptoms (green line) were pulled out and the other where they were not pulled out



d) Management of previous crops remains

Plant Health regulations recommend not leaving crop remains in greenhouses, especially if the are plants infested with TSWV, because of the risk of a massive emigration of viruliferous thrips to nearby crops. Under such circumstances, before removing the crops, chemical, biotechnical or biological methods are required to minimize the emigration risk of viruliferous thrips. The burial of wasted plants and the immediate soil solarization or bio-solarization reduce substantially the remaining thrips population in greenhouse between crops. Depending on the season, solarization has to be applied for approximately 4 or 6 weeks, since nymphs that remain in the soil can survive at 30 ºC or higher temperatures. Besides, flooding soils during 2 or 3 days seems to have a great impact on the survival of nymphs. In winter time, the duration of solarization has to be increased in order to be effective against thrips.

3.3. Biotechnical and genetic methods

a) Biotechnical methods

The capture of adults in yellow, blue (i.e. *F. occidentalis*) or white (i.e. *T. tabaci*) chromotropic traps has been used inside greenhouses to reduce the number of thrips establishing in crops, once they have break through the exclusion meshes of greenhouses' openings. These traps are quite useful with small populations but they are less effective when massive flights, due to prevailing winds, take place.

The colored traps are used to recognize the immigration to greenhouses and to monitor the dynamics of thrips flight. Blue traps are most effective for *F. occidentalis*, although they can also be detected with yellow traps. Thrips natural enemies are also captured with colored cards. This is the reason why it is convenient not to maintain the traps when predators are released or when strategies for the conservation and establishment of native natural enemies are used.

Use of pheromone diffusers in blue cards have been successfully tested to control *F. occidentalis,* with a significant increase in captures in relation to traps without the pheromone. However, no significant reduction of population in plants has been observed.

b) Genetic methods

The response of different varieties, according to their resistance to thrips or susceptibility, has been explored in horticultural (pepper, tomato, cucumber) and ornamental (chrysanthemum, gerbera, statice) crops as a control method. The results have been interpreted in terms of tolerance, not preference and antibiosis mechanisms. Although it is known that color of the flowers and structure can be more or less attractive for thrips, and that the results depend on the thrips species, significant differences have not been found in vegetable crops although some have been found in cut flowers. For instance, *F. occidentalis* population in red varieties of *Gerbera* is larger than in white varieties, although, the incidence of TSWV has no relation with the thrips population level.

A resistance to the virus in vegetable crops such as tomato or pepper has been observed, as well as in some ornamental crops such as *Limonium*. The mechanisms of resistance, and genes involved in the process, have been investigated. In the case of these two vegetable crops, the resistance response were weak; some virulent races of virus were selected in industrial crops soon after the resistant varieties were developed. The short duration of the resistance seems to be a consequence of a poor performance of plants under stress conditions, such as high densities of virus inoculum (= high densities of infective *F. occidentalis* adults), high temperatures, or stress conditions in plants promoting an intensive or sustainable senescence.

3.4. Chemical control

When using chemical methods to control thrips population or to mitigate the damages they produce, it is necessary to keep in mind the general characteristics of the thrips and, particularly, those of each species. The eggs are embedded into the tissues and protected against insecticides; thrips are aggregative and locate in sheltered areas of the leaves (in the underside), flowers (inside) and fruits (contact areas among fruits or between fruits and their leaves, under the sepals). They are insects with high development and population growth rates. In some species (*F. occidentalis*) the nymphal and the pupal stages take place in the soil while in other species they happen in plant-protected areas. Some species, like *F. occidentalis,* are very efficient in virus transmission and, in such cases, the intervention thresholds are very low and very frequently treatments are needed, involving risks of pesticide residues in fruits. In some TSWV- susceptible crops, most of the treatments carried out against *F. occidentalis* but the results are not satisfactory. The virus incidence becomes the most limiting factor for susceptible crops.

Some species, such as *F. occidentalis,* have developed resistance to most of the chemical products, which reduces the number of products that can be used. Resistance mechanisms of *F. occidentalis* are not well understood for all products. In cases where these mechanisms are known, *F. occidentalis* resistance to insecticides depends on many factors such as reduction of penetration, increase of detoxification or esterases sequestration, glutathione s-transferase detoxification, or desensitization or increase of acetyl-cholinesterase levels. A P450 monoxigenase detoxification activity increase in most of the groups of chemical insecticides developed for the *F. occidentalis* population in the south-eastern Spain is also common.

Frequently, crossed resistances between groups of chemical insecticides (carbamates and pyrethroids -if related to *F. occidentalis*-) appear as they use the same detoxification mechanism.

The use of different active ingredients with different working mechanisms is recommended in different generations in order to avoid the selection of resistant *F. occidentalis* populations. Population isolation and the reiterated use of the same active ingredients accelerate the development of resistances.

4. Control strategies in main protected crops

Systems for vegetables production in greenhouses are characterized by a relative isolation and for being favorable to the development of plant and pests Therefore, the goal of the strategies of pest control and, particularly of, thrips control since they are considered as virus vectors, is to reach sustainable strategies.

Sustainable strategies of thrips population in susceptible crops are rarely achieved by chemical methods and very rarely by cultural methods. There are three types of situations that encourage the use or management of natural enemies to control thrips pests, particularly to control *F. occidentalis,* considered to be the main or key pest in many crops.

a) The chemical control gives poor results or it is difficult to be applied. Such deficiencies are a consequence of:

   - Thrips being opportunistic, with high population growth rates and very short generation time at optimum conditions.
   - Thrips being aggregative, taking refuge in plants organs or plant strata which are not accessible to chemicals and also because they develop part of their biological cycle in the soil.
   - The facility with which the selection of population resistant to active ingredients occurs in confined or isolated environments.

   When using products or formulates that are considered to be effective in certain systems, some difficulties appear because of environmental conditions, incompatibility with bio-control agents used to control other pests, limitations in their use due to harvest residues, incompatibility with pollinators; or restrictions of the formulates use in confined environments like those of the greenhouses.

b) The chemical control is effective but it is difficult to maintain thrips population at acceptable levels during long periods of time, just as it happens with *F. occidentalis* control in protected TSWV-sensitive crops in warm climate regions.

c) In ecological crops in which thrips are main or key pests, because thrips are virus vectors or because injuries on fruits reduce the marketable value of harvest.

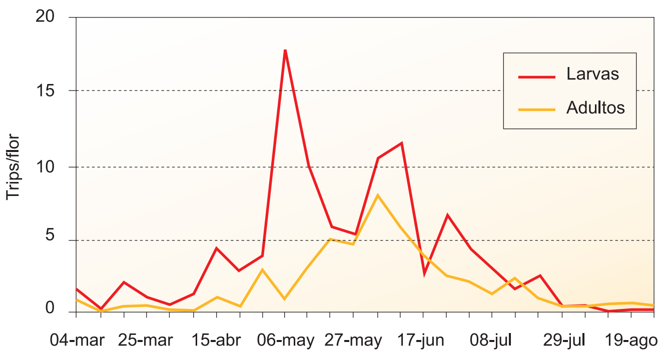
4.1. Peppers

The release and the choice of natural enemies to be used for thrips control are related to the phenology of the crops, environmental conditions, thrips population dynamics and availability of enemies.

Thrips control in protected pepper crops in south eastern Spain is carried out with predators. The strategies aim to achieve prey/predator ratios which allow low levels of thrips population before they reach the demographic explosion risk period; at that point they are uncontrollable and spread devastating viral epidemics. It has to be taken into account that pepper is a TSWV-susceptible crop and that some virus strain may have overcome the resistance conferred by TSWV-genes.

For decision-making and intervention, it is necessary to determine the percentage of infestation of flowers and leaves and the population density by weekly presence/absence (Figure 5).

Figure 5. Average distribution of the Frankliniella occidentalis populations in pepper greenhouses in the Field of Cartagena (Murcia). The critical period of risks of demographic explosions is considered between the middle of April and the beginning of July



The strategies of biological control of thrips in greenhouse pepper crops are focused on the *F. occidentalis* species, since *T. tabaci* is seldom found in this crop in southern Spain. These strategies are based on the release of two natural enemies, selected by effectiveness and adaptation to crop conditions:

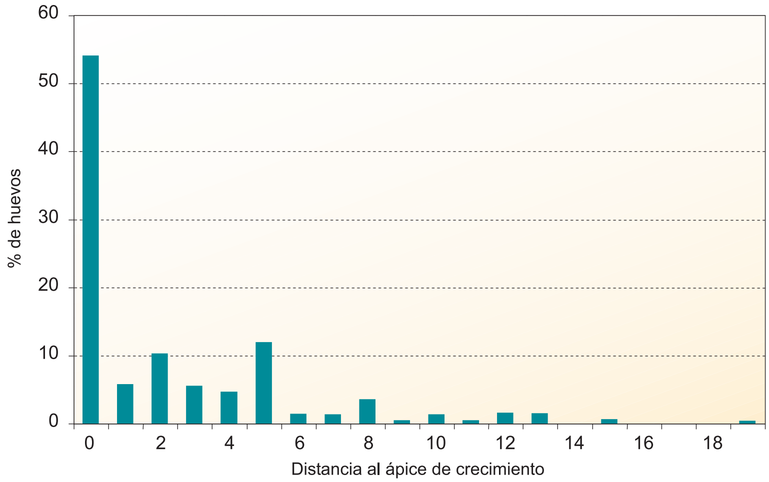
a) Phytoseiids

Neoseiulus (Amblyseius) cucumeris

Adults are pear-shaped, almost transparent or cinnamon colored. They are capable of feeding on small size larvae of several thrips species in several hosts. Their development cycle takes approximately 11 days at 20 °C, 8 - 9 days at 25 °C and 6 or 7 days at 30 °C when they feed on *F. occidentalis* larvae. The fecundity at 20 °C is approximately 15 eggs/day and oviposition spans for 10 days; eggs are attached to leaf hairs along the underside basal veins. The ideal conditions for the development, survival and population growth are between 20 and 25 °C and, approximately, 75 % of RH, with a lower thermal threshold of 8 ºC and upper of 35 ºC; at these extreme temperatures over 50 % of the eggs do not hatch, with 90 % of the larval mortality within the first two days. With a relative humidity below 65 %, only 50 % of the eggs hatch, the mortality being higher with higher temperatures (30 to 35 ºC). The daily average consumption rate for the adult is 2.5 to 6 first-instar thrips larvae. Some *A. cucumeris* "strains" have reproductive diapause when the photoperiod is shorter than 12 light hours, the day temperature is 22 ºC and the night temperature is 17 ºC, although, there are non diapausing "strains" that can be used during the winter period. In the absence of prey or pollen, cannibalism may occur. These phytoseiids are commercially multiplied and they are released in sachets where they multiply. They multiply by preying on some mite species (i.e.*Tyrophagus spp., Acarus spp*) which grow in bran.

It is released in sachets of 500 or 1.000 individuals, hanging them on a leaf petiole on one every 5 plants. Sachets are set in place when at least a flower per plant is opened and the staking basal trellises have been set in place and tied (this is quite helpful for mites to move from one plant to another, when there is no contact between leaves of consecutive plants), and when the temperatures are 10 ºC most of the day and the RH remains above 50 %. *Amblyseius cuccumeris* settles and proliferates well if flowers are available and environmental conditions are at optimum (Figure 6).

Figure 6. Distribution of the lay of Frankliniella occidentalis in pepper plants in the greenhouses of the Field of Cartagena (Murcia)



As day length gets longer, maximum day temperatures increase and humidity decreases, *A. cuccumeris* survival and the thrips control it provides decrease, with population mostly located inside flowers. The presence of this mite in plants makes the establishment of *Orius* easier, as the anthocorid may prey upon the mite when thrips populations are low, which happens at the beginning of the growing season.

Other Phytoseiids

Other Phytoseiids species like *N. californicus* or *A. swirskii* may feed on *F. occidentalis* larvae, although it is not their preferred food. *N. californicus* species prefer tetranychid mites and *A. swirskii* are very good at controlling whiteflies.

N. californicus is native to Spain and appears naturally in pepper greenhouses in southeastern Spanish, while *A. swirskii* is an exotic species that provides good control of thrips and *Bemisia tabaci* in inoculative releases of 50 to 75 mites/m^2. This latter species has its optimum at warm environmental conditions, with lower threshold temperatures over 12-15 ºC.

Other mites

In some crops *Hypoaspis miles* and *H. aculeifer* (family Laelapidae) have been used against thrips pupal stages in soil or in the substratum. They also prey upon mites and sciarid larvae. The duration of their biological cycle is 17 days at 20 ºC, feeding on *Tyrophagus* spp. and the fecundity in these conditions is approximately 69 eggs /female. Adults can survive from 3 to 4 weeks without any food. Thrips do not seem to be their preferred food but they may be useful when used with other predators that explore and occupy plants leaves and flowers. Their light brown colored dorsal plate and their lemon shaped body differentiate these species from the Phytoseiid species.

b) Anthocoridae

Two Anthocoridae species are particularly interesting in the Mediterranean regions.

Orius laevigatus

Adults have black head and thorax, with a seta in each prothorax angle. Hemelytra are black and white, with the tip of the cuneus black. They lay their eggs in vegetable tissue, leaving the operculum outside. The operculum is flat and lightly plucked, and it has serrated edges. Newborn larvae are whitish but they quite soon change their color to yellow or orange. The IV and V instar nymphs have the same color than the tree first instar nymphs, with the only difference of the darker incipient wings.

Photo 32 and 33. Adults and nymphs of *Orius laevigatus* in pepper flowers, where it carries out most of its predatory activity of thrips

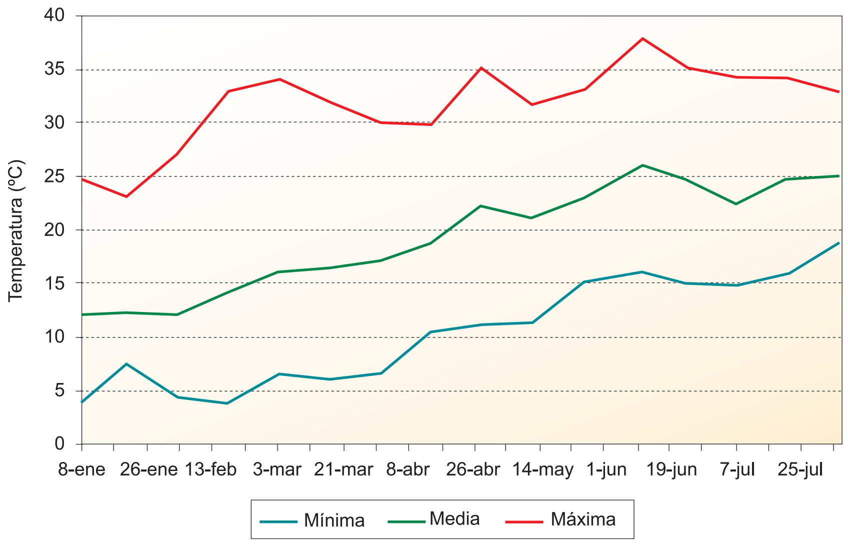
The life cycle lasts approximately: 55 days at 15 ºC, 31-37 days at 20 ºC; 19-21 days at 25 ºC; 14-16 days at 30 ºC; 11-13 days at 35 ºC. At these previously mentioned temperatures and feeding the females on *Ephestia küenhiella*, fecundity is 62, 134-166, 119-147, 74-92 and 15-22 eggs / female, respectively, with a fertility of 78, 75, 87, 85 and 31 %, being the longevity of the females of 78, 66-74, 30-35, 20-23 and 7-9 days, respectively. The lower development threshold is 11-12 ºC and the larvae mortality is very high above 30 ºC temperatures. It is estimated that predator activity reaches its highest levels of efficacy between 20-30 ºC. The Mediterranean area population does not present winter reproductive diapause. For this reason, it can be used when the daylight is short and the temperatures are low.

The capacity for searching and predation of immature stages and adults is high. Each larva needs approximately 44 *F. occidentalis* adults to complete its development. The daily consumption of an *O. laevigatus* larva is over 2.5 larvae or 2 thrips adults. This consumption rate is very similar to the adult's. The pollen is an alternative food that allows adults to survive and larvae to grow although it decreases the development rate, the female fecundity and the adult longevity and survival; therefore, it reduces the population growth rate.

It multiplies in artificial conditions, usually feeding on *E. küehniella* eggs and pollen and using green beans pods as a water source and oviposition substrate. They are generally commercialized in bottles of 500 individuals (only adults or mixtures of adults and last nymph stage, in an inert carrier medium - vermiculite or seeds covers).

The release takes place when the plants have several open flowers, there is some prey (despite their polyphagia they prefer thrips), and temperatures are adequate to maintain the predatory, reproductive and development activities. At the time of the releases, it is convenient to follow the evolution of temperatures and trying to do releases with minimum temperature above 10 °C (Figure 7).

Figure 7. Evolution of the average temperatures in pepper greenhouses in the Field of Cartagena (Murcia)



Orius albidipennis

Adults are slightly smaller (1.7 to 2 mm long) than those of the *O. laevigatus*. They have a dark head and thorax. They do not have setae in the prothorax angles and the cuneus in hemelytra is pale. Eggs have a concave, slightly reticulated operculum with slightly protruding and serrated edges. The larvae and nymphs are similar to those of *O. laevigatus*.

The life cycle and the biotic parameters are similar to those of *O. laevigatus*, although the lower thermal development threshold is higher (13-15 ºC). At 35 ºC the *O. albidipennis* survival is higher than that of *O. laevigatus* as well as the females fecundity (80-100 eggs/ female, compared to 15-23 eggs / female in *O. laevigatus*) and the fertility (49 % compared to 31 % in *O. laevigatus*). The upper reproduction threshold is estimated at 40 ºC.

Photo 34 and 35. Adult and nymph of *Orius albidipennis*

It is also similar to *O. laevigatus* in its predation capacity, searching, prey rate consumption, polyphagy, as well as in the natural host range. In Southeastern Spain agrosystems, it shares hosts and prey with *O. laevigatus* in part of the spring and summer. During summer months, *O. laevigatus* is frequently replace by *O. albidipennis* in some outdoor and greenhouse pepper crop as well as in some wild plants.

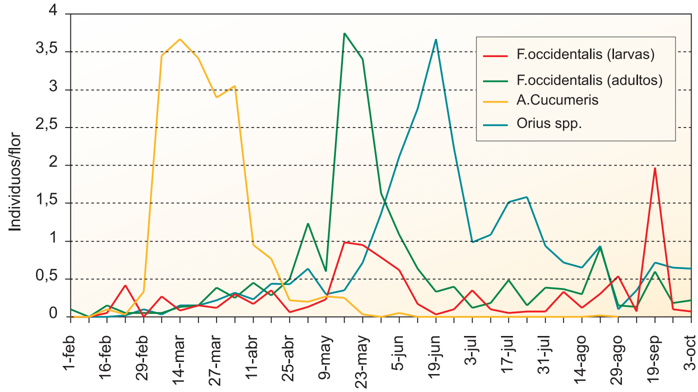
The methods of multiplication, release and management are similar to those described for *O. laevigatus*. It can be used in spring - summer and long duration crops such as pepper crops.

The *Orius* thermal and nutritious requirements are higher than those of the *Phytoseiids*, that have a small food supply in the release sachets. *Orius laevigatus* has lower thermal requirements than *O. albidipennis*, so it is released at the beginning of the growing season in winter-spring, when there is more flowering and the average minimum temperatures in greenhouses are close to the lower development threshold (11-12 ºC), that is, a bit higher than that of the thrips (10 ºC). The release is done by spreading the adults, together with the inert substratum in which they are commercialized, on leaves. The release doses go from 1.5 to 3 individuals/ m^2. The release is carried out once or twice, depending on the period, since there may be very big climatic changes that may affect this predator's survival and establishment. The first release of 1 to 1,5 individuals/m^2 is carried out when there are 2 to 3 flowers opened per plant, repeating the process 2 or 3 weeks later with 0.5 to 1.5 individuals/ m^2. It is convenient for the minimum temperatures to be above the lower development threshold. The first release takes a few weeks to establish. As previously indicated, *O. laevigatus* population establishment and growth is facilitated by the presence of *N. cucumeris*, that may serve as an alternative prey for *Orius* when the pest density are low in the crop. Sometimes, in order to facilitate the establishment of *Orius*, *Ephestia küehniella,* eggs are dispersed on the plants so that the adults may have a highly nutritious food.

The establishment period is the most critical moment, since any perturbation causes delays in population growth, which usually results in a deficient thrips control, which is troublesome when there is a risk of TSWV infections. After the establishment, *Orius* populations respond numerically to thrips densities. It is convenient that the *Orius* population is well established when environmental conditions in greenhouses are suitable for thrips outbreaks (abundant flowering, minimum temperature above 10 ºC, average temperature around 25 ºC and maximum temperatures not higher than 35 ºC).

At the beginning of this period, with optimum conditions for the pest, thrips densities may be high, but the response of *Orius* spp. is usually quick and reduces thrips populations to acceptable levels in a short time (Figure 2). From this moment on, pest densities remain stable and below the admissible density levels (Figure 3). In some greenhouses, the temperatures beginning of the summer are above the upper thermal threshold of *O. laevigatus* (Figure 8). In these cases, the deficiencies that may occur in thrips control may be avoided with the release of *O. albidipennis* at the end of the spring, since the population growth rates of this species remain high at 30-35 ºC temperatures.

Figure 8. Population dynamics F. occidentalis, A. cucumeris and Orius spp. in a pepper crop under greenhouse in the Field of Cartagena (Murcia) where the installation of Orius laevigatus was good but late (after the propitious dates on which demographic thrips explosions are produced: from the end of April)



4.2. Aubergine

Direct damage in aubergines caused by *F. occidentalis* are due to both the oviposition injuries that cause small yellow rings in the fruits, and to the effects of feeding on the underside of fruits, leaves and sepals. Besides, aubergines are susceptible to TSWV, although the damages caused are not as devastating as in other Solanaceae and the frequency of some important epidemics is fairly small.

As it happens with peppers, thrips control can be carried out by biological means, although the trichome density of some varieties makes difficult the establishment and the activity of predators. This is the case of *Orius,* which may have problems in settling in crops even when flowers are abundant, the pest is present and the climatic conditions are optimum. The biological control is based on inoculative releases of the mirid *Nesidiocoris tenuis* at the rate of 0.75 adult individuals per square metre, at the moment of the detection of first damages on the leaves. The *A swirskii* phytoseiid can be used in warm periods and the *N. cucumeris* phytoseiid in mild or cold periods whenever the ambient humidity is not low. When the first flowers open, the introduction of *N. cucumeris* is carried out by hanging a sachet containing 500-1000 individuals on the stem at the top of the plants every 3-5 plants or by hanging a sachet with 100 individuals in each plant, after trellises for guiding plants are set in place. In the *A. swicase* case, doses are much lower, with less quantity than in the case of *N. cucumeris,* so that doses of one sachet per plant are more effective.

4.3. Tomatoes

Tomatoes plants are a bad substrate for *F. occidentalis* and also for their natural enemies, whose activity and survival are reduced due to the nasty characteristics of the plants.

Natural enemies which are effective for the control of thrips in other solanaceae are not that effective at all in tomatoes. Therefore the thrips control, in relation to feeding damages (whitish spots in fruits as a result of oviposition injuries and silvering in leaves underside and colored fruits as a result of feeding) and as a vector of TSWV, it requires the combination of preventive measures and biotechnological methods, and also chemical control in cases of high populations and the risk of virus infections. Neither the phytoseiids nor the *Orius* (sometimes frequent in the low part of the plants), are able to reduce the damages causes by thrips. Generalist mirids (*Macrolophus spp.*, *Nesidiocoris tenuis*, etc.) can reduce thrips populations if they have no other preferred prey (e.g. whiteflies, etc.) although they are not efficient at avoiding virus epidemics.

Photo 36 and 37. *Nesidiocoris tenuis* in tomato leaf, and damage in tomato flowering clusters when populations are very high

In cold seasons and in soil crops, the *Hypoaspis miles* are useful to reduce thrips population. Although it is not a definitive solution, it has been found that releasing the predatory mite at approximately 200-300 individuals/m^2 on the crop lines can reduce thrips populations by 50 % in relation to the control without mite.

Chromotropic traps with pheromone emitters provide good results if used properly. Around 8 traps would be required for a 3000 m^2 greenhouses.

4.4. Cucumbers

Cucumbers are not susceptible to the Tospovirus known to Europe. In some Dutch type varieties, thrips damage in fruits has a big impact on yield producing deformation. These injuries are less important in other short or semi-long fruit varieties.

The characteristics of the cucumber production systems are suitable for the use of *N. cucumeris* and *A swirskiii.* Thrips and predator populations may be monitored using the same methods than in pepper crops. The control strategies in cucumbers are also similar to those used in pepper crops. Nevertheless, the activities of *N. cucumeris* and *O. laevigatus/O. albidipennis* seldom goes together due to the difficulty to establish the phytoseiid and the anthocorids at the same time in most of the varieties.

Neoseiulus cucumeris is released twice, at the rate of 1 sachet/m^2. The first half is released when plants have from 6 to 8 leaves and the other half when the plant has reached the trellises. Releases are repeated when populations are above 3 individuals/leaf. The release levels depend on the variety type and the number of plants per surface unit. High temperatures and low humidities reduce the survival of inmatures and, therefore, the efficacy in thrips control of the species.

Orius can be used in these cases, releasing 1 to 1.5 individuals/m^2 split in two times separated 2-3 weeks. For instance, the first release with 0.75 to 1 individuals/m^2 and the second, with 0.25 to 0.5 individuals/m^2. The scarcity of prey may be compensated adding *E. küheniella* eggs on the leaves.

N. cucumeris is sometime substituted by *A. swirskii* for its double utility in the control of both whiteflies and thrips. Releases are normally carried out when minimum temperatures are next to 12-13 ºC at a rate of approximately 65-70 individuals/m^2. Release in sachets with small quantities located in each plant seems to provide better results due to the homogeneity in the colonization of the plants.

In crops using organic substrates *H.miles* may be used against sciarid flies and thrips. The rate of application is about 300 individuals/m^2 and the predatory mites are released in the substratum shortly after the transplant.

4.5. Other Cucurbits

Melon, watermelon and courgette are sensitive to any of the Tospovirus species known to Spain. Therefore, the damages of *F. occidentalis* are due to plant feeding, which are usually located in the underside leaves or on the fruit surface (i.e. watermelon). IPM is not common in these crops. Nevertheless, the use of phytoseiids and *Orius* spp. has been assayed. Currently, the strategies are directed towards the use of *A. swirskii* because of the easy establishment at suitable environmental conditions. In Galia type melons, *O. laevigatus* releases are carried out at a rate of 1 individual/m^2. The combination of *Orius* with *A. swirskii* at a rate of 40-50 individuals/m^2 provides good results, since the *Orius* tend to colonize the flowers, while the phytoseiids is mostly located on leaves.

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Chapter 9

Aphid management

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1. Generalities about aphids

Belonging to the superfamily Aphidoidea, aphids are Hemiptera insects, many of the species causing economic damage to most cultivated plants. This occurs directly, when they suck the sap and weaken the plants, or indirectly through the transmission of several plant diseases, especially of a viral nature.

Aphids cause this plant damage using their biter-sucker mouth, as all the Hemiptera (MIYAZAKI, 1987b). The labium has been modified, taking the shape of a wide canal mouth (rostrum or proboscis) that encloses four thin stylets, which are developed from the two maxillae and the two mandibles, forming an elongated filament, which contains two ducts. The insect, after piercing the plant with its stylets, will inject the saliva through one of the ducts (the salivary canal) in order to dissolve the sap, while the other duct (the food canal) is used for absorbing the sap once it has been dissolved. This feeding system explains the Hemiptera capacity (and particularly, the aphid capacity) to transmit viruses or other type of pathogens: when they suck the fluids of a sick plant, the insects acquire the virus and later, when they inject the sap into a healthy plant, they infect it with the virus acquired.

Among the Hemiptera, the aphids form a group with very particular morphological and biological characteristics. Figure 1 shows a sketch of an adult aphid (an alate parthenogenetic female, without left wings in order to see some details), and displays the main characteristics of aphids. The presence of siphunculi or cornicles (S) is exclusive of aphids,

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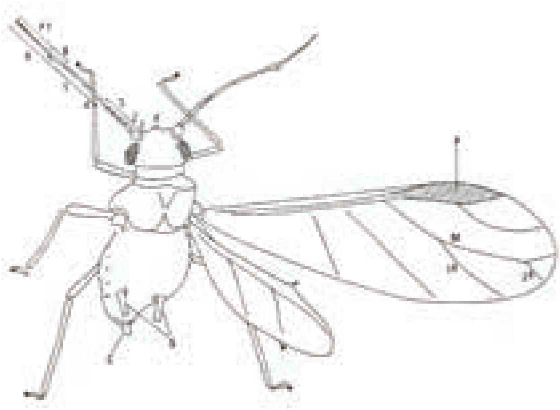

Figure 1. Alate parthenogenetic female of aphid (without left wings)

and although not all the aphid species have them the majority do; they have very different shapes and colours (although constant for each species) and with different functions, among others, the defensive one, because through them, the hemolymph, rich in aphid waxy substances, can be discharged directly outside, and when it is solidified, can glue a predator's mandibles. The cauda (C), situated at the end of the abdomen and with morphological characteristics peculiar for each species, is very typical of aphids, as is the spot or pterostigma (P) of the fore-wing in the alate aphids and the number of bifurcations (1st, 2nd) of the media vein (M) of this wing. Finally, it is useful to classify the aphids the shape of the frons (F) and the number, colour, proportions and characteristics of the antennal segments (1 to 6), especially the last one, which is divided into a base (B) and a processus terminalis (PT).

With respect to the biology of aphids, they show a curious alternation between amphigonic generations (females and males) and parthenogenetic ones (only females), with different apterous (apterae) and winged (alate) adult forms, in addition to the different nymphal instars necessary to produce adults (MIYAZAKI, 1987a). The typical complete cycle of the Aphididae family starts with the winter egg, from which a nymph hatches in spring, and after several molts it will give birth to an apterous female, the fundatrix. It reproduces by parthenogenesis (without male intervention) and by viviparity (it does not lay eggs, but gives birth to nymphs), producing several generations of apterous females (also parthenogenetic and viviparous) until a generation of alate females appears (but always parthenogenetic and viviparous), which migrate to other plants of the same species (if the cycle is monoecious) or to plants of different species (if the cycle is dioecious, then the primary host refers to the vegetal species of origin and secondary host refers to the destination species). Females continue reproducing by parthenogenesis and viviparity on the plants to which they have migrated, giving birth to several generations of apterous females (which remain on each plant) and alate (which infest other plants) during good weather. In autumn, females appear, called sexuparae because they give birth to females and males, generally alate ones, which return to the primary host, when they mate and females lay the winter eggs.

However, this complete cycle only takes place in the holocyclic aphid species, because in the anholocyclic species the amphigonic generation of the cycle is not produced, and they reproduce parthenogenetically during the whole year. Consequently, the only adults that appear are parthenogenetic females, apterae as well as alate. Furthermore, there are holocyclic species that under specific circumstances (for example, in warm climates) can reproduce continuously as anholocyclic, without amphigonic stage. In any case, although this amphigonic stage takes place, it lasts a short time in the year and with fewer individuals. Therefore, in the holocyclic species, as undoubtedly in the anholocyclic species, the parthenogenetic females are usually observed.

With respect to the differences between adults and nymphs, they are evident when the last ones are going to give birth to alate individuals, because the small wings can be distinguished perfectly. However, when the nymphs are going to give birth to apterous adults, it can be difficult to differentiate the last nymphal stages from the adults. For this purpose, characteristics such as the nymph's cauda being less developed than adults' must be considered. Furthermore, we must bear in mind that the morphological characteristics of apterous adults can be different from the alate adults, which could lead to the error of considering different forms of the same aphid species as being from a different species.

2. Aphids on protected crops

The following crops have been considered as main protected crops: within Cucurbitaceae, melon (*Cucumis melo*), watermelon (*Citrullus vulgaris*) and zucchini (*Cucurbita pepo*, v. *oblonga*); within Solanaceae, pepper (*Capsicum annuum*), tomato (*Lycopersicon esculentum*) and eggplant (*Solanum melongena*); and within Papilionaceae, bean (*Phaseolus vulgaris*). These plants are attacked by different species of aphids, the most significant of them being shown in Table 1 (BLACKMAN and EAS-

TOP, 1985, modified). As can be observed, the most widespread aphids are *Aphis gossypii* and *Myzus persicae*, which feed on all the crops reported, and then we can find *Aphis fabae* and *Macrosiphum euphorbiae*, which attack five of them, and *Aulacorthum solani* (three of them). Below, we will study each of these species in depth (HOLMAN, 1974; NIETO *et al*.,1984 y 2005; BLACKMAN y EASTOP, 1985; BELLIURE *et al*., 2009).

Aphis fabae Scopoli

This is a cosmopolite and polyphagous aphid which lives on a great number of plants, cultivated or not, and in principle it behaves as a dioecious holocyclic although it can live as an anholocyclic. The apterous parthenogenetic female (photo 1) has a mat dark colour (intense black or blackish brown, but sometimes with waxy whitish spots), with black siphunculi and cauda of the same colour and with many setae.

Photo 1. Apterous parthenogenetic female of *Aphis fabae*

Besides the crops mentioned in Table 1, it attacks many plants of agricultural interest: avocado, cherimoya, celery, turnip, cabbage, carrot, fennel, parsley, artichoke, lettuce, borage, radish, hemp, hop, chickpea, lentil, lucerne, pea, broad bean, onion, leek, cotton, fig, barley, maize, beet, spinach, strawberry, apple, cherry, plum, pear, pomegranate, orange, sour orange, lemon, mandarin, tobacco, potato and vine.

In addition to the direct damage caused in these plants, it can also cause damage due to its capacity for transmitting more than thirty different viruses, some of them with more harmful effects than physical damage.

Table 1. Most important aphids that attack the main protected crops

Aphid	Melon	Watermelon	Zucchini	Pepper	Tomato	Eggplant	Bean
Aphis fabae			X	X	X	X	X
Aphis gossypii	X	X	X	X	X	X	X
Myzus persicae	X	X	X	X	X	X	X
Aulacorthum solani				X	X	X	
Macrosiphum euphorbiae			X	X	X	X	X

Aphis gossypii Glover

This is also a polyphagous and cosmopolite species, and is particularly harmful in greenhouses. It usually reproduces in the anholocyclic manner although on some occasions has displayed holocyclic behaviour. The colour of apterous parthenogenetic females is very diverse, from almost white to almost black, with a wide range of intermediate colours (pale yellow, brown, light or dark green) (Photo 2), which can make their identification difficult, although the siphunculi are always dark and the cauda has the colour of the body (cauda clear in pale specimens and black in the darker ones), and with a reduced number of setae (seven at the most) in the cauda.

This aphid has been reported on many crops in addition to the crops studied here: avocado, cherimoya, asparagus, carrot, endive, hemp, hop, cucumber, pumpkin, cotton, maize, pomegranate, beet, loquat, strawberry, apple, plum, almond, pear, orange, sour orange , mandarin, lemon, grapefruit , satsuma, clementine, tobacco, potato and vine.

Photo 2. Apterous parthenogenetic females of *Aphis gossypii*

This species transmits more than fifty plant viruses, not only of horticultural crops but also of woody plants: the big epidemic of the citrus tristeza virus which eliminated a large extent of the Spanish citrus plantations from 1957 was basically spread by this aphid. In addition, this species developed resistance to some insecticides around 1985 (MELIÀ and BLASCO, 1990), which made its control difficult and reactivated its presence on the crops, causing, among other things, new epidemics of viral diseases.

Myzus persicae (Sulzer)

As with previously mentioned species, this aphid is also cosmopolite and polyphagous, and it behaves generally in a dioecious holocyclic manner. Several plants of the *Prunus* genus (mainly peach tree) serve as primary hosts, where it spends the winter in egg stage, and many other plants, some of them cultivated, serve as secondary hosts. However, if there are not peach trees or in warm climates, it can reproduce parthenogenetically and continuously as anholocyclic.

The apterous parthenogenetic females are usually yellow or green (Photo 3), although they can also be pink or reddish. They have a very deep groove in the frons, with converging sides, and their siphunculi are a little more elongated than those of the previously mentioned *Aphis* species. They can be differentiated too from those *Aphis* species because the siphunculi of *M. persicae* have a light colour and usually are swollen towards the end, while the cauda also has a clear colour.

Photo 3. Apterous parthenogenetic female of *Myzus persicae*

In addition to the cultivated plants that have been considered in Table 1, it also attacks the following: turnip, cabbage, pawpaw, artichoke, carrot, sweet potato, lettuce, radish, potato, onion, celery, beet, orange, sour orange, mandarin, lemon, satsuma, pumpkin, loquat, strawberry, apple, tobacco, pea, apricot, cherry, plum, almond, peach, pear, wheat, broad bean and maize.

This species is considered as being the most significant species amongst the virus vectors of plants, because it transmits more than 100 viral diseases that affect a high number of crops. Furthermore, as in the case of *A. gossypii*, it is resistant to several insecticides.

Aulacorthum solani (Kaltenbach)

This is a species of European origin, although currently its spread is almost cosmopolite, and it is quite polyphagous (Photo 4). It can reproduce in both a holocyclic and anholocyclic manner. The apterous parthenogenic female can show a yellowish, greenish or brownish colour, and as occurs with *M. persicae*, it has a groove in the frons, but with sides parallel, nonconverging; this female is a little bigger than the preceding species, and its siphunculi and cauda are longer and have a light colour.

Photo 4. Colony of *Aulacorthum solani*

In addition to the crops mentioned at the beginning of this section, *A. solani* can appear on other crops such as lettuce, potato, cucumber and strawberry, and can transmit around 40 viral diseases.

Macrosiphum euphorbiae (Thomas)

Although it is a native aphid of North America, it is spread almost worldwide, and it is very polyphagous. In the United States it behaves as a dioecious holocyclic, with plants of the genus *Rosa* as primary hosts, while in Europe it is mostly anholocyclic (although, occasionally, sexed individuals are observed).

The apterous parthenogenetic females are very big, the biggest ones of all the species mentioned here. In general, they are green, but sometimes are yellowish or pink (Photo 5), with the groove of the frons with diverging sides, and the cauda and the siphunculi also being longer than the other species and of the same light colour as the body. Furthermore, this species can be distinguished from the others because the siphunculi of *M. euphorbiae* adults show reticulated distal parts.

In addition to the crops that have been mentioned in Table 1, this species attacks the following ones: chickpea, cherimoya, beet, cabbage, orange, sour orange, mandarin, lemon, satsuma, pumpkin, artichoke, lettuce, lentil, apple, loquat, tobacco, pea, pear, radish, potato and maize.

Photo 5. Apterous parthenogenetic female and nymphs of *Macrosiphum euphorbiae*

Also it is significant as a vector of viral diseases, because it is responsible for the transmission of more than 40 diseases of this type.

3. Biological control of aphids on protected crops

All these aphid species are controlled, either in a spontaneous way or artificially, by a wide range of natural enemies: parasitoids, predators or even pathogenic agents. Belliure *et al*. (2009) have reported the relationships between enemies and aphids found in Spain, considering a series of bibliographical references, among which the following ones are highlighted: Baixeras and Michelena (1983); González and Michelena (1987); Michelena and González (1987); Michelena and Oltra (1987); Rossmann and Fortmann (1989); Llorens (1990); Bennison (1992); Ben Halima-Kamel and Ben Hamouda (1993); Laubscher and Von Wechmar (1993); Kazda (1994); Marcos-García And Rojo (1994); Michelena *et al*. (1994 and 2004); Alomar *et al*. (1997); Alvarado *et al*. (1997); Castañé *et al*. (1997); Ehler *et al*. (1997); Michelena and Sanchis (1997); Orlandini and Martellucci (1997); Asin and Pons (1998); Völkl and Stechmann (1998); Winiarska (1998); Askary *et al*. (1999); Wojciechowicz-Zytko (1999); El-Arnaouty *et al*. (2000); Hunter *et al*. (2001); Alvis *et al*. (2002); Belliure (2002); García-Marí and Ferragut (2002); Soler *et al*. (2003); Bird *et al*. (2004); Kavallieratos *et al*. (2004); Snyder *et al*. (2004); Deligeorgidis *et al*. (2005); Jansen (2005); Nebreda *et al*. (2005); Van Munster *et al*. (2005);

Pascual-Villalobos *et al*. (2006); Kim *et al*. (2007); Sastre-Vega (2007); ZARPAS *et al*. (2007); Hermoso De Mendoza *et al*. (2008a and 2008b); Pineda and Marcos-García (2008b); Roditakis *et al*. (2008).

3.1. Parasitoids

Table 2 shows all the parasitoids described in Spain on each of the aphid species mentioned before. All of them are Hymenoptera and, although there is a species of the Aphelinidae family, most of them belong to the Aphidiinae subfamily of the Braconidae family. Females lay an egg inside each aphid, from which a larva emerges and feeds on the internal tissues of the aphid, until this one has only the external cover, referred to as the mummy. Finally, the parasitoid carries out pupation in a cocoon that can be internal to the aphid mummy (as it occurs in the genera *Aphidius*, *Diaeretiella*, *Lysiphlebus* or *Trioxys*), or external (as in the genus *Praon*, Photo 6). The colour of these mummies can characterize each genus: in *Ephedrus* they are black (Photo 7), while in *Aphidius*, *Diaeretiella* or *Trioxys* they are brown. Finally, the parasitoid adult emerges from the aphid mummy (Photo 8), after having pierced the cuticle with its mandibles, forming a circle in it.

Photo 6. Mummy of aphid parasitized by *Praon volucre*

Photo 7. Aphid parasitized by *Ephedrus* sp.

Photo 8. Adult of *Trioxys angelicae*

Table 2. Parasitoids (Hymenoptera) mentioned in Spain on the main aphids of protected crops

Parasitoids	*Aphis fabae*	*Aphis gossypii*	*Myzus persicae*	*Aulacorthum solani*	*Macrosiphum euphorbiae*
Aphelinidae					
Aphelinus abdominalis			X		X
Braconidae Aphidiinae					
Aphidius colemani		X	X		
Aphidius ervi			X	X	X
Aphidius matricariae	X	X	X	X	X
Diaeretiella rapae		X	X		X
Ephedrus persicae			X		
Lysiphlebus confusus	X	X			
Lysiphlebus fabarum	X	X			
Lysiphlebus testaceipes	X	X			
Praon volucre	X	X	X		X
Trioxys acalephae	X	X			
Trioxys angelicae	X	X	X		

Among these parasitoids, *Lysiphlebus testaceipes* (Cresson) stands out (Photo 9); probably, it is native of North and Central America and was introduced in Europe during the sixties and in Spain during the seventies, its acclimatization being confirmed during the eighties. Since then, it has been widely spread in the Mediterranean basin, so that nowadays it is one of the most abundant parasitoids on several species of aphids.

Photo 9. Adult of *Lysiphlebus testaceipes* parasitizing aphids

Several of the parasitoids mentioned are commercialised for the control of aphids in greenhouses. As such, *Aphelinus abdominalis* (Dalman), *Aphidius colemani* Viereck and *Aphidius ervi* Haliday are used against aphids such as *Myzus persicae* or *Aphis gossypii*, spread in crops through the use of reservoir plants, usually wheat or barley infested by the aphids *Rhopalosiphum padi* (L.) or *Sitobion avenae* (Fabricius) which are parasitized by the hymenopteran in question so that, when hatching, it will attack the aphids found on the greenhouse crops.

3.2. Predators

In tables 3, 4 and a part of table 5 there appear the main predators reported in Spain on the most significant aphids of protected crops. Table 3 shows the Diptera, table 4 shows the other insects and table 5 shows the mites.

Table 3. Predators mentioned in Spain on the main aphid of protected crops. I: Diptera

Predatory Diptera	*Aphis fabae*	*Aphis gossypii*	*Myzus persicae*	*Aulacorthum solani*	*Macrosiphum euphorbiae*
Cecidomyiidae					
Aphidoletes aphidimyza	X	X	X		X
Syrphidae					
Episyrphus balteatus	X	X	X	X	X
Epistrophe eligans	X				
Eupeodes corollae	X	X	X	X	X
Eupeodes flaviceps	X		X		
Eupeodes lucasi	X				
Eupeodes luniger	X				
Meliscaeva auricollis	X				
Paragus haemorrhous	X	X	X		X
Paragus tibialis	X				
Platycheirus scutatus	X				
Scaeva albomaculata	X				
Scaeva pyrastri	X		X		X
Sphaerophoria rueppellii	X	X	X	X	X
Sphaerophoria scripta	X		X		X
Syrphus ribesii	X		X		X
Syrphus vitripennis			X		X

Table 4. Predators mentioned in Spain on the main aphids of protected crops. II: Other insects

Predators	*Aphis fabae*	*Aphis gossypii*	*Myzus persicae*	*Aulacorthum solani*	*Macrosiphum euphorbiae*
Coleoptera Coccinellidae					
Adalia bipunctata			X		
Coccinella septempunctata	X	X	X		X
Harmonia axyridis		X			X
Hippodamia convergens	X	X			
Propylea quatuordecimpunctata			X		
Scymnus spp.		X			
Neuroptera Chrysopidae					
Chrysoperla carnea	X	X	X	X	
Hemiptera Anthocoridae					
Orius laevigatus		X			X
Orius majusculus		X			X
Hemiptera Miridae					
Dicyphus tamaninii		X			X
Macrolophus caliginosus		X			X
Dermaptera					
Forficula auricularia				X	

Table 5. Mites and pathogenic agents mentioned in Spain on the main aphids of protected crops

Predators	*Aphis fabae*	*Aphis gossypii*	*Myzus persicae*	*Aulacorthum solani*	*Macrosiphum euphorbiae*
Mites					
Erythraeidae	X				
Fungi					
Lecanicillium attenuatum			X		X
Lecanicillium longisporum			X		X
Lecanicillium lecanii	X	X	X		X
Viruses					
Parvovirus			X		

Photo 10. Adult of *Aphidoletes aphidimyza*

Among the Diptera, we can find two families, the Cecidomyiidae and the Syrphidae. Among the Cecidomyiidae we can find *Aphidoletes aphidimyza* Rondani (Photo 10), whose larvae feed on aphids by sucking their internal fluids and are abundant naturally in some crops. Their use is also popular commercially in pepper and tomato greenhouses.

Photo 11. Adult male of *Paragus tibialis*

Amongst the Syrphidae there is a long list of predators of aphids. These dipterans show three larval instars, which feed on aphids by piercing the aphid body surface with their mandibles and lifting them from the plant surface while sucking their fluids out. Adults (Photo 11 and 12) feed on nectar and pollen (Photo 13); females lay the eggs close to the incipient colonies of aphids and, after eggs hatch, larvae begin to feed on aphids until they pupate. Due to the need for nectar and pollen of adult syrphids, the access to flowering plants in the crops or near to them is an important factor in the effectiveness of these dipterans as biological control agents of aphids: the flowers of some specific plants increase the attraction of syrphids

Photo 12. Adult male of *Scaeva albomaculata*

Photo 13. Adult of *Episyrphus balteatus*


Photo 14. Adult of *Adalia bipunctata*


Photo 15. Adult of *Coccinella septempunctata*

in different types of crops, outdoors as well as in greenhouses. On the other hand, the condition of semi-opening, typical of the greenhouses used in the Mediterranean area, is very favourable for the entry of syrphids that are present naturally outside the greenhouses (Pineda and Marcos-García, 2008). With respect to commercialised species, *Episyrphus balteatus* De Geer is available as pupae to be released against aphids.

With respect to Coleoptera, the Coccinellidae (or ladybirds) are the best known predators of aphids, and furthermore, in their two stages: adults as well as larvae of many coccinellid species eat aphids. As in the case of other predators, they lay the eggs next to aphid colonies which are not yet very advanced. Among the most abundant species, found naturally, we can find *Adalia bipunctata* (L.) (Photo 14), *Coccinella septempunctata* L. (Photo 15), *Hippodamia convergens* Guérin-Méneville (Photo 16), *Propylea quatuordecimpunctata* (L.) (Photo 17) and *Scymnus* spp. (Photo 18). Among the species commercialised against aphids we can find *A. bipunctata* and


Photo 16. Adult of *Hippodamia convergens*


Photo 17. Adult of *Propylea quatuordecimpunctata*

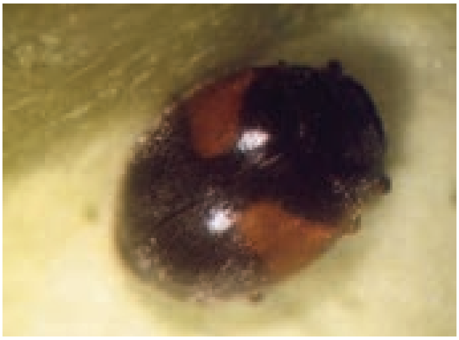

Photo 18. Adult of *Scymnus* sp.

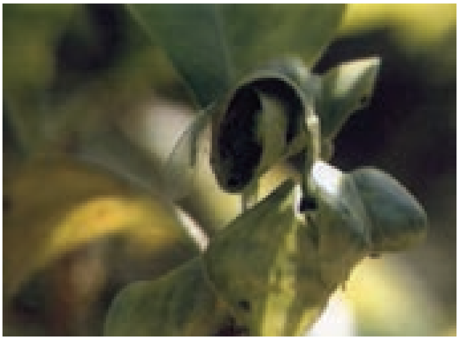

Photo 19. Adult of *Chrysoperla carnea* next to a colony of aphids

Harmonia axyridis Pallas, this last one having been introduced.

Amongst the Neuroptera Chrysopidae, *Chrysoperla carnea* (Stephens) (Photo 19) stands out, whose larvae feed actively on aphids and other arthropods. Its egg laying is very characteristic, with each egg appearing on the tip of a long filament and, generally, grouped close to the aphid colonies. This species is also commercialised for the biological control in greenhouses.

Among the Hemiptera, we can find the Anthocoridae, mainly of the *Orius* genus (Photo 20), which are very polyphagous because they feed not only on aphids, but also on thrips, lepidopteran eggs and larvae, mites and others. Besides, as they can also feed on plants or pollen, they are easy to maintain in crops with low plant density. *Orius laevigatus* (Fieber) and *O. majusculus* (Reuter) are commercialised to be released in greenhouses.

Other hemipteran family with species which prey on aphids is Miridae, among which the genera *Dicyphus* and *Macrolophus* stand out (Photo 21),

Photo 20. Adult of *Orius* sp.

Photo 21. Nymph of *Macrolophus* sp.


Photo 22. Trombidiid mite attacking an aphid

also used commercially against other horticultural pests outdoors and in greenhouses.

Among the Dermaptera, *Forficula auricularia* L. feeds on aphids, amongst other types of prey, and it controls them mainly on the soil.

Finally, within the mites there are several families of the Prostigmata order, such as the Erythraeidae and the Trombidiidae (Photo 22), which attack aphids in a different manner depending on their stage of development: they act as parasites of the aphids in the nymphal stages of the mites, and act as free-living predators in the adult stages.

3.3. Pathogenic agents

There are different species of entomopathogenic fungi of the Entomophthoraceae family which fight against aphids (Photo 23), belonging to the genus *Lecanicillium*, as Table 5 shows.

Some of these fungi, such as *L. longisporum*, are carried by ants from one aphid to the other, transmitting the infection in this manner. There are others, for example *L. lecanii*, which are commercialised to be used in crops, taking into account that the different isolates of this fungus can show different specificity according to the aphid species involved (for example, if it is *Aphis fabae* or *Aphis gossypii*).


Photo 23. Aphid attacked by fungi

With respect to the entomopathogenic viruses, some of these aphids are attacked by Parvovirus (Table 5), with the special feature that the plants invaded by aphids infected by the virus, can transport it through the phloem, infecting other aphids without needing to be in contact with the sick aphids. That is to say, the plant can act as entomopathogenic virus vector using it to defend itself.

4. Integrated control of aphids on protected crops: economic injury level

The integrated control of pests, considering the habitat and the population dynamics of the phytophagous, uses different methods of fight (particularly the biological one), avoiding the excessive use of chemicals to combat it. Therefore, the key point of this system is to find out the economic injury level (EIL), that is to say, the level of the pest over which treatments have to be applied if we do not want to have economic losses, (or, in other words, the amount of pest in which the losses caused are equal to the treatment expenses).

In the case of the crops and the aphids considered here, the formula of the economic injury level for peppers has been obtained in two aphid species: *Aulacorthum solani* (HERMOSO DE MENDOZA *et al*., 2006) and *Myzus persicae* (LA SPINA *et a*l., 2008). These formulae are:

EIL (*A. solani*):

$$\frac{88{,}98 \cdot VP_o K + 3750{,}36 \cdot C}{58{,}16 \cdot VP_o K - 100 \cdot C}$$

EIL (*M. persicae*):

$$\frac{1{,}05 \cdot VP_o K + 359{,}79 \cdot C}{21{,}05 \cdot VP_o K - 100 \cdot C}$$

In both formulae the economic injury level (EIL) is expressed in number of aphids per leaf, and the parameters that intervene in them are:

V: price of the fruit (euros/kg).

P_o: crop yield with the minimum level of pest (kg/ha).

K: efficacy of the insecticide, lying between 0 and 1 (if it is 100 %, K=1).

C: total cost of the insecticide (product + application) (euros/ha).

Under the current economic conditions of the pepper crop in greenhouse, its profitability (as a consequence of the high price and yield of pepper) is so high compared with the treatment cost, that the economic injury level resulting is very low for these two aphid species, so that treatment would have to be applied when the aphid was detected. However, if the profitability circumstances of the pepper crop change, also the economic injury level of both species would change, although it would be calculated in the same manner because the formulae mentioned are valid for each type of economic conditions; it would be enough to apply the parameters (V, K, C and P_o) corresponding to each situation.

On the other hand, these formulae for the calculation of the economic injury level are applicable to all types of insecticides, not only chemical but also biological, that is to say, they are also valid when aphids are controlled by parasitoids or predators. In this last case, the K value should be previously found out (in other words, the efficacy of the natural enemy in question against the aphid involved), as well as its cost (C).

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Chapter 10

Leafminers management

María Dolores Alcázar Alba[1]

1. Introduction

The leafminers belong to the Diptera order, Agromyzidae family, of which 300 species belong to the genus *Liriomyza* and, amongst them, 23 species are economically significant as pests in agricultural and ornamental crops in the warm regions (Parrella, 1987). Among these species, 5 of them in particular display a great economic importance because they are very polyphagous species (Spencer, 1973; Parrella, 1987; Cabello *et al*., 1994).

In the case of horticultural protected crops in Almería, 4 of these species have been found; *Liriomyza bryoniae* (Kaltenbach); *Liriomyza trifolii* (Burguess); *Liriomyza huidobrensis* (Blanchard) and *Liriomyza strigata* (Meigen).

Initially in the horticultural crops of Almería, the autochthonous species present were *Liriomyza bryoniae* and *Liriomyza strigata*, which did not cause damage of economic significance, however with the introduction in 1982 of the American serpentine leafminer *Liriomyza trifolii* and, later in 1991, the introduction of *Liriomyza huidobresis* (South American leafminer) (Cabello and Belda 1992) caused the movement of the two autochthonous species and the appearance of serious damage in the crops as well as problems to be controlled (Cabello *et al.,* 1990). The use of non selective chemical insecticides is considered as one of the most important causes with respect to the problems in the control of leafminers and the increase of damage, the appearance of resistances and the decrease of the auxiliary fauna, because parasitoids are very sensitive to these insecticides.

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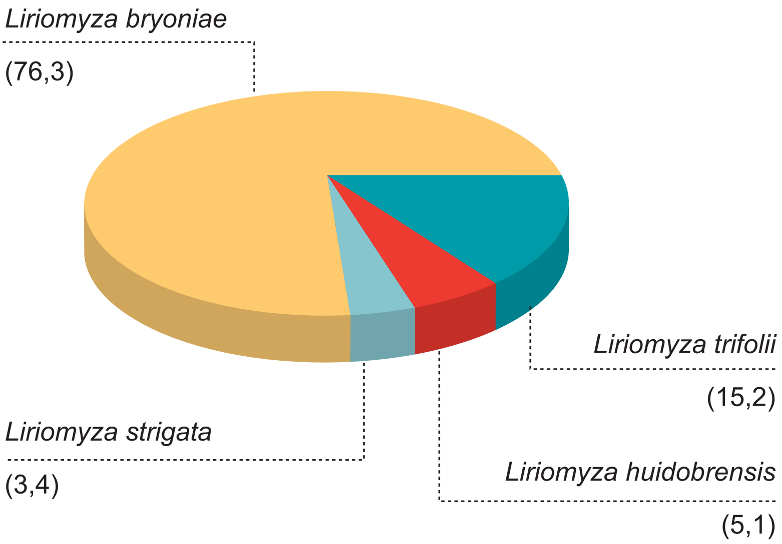
Between 1988 and 1991 the predominant species in the crops under greenhouse in Almería was *Liriomyza trifolii*, replacing the autochthonous species and causing a decrease of the incidence of *Liriomyza bryoniae* centred in green bean, tomato, melon and autumn watermelon crops with respect to a higher incidence of *Liriomyza trifolii*, while in the case of *Liriomyza strigata,* its importance with respect to *Liriomyza trifolii*, was slightly higher, particularly in melon and spring watermelon crops (Cabello *et al*., 1994). This situation changed in 1992 when the *Liriomyza huidobrensis* species appeared having a significant effect on green bean, tomato, pepper and melon crops, especially in the autumn-winter months (Cabello and Belda, 1992; Pascual *et al*., 1992; Cabello *et al*., 1993).

Now the present species with incidence in the horticultural crops in greenhouses are *Liriomyza bryoniae* and *Liriomyza trifolii*, the former appearing as the predominant species, in all the horticultural crops, while *Liriomyza trifolii* appears mainly in tomato and green bean crops (Belda *et al*., 1999; Alcázar *et al*., 2002; Belda 2002; Téllez, 2003; Téllez *et al*., 2004).

Figure 1. Percentage of the species during the years 1999-2001



Source: Belda, 2002.

2. Description of the *liriomyza* species. Distribution and host plants

2.1. *Liriomyza trifolii* (Burgués, 1880)

Synonyms: *Liriomyza allivora*, *Liriomyza pusilla* and *Oscinis trifolii*. It is commonly known as leafminer, American leafminer, or chrysanthemum leaf miner (Sánchez, 1986; Sánchez, 1994).

It is a species of nearctic and neotropical origin. It was first discovered in the state of Florida where it was spread through the distribution of chrysanthemum cuttings. It subsequently spread to several tropical and subtropical regions of America and Africa and came to Europe in 1975, where it spread to some European countries due to the export of chrysanthemum and gerbera cuttings which were infested (Spencer, 1973; Mikenberg, 1988; Sánchez 1994). In Spain it was detected for the first time in the Canary Islands in 1975 and in the Peninsula in 1982 (Sánchez, 1986).

Liriomyza trifolii is a polyphagous species which affects many host species, although its name comes from the relationship it has with the Leguminosae family, and it causes the most significant attacks on the Composite leguminous plants.

With respect to horticultural species most sensitive to attack from this species of pest we find: celery, aubergine, green bean, melon and watermelon. Other horticultural species attacked are: chard, artichokes, potato, courgette, marrow, onion, cabbages, spinach, pea, broad bean, lettuce, cucumber, pepper, leek, beetroot and tomato, and among the ornamental crops affected, we can find, marigold, cornflower, cineraria, chrysanthemums, dahlia, gerbera, gypsophila, zinnia etc., (Spencer, 1973; Sánchez, 1994).

In greenhouses in Almería this species has affected the main horticultural crops: green bean, tomato, cucumber, pepper, aubergine, melon and watermelon; as well as ornamental plants such as gerbera (Cabello *et al*., 1994; Belda *et al*., 1999).

2.2. *Liriomyza bryoniae* (Kaltenbach)

Synonyms: *Agromyza bryoniae*, *Liriomyza citrulli*, *Liriomyza hydrocotylae*, *Liriomyza mercurialis* and *Agromyza solana*. It is commonly known as the tomato leafminer.

It is a species of palearctic origin, which affects several crops of many areas of Europe, as well as the north of Africa (Morocco, Egypt, etc..). In the south of Europe it is a very active species, which affects crops outdoors and also in greenhouses, while in the rest of the continent it appears only in crops under greenhouse.

The main crops affected by this species are: tomato, watermelon, melon, cucumber and lettuce (Spencer, 1973).

In greenhouse crops in the south of Spain it has been reported in green bean, tomato, melon and watermelon crops (Cabello *et al*., 1994; Belda *et al*., 1999, Alcázar *et al*.; 2000 and Tellez, 2003).

2.3. *Liriomyza strigata* (Meigen)

Synonyms: *Agromyza strigata, Agromyza pumila and Agromyza violae*.

It is a species of palearctic origin, very common in Western Europe, although its presence has not been much reported widely in Eastern Europe (Spencer, 1973).

This is a very polyphagous species, it has been found in beetroot, lettuce and pea crops (Spencer, 1973). This same author reports the presence of this species in 187 genera of 31 plant families, among them, the Cucurbitaceae, Leguminosae, Solanaceae, Labiatae, Chenopodiaceae, Euphorbiaceae, Malvaceae, etc..,.

In Spain it has been reported in melon and watermelon crops in greenhouses (Cabello *et al*., 1994).

2.4. Liriomyza huidobrensis (Blanchard, 1926)

Synonyms: *Agromyza huidobrensis*, *Liriomyza langei*, *Liriomyza decora*, *Liriomyza cucmifoliae* and *Liriomyza dianthi*. It is commonly known as the South American leafminer.

It is a species of Nearctic and Neotropical distribution. The origin of its distribution was reported in Argentina, Brazil, Chile, Peru, Colombia, Venezuela and the United States. In Europe, it was detected for the first time in Great Britain in pea plants coming from the United States, as well as in chrysanthemums from Peru and Colombia. It has also been reported in other European countries such as France, Netherlands, Denmark, Italy and Portugal. In Spain, it was detected for the first time in 1992 (Cabello and Belda, 1992).

3. Morphology of the *liriomyza* species

The Agromyzidae, and therefore, the species of the *Liriomyza* genus show an holometabolous postembryonic development with four development stages: adult, egg, larva and pupa.

3.1. Adult

It is a fly of small size between 1.4-2.3 mm long, with different black and yellow shades. Females of all the species are slightly larger than males.

Photo 1: Adult of ***Liriomyza trifolii***

3.2. Egg

The eggs are deposited by females in “laying mines” through their ovipositor. Eggs are kidney-shaped, with an average length and width of 0.25 mm and 0.1mm respectively (Sánchez, 1994). They are opaque white with a smooth and bright surface and when the embryo develops it turns translucent white.

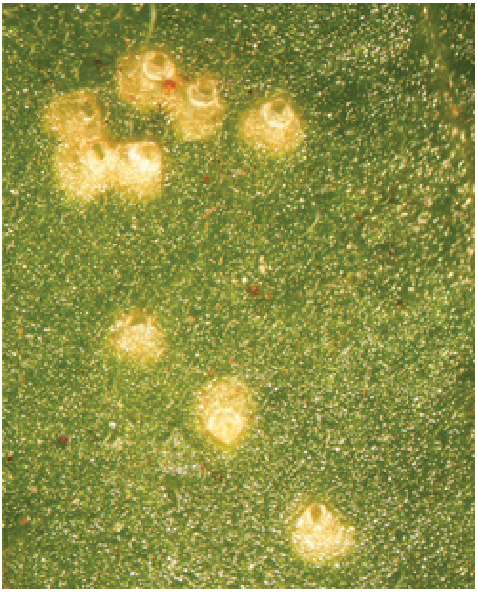


Photo 2. Feeding punctures of ***Liriomyza*** sp.

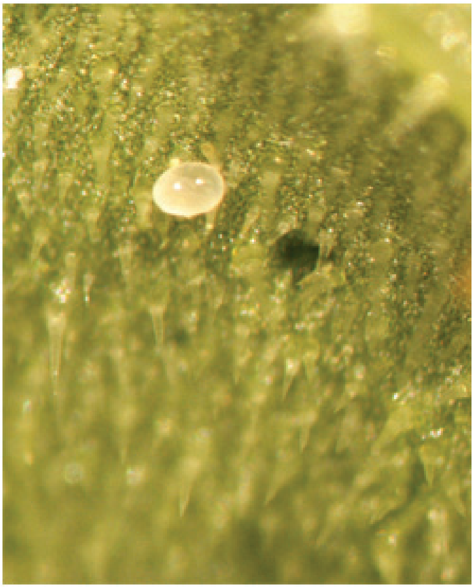


Photo 3. Egg of ***Liriomyza*** sp.

3.3. Larva

The larval stage shows three instars. Larvae are cylindrical, elongated and without apparent segmentation, apodous and acephalous. The newborn larvae are 0.5 mm long and they can reach 3.25 mm at the end of the third stage; with respect to diameter it fluctuates between 0.3-0.6 mm. They show a pair of sawn hook-shaped projections as mouth parts. With respect to the colour, it is green in most of the species, while *Liriomyza trifolii* is yellow.

Agromyzidae show a prepupa stage which develops out of the mine, on the soil or on the surface of the leaf (Sánchez, 1994).

Photo 4. Mines of ***Liriomyza*** sp.

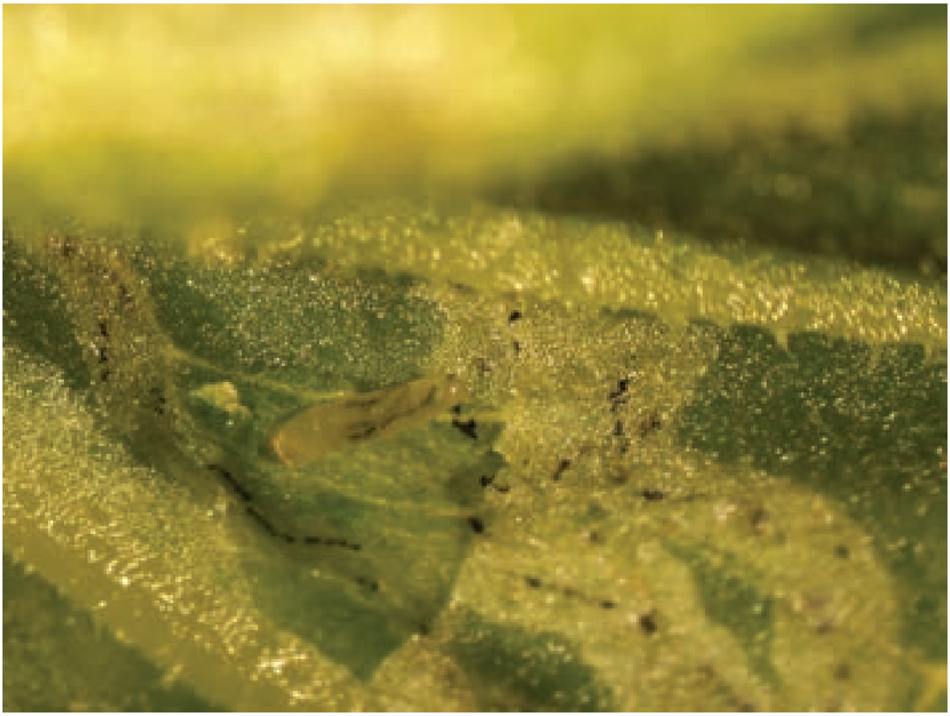


Photo 5. Larva of ***Liriomyza*** sp.

3.4. Pupa

It resembles a “small barrel” shape, and it is between 1.5 and 2.3 mm long and with a diameter of 0.5-0.8 mm. Initially it shows a yellowish colour turning brown when it matures. It shows strongly chitinized walls.

Photo 6. Pupa of ***Liriomyza bryoniae***

4. Damage and economic importance

There are two types of damage caused by the leafminers. On the one hand the damage caused by female with its ovipositor when feeding and laying eggs, and on the other, damage caused by larvae while feeding themselves, known as mines. Mines show different shapes, depending on the species, host plant and number of larvae per leaf, although they usually show a characteristic shape, so that in the case of *Liriomyza trifolii* and *Liriomyza bryoniae* they are elongated and tortuous respecting the nerves, however, *Liriomyza strigata* usually makes the mines follow the main nerves of the leaves with short lateral prolongations. *Liriomyza huidobrensis* makes mines in the central and secondary nerves, as well as in the base and on the underside of the leaf, causing important damage (Cabello and Belda, 1992).

The seriousness of the damage caused by Agromyzidae depends mainly on the size of population and on the species itself, because this influences on the feeding manner of larvae, on the development stage of the plant and the part of the plant that Agromyzidae attack. Also, if the attack is very concentrated after transplanting or sowing the crop it can become a serious problem. In general, damage causes a decrease of the photosynthetic capacity of plants, which can cause drying, necrosis and even the early fall of leaves. Furthermore, agromyzids can cause secondary damage by fungal infections (Broadbent and Matteoni, 1990), and even they can be vector viruses such as the celery, tobacco, soya and watermelon mosaic (Mikenberg and van Lenteren 1986. Sánchez,1994).

Photo 7. Mines in leaves of tomato and aubergine

5. Biology and ecology

The leafminers of the genus *Liriomyza* are polyvoltine insects, this means that we can see specimens in all the development stages at any phase of the crop. However, the location of these stages in the plant varies, the third instar larvae and pupae are usually on the old leaves, while the adults, the punctures and lays and the first instars larvae are usually located on young leaves.

Liriomyza females lay the eggs on the upper side of leaves, although sometimes they also lay them on the underside. This is a characteristic lay because females make a small hole with their ovipositor to deposit the egg on the parenchyma of leaves. The number of eggs that a female can deposit depends on the species; in the case of *L. bryoniae* it can deposit up to 163 eggs with a daily average of 7 eggs. *L. trifolii* can deposit up to 389 eggs, with a daily average of 19 eggs, while for *L. huidobrensis*, the maximum number of eggs that a female can deposit is 131 (Parrella, 1983). This fertility is related with temperature and feeding, the lay being higher if temperature increases. With respect to feeding, females can even duplicate the number of eggs deposited if there is a source of carbohydrates, nectar or honeydew (Peña, 1986; Zoebisch and Schuster, 1987). Other abiotic factors that influence on fertility are brightness and relative humidity, in the case of brightness, when it decreases females are less active and lay a lower number of eggs. With respect to relative humidity, in accordance with Malais and Ravensberg (2006), if it is between 80-90 %, lay is stimulated.

A larva hatches from the egg in approximately 4-8 days, beginning to feed itself by making a mine which increases its size as larva goes through its 3 development stages. The larval stage lasts between 7-13 days. Larvae just before pupating make a semicircular opening in the epidermis, emerging from leaves to pupate. Pupation can be carried out on the soil or on the leaf surface. The duration of the pupa stage changes in accordance with the season, in spring and summer, adults take approximately 3 weeks to emerge, while in winter, emergence can last between 5-9 weeks.

In the tables attached we can see different parameters of the biology of *Liriomyza* species with respect to temperature.

Table 1. Length of the development stages of three *Liriomyza* species at different temperatures and their threshold temperatures

State	Temperature	*L. huidobrensis*	*L. trifolii*	*L. bryoniae*
EGG	15-25 °C	–	4.0-7.7	3.0-6.1
	25-27 °C	2.1-3.0	–	–
LARVA	20-25 °C	5.8-6.7	–	–
	15-25 °C	–	4.3-9.1	5.0-12.3
PUPA	15-25 °C	–	10.0-26.6	9.2-22.2
	20-27 °C	9-12.6	–	–
Thresholds				
Minimum	–	7.3 °C	10.0 °C	15.0 °C
Optimum	–	20.0 °C	30.0 °C	25.0 °C
Maximum	–	30.0 °C	37.0 °C	35.0 °C
Longevity	19 °C	11.42 days	–	–
	15-38 °C	–	3.1-16.7 days	–
	15-25 °C	–	–	6.6-13.6 days

Source: (Barranco, 2003).

6. Control methods

6.1. Preventive and cultural methods

Preventive measures do not lead to the elimination of pests, but they can be a mechanism to delay the presence of pests and decrease their populations.

In line with these measures, or physical methods, it is advisable to place meshes, with a minimum of 10x20 thread/cm^{2,} in the side and zenithal openings as well as in the doors, it is also advisable to place this mesh in the double door and to carry out maintenance of its state, especially on those parts exposed to dominant winds.

The placing of yellow sticky traps from the beginning of the crop is useful as a monitoring method to detect the first infections and also to capture a significant number of adults.

Healthy vegetal material, which comes from authorised nurseries or seedbeds, must be used.

It is advisable to remove and destroy the leaves of the low part of the plant in very severe attacks.

The weeds must be removed from the greenhouse and also from the surrounding areas of the plot, as well as the remainder of previous harvests which can become reservoirs. It is also advisable to sweep the soil between crops to remove pupae.

6.2. Biological methods

6.2.1 Predators

The leafminers have as natural enemies some predatory insects that exert a discreet control. Amongst them we find ant species and a mirid, *Cyrtopeltis modestus*, which preys on larvae (Parrella *et al*., 1982). Also dipteran species are reported such as *Drapetis subaenescens* (Collin), *Tachydromia annulata* Fallen and *Coenosia atenuata* (Zetterstedt) (Freidberg Gijswijt, 1983). This last species has been detected abundantly in protected crops under greenhouse in Almería, playing a control role on *Liriomyza* sp. specimens (Rodríguez-Rodriguez *et al*., 2002).

6.2.2. Parasitoids

The parasitoids are the natural enemies that exert a better control over the different species of leafminers present in horticultural crops, due to their effectiveness as well as their abundance. All these parasitoids belong to the Hymenoptera order. In accordance with Peña (1986) up to 33 parasitoid species that act on agromyzid species belonging to the genus *Liriomyza* have been reported worldwide. Téllez (2003) and Barranco (2003), have carried out each of the revisions of all the parasitoid species detected by different authors regarding the different *Liriomyza* species, and up to now 71 species of hymenopteran parasitoids have been catalogued, belonging to 6 different families.

In protected horticultural crops in Almería, a total of 13 autochthonous parasitoid species have been detected and are shown in table 2 (Cabello *et al*., 1994 and Alcázar *et al*., 2002).

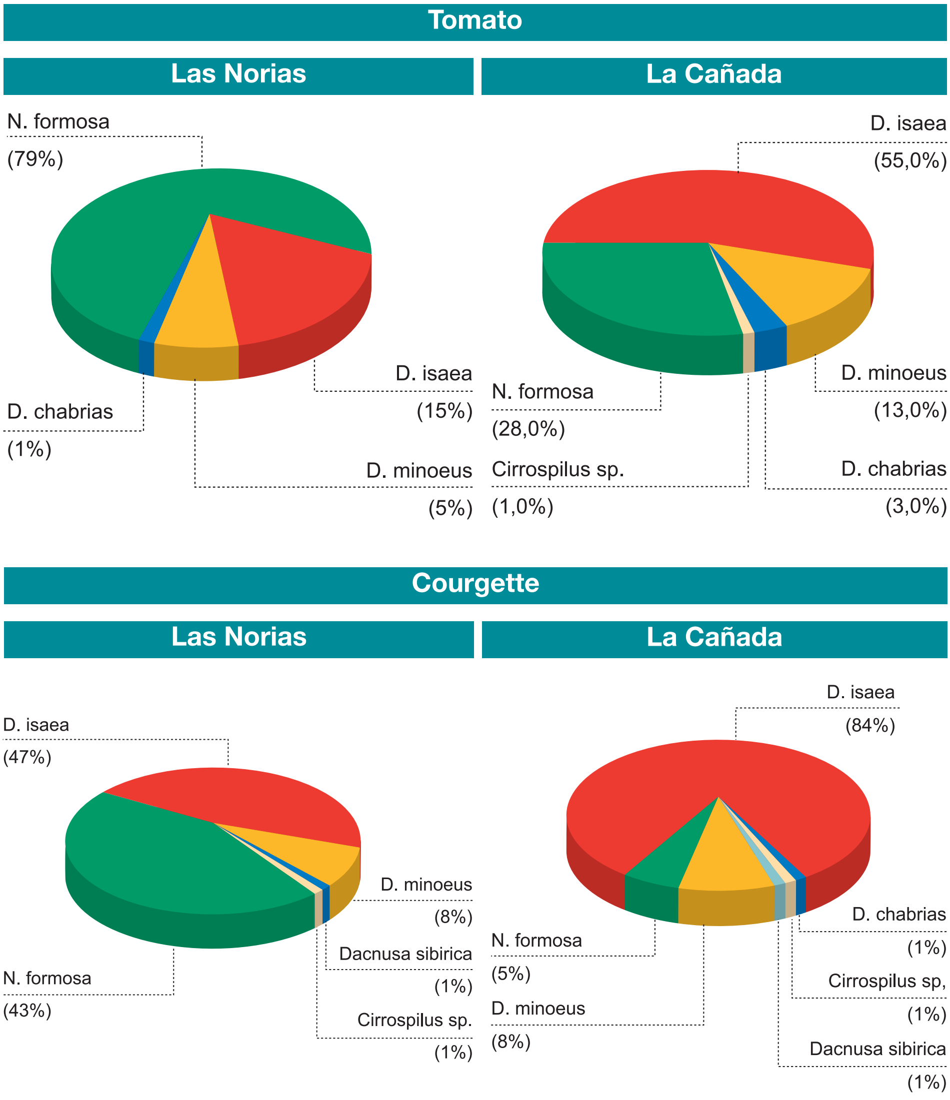
Table 2. List of autochthonous parasitoid species in horticultural crops in greenhouses of Almería

Family	Parasitoid species	Host	Crops
EULOPHIDAE	*Diglyphus isaea* (Walker)	*Liriomyza* spp.	Tomato, green bean, courgette, watermelon, melon, cucumber, aubergine and pepper
	Diglyphus chabrias (Walker)	"	Tomato, courgette, green bean, melon and watermelon
	Diglyphus minoeus (Walker)	"	Tomato, green bean, courgette, watermelon, melon, cucumber, aubergine and pepper
	Diglyphus crassinervis Erdös	"	Tomato, melon and courgette
	Diglyphus poppoea Walker	"	Courgette, melon, watermelon and tomato
	Chrysonotomyia (= Neochrysocharis) formosa (Westwood)	"	Tomato, green bean, courgette, watermelon, melon, cucumber and aubergine
	Cirrospilus vittatus. Walker	"	Green bean, watermelon and tomato
	Hemiptarsenus varicornis	"	Green bean
	Hemiptarsenus zilahisebessi Erdos	"	Tomato, green bean, melon and watermelon
EUCOILIDAE	*Kleidotoma* sp.	"	Courgette, cucumber, aubergine, melon and watermelon
BRACONIDAE	*Dacnusa sibirica* Haliday	"	Tomato, courgette, watermelon, melon and cucumber
	Opius concolor Szépligeti	"	Melon and cucumber
	Opius pallipes Wesmael [1]	"	Courgette,, tomato, melón y sandía.

[1] *Opius pallipes* does not act as parasitoid of *Liriomyza trifolii*, because parasitized larvae encapsulate the eggs avoiding their development.

Of these 13 species, there are two species that stand out, mainly due to the frequency with which they appear in the different crops as well as by the parasitism index they reach; they are *Neochrysocharis formosa* (=*Chrysonotomyia formosa*) and *Diglyphus isaea*. With respect to the rest of species, their appearance and the parasitism index they present are lower, and they are only detected at some specific times and in a particular manner. Furthermore, differences are also observed with respect to the distribution of species by production and crop areas. (Figure 2). (Cabello *et al*., 1994, Alcázar *et al*., 2000, Alcázar *et al*., 2002).

Figure 2. Percentage representation of parasitoids in tomato and courgette crops in Las Norias and La Cañada (Almería)



Source: (Alcázar, *et al*., 2000).

The great diversity of parasitoid species that are present permits that, in specific times and under specific conditions, a control of the pest can be achieved, even without chemical treatments. In addition to this, when chemical treatments have been applied, the parasitism indexes have also contributed to control, especially in the plots where *Neochrysocharis formosa* appears abundantly; therefore, it seems that this parasitoid has higher resistance to traditional treatments carried out in our crops (Cabello *et al*., 1994).

Neochrysocharis formosa is an endoparasitoid, while *Diglyphus isaea* and the rest of eulophids are ectoparasitoids that act on the larval stage.

Neochrysocharis formosa and *Diglyphus isaea,* show preference for the first and second stages to feed on, while they choose the third stage to parasitize. The braconids *Dacnusa sibrica* and *Opius pallipes* are endoparasitoids that lay the eggs mainly inside the 3rd instar larvae, and they are developed during this instar, emerging later from the *Liriomyza* pupa.

In the case of *Neochrysocharis*, it is usually more complex to observe the larvae parasitized by this hymenopteran in field, although dark larvae can be observed as those shown in Photo 8.

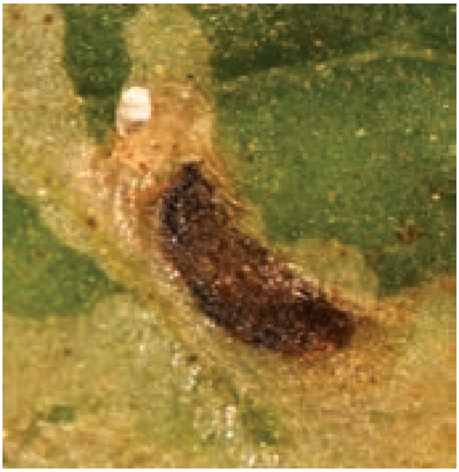


Photo 8. Aspect of the ***Liriomyza*** sp. larva, parasitized by ***Neochrysocharis formosa***

In the case of *Diglyphus*, it is usually easier to observe if larvae are parasitized by this parasitoid because the *Diglyphus* larva or pupa appears next to the *Liriomyza* larva (Photo 9).

The Braconids, as they are endoparasitoids of larvae, it is difficult to detect the parasitism exercised by this species, and it only can be determined dissecting the pupae, therefore, it is necessary to collect the pupae and develop them in the laboratory.

In addition to the parasitoids that appear naturally, there are two species on the market that are produced commercially for the control of *Liriomyza* species. The tables 3 and 4, attached show information about the different commercial companies dealing with natural enemies, about the products and formulations that they have about these two parasitoids, as well as the doses and the application intervals.

Photo 9. A and B Larva of *Diglypus isaea* feeding on a *Liriomiza* sp. larva. C Pupa of *Diglyphus isaea* inside the mine

Table 3. Commercial products of the parasitoid *Dacnusa sibirica*

Biological agent	Brand name	Commercial company	Container	Content	Dose
Dacnusa sibirica Telenga	Dacline s.	Syngenta Bioline.	Bottle 250 ml	250 adults +food	-
	Dacnuplan	Plan Protect S.L.	Bottle 100 ml	250 adults	-
	Dacnusa-System.	Biobest Sistemas Biológicos, S.L.	Bottle	250 adults	When observing the first mines. Release each week 0.25 ind/m2 until observing enough parasitism
	Dacnusa-Mix-System			225 adults *Dacnusa*+25 adults *Diglyphus*	
	Diminex	Koppert Sistemas Biológicos S.L	Bottle 100 ml	125 adults of Dacnusa+125 adults *Diglyphus*	Light curative: <1 larva/10 plants 0.25 ind/m2 per week Heavy curative: > 1larva/10 plants. Treatment with Miglyphus. Minimum 3 treatments
	Minex.			225 adults *Dacnusa*+25 adults *Diglyphus*	
	Minusa.			250 adults *Dacnusa*	

Table 4. Commercial products of the parasitoid *Diglyphus isaea*

Biological agent	Brand name	Commercial company	Container	Content	Dose and application
Diglyphus isaea (Walker)	DIGLY control	Agrobio, s.l.	Bottle 100 ml	250 insects	Release when the first leafminers appear 0.5-1 ind/m2 2 introductions in consecutive weeks
	DigliPAK	Bioplanet	Bottle 100 ml	250 adults	-
	Digline i.	Syngenta Bioline.	Bottle 250 ml	250 adults+food	Release when observing the first mines / first captures of adults in cards 0.5 ind/m2, 2 introductions in consecutive weeks
	Diglyphus-System.	Biobest Sistemas Biológicos, S.L.	Tube	250 adults	0.15 ind/m2. To treat with high infestations and at higher temperature
	Diglyplan.	Plan Protect S.L.	Bottle 100 ml	250 adults	-
	Digsure (i)	Certis Europe B.V. Suc España	Tube 30 ml	250 adults	-
	Miglyphus.	Koppert Sistemas Biológicos S.L.	Bottle 100 ml	250 adults *Diglyphus*	Light curative: (<1 larva/10plants) 0.1 ind/m2 Heavy curative: (>1 larva/10 plants)

6.2.3 Entomopathogens

Several trials have been carried out to determine the effectiveness that some nematode species show for the control of the *Liriomyza* species (Harris *et al*., 1990; Lebeck *et al*., 1993, Sher *et al*., 2000)

In some cases, high mortalities have been obtained, however the effectiveness of these organisms depends on the existence of a high degree of humidity (Hara *et al*., 1996).

There are also trials which evaluate the effectiveness of entomopathogen fungi on *L. huidobrensis* (Solis *et al*., 1998).

With respect to the use of nematodes in horticultural crops in greenhouses of Almería, *Heterorhabditis bacteriophora* is included in the methods of integrated control, within the Regulations of Integrated Production of the Andalusian Regional Government (Official Gazette of the Andalusian Regional Government no. 211, 2007).

6.3. Chemical methods

The use of chemical products of broad spectrum for the control of leafminers belonging to the genus *Liriomyza* has caused important problems, not only due to the appearance of resistances (Peña, 1986), but also due to the effect that these products have had on auxiliary fauna. As we have already seen, these have great importance as control elements of these pests and, for this reason, selective insecticides must be used that decrease the resistances and the negative effect on auxiliary ones.

Table 5 includes the list of chemical products authorised for the control of leafminer species (list updated on 21/05/09, General Direction of Agricultural and Livestock Production. Regional Ministry of Agriculture and Fish. Andalusian Regional Government).

Table 5. List of registered chemical products with authorised uses for the control of the especies of the *Liriomyza* genus in horticultural crops

Substance	Formulation	Crops
Abamectine	Abamectine 1,8 % [ec] p/v	Cou. Me. Pe. Cu. Wat. To.
	Abamectine 3,37 % [ec] p/v	Cou. Be. Me. Pe. Cu. Wat. To.
Azadirachtin	Azadirachtin 3,2 % [ec] p/v	Au. Cou. Be. Me. Pe. Cu. Wat. To
Piperonyl butoxide pyrethrins	Piperonyl butoxide 16 % + pyrethrins 4 % (extr. De pyrethrum) [ec] p/v	Au. Cou. Be. Me. Pe. Cu. Wat. To
Cyromazine	Cyromazine 75 % [wp] p/p	Au. Cou. Be. Me. Pe. Cu.Wat. To
Chlorpyrifos	Chlorpyrifos 5 % [gr] p/p	Au. Cou. Be. Me. Pe. Cu.Wat. To
Oxamyle	Oxamyle 10 % [sl] p/v	Au. Cou. Me. Pe. Cu. Wat. To

Source: General Direction of Agrarian Production. 2009. Regional Ministry of Agriculture and Fish. Andalusian Regional Government.

The abamectine is a natural insecticide-acaricide coming from *Streptomyces avermitilys*, a product that shows a high translaminar movement which comes quickly to the leaf inside and it can act by contact as well as by ingestion. It inhibits female's oviposition and affects larvae during their hatching and development.

The azadirachtin is very effective in the control of different development stages of *Liriomyza*, it has effect on oviposition in the case of *Liriomyza trifolii*, just like it acts on larvae mortality, reaching even 100 % in some cases of *Liriomyza trifolii* larvae and a 98.2 % on *Liriomyza sativae* larvae (Spencer, 1973); when it is applied on the soil in chrysanthemum crops, it shows a mortality of 98 % of pupae of *L. trifolii* (Spencer, 1973 and Mikenberg and van Lenteren, 1996). However, it seems to have a toxic effect on some parasitoid species such as *Hemiptarsenus semialviclava*.

There is another very important group of insect, the insect growth regulators (IGRs). They have a more selective effect, the Cyromazine acting as a growth regulator of larvae which inhibits the chitin synthesis by contact or ingestion and it does not have a direct effect on adults although its fertility decreases.

The oxamyle is a systemic insecticide that is applied by irrigation, and acts on larvae. Its application by irrigation does not have an effect on the parasitoid adults.

It is important to take into account the effect that these products have on the auxiliary fauna, for this reason, the data published by some commercial companies of natural enemies and by the Regional Ministry of Agriculture and Fish (Andalusian Regional Government) can be consulted on their web sites, which show all the authorised active substances and the effects they have, depending on the type of application, on the commercialised natural enemies.

6.4. Genetical and biotechnological methods

Nowadays the genetical methods applied to the control of leafminer species are focused in the study of varieties resistant to damage caused by Liriomyza, Dogimont (1999) has carried out studies in melons and there are also studies about resistant varieties of tomato.

6.5. Methods of Integrated Fight

The Integrated Fight against the leafminer species has been shown as an effective method. In Andalusia, there have been Specific Regulations about Integrated Production for horticultural crops since 1998, which were later updated in 2001 and 2007 (Official Gazette of the Andalusian Regional Government num. 211, 2007). These Regulations establish a set of measures or compulsory practices that are summarised below.

- The biological, cultural, physical and genetic methods shall be put before the chemical methods whenever possible.

- The autochthonous auxiliary fauna must be respected and protected. If releases of biological control organism are made, these must be commercialised in accordance with regulations.
- Chromotropic yellow and blue traps shall be placed for capturing and monitoring, even before the planting of the crop.
- Controls to check the state of pests and useful fauna shall be carried out every 10-15 days.
- In the case of methods described previously not being effective, the active substances specified for each crop can be used adequately in accordance with the manufacturer's instructions, respecting the doses, safety period, etc. In addition to this, active substances of different chemical groups and action mechanisms shall be alternated.

In addition to these general measures, the Specific Regulations of Integrated Production in horticultural crops establish the requirements and recommendations to be followed for each crop. Table 6 shows a summary of those referred to the control of leafminers belonging to the genus *Liriomyza*.

Table 6. Specific regulations of Integrated Production in horticultural crops for the control of Liriomyza spp. Regional Ministry of Agriculture and Fish. Andalusian Regional Goverment

Crop	Intervention criteria	Recommended measures	Active substances of possible use
Aubergine	First releases of BCOs when the first damage appears and when live larvae are observed. Chemical treatments when damage reaches 20 % and the parasitism level is below 70 %.	**TRAPS:** • Monitoring yellow chromotropic. To place them before planting the crop and in the critical points. Keep them the entire crop. • Control yellow chromotropic. To place them before planting the crop with high density. **BIOLOGICAL CONTROL ORGANISMS** • Releases of *Diglyphus isaea*. Dose 0.3-0.75 ind/m2 within 2-3 consecutive weeks, until reaching a parasitism level >70 %. • *Steinernema* spp., Foliar application 5000 ind/m2 weekly intervals before releasing BCOs	Azadirachtin Cyromazine Oxamiyle
Courgette	First releases of BCOs when the first damage appears and when live larvae are observed. It only causes damage in seedling state. There is not risk if damage < 20 %	**TRAPS:** • Monitoring yellow chromotropic. To place them before planting the crop and in the critical points. Keep them the entire crop. • Control yellow chromotropic. To place them before planting the crop with high density. **BIOLOGICAL CONTROL ORGANISMS** • Releases of *Diglyphus isaea*. Dose 0.25 ind/m2 within 2-3 consecutive weeks, until reaching a parasitism level >70 %.	Abamectine Azadirachtin Cyromazine Oxamyle
Green bean	First releases of BCOs when the first damage appears and when live larvae are observed. Chemical treatments when damage reaches 20 % and the parasitism level is below 70 %.	**TRAPS:** • Monitoring yellow chromotropic. To place them before planting the crop and in the critical points. Keep them the entire crop. • Control yellow chromotropic. To place them before planting the crop with high density. **BIOLOGICAL CONTROL ORGANISMS** • Releases of *Diglyphus isaea*. Dose 0.75-1 ind/m2 within 2-3 consecutive weeks, until reaching a parasitism level >70 %.	Summer oil Azadirachtin
Melon	First releases of BCOs when the first damage appears and when live larvae are observed. Chemical treatments when damage reaches 20 % and the parasitism level is below 70 %.	**TRAPS:** • Monitoring yellow chromotropic. To place them before planting the crop and in the critical points. Keep them the entire crop. • Control yellow chromotropic. To place them before planting the crop with high density. **BIOLOGICAL CONTROL ORGANISMS** • Releases of *Diglyphus isaea*. Dose 0,1-0,2 ind/m2 within 2-3 consecutive weeks, curative application.	Abamectine Azadirachtin Cyromazine

Table 6. Specific regulations of Integrated Production in horticultural crops for the control of Liriomyza spp. Regional Ministry of Agriculture and Fish. Andalusian Regional Goverment

Crop	Intervention criteria	Recommended measures	Active substances of possible use
Cucumber	First releases of BCOs when the first damage appears and when live larvae are observed Pest is not a risk if damage does not reach 20 %,	**TRAPS:** • Monitoring yellow chromotropic. To place them before planting the crop and in the critical points. Keep them the entire crop. • Control yellow chromotropic. To place them before planting the crop with high density. **BIOLOGICAL CONTROL ORGANISMS** • Releases of *Diglyphus isaea*. Dose 0.1-0.2 ind/m2 within 2-3 consecutive weeks, curative application.	Abamectine Azadirachtin Cyromazine Oxamyle
Watermelon	First releases of BCOs when the first damage appears and when live larvae are observed It only causes damage in seedling state. Pest under control if there is not a generalised presence of mines and risk of piercing the stem; and parasitism >25 %.	**TRAPS:** • Monitoring yellow chromotropic. To place them before planting the crop and in the critical points. Keep them the entire crop. • Control yellow chromotropic. To place them before planting the crop with high density. **BIOLOGICAL CONTROL ORGANISMS** • Releases of *Diglyphus isaea*. Dose 0.1-0.2 ind/m2 within 2-3 consecutive weeks, curative application.	Abamectine Azadirachtin Cyromazine
Tomato	First releases of BCOs when the first damage appears and when live larvae are observed. Chemical treatments when damage reaches 20 % and the parasitism level is below 70 %.	**TRAPS:** • Monitoring yellow chromotropic. To place them before planting the crop and in the critical points. Keep them the entire crop. • Control yellow chromotropic. To place them before planting the crop with high density. **BIOLOGICAL CONTROL ORGANISMS** • Releases of *Diglyphus isaea*. Dose 0.3-0.75 ind/m2 within 2-3 consecutive weeks, until reaching a parasitism level >70 %.	Summer oil Abamectine Azadirachtin Cyromazine Oxamyle

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Chapter 11

Biological control of phytophagous mites in protected horticultural crops

Francisco Ferragut[1]

1. Red spider mites, *Tetranychus urticae* Koch, *T. turkestani* Ugarov and Nikolski, *T. evansi* Baker and Pritchard and *T. ludeni* Zacher

1.1. Red spider mite species in horticultural crops

Phytophagous mites of the genus *Tetranychus* are commonly known as red spider mites and constitute some of the most significant pests of vegetable production in Spain's warm areas, both in greenhouses and outdoors. On these crops, four species of similar appearance can be found: *Tetranychus urticae* Koch, *Tetranychus turkestani* Ugarov and Nikolski, *Tetranychus evansi* Baker and Pritchard and *Tetranychus ludeni* Zacher, which may appear together on plants and develop high populations at any year and season (Escudero and Ferragut, 1998; Ferragut and Escudero, 1999). This situation is different to that of other European countries, where damage in vegetables is almost exclusively caused by *T. urticae.*

These species are characterized by a series of attributes associated to their biology and geographical distribution. First of all, they are, in most cases, native not-introduced species, that probably have colonized the crops for many years, and, therefore, show a great adaptation to the region's weather conditions and to the cultural practices, as well as to the cultivated or spontaneous vegetation that serves as food for them. Secondly, they are capable of feeding and reproducing on many different vegetable species, so that their populations in agricultural areas are very dynamic and unstable. Thanks to the weather conditions, which are usually mild throughout the region, they reproduce actively all year long and spread quickly and efficiently from some plants to others, creating new

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colonies that, in a short time, reach a high number of individuals until they finish a food supply and spread in order to search new nutrition sources. Finally, they show a gregarious behaviour that promotes the creation of colonies and aggregates. All these species have glands producing silk, with which they build the dense structures that we call “webs“ and that cover the physical space where the members of the colony are located.

T. urticae and *T. turkestani* are, in this order, the most frequent and abundant species in horticultural crops and in non-cultivated vegetation in agricultural areas. Both species are known since the studies on these pests began and they can be found in all Spanish regions, including the Balearic and Canary Islands, both in coastal regions as well as in the interior of the Peninsula. *T. ludeni* is less common and its distribution area is limited to the warmest and humid seaside regions. It is more common on spontaneous vegetation than in crops, probably because of its high sensitivity to pesticides. It is probably an introduced species of tropical and subtropical origin, although the moment of its arrival is not known. Finally, *T. evansi* is an invasive species that was detected in Spain in 1995 (Ferragut and Escudero, 1999). Since then, it has spread quickly and, at present, it is possible to find it all over the Mediterranean coast and the Balearic and Canary Islands. It prefers plants of the Solanaceae family and it develops quickly in tomato, aubergine and potato outdoor crops. It is less likely in greenhouses, especially if they are treated, because it is very sensitive to acaricides and other insecticides. Two characteristics may be highlighted about this species. The first one is that it is very common on weeds. Its colonies on the black nightshade *Solanum nigrum* L. (Solanaceae) are very striking, developing high populations that may kill the plant (Photo 1), but it is, also, frequent on *Amaranthus, Chenopodium, Convolvulus, Diplotaxis, Lavatera* and *Sonchus* species. On all these plants, eggs and immature individuals can be found through the whole year, indicating that it feeds and reproduces in these plants. Studies carried out in recent years have proved that, in citrus orchards, *T. evansi* is the predominant species

Photo 1. ***Solanum nigrum*** plant affected by the tomato spider mite ***Tetranychus evansi***. The mites have dried up the plant and they are arranged in the upper part forming groups of numerous individuals

in the ground cover (Aucejo *et al*., 2003; Pascual, 2007). There also exists data that indicates that it is capable of competing with and displacing the native spider mite *T. urticae* on weeds of agricultural areas (Ferragut *et al*., 2007). The second feature is that, unlike others red spider mites species, it is not an adequate prey for phytoseiid mites, the main predators of spider mites. It is surprising the absence of phytoseiids into *T. evansi* colonies, despite being abundant in other nearby plants with other red spider mite species. The only predators that are usually found in these colonies are larvae of the cecidomyiid *Feltiella acarisuga* (Vallot) (Dipteral: Cecidomyiidae) and the coccinelid *Stethorus punctillum* Weise (Coleopterous: Coccinellidae), although in low densities. The scarce phytoseiids that can be seen inside the colonies show a whitish colouration, demonstrating that they do not feed on *T. evansi*, even plants present high populations of prey. The absence of phytoseiids in the field confirms that this red spider mite is not a suitable prey and that when predators reach the *T. evansi* colonies, they spread quickly in search of other prey that may allow them to express all its biotic potential with a higher efficacy.

1.2. Morphologic characteristics and diagnosis

The external appearance of the four species of red spider mites is very similar. The colouration of the individuals depends on the age, being the youngest either clearer or almost colourless and the adults of reddish colour with different tonalities, according to the species. These species are genetically different, their behaviours are different, they do not have the same sensitivity to pesticides and they cannot be controlled by the same natural enemies. Therefore, from a practical point of view it would be advisable to be able to distinguish these species in field by their external appearance. That would permit the development of a more precise control of the pest, choosing the most suitable products or the most effective natural enemies.

Although it is not a completely rigorous criterion from a scientific point of view, at a practical level these four species can be distinguished by adult female colouration, the biggest form in the population. Based on experience, we have realized that, in most cases, this criterion is fulfilled and can be used in field for taking a decision about the pest control.

T. urticae females are reddish brown coloured, similar to that of a brick, sometimes more intense and some others muter or darker, in a dull tone; *T. turkestani* females are honey-coloured, caramel-coloured or even greyish, in a pale and always mute tone; *T. ludeni* females are intense red coloured, a similar colour to that of *Panonychus citri*, the citrus red mite, and *T. evansi* females are orangey coloured. In comparison to other species the latter's first pair of legs are longer (Photos 2-5). It is also necessary to bear in mind that in some crops, like strawberry crops, the existence of *T. urticae* individuals in diapause in winter is common; that is to say, individuals which stop their reproductive activity during this season. These forms may be easily distinguished due to their bright orangey red colour. Usually, only one part of the population enters in diapause in the Mediterranean area and that is why in the same colonies normal colouration individuals mix with others with diapause typical colour.

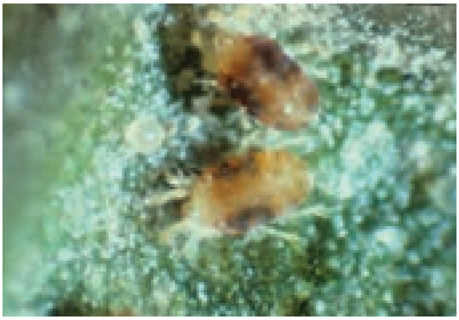


Photo 2. Common spider mite females, *Tetranychus urticae*

Photo 3. External aspect and colour of *Tetranychus turkestani* female

Photo 4. *Tetranychus ludeni* female (left) and male

Photo 5. External aspect and colour of *Tetranychus turkestani* female

1.3. Symptoms and damages

Another possibility is to distinguish the species by the symptoms and the damages that they produce in plants. Apparently all of them cause a similar damage, diffuse discolouration that from a closer view are, in fact, small whitish spots that correspond to the epidermal cells sucked in the leaves and the silk production that covers the colonies. Nevertheless, a more detailed analysis of *T. urticae* and *T. turkestani* on beans has proved that the symptoms are different and can be used to diagnose the species causing them (Soler-Salcedo *et al*., 2006).

The results of that work indicate that a similar number of individuals of each species, in the same period of time, give rise to different damage because of the different distribution on the leaves. *T. urticae* spreads evenly showing no predilection for any determined area of the upper side or underside surface. For this reason, stings are more or less uniformly distributed in the leaf, or concentrated on the areas where the colony is located, but without showing any kind of pattern. On the other hand, *T. turkestani* is usually located preferably along the leaf nerves, either the lateral nerves or the central one. The feeding takes place there and the discolouration is evident in the nerves' sides. Additionally, it has been observed that bean leaves attacked by *T. turkestani* are often deformed, and brown or yellowish spots may appear inside these deformities. The deformities appear in the leaves' margin, making them bend and form concave structures that are occupied by the colony and covered with web. These deformities can be small or can enormously alter the leave development, and some of them are evident in very small leaves and with few mites. Apparently, this species introduces in the plant some chemical substances, via its saliva, which modify leaf development and provokes these alterations.

1.4. Biological control

Generally these mites are controlled by chemical means, representing significant costs in economic and ecological terms and not always guaranteeing the suppression of the pest. Another feasible alternative is the biological control with the release of phytoseiid mites distributed in plants, where they feed on the spider mites. This technique of biological control has been used for decades in other European countries and its efficacy has been proved, especially in crops protected against *T. urticae*

(Scopes, 1985; Lenteren and Woets, 1988; Gerson *et al*., 2003; Zhang, 2003). However, there exists much less information on predator capability to control other red spider mite species that can be found in Spain.

Spanish horticultural crops are characterized by having a rich fauna and, sometimes, a high density of phytoseiids, the most representative species being: *Neoseiulus californicus* (McGregor) and *Phytoseiulus persimilis* Athias-Henriot (Escudero and Ferragut, 1998). These are particularly common in outdoor crops and they are also abundant on non-cultivated vegetation, being generally found on plants with red spider mite colonies. Both are native species, or at least, have been known for ages in Spanish crops and they are commercialized by some companies to be used in inoculative releases in protected crops (Photos 6 and 7).

Photo 6. Female of the phytoseiid *Neoseiulus californicus* (left) next to a red spider mite female

Photo 7. *Phytoseiulus persimilis* catching a red spider mite female

It would be desirable that these predatory mites were capable of controlling all the *Tetranychus* species, since the presence of these pests in the field can be easily detected, but the identification of the exact species responsible for the damages is never done before applying the control measures. In a series of studies, the efficacy of these predators on each of the red spider mites in laboratory conditions has been evaluated. The results obtained indicate that both phytoseiids species are capable of feeding and increasing their abundance when they feed on *T. urticae*, *T. turkestani* and *T. ludeni*, suggesting that they can be also effective in field. Nevertheless, when they feed on *T. evansi* their development is very slow and the laying of eggs is scarce, which suggest its inefficiency in the control of this pest in commercial plots (Escudero and Ferragut, 2005; Escudero *et al*., 2005).

These results, obtained on fragments of leaves in controlled and ideal conditions for predators, have been confirmed in semi-field tests carried out on whole plants in greenhouses (Gómez-Moya and Ferragut, 2009). Figure 1 and 2 show the response of *N. californicus* and *P. persimilis* when they are released in plants with *T. urticae* and *T. evansi*.

The results obtained are illustrative for the effectiveness of these predators at different release doses, measured as the ratio between the number of predators and prey. *P. persimilis* was very effective since it completely eradicated *T. urticae* and *T. turkestani* populations at all the release doses, greatly reducing their number from the first or second week, after the release, onwards. Nevertheless, *N. californicus* is not capable of completely eliminating this prey, and it only significantly reduces their number, with respect to the reference, in a 1:4 ratio. The differences in effectiveness are due to the predators' mobility. Red spider mites move to the higher parts of the plant searching for new leaves. Phytoseiids follow their prey populations and this behaviour determines their capability to reduce the prey number or to eliminate them. In this research, *P. persimilis* quickly moved to the top part of the plant. In the 3rd week, more than half the females were already at this level, while *N. californicus* did it slowly and in an incomplete form, since at that moment between 80-100 % of females were still in the lower parts of the plants. In short-sized plants, like horticultural ones, the efficacy of these predators depends on their ability to spatially spread according to their prey, gathering together in leaves or parts of the plant where the population of spider mites is higher.

Figure 1. Effectiveness of the phytoseiids Neoseiulus californicus and Phytoseiulus persimilis in the control of the common spider mite Tetranychus urticae in green bean plants. Data of the predator-prey ratios 1:12, 1:8 and 1:4. T. urticae ratio = plants with phytoseiids; T. urticae control = plants without phytoseiids

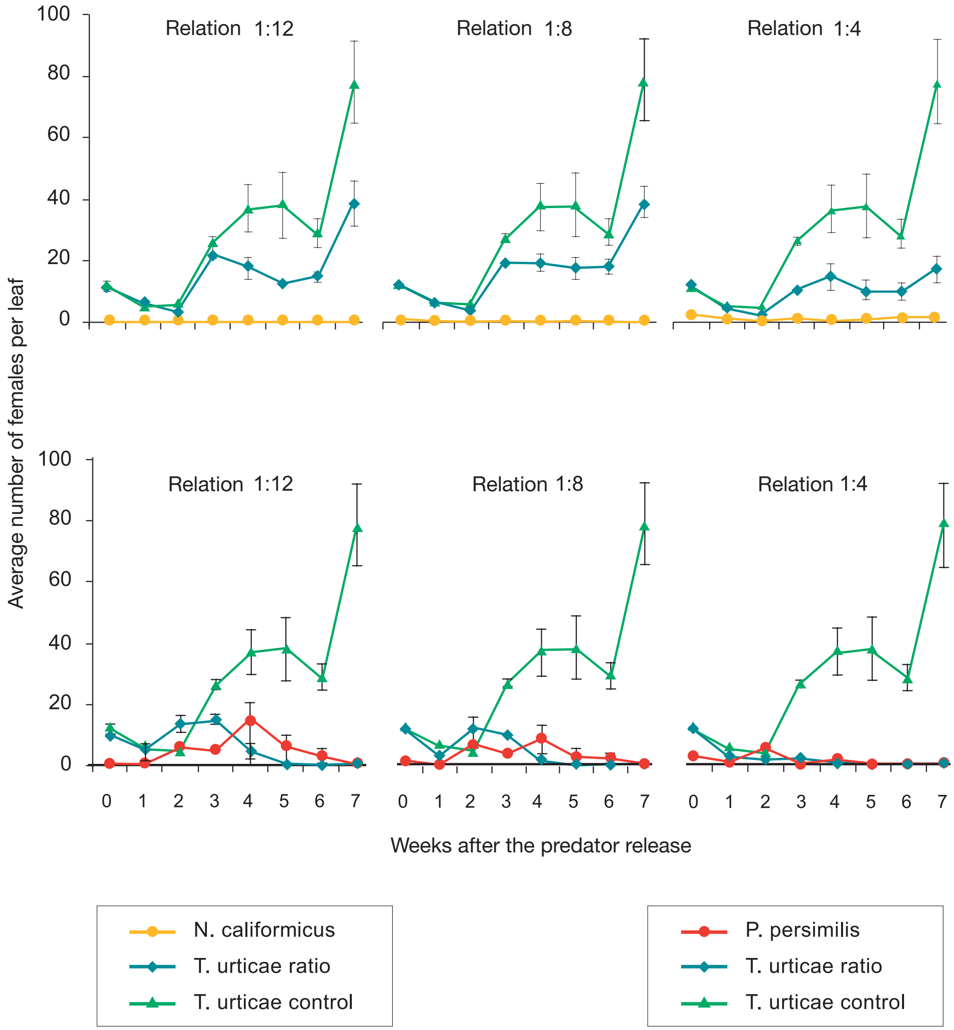
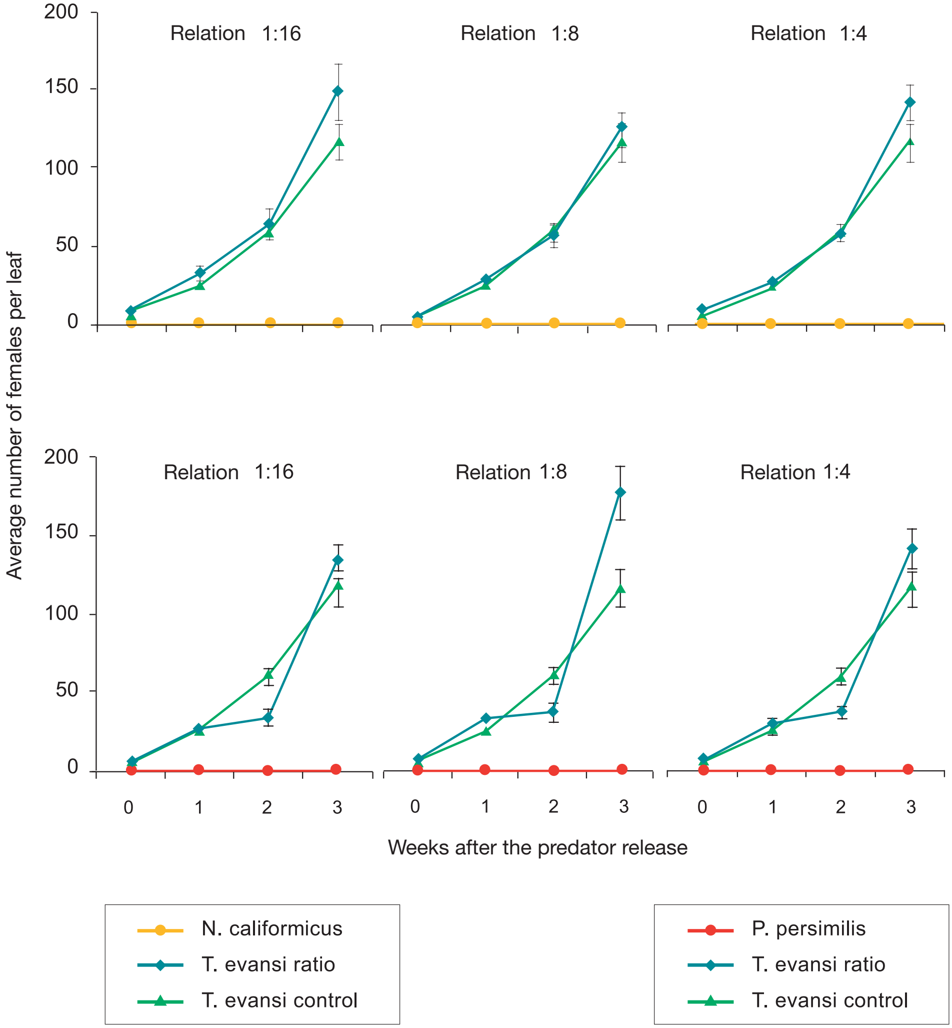


Figure 2. Effectiveness of the phytoseiids Neoseiulus californicus and Phytoseiulus persimilis in the control of the tomato spider mite Tetranychus evansi in potato plants. Data of the predator-prey ratios 1:16, 1:8 and 1:4. T. evansi ratio = plants with phytoseiids; T. evansi control = plants without phytoseiids



When *T. evansi* was the prey, the phytoseiids were unable to control or to reduce their populations, since their abundance at the end of the tests and for the three release doses was higher than that found in the control test plants. There has been much speculation for the reasons why *T. evansi* is not an adequate prey for phytoseiids used in *T. urticae* biological control. Back in the 80s, Moraes and McMurtry (1986) suggested that this prey must contain some substance with a feeding inhibiting effect for phytoseiids. Recently, Koller *et al*. (2007) have presented the hypothesis that the *T. evansi*-phytoseiids relationship is determined by the plants consumed by the red spider mite. Their experiences with *N. californicus* show that the phytoseiid is negatively affected when it feeds on *T. evansi* grown on tomato, but not on beans. Apparently, this red spider mite accumulates, in its interior, toxic substances synthesized by tomatoes and other Solanaceae that affect their predators.

In recent years, *T. evansi* has spread along the countries of the Mediterranean basin and there are concerns about its arrival in horticultural production greenhouses in Central and Northern Europe, where *T. urticae* is mainly controlled by phytoseiids. Promising results for the biological control of *T. evansi* have been recently obtained with the discovery of a population of the phytoseiid *Phytoseiulus longipes* Evans in the south of Brazil that feeds on *T. evansi* in Solanaceae (Furtado *et al*., 2007). Preliminary studies indicate that its development and fecundity is very high when feeding on *T. evansi* on tomatoes. This would allow its use as a biological control agent in Europe and Africa (Furtado *et al*., 2007; Ferrero *et al*., 2007).

1.5. Practical use of phytoseiids in red spider mite biological control

From the abovementioned, it follows that phytoseiids commercialized for the control of red spider mite in horticultural crops are effective against *T. urticae*, *T. turkestani* and *T. ludeni*, but not against *T. evansi*. At this moment, there does not exist any produced and commercialized phytoseiid effective against this pest.

In the case of remaining red spider mite species, the biological control is based on the release of *Phytoseiulus persimilis* and *Neoseiulus californicus* (the last one commercialized by different producing companies with the name of *Amblyseius californicus*). These two predators have different characteristics and predatory behaviour and, sometimes, their joint release is recommended, in order to make use of each of their advantages. *P. persimilis* exclusively feeds on red spider mites, has a bigger size and its capability for prey consumption is high if compared to *N. californicus*. On the other side, *N. californicus* can feed on other mites apart from spider mites, on small insects and even on pollen; its size and mobility are smaller and its daily capability of prey consumption is lower than that of *P. persimilis*. Therefore, *N. californicus* may be preventively introduced in crops, before the emergence of red spider mite or whenever they are found at very low levels. *P. persimilis* releases may only be carried out in the presence of the pest, releasing this predator alone or together with *N. californicus* or the cecidomyiid *Feltiella acarisuga*, especially when red spider mite populations are already significant. In general, the joint release of these predators tries to take advantage of their characteristics for a better control of the pest. *P. persimilis* is more voracious and it has a higher growth rate, therefore producing a short time effect; however, *N. californicus* is capable of staying on plants longer, at very low pest densities, which guarantees a more lasting control. Besides, *N. californicus* is more tolerant to some pesticides and it resists environmental conditions of high temperatures and low moisture better than *P. persimilis*.

2. Greenhouse broad mite *Polyphagotarsonemus latus* (Banks)

This species is known by the name of greenhouse broad mite and it is a very significant pest on a global scale, especially in tropical regions, where it affects many crops, such as cotton, tea, citrus, cultivated Solanaceae and many ornamental crops. The mite attacks young vegetable organs and is generally located in the underside of the leaves, whose margins become rigid and deformed as a consequence of its feeding activity. In our region, it affects diverse crops, especially pepper. On pepper plants it produces weakness and a low development due to the continuous production of sprouts and buds that are affected by the mite. Symptoms are very characteristic and they consist of deformations, curls and necrosis of young leaves, which may dry up in case of severe attacks.

Distributed in tropical and subtropical regions around the world it is common in outdoor Mediterranean crops and in the greenhouses of milder regions of colder weather areas. As its generic name indicates, it is a very polyphagous species that has been reported on more than 90 plant families. In Spain it can be found in several horticultural crops, amongst others, in pepper, tomato, aubergine and cucumber crops, also being a pest species in floral and ornamental crops and sometimes in citrus trees, where it affects, especially in young potted trees, in which, besides the abovementioned symptoms, it can also produce multiple gemmation.

2.1. Physical appearance and dispersal behaviour

The broad mite is small-sized and difficult to detect in the field. Its size is situated between that of red spider mites, significantly more voluminous, and eriophyids, even smaller. In order to get a correct observation it is necessary to use a quality hand lenses or binocular magnifying glasses. Colouration is variable depending on the plant on which it feeds, but it is usually whitish, amber or greenish. Eggs are oval-shaped, long and translucent and males, a bit smaller than females, carry female nymphs on their bodies until they complete their development and can mate.

Some years ago it was proved that broad mite spread is carried out by insects that act as vectors when transporting the mite on their legs or on their bodies. The most involved species in mite distribution along crop areas is the whitefly *Bemisia tabaci* (Gennadius), so that, as soon as the broad mite enters a greenhouse infected with whiteflies, it can spread quickly and produce significant damages. All control measures that may be effective against whitefly also favour low population levels of *P. latus*.

2.2. Biological control

Several acaricides and insecticides with acaricide action are available for the control of this pest. Furthermore, in some countries treatments with hot water at 43-49 ºC, wetting well plants for several minutes to eliminate mite populations, are applied.

The search of effective natural enemies of the broad mite began some years ago, looking for phytoseiids that would guarantee its control in greenhouse crops. The most promising species used have been those of the genus *Neoseiulus*, especially *Neoseiulus cucumeris* (Oudemans) and *N. californicus*. In tests performed in Israel, *N. cucumeris* effectively controlled the broad mite in two greenhouse pepper varieties, its effect being similar to that produced by treatments with sulphur (Weintraub *et al*., 2003). *N. californicus* has also proved its capability to reduce broad mite populations in commercial crops. Castagnoli and Falchini (1993) observed that this phytoseiid is able to feed, develop and reproduce with an exclusive diet of *P. latus*. When it feeds on broad mites, its development is similar to when fed on red spider mites and its populational growth, although a bit smaller, is enough to increase its number in a short period of time.

On a practical level, *N. californicus* is commercialized by some companies to be used against broad mite in protected crops. Likewise, *N. cucumeris* and, even, *Amblyseius swirskii* Athias-Henriot, used especially as a biological control agent for whiteflies and thrips, can carry out a good control of *P. latus*.

3. Tomato eriophyids *Aculops lycopersicii* (Massee) and *Aceria lycopersici* (Wolffenstein)

Tomato crops have in Spain, and in other tomato producing countries, two species of eriophyids that are difficult to distinguish in the field and that can seriously affect the vigour of plants and their production. One of them has been well known amongst producers for many years, since it was reported in Spain in the 1940s. This is *Aculops lycopersicii* (Massee), also known as “The tomato russet mite” and “Vasates”, because formerly this species was included in the genus *Vasates*. The other one is *Aceria lycopersici* (Wolffenstein), much less known and widespread than the previous, although it is widely distributed along the whole Peninsula and the Canary Islands. It can often be confused in field with *Aculops lycopersicii*.

Aculops lycopersicii is a common tomato pest around the world that has increased its damage in many European countries over the past few years. It preferably develops on Solanaceae plants, both protected crops and outdoors, and it is very polyphagous, which is not very common among eriophyids. Besides, unlike other eriophyids, it has free life (it does not live protected in galls or erinea) and it is tolerant to low relative humidity, below 50 %; therefore the biggest damages take place in summer, when the conditions of high temperature and low humidity are more favourable for its development.

Damages are, also, very characteristic. Damage starts in the lower parts of plants, lower leaves curling and acquiring a silver tone before becoming darker, with an appearance like parchment. Populations grow and rise to the upper part of the plant, also affecting top leaves. Stems become brown-coloured and their surfaces crack longitudinally, taking on a characteristic appearance. If damage continues, defoliation that affects fruits production occurs.

Studies of population dynamics carried out in tomato crops of the Ribera region in Navarra, indicate that infestations in this area begin at the end of April or the beginning of May. From this moment on, populations grow up to the highest values at the end of the crop, that is, at the end of July or the beginning of August (García-González, 2003).

Aceria lycopersici is also a polyphagous species that preferably feeds on cultivated and wild Solanaceae. It is a common mite in tropical regions and it can be found in greenhouses of mild temperate areas. It produces hair hypertrophy in tomato stems and leaves, a symptom known as erinea. Mites can be found amongst the hair mass protected from predators and in more favourable weather conditions for their development. Therefore, the symptom produced by this species is clearly different from that of *Aculops* damage. Erinea in stems and leaves provides the plant with a silver and whitish appearance, which is a reason why this mite has been popularly referred to as “ash“.

García-González (2003) has discussed the effect of sulphur treatments on both species. Apparently, *Aculops* and *Aceria* compete whenever they are on the same plants, the first species being a superior competitor and, therefore, more abundant in the crop. Sulphur treatments might alter this relation. *Aceria lycopersici* populations seem to recover from the treatment before those of *Aculops lycopersicii*. They are predominant for a while until *Aculops* populations recover and become a majority again.

3.1. Microscopic separation of Aculops and Aceria

Eriophyids are the smallest known arthropods and, certainly, the smallest mites that live in horticultural crops. Their length varies from 150 to 200 micrometers (0.15 – 0.20 mm). For this reason its detection, recognition and diagnosis is difficult and generally needs a previous preparation and a microscopic observation. Nevertheless, separation between *Aculops* and *Aceria lycopersici* can be done by a simple microscopic preparation between slide and coverslip, and observation with a microscope. Eriophyids have long bodies, covered with transverse rings. In *Aculops lycopersicii* the dorsal and ventral parts of the rings are different. In the dorsal part, the rings (tergites) are thick, so that it has only 27 tergites, while in the ventral part, the rings (sternites) are thin; therefore, it has about 60 sternites (Figure 3). However, in *Aceria lycopersici,* the transverse rings have the same thickness in the dorsal and ventral parts of the body, so that the mite appearance is different (Photo 8).

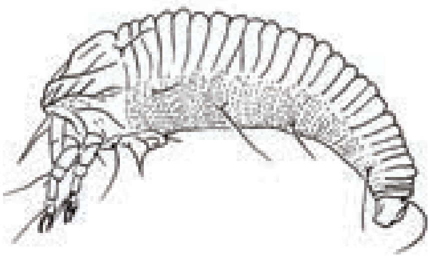


Figure 3. External aspect of the eriophyid *Aculops lycopersici*

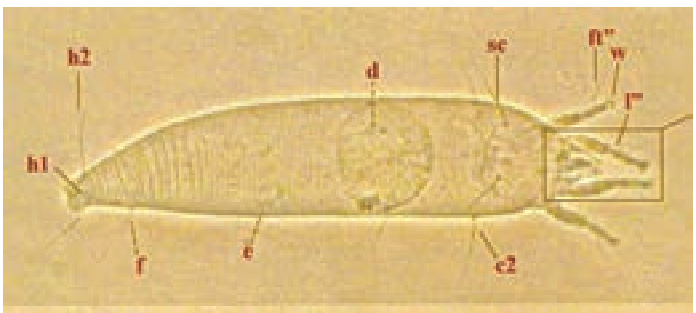


Photo 8.- External aspect of *Aceria lycopersici*. (Photo by María Lourdes Moraza)

3.2. Possibilities of biological control

Some tomato eriophyid predators are known, especially for *Aculops lycopersici*. Nevertheless, no species is available at the moment to be used in commercial greenhouses. Studies have been carried out to know the efficacy of several species of phytoseiids. Some of them feed on the pest, but they cannot complete or reach the adult state, neither can lay eggs when feed on this prey. Only *Neoseiulus fallacis* (Garman) seems to be a good biological control agent with possibilities to offer good results in commercial crops (Zhang, 2003).

In the region of La Ribera (Navarra), *Aculops lycopersicii* is associated to the tydeid mite *Homeopronematus anconai* (Baker), a predatory mite that García-González (2003) considers to be a good biological control agent of pest, although laboratory studies have proved that it cannot complete its development when it exclusively feeds on *Aculops* (Zhang, 2003). Sulphur treatments are very toxic for this predator.

4. Short-term challenges

The basic research regarding pest mites of protected horticultural crops and their predators has advanced a lot in recent years and the results have been evident, with a better knowledge of the behaviour of these natural enemies and their practical effectiveness. However, there still remain some questions that will deserve attention and a special effort in the short-term. These are some of them.

Distribution, nutritive preferences and economic importance of some pests are not yet well known. This is the case with the red spider mite species, since at this moment the host plants of each of them, their crops preferences, or the damages that each of them causes are not known in detail. The extension and economic impact of *T. evansi* is neither well known, although observations carried out up to now suggest that it is not a significant problem in greenhouse crops. Likewise, the presence and importance of *Aceria lycopersici* are not established enough, because it is often unnoticed or it is confused with other tomato eriophyids.

Biological control of mites in tomato plants is far from being a solved question. The tomato, like other Solanaceae, produces toxic compounds to defend itself from phytophagous mites and insects and also has defensive structures in leaves and stems in the form of acute and glandular hairs that produce sticky or poisonous substances. These defensive barriers against phytophagous also affect natural enemies that, under these circumstances, cannot exercise their control function. This is the case with red spider mite predators such as *P. persimilis*, of which there exists a commercialized special race adapted to tomato, or *Amblyseius swirskii* which is very effective in the control of whiteflies and thrips in other crops, but that does not settle in tomatoes. It is necessary to search out other predators that live in Solanaceae and, therefore, that are well adapted to develop in this environment. Biological control of the tomato eriophyids is also a problem which has not been addressed yet since, at this moment, there is no possibility of their control with predators.

Finally, there remains the preparation of an international evaluation protocol on the environmental impact of introducing exotic predatory mites in our crops. This is a demand of European regulations that, in short time, will request the execution of a series of tests that may guarantee that exotic predators, introduced or released in crops, are not going to cause environmental problems; such as depredation on native species or invasion of natural ecosystems, like those that happened recently in North America and Europe with the ladybug *Harmony axyridis* (Pallas) a voracious predator of aphids.

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Chapter 12

Predatory arthropods in agrosystems in almería

*Marta Goula, Luis Mata**

1. Introduction

Almería is the main exporter of extra-early vegetables (Rodríguez-Rodríguez, 1988), which gives the region special characteristics with respect to the crop cycles. Biological control implementation in Almería has been more difficult than in other regions because the main crop, capsicum pepper, of which more than 50 % of the total national crop is produced in Almería (National Statistics Institute, 2009; Andalusian Regional Government, 2009), begins in summer, thus small plants bear big pressures due to pests. Other unfavourable regional elements to be taken into account are the closeness of greenhouses and crop overlapping (Blom, 2008a). Also, biological control implementation depends on the increasing demand of a product free of phytosanitary residues, increasing ineffectiveness of treatments with synthetical chemical products, and improvements in the establishment of technical protocols (Castañé, 2002). These drivers, plus the farmer's wish to adopt more adequate control methods (Sánchez *et al*., 2000), have favoured a change in the pest control strategies of the protected crops regions in south-eastern Spain. Consequently, it is estimated that, in Almería, crops under biological control have reached an average of 36 %, specifically 90 % in capsicum pepper, 27 % in aubergine and 15-20 % in tomato-cucumber (Blom, 2008a); moreover, it is foreseen that biological control implementation will continue to grow in the following years. The Regional Department of Agriculture and Fisheries of the Andalusian Regional Government interest for biological control is clearly manifested on their web site, where, for example, through their Alert and Phytosanitary Information System (RAIF, 2009), information on the phytosanitary state of crops is given, as well as details about the pests that attack them and the most appropriate

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auxiliary agents to implement biological control. For the Almería region, biological control strategies for capsicum pepper, tomato, cucumber, aubergine, green bean, melon and watermelon crops were last summarised by González García (2009).

Aiming at identifying the biodiversity and potential uses of biological control agents, this work compiles the predatory arthropod species present in agroecosystems and crop adjacent habitats in Almería, as well as the vegetal hosts where they live.

2. Predatory arthropods of pests in spain

Predatory arthropods of insects and mites in Spain are reported by Urbaneja *et al*. (2005a) and Jacas *et al*. (2008). According to the latter authors, there are 7 orders and 25 families of insects, and 2 orders and 13 families of arachnids (Jacas *et al*., 2008; table 4.1., page 44). These works give an account of the biological characteristics, biological control strategies and appropriate usage, of these predators. Jacas & Urbaneja (2008) compiled the exotic predators introduced to Spain (table 1.2, page 11), as well as the important groups of predatory invertebrates in agroecosystems in Spain, specifying genera and species (table 2.1, page 17). Likewise, Albajes & Alomar (2008) reflected on the general ecology of natural enemies, particularly aspects applicable to predators, such as biological control at the landscape level (metapopulations and shelters), omnivorous capacity, intraguild predation, and impact risks of introducing natural enemies into agroecosystems.

2.1. Predatory arthropods of pests in Almería

Information to prepare this work was mainly obtained from an inventory of arthropods in agroecosystems in Almería (Rodríguez-Rodríguez, 1988). This works describes useful and harmful arthropods captured in horticultural, ornamental, or adjacent wild habitats. This inventory was completed with the contributions from Urbaneja *et al*. (2001), regarding *Creontiades pallidus* (Rambur); Rodríguez-Rodríguez & Aguilera-Lirola (2002), concerning the “hunter fly” *Coenosia attenuata* Stein; Lara & Urbaneja (2002), in respect of the syrphid and cecidomyiid dipterans

Aphidoletes aphidimidyza (Rondani) and *Episyrphus balteatus* (De Geer), respectively, and the phytoseid mite *Neoseiulus cucumeris* (Oudemans); Rodríguez-Rodríguez & Gómez-Ramos (2005), on the subject of four species of hybotid dipterans; Calvo *et al*. (2006), in the matter of the phytoseiid *Amblyseius swirskii* Athias-Henriot; and González-García (2009) with reference to the predators used in capsicum pepper, tomato, cucumber, aubergine, green bean, melon and watermelon crops.

Furthermore; the authors studied 400 specimens of heteropterans out of 62 samples collected in different municipalities of Almería, as a part of a project developed by CIFA La Mojonera, in which samples were drown every two weeks between November 2003 and December 2006 from commercial plots of horticultural crops (tomato, pepper, aubergine, courgette, cucumber, watermelon, melon and green bean) and their associated adjacent habitats. As a result of this study, the mirids *Dicyphus hyalinipennis* (Burmeister) and *Macrolophus pygmaeus* (Rambur), and the lygaeid *Geocoris melanocephalus* (Rossi) are incorporated to the inventory.

Table 1 synthesizes the information gathered from the above-mentioned studies. It shows the plants hosts where arthropod predators have been reported in Almería. As it can be seen, a total of 34 species or genera have been reported: 7 mites, 3 thysanopterans, 8 heteropterans, 7 dipterans, 2 neuropterans, 7 coleopterans, and one hymenopteran family. Their presence in the crops and relevance for biological control are very different from one species to another. Information is provided below on the 9 predators reported in González-García (2009), which are considered as the most abundant, frequent and useful: the mites *Amblyseius swirskii* Athias-Henriot, *Amblyseius andersoni* (Chant), *Neoseiulus cucumeris* (Oudemans), *Neoseiulus californicus* (McGregor) and *Phytoseiulus persimilis* Athias-Henriot; the heteropterans *Nesidiocoris tenuis* (Reuter) and *Orius laevigatus* (Fieber); the neuropteran *Chrysoperla carnea* (Stephens); and the dipteran *Coenosia attenuata* Stein. However, it must be highlighted that other predators shown in Table 1 are also very important pests controlling agents in other horticultural regions.

Nicoli & Burgio (1997) reviewed the importance of Mediterranean biodiversity, including biological control auxiliary species in protected crops with ranges that extended beyond their native geographic regions. Most of the following comments come from the reviews by Urbaneja *et al*. (2005a) and Jacas *et al*. (2008).

Table 1. Main predatory arthropods in agroecosystems in Almería

			Cheliceromorpha							Hexapoda																											
			Acari							Thysanoptera			Hemiptera								Diptera							Neuroptera		Coleoptera							Hymenoptera
			Argestidae	Phytoseiidae						Aeolothripidae		Thripidae	Anthocoridae		Miridae					Lygaeidae	Cecidomyiidae	Hybotidae				Muscidae	Syrphidae	Chrysopidae		Carabidae			Coccinellidae				Formicidae
			Agistemus cyprius Gonzalez	*Amblyseius andersonii* (Chant)	*Amblyseius swirskii* Athias-Henriot	*Euseius stipulatus* (Athias-Henriot)	*Neoseiulus californicus* (McGregor)	*Neosieulus cucumeris* (Oudemans)	*Phythoseiulus persimilis* Athias-Henriot	*Aeolothrips intermedius* Bagnall	*Aeolothrips tenuicornis* Bagnall	*Scolothrips longicornis* Priesner	*Orius albidipennis* (Reuter)	*Orius laevigatus* (Fieber)	*Creontiades pallidus* (Rambur)	*Deraeocoris serenus* (Douglas & Scott)	*Dicyphus hyalinipennis* (Burmesiter)	*Macrolophus pygmaeus* (Rambur)	*Nesidiocoris tenuis* (Reuter)	*Geocoris megacephalus* (Rossi)	*Aphidoletes aphidimyza* (Rondani)	*Platypalpus cf. annulitarsis* Kovaler	*Platypalpus morgei* Chvála	*Platypalpus ostiorum* (Becker)	*Platypalpus pallidiventris* (Meigen)	*Coenosia atenuata* Stein	*Episyrphus balteatus* (De Geer)	*Chrysoperla carnea* (Stephens)	*Chrysopa formosa* Brauer	*Amara fulva* (Mueller)	*Lionychus albonotatus* (Dejean)	*Syntomus fuscomaculatus* (Motschulsky)	*Coccinella septempunctata* L.	*Hippodamia variegata* (Goeze)	*Scymnus* sp.	*Stethorus* sp.	
Asteraceae	*Chrysanthemum sp.*	Chrysanthemum									1																										
Asteraceae	Several spp.	Thistles									1																										
Caryophyllaceae	*Dianthus sp.*	Carnation											1																								
Caricaceae	*Carica papaya* L.	Pawpaw					1					1																									
Cucurbitaceae	*Citrullus lanatus* (Thunberg.)	Watermelon			7		7		1, 7										7							3, 7		1					1	1			
Cucurbitaceae	*Cucumis melo* L.	Melon			6, 7														7							7			1								1
Cucurbitaceae	*Cucumis sativus* L.	Cucumber			6, 7			7	7				1	7					7																		
Cucurbitaceae	*Cucurbita pepo* L.	Courgette															8		8					5					1								
Cucurbitaceae	*Cucurbita maxima* Duchesne	Marrow																	8																		
Fabaceae	*Phaseolus vulgaris* L.	Green bean			6, 7		1		7				1	1		1, 8						5		5		3		1							1		
Fabaceae	*Pisum sativum* L.	Pea																								3											
Fabaceae	*Vicia faba* L.	Broad bean																					5	5													
Geraniaceae	*Pelargonium zonale* (L.)	Geranium											8																								
Lamiaceae	*Vitex agnus-castus* L.	Chasteberry												8																							
Lamiaceae	*Mentha sp.*	Mint												8						8																	
Malvaceae	*Malva sp.*	Mallow																						5													
Marantaceae	*Calathea sp.*	Calathea	1																																		
Moraceae	*Ficus retusa* L.	Indian laurel					1					1																									
Pittosporaceae	*Pittosporum tobira* (Thunberg)	Japanese pittosporum												8																							
Rosaceae	*Prunus persica* (L.)	Peach tree									1																										
Rosaceae	*Rosa sp.*	Rosebush								1				1														1	1								

2.1.1. Mites

The information given below was compiled from Abad-Moyano *et al*. (2008) and from the Alert and Phytosanitary Information System (RAIF, 2009). The most common and effective species in Almería belong to the family Phytoseiidae, and detailed information can be found in McMurtry & Croft (1997). As occurs with other mites, the differences between species cannot be appreciated at first sight, and require the microscopic study of the female's spermatheca and ventrianal shield. Mites can be polyphagous predators, feeding on tetranychids, thrips eggs and larvae, scale insects, and whiteflies, although some of them are very specific (Jacas *et al*., 2008). They can also feed on nectar, which has an influence on the establishment and continuity of their populations on their host plant. The review made by Gerson & Weintraub (2007) provides detailed information on the main predatory mite species in protected crops, their prey, and the crop where they are found, as well as comments regarding their interaction with other biological control agents and the influence of new technologies.

*Amblyseius swirskii*_Athias-Henriot. It preys on different thrip species, as well as eggs and larvae of *T. vaporariorum* Westwood and *B. tacaci* Gennadius, and the broad mite *Polyphagotarsonemus latus* (Banks) (Blom, 2008b). Its optimum temperature ranges from 25 to 28 ºC, and below 15 ºC it is inactive. It is a very effective biological control agent in different horticultural crops (e.g., aubergine, capsicum pepper, cucumber, melon, green bean) and ornamental crops, where its potential to control *B. tabaci* (Gennadius) (Calvo & Belda, 2007) or mites (Castañé *et al*., 2008) has been demostrated. When used against thrips, its action must be combined with *Orius laevigatus* (Fieber) (Calvo & Belda, 2007), and in respect of whitefly, it is complementary of *Eretmocerus mundus* (Mercet) (Belda & Calvo, 2006). Due to the broad spectrum of its prey, including the thrips *F. occidentalis* (Mercet) (Houten *et al*., 2005), and the different crops where it can live, *A. swirskii* is a totally polyvalent biological control agent (Calvo & Belda, 2007). In addition, as a consequence of its pollenophagy it can be introduced preventively in the crops (Calvo *et al*., 2006).

Amblyseius andersoni (Chant). This mite is a generalist predatory species that feeds, among others, on red spider mite, broad mite, tomato russet mite, thrips, and eriophyids. It has the potential to develop resistance to different pesticides. It can endure starvation and extreme temperatures. Very mobile, it is useful outdoors, as well as in protected greenhouse crops. It is known to feed on pollen. Several vegetal hosts have been documented, including conifers, ornamental crops, fruit trees, horticultural crops, and adventocious vegetation. Houten *et al*. (2005) studied its predatory potential on the thrips *F. occidentalis* Pergande.

Neosieulus cucumeris (Oudemans). Release of this polyphagous mite, in combination with the anthocorid *Orius laevigatus* (Fieber),, controled populations of *Frankliniella occidentalis* Pergande in capsicum pepper (Urbaneja *et al*., 2001; Houten *et al*., 2005; Lacasa-Plasencia *et al*., 2008). Optimal conditions for its development are 18-20 ºC and 50 % humidity. Unlike other species of the genus, it enters in diapause when photoperiods are short. According to Lacasa-Plasencia *et al*. (2008), it preys mainly on hatched eggs and first instar larvae of thrips (e.g., *T. abaci* (Gennadius) and *F. occidentalis*) Pergande, but also on the broad mite *Polyphagotarsonemus latus* (Blom, 2008b).

Neoseiulus californicus (McGregor). It preys on different species of tetranychids, particularly of the *Tetranychus* genus, although it also occasionally feeds on *Frankliniella occidentalis* Pergande and the broad mite *Polyphagotarsonemus latus* (Banks)(Blom, 2008b). It is the most widespread phytoseiid in crops and wild flora in Almería. Due to its potential to feed on pollen, it can be introduced preventively to crops, and be kept on them longer. It tolerates some acaricides and resists warm and dry conditions. It is especially effective in combination with other biological control agents. Prospection of wild habitats would be needed to know *N. californicus* refuges. (la frase en castellano dice “Convendría prospectar detenidamente la flora silvestre para conocer dónde se refugia”).

Phytoseiulus persimilis Athias-Henriot. Native and monophagous species, it preys on the genus *Tetranychus*. Its most favorable conditions are humidity over 60 % and low temperatures ranging between 15 and 25 ºC, being replaced by other phytoseiids when conditions turn drier and warmer. As with *N. californicus* (McGregor)*,* it is frequently found in greenhouse-growned horticultural and ornamental crops (Jacas *et al*., 2008). It reproduces easily, being very voracious and mobile.

Photo 1. *Phytoseiulus persimilis*. Mike Pser (Personal donation)

2.2.2. Heteroptera

In the Mediterranean basin, the biodiversity of agroecosystems favours the presence of mirids, which can be established in crops as well as in wild vegetation, even in winter (Alomar *et al*., 1994). Some species are well documented predators of red spider mite, thrips and whiteflies (Jacas *et al*., 2008).

Nesidiocoris tenuis (Reuter). As a consequence of its zoophytophagous behaviour (Sánchez, 2008), which would depend on spatial (e.g., geographic area) and temporal (e.g., colonisation time) factors (Malausa, 1989; Calvo & Urbaneja, 2003; Vacante & Garzia, 1994), this mirid will act either as a useful or harmful species. Calvo *et al*. (2009) studied the role of *N. tenuis* (Reuter) in the control of *B. tabaci* (Gennadius) in tomato crops in the Murcia region, specifying the optimal predator/prey ratio to avoid crop damage. Sánchez & Lacasa (2008) reported tomato harvest losses when the mirid reached levels of 32.11 accumulated individuals per leaf and its inflicted punctures generated a flower abortion greater than 27 %. This mirid colonizes different crops, but its survival and development on each crop will be different, ultimately depending on prey availability (Urbaneja *et al*., 2005b). Sánchez *et al*. (2009) studied the influence of temperature on the species life cycle, concluding that the optimal range falls between 20 and 30 °C., and pointed out that it is the most thermophile dicyphine mirid of horticultural crops in the Mediterranean region. It can prey on different pests: aleyrodids (e.g., *Trialeurodes vaporariorum* Westwiid and *Bemisia tabaci* (Gennadius)), agromyzids (e.g., *Liriomyza trifolii* (Burguess)), thrips (e.g., *F. occidentalis* Pergande), small aphids (e.g., *Myzus persicae* (Sulzer)), red spider mites, thrips and lepidopteran eggs, as well as the recently introduced tomato borer, *Tuta absoluta* Meyrick (Urbaneja *et al*., 2008). Less frequent feeding behaviours include cannibalism and necrophagy (Wheeler, 2000b). Crop damages generated by this species have been documented

Photo 2. *Nesidiocoris tenuis*. WonGun Kim (Personal donation)

generally in the family Solanaceae (Wheeler, 2000a), and were caused principally by lack of prey, but also by the insect water needs which varies with environmental conditions (Sánchez, 2008).

Orius laevigatus (Fieber). As mentioned by Lattin (2000) and Cabello (2008), this polyphagous anthocorid can feed on mites, thrips, aphids, aleyrodids and noctuids (e.g., *Spodopera exigua* (Hübner)). As with other species of the genus, it can also feed on pollen. However, the number of fruits setting must be managed appropriately to guarantee this pollen supply (Blom, 2008b). Since 1993 it has been produced on a large scale for the control of *F. occidentalis* Pergande (Nicoli & Burgio, 1997), and used in different horticultural crops, as for example, capsicum pepper (Riudavets & Castañé, 1994, Sánchez *et al*., 2000) and cucumber (Riudavets & Castañé, 1994). It is also systematically used in the control of *Thrips tabaci* Lindeman. Sánchez *et al*. (2000) and Lara *et al*. (2002) evaluated the effectiveness of this species in greenhouse capsicum pepper, and compared it with that of *O. albidipennis* (Reuter), concluding that *O. laevigatus* (Fieber) is more effective in the crop's first phenological stages, and that its biological control function can be complemented with that of *O. albidipennis*, a more thermophilous species. On capsicum pepper, the

Photo 3. *Orius sp. Eduardo Mateos (Department of Animal Biology, University of Barcelona)*

mite *N. cucumeris* (Oudemans), which can control initial populations of thrips, can later become prey for *O. laevigatus* (Fieber), making the establishment of the latter much easier, especially in the absence of other prey (Urbaneja *et al*., 2003b). A similar situation occurs with the phytoseiid *A. swirskii* Athias-Henriot (Blom, 2008b). Optimal temperature and relative humidity for predatory activity fluctuates between 20-30 ºC and 45-50 %, respectively. Taxonomic characters to identify *O. laevigatus* (Fieber) can be found in Ferragut & González Zamora (1994). *O. laevigatus* effectively controls thrips when it is present in more than 25 % of the crop flowers (Blom, 2008b).

2.2.3. Neuroptera

Chrysoperla carnea (Stephens). This lacewing is a voracious polyphagous predator that shows effective predation activity between 12 and 35 ºC. It preys on aphids, leafminers, scale insects, thrips, whiteflies, small lepidopterans (including *Spodoptera exigua* (Hübner)) and mites (Jacas *et al*., 2008; Belliure *et al*., 2008; Cabello, 2008). Its three larval stages are very effective aphid predators, and have been shown to respond to pheromones emitted by their prey. The wide range of prey available to this species facilitates its ability to remain upon crops.

Photo 4. *Chrysoperla carnea. Fritz Geller-Grimm (Creative Commons ASA)*

2.2.4. Diptera

Coenosia attenuata Stein. Although being smaller in size, adults of this muscid species are very similar to the common fly. They are effective predators of adult whitefly (e.g., *T. vaporariorum* Westwood and *B. tabaci* (Gennadius)) in both horticultural and ornamental greenhouse protected crops (Castañé *et al*., 2008), where they exert a complementary action to other biological control agents that feed on whiteflies (Rodríguez-Rodríguez, 2002). They also prey on agromyzids (e.g., *Liriomyza* and *Chromatomyia*) and on many other dipteran families (Rodríguez-Rodríguez, 2002) Since 1996 they have been bred massively to be used as greenhouse biological control agents in Germany, where they are combined with the action of predatory hybotids. In Italy, several *Coenosia* species, including *C. attenuata* Stein have been studied for their future implementation (Rodríguez-Rodríguez, 2002). *C.attenuata* Stein lays its eggs on different substrates, its larval stages live on the soil and feed on sciariid flies (Jacas *et al*., 2008), and the adults are found upon the crop's leaves. In the greenhouse *C.attenuata* Stein can remain year around.

Photo 5. *Coenosia attenuata*. Muhammad Mahdi Karim (Creative Commons ASA)

3. Other predators present in agroecosystems in Almería

3.1. Mites

Rodríguez-Rodríguez (1988) reported the phytoseiid *Euseius stipulatus* (Athias-Henriot) and the stigmaeid *Agistemus cyprius* González on orange tree and calathea, respectively. *E. stipulatus* (Athias-Henriot) preys on different mite and insect pests, but can also feed on pollen. It is an important predator on fruit orchards and vineyards (Abad-Moyano *et al*., 2008; Jacas *et al*., 2008). Stigmaeids are less mobile than phytoseiids. Studies on *A. cyprius* González have shown that it can feed on all stages of *Panonychus citri* (McGregor), or on pollen from the ice plant *Malephora crocea* (Jacquin) (Goldarazena *et al*., 2004).

3.2. Thysanoptera

Although thrips are best known for their harmful rather than their beneficial effects on crops, up to 23 documented genera, including several species of *Aeolothrips*, are predators (Trdan *et al*., 2005). Three species are found in Almería. Collected in rosebushes, *Aeolothrips intermedius* Bagnall preys on at least 44 different thrip species (Riudavets, 1995), as well as other small arthropods (e.g., mites, psyllid eggs and larvae, whiteflies and aphids). It also feeds on pollen, and can complete its life cycle exclusively feeding as a pollenophage (Trdan *et al*., 2005). *Aeolothrips tenuicornis* Bagnall, which can also feed as a pollenophage, was found on chrysanthemum, peach tree, orange tree, thistles and adventicious plants. *Scolothrips longicornis* Priesner, known commonly as the "six-spotted thrip" (Lacasa-Plasencia *et al*., 2008) was found on pawpaw and capsicum pepper. The latter species preys, with some degree of specificity, on tetranichid mites in any stage of development (Jacas *et al*., 2008).

3.3. Heteroptera

As described by Lattin (2000) and Cabello (2008), the anthocorid *Orius albidipennis* (Reuter) is a polyphagous predator of aphids, leafhoppers, whiteflies, mites, thrips, and the first stages of moths (including *Spodoptera exigua* (Hübner)). Lara *et al*. (2002) and Urbaneja *et al*. (2003a) reported that, in south-eastern Spain, *O. albidipennis* (Reuter) can have a beneficial role complementary to that of *O. laevigatus* (Fieber), when, for example, the latter does not spontaneously colonize greenhouses or its population decreases as a consequence of high temperatures (Sánchez *et al*., 2000). Taxonomic characters to identify *O. albidipennis* (Reuter) can be found in Ferragut & González Zamora (1994).

In addition to *N. tenuis* (Reuter), another four mirid species were collected. *Deraeocoris serenus* (Douglas & Scott) is a Mediterranean species that, as other species of the same genus (Wheeler, 2000b), has generalist predatory behaviour (Urbaneja *et al*., 2005a). Citation of the eurosiberian species *D. punctulatus* (Fallén) (Rodríguez-Rodríguez, 1988), must be attributed to *D. serenus* (Douglas & Scott). *Dicyphus hyalinipennis* (Burmeister) was collected on Cucurbitaceae, mainly on courgette, but also on tomato. Dormanns-Simon *et al*. (1997) indicate that this species may be potentially useful in Hungary for the control of *Trialeurodes vaporariorum* Westwood and *Helicoverpa armigera* (Hübner), both in greenhouse and outdoor crops. Ceglarska (1999, 2003) studied the biology of *D. hyalinipennis* (Burmeister) and valued its possible use as a biological control agent. *Macrolophus pygmaeus* (Rambur) is a polyphagous predator found in tomato, cucumber, green bean and aubergine (Perdikis & Lykouressis, 1997). It generally preys whiteflies (e.g., *T. vaporariorum* Westwood and *B. tabaci* (Gennadius), but can also survive by feeding on larval and grown thrips, mites, and eggs of aphids or lepidoterans. Since 1992 *M. pygmaeus* (Rambur)has been commercialized for the control of pests in greenhouse protected crops. As showed by Lefant *et al*. (2000), the most suitable conditions for *M. pygmaeus* biopropagation is food suplementation with eggs of *E. kuehniella* Zeller. Unlike other mirids in Mediterranean environments it does not cause economic damage when prey numbers decreases (Nicoli & Burgio, 1997). The difficulty of separating *M. pygmaeus* (Rambur) from *M. melanotoma* (A. Costa) (= *Macrolophus caliginosus* Wagner) with morphological characters (Goula & Alomar, 1994) has led to their study with molecular techniques, which have confirmed the existence of both species (Martínez-Cascales *et al*., 2006). This difficulty has usually leaded to one species being reported as the other, contributing to the lack of consistency among results obtained by different authors. Martínez-Cascales *et al*. (2006) studied the biology, taxonomy and economic importance of *M. pygmaeus* (Rambur), *M. melanotoma* (A. Costa) and *M. costalis* Fieber. Finally, *Creontiades pallidus* (Rambur) was found in greenhouse capsicum pepper crops (Urbaneja *et al*., 2001). It is an omnivorous species that can feed on *B. tabaci* (Gennadius), although it cannot complete its life cycle with a monophagous diet of this aleyrodid. The lack of prey can cause economic damage, for example, by feeding and ovopositing, *C. pallidus* (Rambur) can produce scars in capsicum peppers (Blom, 2008b), which deform fruits. Moreover, it is known to be a minor pest of cotton, sorghum and sweet corn (Wheeler, 2000a).

The lygaeid *Geocoris megacephalus* (Rossi), as all other species of the *Geocoris* genus, is a generalist predator that depends on the host plant for reproduction and development. As noted by Sweet (2000), it can display cannibalistic behaviours. *Geocoris* spp. does not tolerate broad spectrum pesticides.

3.4. Diptera

Throughout agroecosystems in Almería some Hybotidae species have been documented. Rodríguez-Rodríguez & Gómez-Ramos (2005) provided complete information on the subject. To synthesize: (1) the family has predatory habits; (2) larval stages feed on larvae and juveniles of other insects, and as adults they feed on insects and arachnids, and can display cannibalism; (3) in horticultural crops in Almería, two genera have been found, including the species *Platypalpus ostiorum* (Becker), *P. pallidiventris* (Meigen), *P. morgei* (Chvála), *P. cf annulitarsis* Kovalev, and *Crossopalpus* sp., which can be identified following the descriptions and illustrations given by the authors; (4) presence of hyobotids increases when a rational control of pesticides is undertaken, and when the vegetation cover permits them to complete their life cycle, which requires different habitats; (5) the Hybotidae can be used as ecological quality indicators; (6) species of *Platypalpus* prey on *Liriomyza trifolii* (Burguess), *L. bryoniae* (Kaltenbach) and other leafminers of the same genus, as well as dipterans, drosophilids and thrips; and (7) hybotids can potentially exert biological control in greenhouses and outdoors crops because during their whole cycle they are active predators and, as a consequence of their parthenogenetic reproductive behaviour, can reach high population levels. This last characteristic could fuel intensive commercial breeding, which has in fact already been attained for some species.

Lara & Urbanjea (2002) reported the presence of the syrphid *Episyrphus balteatus* (De Geer) in capsicum pepper crops. The adults feed on nectar and fluids, while larvae are very effective preying on aphids, although they do not reject other prey.

Photo 6. *Platypalpus*. sp. Sareto (Creative Commons ASA)

Regarding cecidomyiids, *Aphydoletes aphydimyza* (Rondani)was also found in capsicum pepper crops (Lara & Urbaneja, 2002). This is one of the few cecidomyiids that feed on aphids and other prey. It uses spider webs to mate, and responds positively to the honeydew secreted by aphids. Belliure *et al*. (2008) noted that If there is an excess prey availability, the syrphid kills more aphids than it can actually feed on.

3.5. Neuroptera

Chrysopa formosa Brauer. In Almería, this chrysopid has been found in melon, courgette and rosebush plants, but information about this species in agroecosystems throughout the Mediterranean region is scarce (Duelli, 2001). It has been reported on green bean, broad bead and amaranth crops (Szentkirályi, 2001). As with other chrysopids, it is a well-known predator, including *Spodoptera exigua* (Hübner) among its prey (Cabello, 2008).

3.6. Coleoptera

In general, coccinelids are well-known pest predators that can also feed on pollen and nectar. In Almería, Rodríguez-Rodríguez (1988) found *Coccinella septempunctata* Linnaeus, *Hippodamia variegata* (Goeze) and *Scymnus* sp. to prey on aphids. At least once, *Sthetorus* sp. was recorded on strelitzia. This genus is known for its potential to prey on tetranichids (Chazeau, 1985), although it requires high prey densities to colonize the crop (Urbaneja *et al*., 2008).

Carabids live on the soil, and can be strict or facultative predators. They prey on underground arthropods, and are found very often in larval or pupal stage. In Almería, Rodríguez-Rodríguez (1988) reported *Lyonychus albonotatus* (Dejean) on adventicious plants, *Amara fulva* (Mueller) on horticultural crops, and *Syntomus fuscomaculatus* (Motschulsky), without specifying the vegetal hosts.

Photo 7. *Symnus* sp. Entomart (Green Copyright)

3.7. Hymenoptera

In forest ecosystems, Urbaneja *et al*. (2005a) highlighted the importance of the ant *Formica rufa* Linnaeus for the control of some pests. Rodríguez-Rodríguez (1988) reported the presence of formicids in some agroecosystems in Almería, without specifying the species. Some authors, for example Jacas *et al*. (2008), consider that the protective role of ants over aphids can be harmful.

4. Conclusions

As noted by Blom (2007), biological control is an open and dynamic system where pests and auxiliary predatory agents follow one another in time and space as a function of crops, abiotic conditions and agronomic practices. A broad conclusion of this report is that predatory biological control agents are very diverse throughout agroecosystems in the Almería region. It is important to know this diversity, as it may be the case that some of these species, which presently have a less relevant role, could become significant biological control agents in the future. Of great relevance is the potential for ecological adaptation displayed by some of these species, for example, *A. swirskii* Athias-Henriot, which was first reported mainly in fruit trees, has adapted to horticultural crops, and is having a remarkable positive effect due to its wide range of both preys and host crops. As previously stated by Lefant *et al*. (2000), the possibility of implementing the use of some of these potential biological control agents will still require their successful early establishment on targeted crops, the reduction of pesticide treatments before their release, and a general decrease of implementation costs. The authors agree with Blom (2007) in that future research for the development of biological control strategies could concentrate on selecting adequate predatory species, disentangling the relationships between plants, pests and predators, and conservation of potential biological control agents through nectar producing plants. It is foreseeable that biological control of pests in Almería will continue to increase, extending to a wider variety of crops, and hopefully satisfying the requirements of modern horticulture.

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Chapter 13

The toxicity of pesticides on beneficial arthropods and pollinators

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1. Introduction

The concept of Integrated Pest Management, IPM, was defined 50 years ago (Stern et al., 1959). However, it has not lost its initial elegance and sharpness: the harmonious use of different control methods. The main novelty of the concept was the substitution of pest elimination as final purpose, for population management. That is to say, it is no longer about "cleaning" the crops from the pest, but keeping their populations below the levels economically acceptable (economic damage threshold). This requires monitoring of the variation of populations through different techniques (sampling, traps, etc…), but it has two main advantages: there is a continuous availability of food (pests as prey) to keep populations of natural enemies, and the control pressure does not have to be so high, which permits using pesticides more rationally. Therefore, IPM provides higher compatibility of the biological and chemical control, but at the same time, this point is usually misunderstood. In principle, there are not better or worse options, science and technology mark the opportunity and effectiveness of the appropriate type of control in each case. Some characteristics of the crops and areas influence, to a large extent, the development and evolution of populations of insects and mites, determining the success of a type of control or its failure. The incidence on the cycles and the reproduction of pests, of cultural practices, the characteristics of the varieties used, the crop cycles, among other factors, ensures the control protocol has to be implemented in each area and for each crop.

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IPM, based on biological control, is being used successfully in intensive horticulture in the southeast of Spain. However, neither in all crops, periods, nor areas, is it possible to carry out a pest control through biological control agents alone, being necessary to intervene with insecticide applications on some pests, either throughout the crop or at some key moments.

Furthermore, even in the well-established systems and those which work properly it is possible that some change can destabilize the system. Some new pest, a new virus transmitted by insects or a change of the incidence of some of the secondary or potential pests, could break the balance of the system. In that manner, some selective phytosanitary applications could be used to stabilize it.

For all these reasons, it is essentially important for the future of IPM to deeply understand the compatibility of pesticides with natural enemies and pollinators.

2. Type of effects

The influence of pesticides on the populations of auxiliary insects is referred to as having generic side effects. Auxiliary fauna or beneficial organisms are mainly the natural enemies of pests (predators and parasitoids), and pollinators.

Most of the studies about side effects of pesticides have been focused on their lethal effect, estimating the mortality caused. However, sometimes, the effects of pesticides are not lethal, but can produce sublethal effects. Recently, a revision of the sublethal effects of pesticides on beneficial arthropods was made (Desneux et al., 2007), and these effects have been classified in physiology and behaviour. We will refer later to this revision and the quotations included in it.

Within the effects on the insects' or mites' physiology, we can classify them in terms of effects on general biochemistry and neurophysiology on development, longevity, immunology, fecundity and sexual ratio. Pesticides, mainly insecticides, can affect the general metabolism of insects in a significant way, even when they not being lethal. Likewise, the enzymes produced by the insect to detoxify pesticides can have an effect on other biochemical routes, and can cause physiological malfunctions.

These effects on general biochemistry of insects are difficult to study in isolation. The sublethal effects that insecticides can have on the development, longevity and fecundity are clearer. These effects are usually more marked in the insecticides called bio-rational (IGR, insect growth regulator), than in those called neurotoxic conventional ones.

Another sublethal effect, within the physiological ones, which is more unknown and less studied, is the effect on the immunological capacity of the beneficial organism. Pesticides can increase or depress the immunological capacity of insects, making them more or less sensitive to entomopathogens. This effect can be used to synergize the action of some pathogens of pest insects, through the use of sublethal doses of some pesticides.

Within the other group of sublethal effects, called behaviour, we can highlight the effects on mobility, orientation, feeding, oviposition and learning. The capacity for moving and finding prey is fundamental for the success of biological control in predators as well as parasitoids, especially at low densities of pest. However, the sublethal effect of pesticides is not always negative; it can increase the mobility and the search of prey of natural enemies.

The repellency effects of some pesticides, even at very low doses, can also have important effects on the predatory or parasitic capacity of beneficial organisms. However, this quality can be used to avoid exposing the natural enemies (more mobile) to localised treatments against some pests (less mobile).

3. Measure of effects

A great effort has been made to standardise the assessment assays of evaluation of the pesticide effects on beneficial organisms. This standardisation is very necessary because in some cases the results change a lot depending on the bioassay methodology, and therefore, the results obtained are more stable and repeatable. However, this standardisation has been intended for minimising risks, reflecting the possible “worst cases”. In that manner, the compatibility of the products considered as innocuous is guaranteed, although the products that could be a very useful tool in some cases are rejected, as are not compatible in the “worst case” scenario.

The assay methods have been greatly developed and a basic evaluation outline of compatibility has been obtained, which is quite solid but much too restrictive. The evaluation outline is the following one (Sterk *et al*., 1999):

In a first stage of laboratory, the incidence of the maximum field dose of a product on the most susceptible development stage of the beneficial organism is evaluated. The bioassay methodologies are different, but they mainly consist of the exposure of the organism to the fresh residue of the product on a substrate (leaf, glass, soil, etc…). After an adequate period (generally 24-48 hours) mortality is estimated, and in some cases, the beneficial capacity (eggs laid, parasitism, etc…). The results are classified in one of the following categories: 1 = innocuous (<30 % of mortality), 2 = slightly toxic (30-79 %), 3 = moderately toxic (80-99 %), and 4 = toxic (>99 %).

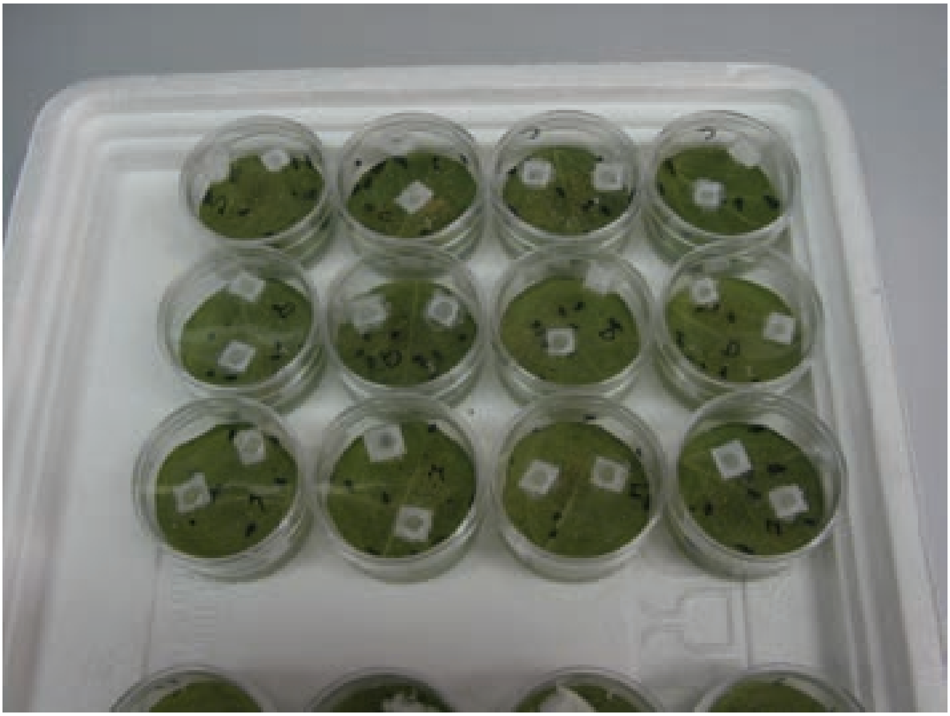


Photo 1. Laboratory bioassay to determine the compatibility of a pesticide with larvae of the coccinelid *Adalia bipunctata*

Given that the possible worst case is studied (maximum field dose and most sensitive stage) and that the exposure to the product in the laboratory is considered as being more constant than in the field, it is estimated that the products classified as 1. innocuous, are going to be perfectly compatible in field, and no more studies are made.

In this first stage of laboratory, studies are also made regarding other less sensitive development stages, or by simulating conditions more similar to the field, or estimating the persistence of the toxic activity of the product. In this last case, the products are classified as: A = non persistent (<5 days), B = slightly persistent (5-15 days), C = moderately persistent (16-30 days), and D = persistent (>30 days). It is supposed that the persistence of the toxic action of the product on the beneficial organism gives information about the risk of use of the product. Furthermore, it is estimated that non persistent products, although initially being toxic, can be used in integrated control of pests. This persistence can indicate to us the safety period between a treatment and the introduction of a natural enemy, or the capacity of recovery of a beneficial organism population after a phytosanitary application.

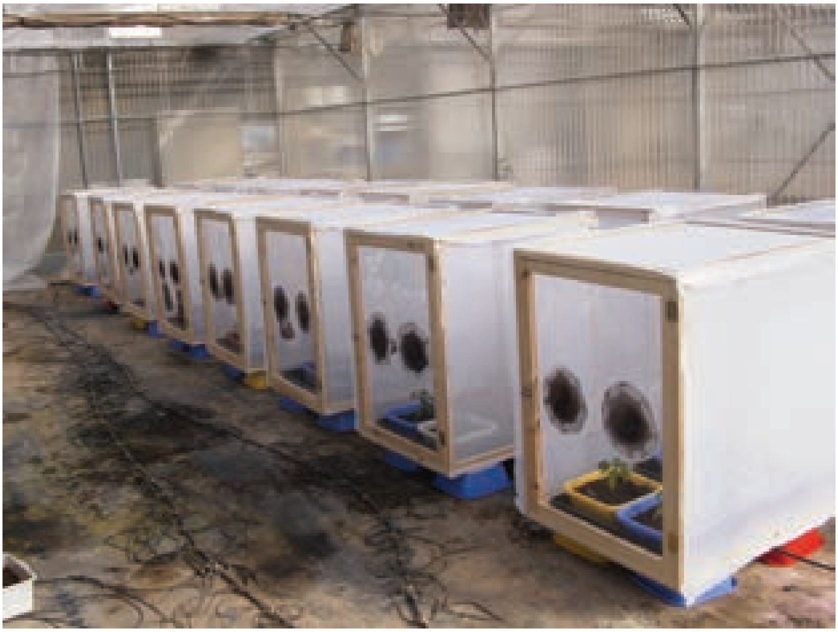


Photo 2. Semi-field assay to evaluate the effect of a pesticide on the parasitoid *Eretmocerus mundus*

In a second stage, semi-field, studies are made while trying to keep the same climatic conditions as in the crop (except for rain), but applying also “the worst case”. In this stage, the risks and other practical information are considered in the most realistic way, but still trying to control some variables, and especially, estimating mortality and the effects on beneficial capacity of organism (oviposition, parasitism, prey consumption, etc…), accurately.

Photo 3. Field assay to evaluate the compatibility of a pesticide with phytoseiids

In the third stage, field, studies are made directly in the crops and are repeated in several places. These studies not only reflect the real conditions in which the phytosanitary product is going to be applied but, mainly, the study of natural populations of beneficial organisms (which are going to be introduced). This last one is an essential difference in the evaluation outline. The differences of susceptibility or tolerance to a toxin that exist between different insect populations of the same species are well-known. This is well studied in pest populations, in different studies about resistances to insecticides, but it also occurs with beneficial organisms.

In the semi-field and field stages, the products are classified according to four categories: 1 = innocuous (<25 % of mortality), 2 = slightly toxic (25-50 %), 3 = moderately toxic (51-75 %), and 4 = toxic (>75 %).

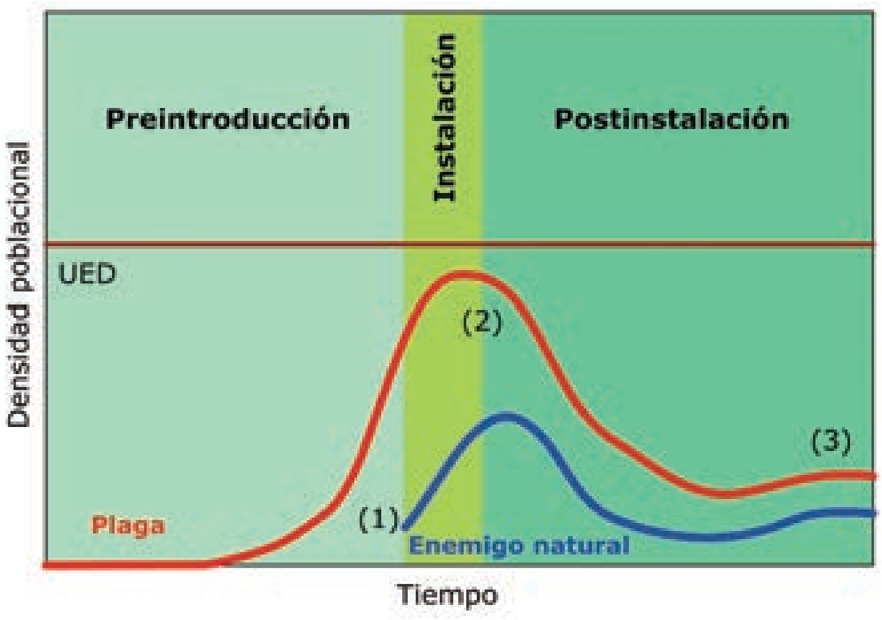
4. Effects on populations

The toxic effects of pesticides have always been considered at an individual level, that is to say, the pesticides are classified as has been described previously, according to the toxicity they provoke (mortality) in the individuals of the beneficial organism involved. However, the integrated management of pests is a question of populations, and therefore, the effect on the population of the beneficial organism is what must be really considered. In fact, the final effect on the beneficial action (control of the pest population or pollination) will determine whether the variation of the population of a natural enemy or pollinator caused by a pesticide is harmful or not.

In biological control, populations of natural enemies are managed by their controlled release or favouring their natural populations, which will control the pest populations through their predatory or parasitic action. Therefore, the biological control consists of the relative dynamics of populations of the beneficial agent and the pest. After all, we are interested in its global controlling action over the pest population; just as in the case of pollinators we are interested in the global pollinating activity on the crop. Consequently, the effect of pesticides on the biological control agents must not be considered at individual level, but at population level. Therefore, it is necessary to distinguish the state of the population of the beneficial agent when a treatment is applied in the field, and the effect of this treatment on the organism population depending on the pest population. Therefore, we can distinguish three different periods:

1. Pre-introduction: Before the introduction of the biological control agent.
2. Establishment: After the introduction of the biological control agent and during its establishment in the crop.
3. Post-establishment: Once the population of the biological control agent is well established in the crop.

Graph 1. Stages of development of biological control



A) Preintroduction. The natural enemy is not present. The pest population is very low. B) Establishment. The pest population begins to increase approaching to the Economic Damage Threshold (EDT), and the release of the natural enemy is carried out (1). C) Post-establishment. The pest population begins to decrease when controlling by the natural enemy (2). The population of the natural enemy is established. The pest population decreases due to the control exerted, and the natural enemy due to the lower population of prey, until the system is balanced (3)

In the pre-introduction stage the beneficial organism is not present, therefore, there is not a direct toxicity of the product due to application. However, we have to consider the persistence of the pesticide applied, that is to say, the period of time that must elapse after the treatment to introduce a beneficial organism, without risk of toxic, lethal or sublethal effects. This safety period, which we will call “period of introduction”, or time we must wait for the introduction after the treatment, will be modified by several factors; in addition to the active matter, also by the doses, the volume of water applied, the formulation of the product, the mode of application, temperature and solar radiation. Although this persistence will be related with the persistence in terms of effectiveness on the pest, and with the persistence or safety period with respect to residues, this is not exactly the same concept. An applied insecticide could be degraded within a given period, so that the residue on the plant might not have enough effectiveness on the pest. However, it could have a toxic, lethal or sublethal effect on a biological control agent if it is more sensitive to that insecticide than the own pest. Therefore, this period of introduction has to be determined specifically for each product and formulation and for each biological control agent and for each pollinator.

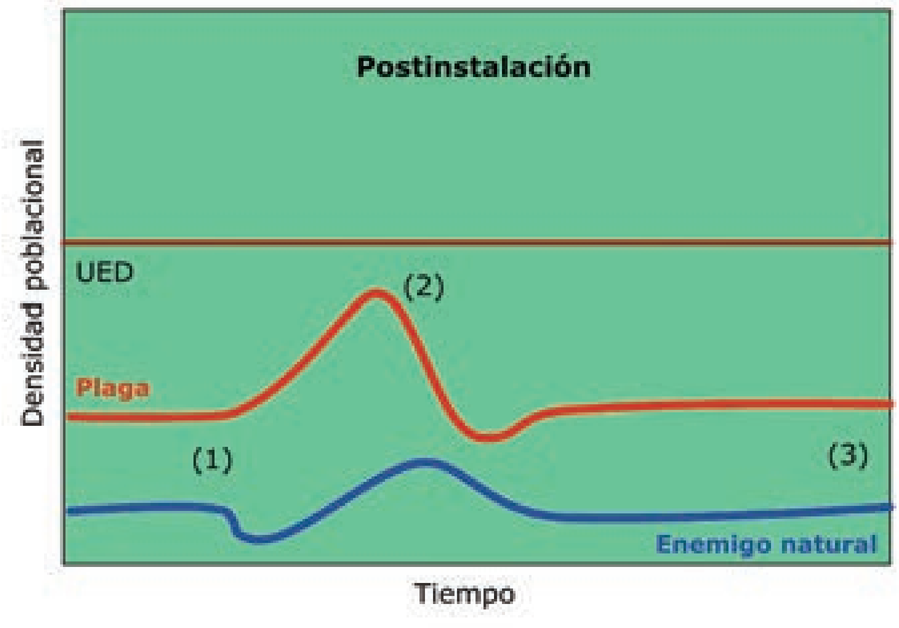
If this period of introduction is observed, some products, which could be very toxic when applying them directly, but have a reduced persistence, could be used. This period of introduction can also be used to estimate the waiting time for a re-introduction when a treatment has been made, or when the beneficial organism is present, and the population has been affected. Also, when a treatment has to be made, this period of introduction can be used when choosing among several products. If all of them have a similar toxicity for the beneficial organism, the product with a lower period of introduction might be chosen, because it will specify the period that the population of the beneficial organism will need to begin its recovery.

The establishment stage is the most delicate, and it is estimated that it lasts from the introduction of the natural enemy until it has been established successfully in the crop. The biological control agent can be considered as established when at least one generation has been reproduced in the crop, and there is enough population to control the pest population. This establishment stage can last several weeks, but the most common period ranges from 4 to 6 weeks.

The success of the release, or introduction, of the natural enemy is seen in the establishment stage. This is the most critical moment, since the population introduced arrives after a period of transport and is released in a different environment to where it has been bred. Therefore, in this stage the population will show signs of weakness, more or less severe depending on the quality of the reproduction process and distribution. Furthermore, the introduced population is not usually enough to control the pest population itself, but the population derived from it, usually in one or two generations, will be capable of keeping the pest populations under control for a considerable period of time. Therefore, it is essential that this establishment on the crop and the consequent reproduction of the population of beneficial organisms should be as effective as possible, which implies better care taken with the chemical treatments that are applied. In this stage the sublethal effects on the natural enemies are especially harmful, because they may negatively affect the reproduction, so that the formation of enough population in the crop can be slowed down. In this establishment stage, small sublethal effects, as a reduction of fecundity (number of eggs) or of fertility (number of emerged larvae), or even an increase of the cycle length from egg to adult, can severely jeopardise the success of the control. We must take into account that in this establishment stage, the population of the biological control agent will not be very high, therefore, small sublethal effects on reproduction or the predation capacity, or search of prey, can significantly reduce the capacity to control the natural enemy.

The post-establishment stage occurs when it is considered that the population of the beneficial organism is established. In this stage, there will be enough population of natural enemy to control the pest, with generations adapted to the crop and the pest, and with a fully expanding population. Therefore, treatments can be tolerated with some mortality percentage, because the reduction of the population of the beneficial organism will not affect the effectiveness of its control. Furthermore, the pest population will be under control, even far below the economic damage threshold, for this reason, probably a small increase of pest population will not have a negative effect on the crop.

Graph 2. In the post-establishment stage

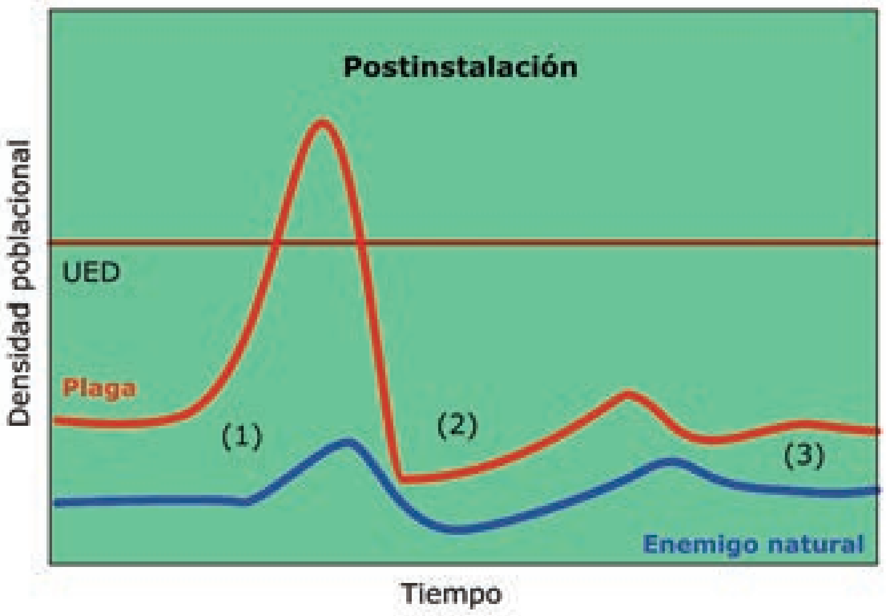


when population has already been installed, a reduction of the natural enemy population can be tolerated (1) due to a treatment with a pesticide, necessary against other pest or disease. The pest population can undergo a recovery, but due to it was under the economic damage threshold (EDT), it does not affect significantly the crop (2). The natural enemy population returns to control pest and the system is balanced again (3).

In this post-establishment stage, when the population is established and the pest controlled, the sublethal effects of pesticides do not have so many repercussions. It is easy to understand that under those conditions and within some limits, the reduction of reproduction or an increase of the cycle length of the beneficial organisms will not have a significant effect on its capacity to control the pest. We must not forget, as it has been already suggested, that the compatibility of pesticides with auxiliary fauna must not be considered in terms of its effect on the reduction of auxiliary populations but its effect on the reduction of its beneficial action, predatory or parasitic capacity for the biological control agents, or pollinating capacity for pollinators. “Action population threshold” can be defined as the minimum population of beneficial organism required to carry out satisfactorily its beneficial action. So the action population threshold of a pollinator will depend on the number of flowers per unit of area, which may vary throughout the crop. The action population threshold of a biological control agent will be the minimum population density to exert its predatory or parasitic action with enough effectiveness to control the population density of pest. Therefore, it will depend on the population density of pest, that is to say, what is really important is the ratio between the population of the natural enemy and the pest population. When pest pressure is high and there are also high pest populations, the action population threshold of the biological control agent will be high. However, with an established population of the beneficial organism and a controlled pest population, this ratio will be over the action population organism. Therefore, a limited decrease of the population of beneficial organisms due to a phytosanitary treatment in this stage will not affect its beneficial action.

Another consideration to be noticed it is that the final effect of all the control methods on the pest must be taken into account. The aim of the integrated control is the maintenance of the pest population below the economic damage threshold. Therefore, the control action must be considered according to its effectiveness on the pest population, and the long-term effects. When a treatment is applied against a pest, its population will be reduced. Therefore, the population of its natural enemy may be also reduced as a consequence of the reduction of the prey population, that is to say, its food source. But the balance between pest (prey or host) and the natural enemy (predator or parasitoid) must be maintained. So a reduction of the population of the natural enemy will not be negative if the proportion with the pest population is kept. So that, when considering the effects of a phytosanitary, intended for a specific pest, on the natural enemy which preys or parasitizes this same pest, it is more important to make sure that the relation between pest/natural enemy is kept than the possible reduction of the population of the natural enemy, whether caused directly by a toxic effect of the pesticide or, indirectly by a reduction of the prey population. In conclusion, the final aim should be a good control over the pest population, maintaining a decent level of population of the natural enemy, that is to say, keeping the action population threshold.

Graph 3. In the post-establishment stage



A recovery of the pest due to different causes can be shown (pest immigration, natural enemy emigration, reduction of population, etc…), reaching the economic damage threshold (EDT). A phytosanitary treatment can solve the problem reducing the pest population (2). The resulting reduction of population of natural enemy, because of the decrease of food source, is not a problem if the relation pest/ natural enemy is kept, balancing the system again (3).

As can be deduced from the previous paragraphs, the compatibility between the pesticides and the natural enemies cannot be reduced to some tables about their intrinsic toxic properties, independently from the effect on the balance between the populations of pest and natural enemy. Therefore, better quality information must be demanded, insisting on the global effects in the field on the effectiveness of the effective control of the pest. The compatibility of the products must be known according to the periods through the development of the biological control goes, that is to say, their effects in the pre-introduction, establishment and post-establishment stages.

5. Proactive compatibility

As has been already discussed, it is extremely important for the future of IPM to study in depth the compatibility of pesticides with natural enemies and pollinators. But this study must not be restricted to a descriptive knowledge of the incidence of the different pesticides and formulations on the populations of each species of auxiliary fauna and its beneficial action. If we remain in this passive position, we will be moving away from the technological development that modern agriculture demands. Science and technology must respond to the challenge of achieving agriculture that is respectful to nature, economically viable and supplies safe and healthy food. Science and technology must work together to actively seek an effective PM in all the crops and areas, and this implies the development of techniques and methods that make pesticides more compatible with natural enemies and pollinators.

As we have mentioned before, the integrated control of pests cannot be reduced to a description of the intrinsic toxicity of pesticides over the individuals of beneficial organisms. In previous paragraphs, the basis to pass from the simple study of the mortality caused to the most complete and practical study of population effects has been explained, the latter including the lethal and sublethal effects, and the effects on the beneficial action. The following challenge is to study how dealing with an active compatibility, through techniques that allow a more versatile use of auxiliary fauna as well as pesticides. In other words, a proactive attitude to achieve higher possibility of use of pesticides compatible with natural enemies, which will benefit a higher possibility of application of biological control, spreading it to crops or areas with implementation problems. Furthermore, in this way, the action protocols will be more balanced and stable, as allow biological control protocols less vulnerable to changes of the incidence of pests and diseases, or to changes of productive structures.

The active compatibility can be achieved through different strategies, using pesticides as well as beneficial organisms. Within the strategies of use of pesticides, the tactic of applying the treatments via an irrigation system is already used, and is known as chemigation (because it is similar to fertirrigation). As the treatment is not applied on the crop, the natural enemies are not exposed to a direct contact with the pesticide, neither directly during the application, nor as a result of remaining residue on the plants. To a large extent, this decreases the exposure of beneficial organisms to the pesticide. In the case of systemic pesticides, this mode of application could be carried out for the control of many pests, decreasing its incidence on many natural enemies. For example, while the use of imidacloprid in spraying can be very toxic, if it is applied by drip irrigation it is totally compatible with bumblebees in tomato crops (*Bombus terrestris*) (Bielza et al., 2001, 2005). However, the systemic products could keep affecting some natural enemies. Some predators, like most of the predatory hemipterans (anthocorids such as *Orius*, and mirids as *Macrolophus*) usually feed on plants, or, at least, suck their fluid, so that a systemic product applied on the soil could also affect them. Likewise, indirectly, the acquisition of the insecticide by the pest when feeding on the plant, could affect the natural enemy which preys or parasitizes it.

Chemigation could also be used with non-systemic products, but on pests that have some development cycles on the soil, such as thrips or some lepidopterans (many noctuids as *Spodoptera* or *Helicoverpa*, or from other families as *Tuta absoluta*). This is the case of thrips *Frankliniella occidentalis*, which undergo the last nymphal stages on the soil. Some products applied by irrigation, significantly reduce the emergence of adults, which contributes to a reduction in its population. Maybe, this type of treatment does not achieve a high effectiveness, but as it is compatible with natural enemies, it can be an excellent control complement. An additional possibility to come out of the active search for compatibility, would be to add a pest attractant to some insecticides that are applied by irrigation, so that it may affect the pest more than the natural enemy or pollinator.

Another strategy to reinforce the compatibility of pesticides could be the use of the repellent effect of some products to move the natural enemies away from the treated area. In such a way, if a localized treatment has to be applied to control a little mobile pest, very low doses of a repellent product could be added, which prevents or reduces contact with the toxic substance by the natural enemies or more mobile pollinators.

A classic strategy, which would be advisable to use again, is the use of baits that attract differentially pests and natural enemies. Such a method would lead to the effect on pests being maximized and while minimizing the effect on the beneficial organism.

The treatments applied by crop areas, or focused expressly on some parts of the plant, could have a significant effect on the reduction of the side effects of pesticides.

The side effects of some formulations are not caused directly by the active matter, but by solvents, co-adjuvants, etc. So, new formulations that reduce or remove these effects could be developed.

Among the management strategies of the beneficial organisms to seek the active compatibility with pesticides, auxiliary populations which were more tolerant to pesticides could be established. Populations of natural enemies have been described with a susceptibility to pesticides significantly lower (Hoy, 2003). If we use the technology in agriculture, turning to plant improvement to achieve varieties with better characteristics, it is admissible to think about the improvement of natural enemies seeking better qualities, among them, a higher resistance to pesticides. This improvement, which could be developed through classic systems, or through genetic engineering, could achieve a significant extension of the application of biological control (Hoy, 2003). The studies about resistance to insecticides in the pests give us the scientific bases to carry out this adaptation of the auxiliary fauna towards the compatibility with pesticides.

In some cases, this genetic improvement is not possible, or can cause environmental problems. In these situations, the strategy of induced resistance can be used. This is a well-known phenomenon in the resistance to toxic substances, some compounds induce the production of detoxification enzymes. This overproduction of enzymes will cause an induced resistance to the toxic substances which will be applied later. This induced resistance may be more or less lasting, depending on the exposure time, development stage, involved enzymes, etc. So, in some specific situations where a release of natural enemies would fail due to an exposure to pesticide residues or external pollution, the introduction of natural enemies with induced resistance could mean the difference between success and failure.

6. Effects on groups

6.1. Phytoseiids

The predatory phytoseiids, like *Amblyseius californicus*, *A. cucumeris*, *A. swirskii* and *Phytoseiulus persimilis*, are, in general, very susceptible to pyrethroids. Likewise, the carbamates and organophosphates are very toxic for these beneficial mites. However, some carbamates, such as pyrimicarb, show a better compatibility, like some phosphates as chlorpyrifos-methyl. The neonicotinoids (imidacloprid, acetamiprid, thiamethoxam, thiacloprid) have a different compatibility with phytoseiids, with some products, such as thiacloprid and thiamethoxam, being more compatible than others. However, their compatibility improves substantially when applications are made on the soil, seeking their systemic effect.

The growth regulator insecticides, such as benzoylureas (diflubenzuron, hexaflumuron, lufenuron, etc…), or the inhibitors of the chitin synthesis (buprofecin), or those similar to the juvenile hormone (pyriproxyfen), or products with a multiple mode of action like the azadirachtin, have, in general, a good compatibility with phytoseiid mites.

New groups of insecticides are coming on to the market like the products derived from tetronic acids (spiromesifen) which also have good compatibility with phytoseiids.

Within the acaricides, although it could seem otherwise, there are some products with good compatibility with the phytoseiid mites, like the hexythiazox or tetradiphon, among others.

6.2. Predatory insects

Within this group we will include the main predators, such as *Chrysoperla carnea*, the coccinelids (*Coccinella septempunctata*, *Harmonia axyridis*), and the hemipterans (*Macrolophus caliginosus*, *Orius laevigatus*).

In general, the pyrethroids, as well as the organophosphorates and carbamates show a high direct toxicity on most predators. Even the pyrimicarb, though less toxic than the rest, is more toxic for predatory insects than predatory mites.

The neonicotinoids are also toxic for this group of predatory insects, however their persitance is lower. The applications of these products on the soil are more compatible, although we must be careful with the predatory hemipterans (*Macrolophus*, *Orius*) because they suck the sap of the plants to a larger or lesser extent.

The growth regulators insecticides and similar ones, due to their own mode of action, show, in general, a good compatibility with adults but this is worse with the larval or nymphal stages.

The new products that arrive to the market show excellent compatibility properties, such as the spiromesifen, with a good compatibility with *Orius* (Bielza et al., 2009).

The acaricides are very compatible with the predatory insects, except for those with a known insecticide action, like the pyridaben, which show a lower compatibility.

Parasitoids

Within the group of parasitoids, we consider hymenopterans such as *Aphidius colemani*, *Diglyphus isaea*, *Eretmocerus mundos, Encarsia formosa*, or *Trichogramma brassicae*.

As in the previous groups of natural enemies, the pyrethroids, organophosphorates and carbamates are very toxic for these insects, except for the carbamate pyrimicarb, which is a well-knwon aphicide, and it is especially compatible with the aphid parasitoid *A. colemani*.

The neonicotinoids have a toxic effect on the parasitoids, although there are differences between them. For example, the thiamethoxam is less toxic than the imidacloprid for the ectoparasitoid *D. isaea*. However, all the neonicotinoids applied on the soil are more compatible with parasitoids.

The growth regulator insecticides are very compatible with parasitoids, except for diafentiuron, which is toxic for some of them.

Among the acaricides, only those with immediate action, like the piridaben, can have significant toxic effects on parasitoids.

As the beneficial organisms, one of the main characteristics of the new products which are developed is a low toxicity for the auxiliary fauna, and, specifically for parasitoids. For example, the spiromesifen is very compatible with *Eretmocerus mundus* (Bielza et al., 2009). This is a typical example of the importance of keeping the proportion between the pest and the natural enemy, this is more important than keeping the parasitoid population. Due to the effectiveness of the treatment with spiromesifen on whitefly, the population of the parasitoid can be reduced because the food source decreases. However, the parasitisim percentage is not affected, keeping the level of beneficial action and making sustainable the effectiveness of the insecticide treatment and its compatibility with the natural enemy across time.

7. Reports about effects

As has already been discussed, the lethal and sublethal effects must be defined for each species of beneficial organism and for each pesticide. In fact, different formulations of the same pesticide can have very different effects on the populations of natural enemies and pollinators; therefore, each commercial formulation has to be studied. In addition to what has been already explained, we must stress the importance of considering the effects on the beneficial action, that is to say, on the relation between the pest population and the natural enemy population, more than the intrinsic toxicity of each pesticide on the individuals. For all these reasons, a detailed list of the pesticide effects on the different species of beneficial organisms is outside of the scope of this chapter.

As a source of information about the side effects, some websites are provided:

- Biobest Biological Systems. http://www.biobest.be/v1/sp/index.htm
- Koppert Biological Systems. http://efectos-secundarios.koppert.nl/

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Chapter 14

Soil bio-disinfection for mycosis control of edaphic origin, “fatigue” correction and effect on the chemical-physical properties of the soil

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1. Introduction

The purpose of this chapter is to show that biodisinfection can be a remedy to control some fungal soil pathogens, which directly affect final crop yield, acting as limiting factors. These problems, as it is specified in the title, can be grouped into different sections: a) “Fatigue” or “tired soils” cases, b) Satisfactory control of two very different groups of diseases: Root and neck rot caused by *Phytophthora capsici* on pepper, and the carnation Fusarium wilt whose causal agent is *Fusarium oxysporum* f. sp. *dianthi*, c). Improvement physical-chemical soil properties favoured by biodisinfection using fresh organic matter.

Just a few years ago, it was unbelievable to think of a soil disinfection procedure that served for so many different things. However, the Montreal Protocol has led to the removal of methyl bromide soil fumigant. This removal was accepted by practically all the countries of the World. This action was taken because of the proven role of bromine in the destruction of the lower stratosphere of the ozone layer, combined with the health problems such a situation implies (cataracts, melanomas, etc.). Developed countries removed the use of the fumigant in 2005, with the remainder obligated to remove it by January of 2015. The widespread use of this biocide was not a mere coincidence. Its use was due to the fact that it was shown to be the best chemical soil disinfectant during almost 50 years of general application, antimicrobial capacity allowing it to extend its benefits to ships´ holds, barns, museums, etc.

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Spain was one of the pioneering countries in trying out alternatives to substitute the methyl bromide thanks to a national research project, which gathered a significant number of experts, which has been operating for 14 years. This chapter is the consequence of some of the tasks developed in our country and the experience some of the tasks' authors acquired in different countries of the world.

In order to provide a clear text it is convenient to explain the biodisinfection term, which is starting to be accepted in lots of technical and scientific works: different types of organic matters (harvest remains, manures that were not properly prepared and other vegetable residues) that, once they are decomposed in the soil, release toxic molecules for arthropods, nematodes, fungi, bacteria, virus. The molecules mentioned can have a double effect: on one hand on the pathogen microorganisms and, on the other, fostering antagonists' microbian populations.

Within that concept of biodisinfection, two techniques have been developed, on one side biofumigation and on the other biosolarization. They differ in the way solarization is applied. While in biofumigation the compost of only slightly decomposed organic matter is buried in presence of permanent humidity to the capacity of the field, in biosolarization such a compost is accompanied by 4 weeks of solarization. There are some variants. Thus, as there was not a strong solar radiation, the soil has been covered with plastic (transparent or opaque), or it has been paddled with crop residues and the effect has been comparable to a biosolarization, in which, as it is well known, the solarization disinfection effect is added.

We have to recognize an added value to these techniques, apart from its proved efficiency: its use without intermediaries. Anyone can practice these techniques and if the farmer is well organized, there is no cost at all. But, at the same time, these techniques are environmentally friendly as they clean the field of any crop residues and avoid a powerful source of pathogenic inocula for new, old, owned and neighbouring plantations. As far as we know, the diseased plants used for biodisinfect do not contain any pathogens as they have been removed during the process in the soil.

2. Correction of soil fatigue

Not many years ago, TELLO and LACASA (2004) published a historical retrospective (1979-1985 sexenial) about their experiments to control *Phytophthora capsici*, a causing agent of the pepper tristeza. The conclusions convene with the intent this section was written for, and they could be summarized in the following way: Although none of the evaluated disinfection treatments allowed a complete control of the parasite, they recommended the use of methyl bromide to increase production in a considerable manner. This effect is known among farmers and technicians from the area as the “bromide effect”. That relation between the soil disinfection and the production increase has been highlighted in the Murcian pepper harvests by Dr. Lacasa’s team. The assessment of the production decrease in a greenhouse dedicated to just a single crop for 12 years, with no soil diseases that justified disinfection, threw up values higher than 60 %. Comparatively, when the harvest was assessed in a greenhouse dedicated to just one crop for 2 years that decrease was about 25 %. When the soil was disinfected with methyl bromide the production increased in the proportions measured for the losses. The “bromide effect” was indeed a way to mask the soil “fatigue”. The phenomenon was reproduced under controlled conditions, achieving some really interesting results: 1) the fatigue phenomenon was specific for the pepper crop and it seemed to have a microbiological origin, 2) when celery, onion and lettuce was harvested in those soils, the fatigue phenomenon did not appear, and 3) the celery, onion and lettuce plants were less strong and productive when the soil was disinfected with methyl bromide or with water steam (autoclave, 120 °C, 30 min.).

In that same sense, the results obtained in the trials can be interpreted to look for alternatives to the methyl bromide use in the strawberry crops in Huelva.

Table 1 shows the results relating to different fumigants used in soils cropped with strawberries. We must remember that authors declare that during the years of experimentation, plantations had an enviable health without soil pathogens that needed to be controlled (*Phytophthora cactorum, Verticillium dahliae, Colletotrichum acutatum* and nematodes).

Table 1. Total accumulated yield (g•plant-1) related to large strawberry plants, as response to different techniques of soil disinfection

Treatments tested	Accumulated yield			
	Plot Occifresa		Plot Cumbres Malvinas	
	Total	Relative (*)	Total	Relative (*)
Telopic VIF	1081 a	104,3 a	1038 a	97,0 a
BM-pic (50-50)	1036 ab	100 ab	1070 a	100,0 a
Chloropicrin VIF	1008 ab	97,3 ab	1068 a	99,8 a
BM-pic (33-67) VIF	1009 ab	97,4 ab	1062 a	99,3 a
Dazomet-dir VIF	965 ab	93,2 ab	1084 a	101,3 a
Dazomet rot VIF	994 ab	96 ab	1034 a	96,6 a
DMSD VIF	930 abcd	89,7 abcd	1071 a	100,1 a
Propozone	916 bcd	88,4 bcd	1055 a	98,6 a
DMSD PE	812 cd	78,3 cd	986 a	92,2 a
Control test	791 d	76,4 d	989 a	92,4 a

* The 100 to compare is the generalised treatment in the area (BM-pic(50-50))
PE=polyethylene; VIF= virtually impermeable film.
Numbers with the same letter in each cell are not significantly different (P>0,05)

Source: López-Aranda *et al*.,2004.

If there are no parasites of the strawberry crop, it is evident that the production increase observed after the use of some fumigants could be explained by the fatigue phenomenon. This fatigue was also evidenced for the same crop in the same zone, during the 2001-2002 and 2002-2003 seasons, by CASTILLO and LÓPEZ BELLIDO (2003). This topic was widely reported by TELLO *et al.* (2006). The correction of "tired soils" phenomenon through soil disinfection has been proposed by CEBOLLA ROSELL and MAROTO BORREGO (2004).

The fatigue soil phenomenon has been corrected by the use of bio-fumigation and biosolarization in pepper crops in the region of Murcia.

Essays based on a plant pathology point of view provide very little information, about this fatigue soil phenomenon. This deficiency is also appreciated in periodic publications. Only the 3rd Meeting of French Association of Phytopathology celebrated 30 years ago could be consulted. The title was: "*La fatigue des sols. Diagnostic de la fertilité dans les systémes culturaux".* It took place in Versailles (France) in 1982. However, the experts that met there did not manage to provide a unique definition of the phenomenon. Maybe the most accepted definition was provided by BOUHOT as follows: "*soils fertility disturbance is due to multiple causes that can be accumulative, successive and simultaneous causes in the field"*. In relation to tired soils BOUHOT stayed: *Nowadays, the farmer observes that he does not get the yield he expected and nobody knows why.*

Some fatigue cases have been reported for different crops: sugar beet, wheat, asparagus, strawberry, tomato, celery, cauliflower, escarole, parsley, artichoke, scorzonera or Spanish salsify, potato, apple trees and citrus trees.

Three types of fatigue have been described:

1. Physical fatigue due to a bad or defective soil structure.
2. Chemical fatigue due to a phytotoxin.
3. Microbiologic fatigue due to "paratisism of weakness".

Soil bio-disinfection acts by correcting these three types of fatigue as it acts on the microbiological fraction and on the physical-chemical properties of the soil.

3. Disease control of soil origin

Telluric, soil or edaphic diseases produced by fungi are limited, among other procedures, through soil disinfection, normally using chemical fumigants. This section includes data relating to two well-known pathogens occurring in under plastic intensive crops. The first model refers to the carnation Fusarium wilt, whose causal agent is the fungus called *Fusarium oxysporum* f. sp. *dianthi.* This can be a model for other Fusarium wilts. The second model refers to the oomycete pathosystem (for some experts this is not included any longer in the fungus kingdom), *Phytophthora capsici* in pepper crops. This model can be useful for other models where the phycomycetes causes root rot causing the infected seedlings to collapse and eventually die.

3.1. Control of carnation fusarium wilt

The model taken for study has been developed in the carnation crops for flowers cut in greenhouses on the northwest coast of the Cadiz province, where the main concentration of this crop appears (around 700 hectare) in our country and takes place in the municipalities of Sanlúcar de Barrameda, Chipiona and Rota. The assessments that were recently carried out by GARCÍA RUIZ (2008) showed that the mycosis appeared in all the tested greenhouses (6 % of the total of harvested area). The totality of greenhouses agricultural holdings were disinfected before planting by using chemicals fumigants (methyl bromide, metam sodium and 1-3 dichloropropene) and, among them, the methyl bromide was applied on 28.97 % of greenhouses. A total of 94 varieties of carnation are used, 44 had the maximum level of resistance to pathogens and, in all of them, diseased plants were found. These results are comparable with the ones submitted by TELLO MARQUINA and LACASA PLASENCIA (1990) for the south of Spain and by ANDRÉS ARES (1995) for Galicia. To sum up in a few words, carnation Fusarium wilt is a disease whose control is difficult and imperfect when using varietal resistance and soil chemical fumigation in a combined way. This control is even more imperfect if we take into account that in order to obtain an acceptable yield it is required to keep it in the same soil for at least two years.

Soil disinfection comparison with chemical fumigants and with different decomposed organic materials appears in table 2, six hundred and forty one days after the plantation took place, that is to say, once the crop was practically finished.

Table 2 allows some clarifications. Plant material used were slightly decomposed when they were added to the soil. So, for the rest of the chrysanthemum crop, the C/N proportion was 31.78, while for carnation the C/N value was 30.71. It is necessary to take into account that the remains of the carnation plants had a minimum of 30 % of plants affected with Fusarium wilt. The antagonist *Trichoderma asperellum* (stump T34) was added with $24 \cdot 10^9$ UFC per test plot before planting and, in 5 cases, at the base of the plants during the two years that the crop lasted. Finally, solarization was applied for 4 weeks.

Results show that the best treatment was the combination of chrysanthemum and carnation crop remains with hen manure and solarization (CL+CR+HEN+BIOS) which was, with respect to mycosis control, more effective than methyl bromide. It is necessary to clarify two aspects about this treatment: 1) The difference is considerable if we compare this treatment with that used with the same ingredients, but without solarization (it was a biofumigation) coded as CL+CR+HEN+BIOF. We should consider that solarization determines efficiency; nevertheless, soil temperatures did not significantly differ from air measurements, so it could be suggested that “retention” of fungicide molecules was the reason for the difference. This observation can be seen in Figure 1 and Table 3 showing air and soil temperatures.

Table 2. Severity of carnation Fusarium wilt and production of flower stems 641 days after transplanting. (Season 2004-2006)

	641 Days after planting			
Treatment	**% Diseased/dead plants**	**Pt (Stems•m-2)**	**Pc (Stems•m-2)**	**Pnc (Stems•m-2)**
Control test	86,46 a	252,18 a	213,73 a	38,46 a
Alp+biof	50,00 ab	299,53 a	277,89 a	21,64 ab
Alp+bios	54,17 ab	313,83 a	284,65 a	29,18 ab
Cl+cr+biof	100,00 a	214,50 a	198,46 a	16,04 b
Cl+cr+bios	89,58 a	276,53 a	255,86 a	20,68 ab
Cl+cr+hen+biof	98,96 a	229,38 a	206,77 a	22,61 ab
Cl+cr+hen+bios	13,54 b	370,26 a	351,90 a	18,36 b
Dicl+cl	54,17 ab	327,16 a	295,47 a	31,69 ab
Dicl+cl+t34	85,42 a	330,45 a	310,16 a	20,29 ab
Metam sodium+sola	72,92 a	313,06 a	286,97 a	26,09 ab
Metam sodium+sola+t34	70,83 a	255,08 a	226,10 a	28,99 ab
Bm	47,92 ab	334,12 a	320,01 a	14,11 b
P-value	0,0002	0,0561	0,0671	0,0044

The averages followed by different letters differ significantly ($P < 0,05$), variance analysis followed by the Tukey test for homogeneous groups. The variance analysis has been made with the data transformed of the arcsine of the square root of the percentage of diseased plants in parts per unit. % diseased plants and/or dead: they express the seriousness of the disease progress of the different disinfection treatments since planting to the end of the second season. AL+BIOF: alperujo compost (12kg:m-2) + biofumigation; ALP+BIOS: alperujo compost (12kg•m-2) + biosolarization; CL+CR+BIOF: carnation compost + chrysanthemum (12kg•m-2) + biofumigation; CL+CR+BIOS: carnation compost + chrysanthemum (12kg•m-2) + biosolarization; CL+CR+HEN+BIOF: carnation compost + chrysanthemum (5Kg•m-2) + hen manure (5Kg•m-2) + biofumigation; CL+CR+HEN+BIOS: carnation compost + chrysanthemum (5Kg•m-2) + hen manure (5Kg•m-2) + biosolarization; DICL+CL: dichloropropene + chloropicrin (50g•m-2); DICL+CL+T34: dichloropropene + chloropicrin (50g•m-2) + Trichoderma T34; MET+SOL: metam sodium (120cc•m-2) + 4 solarization weeks; MET+SOL+T34: metam sodium (120cc•m-2) + 4 solarization weeks + Trichoderma T34; TEST: control test without treatment; BM: methyl bromide (30g•m-2). PC: commercial production nº stems•m 2 ; PNC: non-commercial production no. stems•m 2 . PT= PC+PNC.

2) The treatment that seems to be needed in the process is hen manure. This is what the results show when this ingredient is the only one that is missing in the treatment, coded as CL+CR+BIOS.

Table 3. Soil temperatures (ºC) taken at the same sunlight hour at 20 cm depth, leaving days intervals during the 31 days that solarization lasted

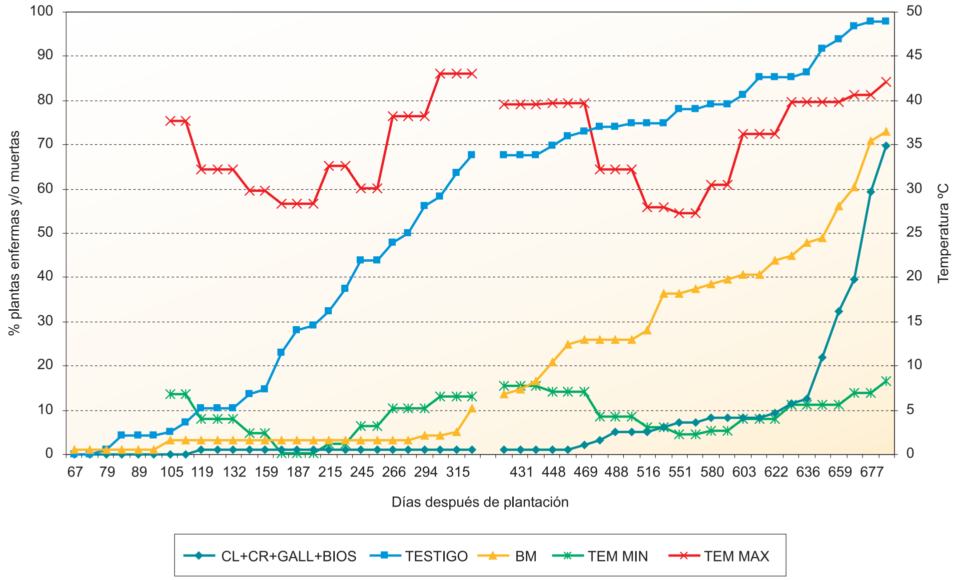
Treatment	Days after disinfection								
	3	8	12	15	20	25	29	31	Average
Alp+biof	21,7 b	22,9 b	22,9 a	25,6 b	32,0 b	32,9 b	28,4 a	28,5 b	26,9 a
Alp+bios	23,0 a	23,6 ab	23,6 a	28,1 a	36,8 a	38,7 a	31,7 a	30,8 a	29,5 a
Cl+cr+biof	21,7 b	23,0 b	23,0 a	25,6 b	31,8 b	32,4 b	28,0 a	27,8 b	26,7 a
Cl+cr+bios	23,3 a	23,8 ab	23,8 a	28,5 a	36,8 a	38,7 a	28,9 a	31,3 a	29,4 a
Cl+cr+gall+biof	23,5 a	23,6 ab	23,6 a	26,1 b	32,6 b	33,2 b	28,4 a	28,1 b	27,4 a
Cl+cr+gall+bios	23,2 a	24,3 a	23,8 a	28,9 a	37,3 a	39,2 a	32,5 a	32,1 a	30,2 a
P-value	0,0001	0,0035	0,0439	0,0000	0,0000	0,0000	0,0333	0,0000	0,01

The averages followed by different letters differ significantly ($P < 0,05$), variance analysis followed by the Tukey test for homogeneous groups. The data show the temperature registered during the disinfection process of the treatments with organic matter supply. AL+BIOF: alperujo compost (12Kg•m-2) + biofumigation; ALP+BIOS: alperujo compost (12Kg•m-2) + bisolarization; CL+CR+BIOF: carnation compost + chrysanthemum (12Kg•m-2) + biofumigation; CL+CR+BIOS: carnation compost + chrysanthemum (12Kg•m-2) + biosolarization; CL+CR+HEN+BIOF: carnation compost + chrysanthemum (5Kg•m-2) + hen manure (5Kg/m2) + biofumigation; CL+CR+HEN+BIOS: carnation compost + chrysanthemum (5Kg•m-2) + hen manure (5Kg•m-2) + biosolarization; DICL+CL: dichloropropene + chloropicrin (50g•m-2); DICL+CL+T34: dichloropropene + chloropicrin (50g•m-2) + Trichoderma T34; MET+SOL: metam sodium (120cc•m-2) + 4 solarization weeks; MET+SOL+T34: metam sodium (120cc•m-2) + 4 solarization weeks + Trichoderma T34; TEST: control test without treatment; BM: methyl bromide (30g•m-2).

In treatments where, *Trichoderma asperellum* was applied, no benefits were shown regarding Fusarium wilt control. Its presence in the soil was tested throughout the whole crop process. Although it was present in all tests, inoculum density was lower than the one rated for initial inoculations.

Concerning stem production accumulated throughout the whole crop, statistic procedure only shows significant differences for non-commercial flower sticks with the control, the methyl bromide and biosolarization with carnation, chrysanthemum and hen manure treatment. Total and commercial productions do not show any significant statistical differences. This fact, from the farmer's point of view, can be irrelevant when the difference in sales is more than 120 stems. At the end of the crop, results showed significant differences in favour of biosolarization treatment

Figure 1. Progression curve of the epidemic of carnation fusarium wilt for the control test without treatment and maximum and minimum temperatures of air in the greenhouse. (Seasons 2004-2005 and 2005-2006)



Source: CL+CR+GALL+BIOS: carnation compost + chrysanthemum (5Kg•m-2) + poultry manure (5Kg•m-2) + biosolarization;TEST: control test without treatment; BM: methyl bromide (30g•m-2).

which proved to be the best for disease control (CL+CR+HEN+BIOS). Finally, it should be pointed out that the amount of organic matter used was very high. Nowadays, tests that are being carried out show that bio-solarization could have the same efficiency if just a fourth part of the one used in this experiment is applied. This procedure has been implemented by a large number of farmers from the area.

Ultimately, chemical treatments, with and without solarization, did not provide a sufficient protection to support the process from an economic point of view. To this point, it is convenient to highlight that compared costs between treatments showed that the best treatment with bio-solarization cost 0.27 €·m^{-2}, and it cost 0.47 €·m^{-2} for the methyl bromide.

3.2. Control of pepper tristeza

As mentioned above, diseases caused by *Phytophthora capsici* and *P. parasitica*, constitutes a limiting factor for pepper crops. This fact has been widely studied in Murcia and Extremadura crops. The model developed in Murcia is taken here for analysis below in this document (in the pepper crops at Field of Cartagena for the last fourteen years). This model will allow the checking of minimum quantities of slightly decomposed organic matter, which is required to carry out biosolarization, and that can be repeated every year in the same soil. The selected example corresponds to a monocrop harvested for 30 years, with duration of 7 and 9 months every year. The common disinfection procedure was, until 3 years ago, fumigation with methyl bromide, taking place every year just before planting. This data were published by GUERRERO *et al.* (2004) and reported later by TELLO *et al.* (2006).

A first approach on using high organic matter quantitiesis compared for two different greenhouses (Table 4).

As happens every time tests take place in the field, there are some slight differences between the two greenhouses. In spite of that, *P. capsici* control and crop production are good, when they are compared to the control with methyl bromide (that was the fumigant to be replaced). An objection could be made to these results: the high amount of organic matter to be used, around 100 $t \cdot ha^{-1}$, which cannot be supported from two points of view, on one side the economic balance and on the other side a possible contamination by nitrates in phreatic waters. This previous research was completed with a test in order to assess how biosolarization could control tristeza. To do that, crop remains containing plants affected by *Phytophthora capsici* were used in the process as biofumigant material (Table 5).

As was specified for the control of carnation Fusarium wilt, it is hereby reiterated how using crop remains with diseased plants does not represent an increase of disease severity. These observations have got a lot of importance and, against the common opinion of experts, crop remains can stop being an inoculum source by action of the same means where the pathogen appeared. This importance also refers to the need of having a “clean plot” in protected intensive crops.

Table 4. Effects of the first year of biosolarization with different organic matters on the control of Phytophthora capsici in pepper crop

Treatment	Dosis	% plants with P. capsici	Plant height: strength (cm)	Commercial production	Greenhouse code
Methyl bromide (98:2)	30 g•m-2 VIF	0,0 a	133, 2 a	9,6 a	C
Biosolarization	7kg•m-2SM+3kg•m-2 HM	0,8 b	94,5 b	8,7 ab	
Biosolarization	7kg•m-2SM+0,5kg•m-2 SF	0,4 ab	93,2 b	8,1 b	
Methyl bromide (98:2)	30 g•m-2 VIF	0,0 a	108,0 a	9,8 a	J
Biosolarization	7kg•m-2SM+3kg•m-2 HM	3,1 b	92,5 b	8,6 b	
Biosolarization	7kg•m-2SM+0,5kg•m-2 SF	0,0 a	89	9,7 a	

VIF=Virtually impermeable film; SM=sheep manure; SF=soya flour; HM=Hen manure.

Source: Adapted from Guerrero *et al*., 2004.

Table 5. Effect of biosolarization applied in July for the control of Phytophthora capsici in soil and the disease severity at the end of the crop

Tested treatment	Density of the inoculum of P. capsici in the soil (UFC•g-1 soil)		%Plants dead at the end of the crop
	Before treatment	After treatment	
Biosolarization without rests of diseased pepper plants. Treatment in July.	0,028 a	0	9,9 a
Biosolarization with rests of diseased pepper plants. Treatment in July.	0,013 a	0	9,8 a
Control test (no treatment)	0,034 a	0	72,1 b

Source: Adapted from Guerrero *et al*., 2004.

Thus, the research team headed by Dr. LACASA tested dose reduction and replication in the same soil. A summary of results is shown in table 6.

The first consequence that can be raised from their results is that the produced effect per 25000 kg·ha^{-1} of just a slightly decomposed organic matter is comparable to the one obtained with 100 t (Table 6). It must be observed, as it was already stated for the carnation Fusarium wilt model, that hen manure was present. Its role is not well known but its ef-

Table 6. Effect of biosolarization applied repeatedly in pepper crop in the Field of Cartagena (Murcia)

Treatment	Doses	Índex of weeds	Meloidogyne incognita		Plant height: strengh	Commercial production
			% diseased plants	Nodulation index		
Methyl bromide (98:2)	30 g•m-2	0,04 a	0,00 a	0,00 a	142,0 a	9,4 a
Biosolarization 2nd year	5kg•m-2SM+2,5kg•m-2 HM	0,71 b	53,33 b	2,7 c	144,0 a	8,8 a
Biosolarization 4th year	3kg•m-2SM+1,5kg•m-2 HM	0,33 b	20,00 ab	0,7 ab	155,0 a	8,9 a
Biosolarization 5th year	2kg•m-2SM+0,5kg•m-2 HM	0,17 a	33,33 ab	1,0 b	141,0 a	9,1 a
Biosolarization 6th year	2kg•m-SM+0,5kg•m-2 HM	0,37 ab	13,30 ab	0,3 a	144,0 a	9,6 a
Control test		1,68 c	100	3,8 d	125,0 b	7,2 b

sm=sheep manure; hm=hen manure; Significance level $P<0,05$.

Source: Guerrero *et al*., 2004.

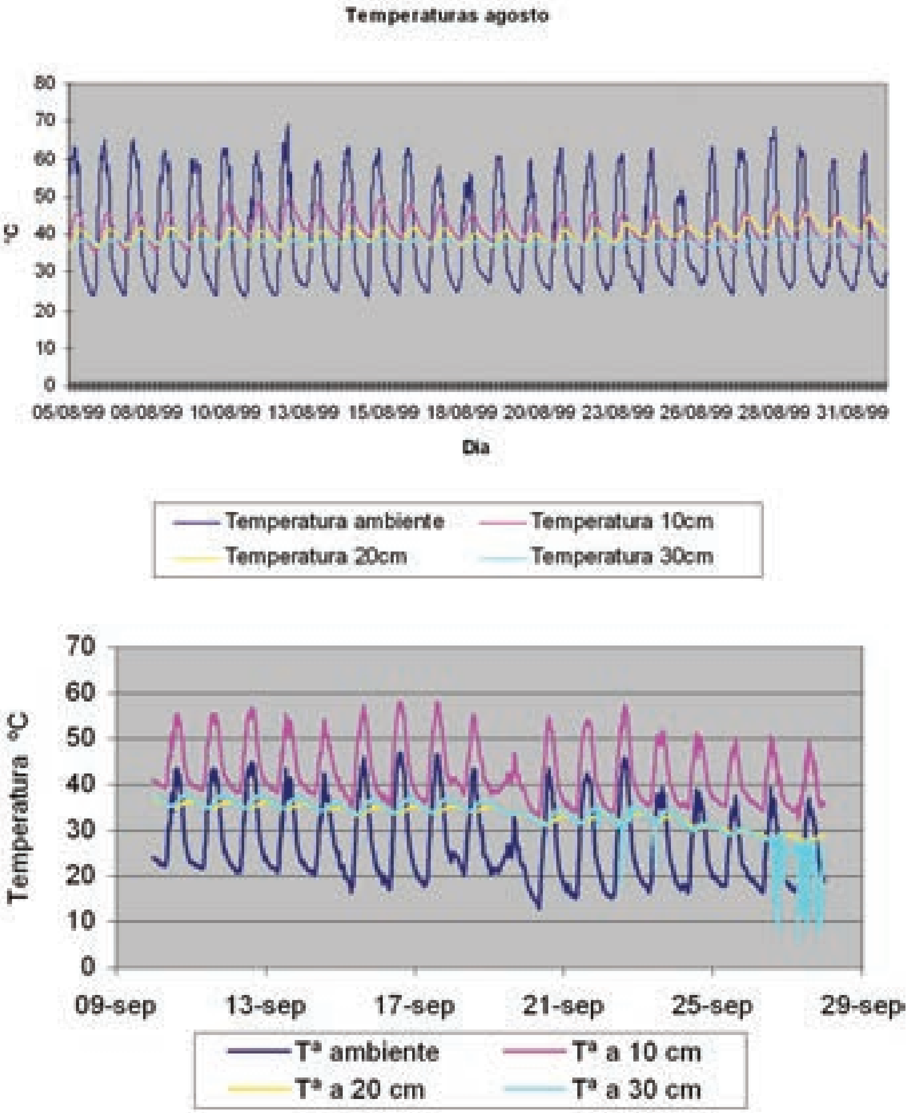
fectiveness is evident. It is known that it produces, among other things, ammonia which is toxic for a large number of microorganisms, including nematodes. It also covers the "nitrogen hunger" that is observed in the plants when biofumigation is applied. This lack of nitrogen is favoured by soil microbian communities' consumption that seems to be mainly transformed in cellular compounds. With respect to fresh sheep manure consumption, it must be clarified that manuring with 20 $t{\cdot}ha^{-1}$ is practised in this crop every year as a mandatory task.

A second consequence, no less important, is the herbicidal role of biosolarization. Another one is plants' strength and commercial production, comparable to the one obtained with the fumigant to be replaced.

It is convenient to underline that the control of nematodes is quite efficient and an important practice: more significant than the number of plants with nodules it is interesting to assess the nodulation level of roots; as this is evidence that has a bigger impact in strength and commercial production.

It is important to know the biosolarization effect practised in pepper plots in Murcia and the resulting residual microbiota after applying that procedure. MARTÍNEZ FRANCÉS (2008) studied and compared general fungal microbiota and, in a special way, the one formed by fungus of

Figure 2. Soil temperatures at different depths and in the aerial environment of a greenhouse in the Field of Cartagena (Murcia), during the biosolarization process in August and September



Source: Guerrero *et al*., 2004.

Fusarium genus. No important differences were found among uses with chemical agents (methyl bromide, 1.3 dichloropropene+chloropicrin) and biosolarization (2 kg·m^{-2} of sheep fresh manure + 0.5 kg·m^{-2} of hen manure). In this model, biosolarization raised soil temperatures in a sustainable way (Figure 2).

4. Effects on physical-chemical properties of soil

This section has been drafted based on the results submitted by FERNÁNDEZ *et al.* (2004). The experiment took place in two greenhouses located in the Field of Cartagena and dedicated to pepper crops. In one of the greenhouses, it was the first time peppers had been planted, while in the other a monocrop was harvested for 20 years. In the oldest greenhouse, biosolarization was applied using sheep manure (7 kg·m^{-2}) + hen manure (3 kg·m^{-2}). In the other greenhouse, sheep manure was applied (3 kg·m^{-2}). Results were as follows:

a) Apparent soil density

Porous space in the soil is evaluated with this parameter. Increase of this porous space has, as a consequence, more significant water dynamic, which is really important in root development and decreases root asphyxia, something the pepper is very sensitive to. As it is well known, *Phytophthora* and *Meloidogyne* are aquatic organisms. Biosolarization application improved apparent soil density, increasing infiltration speed of water.

b) pH

This parameter is well known as it indicates chemical reactions that take place in soil. PH role in soil microbiota has been reported for many years. Therefore, it is a really important value. Biosolarization contributed to slightly decreasing it at the end of the crop.

c) **Electric conductivity**

This parameter allowed the assessment of the increase of salts in soil, especially when fresh manures are applied. Results showed that level did not vary in a significant manner, in fact the opposite, as it was evidenced that there a decreasing trend of that value occurred with biosolarization.

d) Sodium and chlorides

Ions that have got a narrow relationship with electric conductivity. There was not a significant increase in soil with a bigger concentration, but this increase could be found in soil where chlorides and sodium were in a lower concentration.

e) Organic matter

his is important for its role in forming stable aggregates and improving physical soil conditions. This parameter has a lot to do with C/N relation of added manures. It was noticed that this value increased in soils taken for research.

f) C/N relation

It provides information about the mineralization state of organic matter. Values above 20 indicate a low mineralization process. Values under 10 are the evidence of a high mineralization speed. It is important to raise carbon as primary transformation source to a nitric form. In the case of biofumigation, values around 8 are achieved at the end of the crop, which indicates a fast mineralization process. This result should be studied in more depth in order to know the nitrate proportion.

g) **Nitrogen**

The biological activity of soil provides a permanent transformation of organic nitrogen to other forms, ammoniacal at first, and in nitric form at the end (nitrification). The speed of these processes depends mainly on the carbon availability (that is why it is so important the C/N relation). With the tested biosolarization, nitrogen quantity was increased at the end of the crop.

h) Calcium and magnesium

Tests were carried out in soils with a high content of calcium and magnesium coming from the mother rock that generated them. Calcium and magnesium are flocculating cations of organic matter and provide linkage between it and the clay part leading to a clay-humic compound, mainly for physical-chemical and chemical soil properties. Both cations increase their presence with biosolarization treatments, having a positive impact in the crop development.

i) Potassium

Biosolarization considerably increases the presence of potassium.

j) Phosphorus

Since soils where tests were carried out have got a high content of calcium carbonate and active limestone, the main part of phosphorus appears insoluble, mainly as tricalcic phosphate. Biosolarization treatments significantly increased soluble phosphorus in soil. It has been suggested that the effect is due to microorganisms and their enzimatic activity multiplied by the organic matter that intervenes in organic phosphate formation (phosphatase activity) of a high molecular weight that are not precipitated as tricalcic phosphate.

k) Iron

Biosolarization treatments considerably increase soluble iron content in soil (ferrous form); an increase which is maintained throughout the crop's lifetime. Two complementary causes have been suggested to explain these analytic observations: on the one hand, soil temperature increase seems to have an impact on solubilization. On the other hand, possible application conditions, with respect to humidity and the plastic that it keeps it away from, can decrease solubilization due the existing difficulties generated from gaseosus exchange. Phenomena of manure chelation cannot be excluded with respect to inorganic iron.

l) Manganese

A similar case occurs to that of iron, described above. High temperatures and organic matter of biosolarization significantly increase the manganese content in manganese form (Mn^{++}). Once the biosolarization treatment of manganese is finished in assimilable form, it is insolubilized to manganic form (Mn^{+4}), taking soil microorganisms part in this process.

m) Copper and zinc

The same situation takes place for iron and manganese; that is to say, biosolarization treatment increases its presence reaching normal values when treatment application is finished.

n) Boron

Its presence was not affected by solarization application.

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Chapter 15

Non-chemical alternatives for the management of phytoparasitic nematodes

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1. Introduction

Plant protection has traditionally been focused on the concept of fight and control, making use of chemical or biological “arsenals” and lately, biotechnological. The application of ecological criteria in vegetal protection has led to the development of techniques such as **soil biofumigation** or **biodisinfection**, which are based on the use of gases released during the decomposition of organic matter for managing plant pathogens. It is expected that in the future agriculture will show a higher concern for the environment, conservation of natural resources, development of agrofuels, human health and also towards becoming a solidary agrarian model (Bello *et al*. 2003, 2008, 2010, 2011a,b, Castro Lizazo *et al.* 2011, Díez Rojo *et al.* 2009, 2011, González López *et al*. 2011).

The non-chemical alternatives to soil disinfection, based on the use of organic matter, have become recently more feasible for growers and have been specially analysed by the Methyl Bromide Technical Options Committee (MBTOC 1995, 2007, 2009, 2011a,b). The review of bibliography about the management of phytoparasitic nematodes by using organic matter shows that some authors do not differentiate whether the organic matter acts as an amendment or a disease suppressor (Cook and Baker 1983, Hoitink 1988, D´Adabbo 1995). The nematicide effect may occur through different mechanisms; Stirling (1991) highlights that it can be caused by the release of biocidal or biostatic compounds, or by the increase in the antagonist microorganisms, as organic matter favours the development of soil microflora and microfauna.

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Soil biodisinfection, as well as biofumigation is based in the induction of processes producing volatile substances (decomposition of organic amendments and agroindustrial residues) that can act as fumigants to control plant pathogen organisms (Kirkegaard *et al.* 1993, Matthiessen and Kirkegaard 1993, Bello 1998, Bello *et al.* 2003, Díez Rojo *et al*. 2008). The effect of biodisinfection on the microbial activity is selective, favouring the antagonistics and decreasing, e. g. populations of phytoparasitic nematodes. The susceptibility of pathogens to volatile compounds, which are released during the decomposition of organic matter, increases when soil temperature rises. On the other hand, the addition of organic matter can increase 2-3 ºC soil temperature, as well as the soil depth to which the disinfection effect reaches (Bello *et al*. 2000, 2001, 2003). Biodisinfection with solarization (**biosolarization**) has been shown to be an effective method to regulate nematode populations, fungal pathogens and weeds. Ros *et al.* (2002) highlight that repeated use of biosolarization does not have negative effects on soil, but compared to soils without organic matter supply, improves soil fertility, increases organic matter content and enhances the microbial activity and the biogeochemical cycles. It is even more effective when it is included within an integrated management programme instead of being applied alone (Bello 1998, León de *et al.* 2002).

2. The phytoparasitic nematodes and their management

Phytoparasitic nematodes are characterised by having one **stylet**, which is similar to a hypodermic needle, provided with an internal duct, and muscles that permit the organ being retractile and to be introduced within the root and the plant tissues to feed it. Among the **phytoparasites** there are two big groups: **ectoparasites**, some of them feeding on root hairs and epidermal cells of the root, with a weak stylet (*Tylenchus*), and others feeding on deeper cells with a long stylet, such as the **virus transmitting nematodes** (*Longidorus* and *Xiphinema*); other phytoparasites are **endoparasitcs,** either sedentary, mainly those of spherical shape (*Heterodera* and *Meloidogyne*), or mobile (*Pratylenchus*) nematodes (Siddiqi 2000).

In the past synthetic nematicides were the main management technique used for these parasites, particularly in the case of development of severe diseases. Nowadays, most of the synthetic nematicides have been banned or their use has been limited to some geographical areas, crops and technological conditions (greenhouse, drip irrigation …) because of their impact on human health and the environment. Due to the phytonematological problems that have arisen and the difficulty to control them with chemical alternatives, it is necessary to find non chemical alternatives adapted to the agroecological characteristics of each area and crop. At present, consumers demand environmental and health-friendly agriculture, which has led to a change in the approach of agrarian production towards new crop models, especially for the management of pests and diseases. For this reason, the available alternatives for managing the problems caused by nematodes in the crops, mainly by the genus *Meloidogyne* (MBTOC 1995, 2007, 2009, 2011a,b), are analyzed.

Some problems arising from the management of nematodes have been analysed by Bello (1983), who first highlights the fact that the plant parasitic nematodes, especially those which are soil-borne, cannot be easily seen. Another important problem in the study of the soil nematodes is that the collected samples may not be representative of the real conditions of soil and crop. Therefore, samplings must be planned taking into account the biological and ecological characteristics of phytoparasitic nematodes, to allow the assessment of the population with an acceptable level of accuracy. One of the key factors when planning a sampling is the space-temporary distribution pattern that nematodes display. Although nematodes can be distributed in a regular, random or aggregate pattern, the typical spatial distribution of phytoparasitic nematodes is a consequence of the micro and macro distribution patterns related to their biology and food source. The temporary distribution and the seasonal fluctuations of plant parasitic nematodes are determined by their biology, life cycle and population dynamics, as well as by host-parasite relations and the interactions with environment. The basic knowledge of its biology is essential to consider the results of a representative sampling of population, being necessary sometimes to carry out samplings in different periods of the year to determine the seasonal fluctuations (Bello *et al.* 2003). Knowledge of the host-parasite relationship is also essential to localise and estimate the population density of many species correctly.

Soil nematodes are sometimes distributed in spots, making their location and characterization more difficult. For that reason, earlier stages of sampling could lead to errors, if not made by experts and following the sampling rules according to crop characteristics and the sampling time. The nematological analysis, identifications based on the morphology of the digestive system, the accurate study of morphometrics, biology and biotechnology, the characteristics of the host plant, the study of the spatial and temporary distribution of nematodes, the phenological state of the crop and the season of sampling, should be studied and developed by experts.

2.1. Symptoms caused by nematodes

Plant parasitic nematodes´ symptoms can be mistaken with other problems in the initial stages of plants. Therefore, diagnosis can sometimes be wrong or difficult to establish. For example, the plants affected by phytopaparasitic nematodes in their root system, display symptoms in their leaves and stems similar to those caused by nutritional deprivancy. Depending on the nematode species parasitizing the plant, specific symptoms can be observed in the roots, such as the formation of galls or root-knots, when they are parasitized by *Meloidogyne*; stubby roots and small knots in the root apexes, in the case of virus vectors nematodes of the genera *Xiphinema, Longidorus* and *Trichodorus*; small knots in the secondary roots, in the case of parasitism by *Tylenchulus semipenetrans* (citrus tree nematode); excessive proliferation and unusual growth of secondary roots due to the presence of pathogens of the genus *Heterodera* and *Meloidogyne*; and finally, necrosis in the feeding area, in the case of ectoparasitic nematodes; and rot when nematodes favour the entrance of bacteria or fungi in the plant tissues (Fig. 1).

Once the correct identity of the species has been verified, a good knowledge of the life cycle of these organisms is necessary, their dynamics, level of presence, ecology, symptoms, crops and weed flora which parasitize, as well as the available management techniques. This knowledge is essential to adopt the right preventive and management alternative to each case, without affecting other beneficial organisms for the crops (Barres *et al*. 2006).

Photo 1. Symptoms of Meloidogyne in crops: A. Cucumber crop affected by M. incognita; tomato roots parasitized by Meloidogyne from B. sandy soil, C. clayey solarized soil

A main aspect for a correct use of the management alternatives is related to **the interaction between nematodes and plants**. The biological and ecological characteristics of migratory or sedentary nematodes, either ectoparasitic or endoparasitic, are essential for the selection of the most appropriate sampling and management methods in each case. The ectoparasitic and the endoparasitic migratory nematodes can be controlled, in general, with non chemical treatments. On the contrary, the management of the endoparasitic sedentary nematodes requires an appropriate harmonization of techniques that must be contrasted for each specific situation (Barres *et al*. 2006). Furthermore, the possibility that different species are present simultaneously in the crop under some conditions must be taken into account. In this case, it is advisable to know the possible interactions between the nematode groups, as it can affect the effectiveness of the management techniques (Noling 2000).

3. Non chemical alternatives

Taking into account the relevance of plant parasitic nematodes problems arising and the difficulty in controlling them with chemical alternatives, it is necessary to find non chemical alternatives adapted to the agroecological characteristics of each area. For this reason, the non chemical alternatives available to manage the problems caused by nematodes, especially those belonging to *Meloidogyne* genus in the crops, are analysed below following the proposals made by MBTOC (1995, 2007, 2009, 2011a,b).

3.1. Sanitary methods

The first action is the assessment of the phytonematologic state of the previous crop, in order to prevent the possible damage caused by the nematodes present in the next crop. It must be especially taken into account the irregular chlorosis symptoms in the crop, which often indicate the presence of pathogen nematodes. If a nematological problem is suspected, a soil and root analysis must be carried out in a specialized laboratory which permits a precise knowledge of the characteristics of the problem. Once a severe phytonematological problem has been identified, extreme care must be taken to avoid the spread of nematodes by humans, animals, farm tools or water, and particularly trying to avoid contaminated land allocation.

Plant propagation material (seeds, bulbs, seedlings, etc.) must be **free of pathogens** to avoid its possible introduction into the soil to be cultivated. Sanitary inspections and quarantine measures must be established and they must be perfectly standardised. In some cases, the symptoms can be observed at first sight, but in most cases a laboratory analysis is necessary (Díez Rojo *et al.* 2006). Furthermore, for quarantine pathogens, the analysis and phytosanitary certifications must confirm previously that this species does not exist in the places where the propagation material was multiplied, that the seed or parts of the plants used for its crop are free of the pathogen, and that the plant material was protected through standardized plant health protocols. These measures must be combined with actions to avoid the nematode spread and to reduce as far as possible the propagation of the parasite (Barres *et al*. 2006).

3.2. Treatments with hot water

The treatments of propagation material with hot water are a very common and old practice in several crops. Heald (1987) proposes them for the control of nematodes in infected plant material and substrates. Westerdahl *et al.* (2003) have highlighted the interest of the management of *Pratylenchus penetrans* in ornamental bulbs in nurseries by treatment of bulbs in hot water at 49 °C for 35 minutes or at 46 °C for 90 minutes, to reduce the nematode populations. This treatment has also been used for the control of nematodes forming knots on vine rootstocks. This practice can be combined with the addition of synthetic products such as sodium hypochlorite (Barres *et al*. 2006), provided that they are authorised by the corresponding legislation.

The treatments with hot water have an important function for the control of nematodes in quarantine programs. This alternative is being used in protected crops in Japan (Kuniyasu and Takeuchi 1986), mainly for the control of *Monosporascus* in melon roots, which cannot be controlled with solarization (Sakai *et al.* 1998). This technique consists of introducing 250 L/m^2 of hot water at 70-75 ºC, which lead to improvements in yield, in many cases higher than 30 %, due to the change of physical or chemical soil conditions such as desalination, and nitrogen mineralization from dead soil microorganisms (Nishi 2002). Another disinfection method is the use of hot air, by blowing extremely hot air into rotavating humid soil, but despite the positive effect on crop production, root assessments showed that after hot air treatment the root-knot nematodes were still able to infect plants and cause galling damage (Runia *et al.* 2006).

3.3. Steaming

This treatment consists of the introduction of water vapour into the soil, producing a lethal action on pathogens through the heat released when the vapour is condensed. In order to be effective to control plant diseases and weed seeds, it is necessary to keep a temperature of 70 ºC for at least half an hour, although some treatments can be applied at between 60-80 ºC for approximately one hour (Runia 1983).

Soil temperature and treatment duration determine whether elimination of soil pathogens is total (sterilization) or only partial (pasteurization). Pasteurization with vapour at 70-80 ºC is as effective for pathogen control as methyl bromide (MB), with the advantage that it keeps a significant part of the microflora, which acts as "biological barrier" against a possible re-infection by pathogen organisms (Runia 1983). If this temperature is exceeded, it gives as result a "biological vacuum" where any organism, including pathogens, can re-colonise the soil. To amend the "biological vacuum", after the application of vapour to soil, compost or any beneficial organism such as *Trichoderma*, a beneficial bacteria (Pizano 2004) must be added immediately.

On the other hand, if the treatment at high temperature (80-120 ºC) is prolonged, it can negatively affect soil structure, as well as release heavy metals, accumulate soluble salts (particularly Mn), cause toxicity by ammonia, and favour the formation of phytotoxic substances from organic matter in soil. However, if the vapour temperature and treatment time are controlled, this is an alternative that can be used effectively for the control of pathogens, without any phytotoxic effects. Therefore, it is not necessary to wait for a specific period of time to the next crop. From the economic point of view, this is an expensive treatment, due to the required energy consumption as well as the necessary investment and the limitations of application in some type of soils. In consequence, it is a soil treatment that would be recommended only for some countries. Conversely its use is more frequent to disinfect seeds, bulbs or substrates (MBTOC 1995, Pizano 2004).

3.4. Solarization

Solarization is a non chemical alternative for the control of soil pathogens of plants and the weeds which has been first described by Katan (1981). It is based on trapping heat from solar radiation under clear polyethylene plastic sheeting to elevate the temperature of moist soil for prolonged periods (≧ 4 weeks), to a level that is lethal to pathogens (Katan 1993). Its action is related with the direct effect that the increase of temperature has on pathogens as well as the stimulation of beneficial organisms (MBTOC 1995).

On the other hand, the changes produced in the soil microbiota favour the increase of plant growth and production (Stapleton and De Vay 1984, Medina 2002). Its efficiency in the control of pathogens of edaphic origin is improved when it is combined with other alternatives. The advantages of combining solarization + biofumigation are mainly that the reduction of the time required for solarization, the increase in the efficacy and consistency of solarization against pathogens, broadening its spectrum of activity to include phytoparasitic nematodes and allow its use in cooler conditions. They can also be applied to perennial or long season crops, as olive (Tjamos 1998), cherry tomato, almond and pistachio nut crops (Piedra Buena 2004) and vineyards (Díez Rojo 2006).

Solarization alone can be a management alternative in those aeras with hot climate and long sunlight daily periods. Although it was first used in arid and semi-arid regions with intense sunshine and minimal rainfall (MBTOC 2007), recent advances in technology have extended its use to other regions where it was once regarded as impractical (Horiuchi 1991, Chellemi *et al*. 1997a,b, Lamberti *et al.* 2001, Ozturk *et al.* 2002). Furthermore, its effectiveness can be enhanced by using a double sheet of an impermeable, black polymer plastic (Arbel *et al.* 2003) or a double plastic or VIF (*virtually impermeable film*) plastic cover. New technologies to improve the efficacy of solarization are under development including sprayable mulches or new plastic formulations that increase soil temperature (Chellemi *et al.* 1997a,b, Tjamos 1998, Stapleton 2000, Gamliel *et al.* 2001, Cebolla 2002). In the case of root-knot nematodes of the genus *Meloidogyne*, sedge (*Cyperus* spp.), *Monosporascus* and *Macrophomina* spp. inconsistent results have been obtained in some cases.

Solarization alone as a method is not always effective, particularly in the control of mobile organisms like nematodes. Escuer *et al.* (2004) report that solarization has limitations in the mobile stages of nematodes, because when soil gets warm they move deeper and the treatment is not effective. In general, solarization, enhanced with complementary techniques (plastics, chemical fumigants or pesticides, biological antagonists, organic amendments, appropriate cultural practices, etc.), can be a potential alternative to chemical fumigants, but solarization alone has its drawbacks (Barres *et al*. 2006).

3.5. Resistant cultivars and grafting

The use of cultivars with resistance genes has the advantage of being an effective, environmentally friendly and with low cost practice. It allows growers to keep low nematode populations and to reduce crop rotation periods and, in addition, it does not need special techniques to be applied and can be obtained through traditional improvement techniques.

Nowadays, there are commercially available cultivars of tomato and pepper resistant for the nematodes of the genus *Meloidogyne*. Amongst its main disadvantages, it is the arising susceptibility against virulent populations, generally selected by the repeated use of the same cultivars or resistant rootstocks. Therefore, the use of resistant varieties would be effective in soils where the nematode populations are not virulent. Otherwise, virulent populations can be selected and affect the plant resistance in a more or less short period of time (Lacasa *et al.* 2002, Ros *et al.* 2004, Robertson *et al.* 2009, López-Pérez *et al.* 2011). On the other hand, Trudgill (1991) and León de *et al.* (2002) observed that resistance is lost when soil temperature is high and when roots are parasitized by fungi.

The major limitations to the use of resistant cultivars and varieties are the wide range of pathogens and the different populations or biotypes to which is not possible to develop complete resistance. Also the agronomic characteristics of the resistant varieties are usually poorer to those of the traditional varieties, although this can be corrected by grafting, where the resistant cultivar is used as rootstock, and the traditional variety with the agronomic characteristics wanted is used as scion. Tello and Lacasa (1997) reported that after three years of using resistant pepper rootstocks on the same soil, the selection pressure led to established virulent populations. Ornat *et al*. (1999) reported that the use of resistant tomatoes affected negatively to fruit quality and that the resistance was not stable under high soil temperatures (>28 ºC). It is also known the differential response of *Mi* gene-resistant tomato rootstocks to root-knot nematodes (López-Pérez *et al*. 2006). Therefore, the use of varieties with resistance genes can be recommended provided that nematode populations are low enough not to select virulent populations (MBTOC 2007).

3.6. Induced resistance

The systemic acquired resistance (SAR) is a natural plant defence mechanism in which plants activate their defences in response to a pathogen or parasite attack. A plant expressing SAR can be protected against a wide range of pathogens for a period of time, from weeks to many months. However, there are some pathogens against which the mechanisms have little effect (Walters *et al.* 2005).

There are a series of soil organisms which act to induce resistance to plants through different pathways. For example, the bacteria of **ge-**

nus *Rhizobium* in leguminous plants competes with *Meloidogyne* in root areas, which leads to higher population of the bacteria and decreases the nodulation indexes produced by nematodes. Also **mycorrizhae,** which are symbiotic associations between specific fungi and the roots of some plants, and is generally beneficial. The vesicular arbuscular mycorrizhae (VAM) are the most studied group, because they improve phosphorus and other nutrients absorption by plants from the soil. This facilitates the nodulation by rhizobacteria in leguminous plants as well as plants growth. At the same time, mycorrizhae establish a physical barrier that makes difficult the nematodes to access the root providing some tolerance against *Meloidogyne* to the plants. In any case, the increase of phosphorus in soil decreases VAM colonization and spore production. Other beneficial group is **endophytes,** organisms that usually develop in most plant species, inducing resistance. The use of endophytes removes one of the main disadvantages of biocontrol agents, the dependency on specific environmental conditions, widening the range of conditions suitable for those plants (MBTOC 1995). Field research with cucumbers has shown that endophyte-inoculated plants are resistant against a variety of plant pathogens and had higher yields than cucumber plants raised conventionally (Ryder *et al.* 1994).

3.7. Biological control agents

Biological control of nematodes consists of the use of antagonistic organisms, such as fungi, actinomycetes, other nematodes or microarthropods to reduce the nematode populations. There are multiple mechanisms, including antibiosis of metabolites -specific or not- of microbial origin, parasitism, predation and competence. Under balanced environmental conditions, the biological control of nematodes would be produced naturally. In situations where such a balance is changed, the main objective of the biological control techniques would be the modification of environmental conditions, to achieve new status in which nematodes do not cause problems for the crop (Barres *et al*. 2006).

Several predatory, parasitic and pathogen organisms are natural enemies of nematodes, and they can reduce their populations. In general, biological control agents have a narrow spectrum of activity and high specificity with the host, and its efficacy is variable under different crop conditions (MBTOC 1995). The effectiveness of the application of biocontrol agents has not been very satisfactory in soils with high biodiversity, but its use is recommended to recover those soils affected by the intensive use of agrochemicals in which there are low or no biodiversity. In general terms, it is considered that instead of introducing biological control agents it is better to favour their presence and the increase of their populations, by using ecological criteria which promote the self-regulation capacity of the soil system.

The MBTOC (1995) made a revision of the most effective biological agents for the control of *Meloidogyne* nematodes, and highlighted the following organisms:

- ***Paecilomyces lilacinus.*** Antagonistic actinomycete of *Meloidogyne*. Its mode of action is the penetration of the hyphae into the nematode. This organism acts as an efficient parasite of eggs-hells and juveniles inside the egg, and decreases the nematode populations (Hewlett *et al.* 1990). It also parasitizes females, although in a lower proportion (40 *vs* 70 %). The parasitism of eggs and juveniles begins with the growth of the fungus hyphae inside the gelatinous matrix that surrounds the eggs, while females are parasitised through the anus (Gautam *et al.* 1995). It requires high soil temperatures and a high number of propagules to be effective. It can be multiplied in leaf remains (Siddiqi *et al*. 1995), therefore, the crop leftovers favour its development. However it does have the drawback that some isolates can pathogenic for humans.
- **Bacteria of the *Pasteuria penetrans* group*.*** They are gram-positive bacteria, endospore formers, which gives them resistance to adverse conditions such as heat, desiccation or some soil treatments such as solarization. *Pasteuria* group bacteria are obligate parasites of phytoparasitic nematodes, being important potential agents of biological control for the main nematodes´ species. They act by adhering to the cuticle of second stage juveniles (J_2) and females of *Meloidogyne* genus (Davies and Danks 1993). Their host range covers 102 genera and 236 nematode species (Ciancio *et al*. 1994). They cause degeneration of the nematode reproductive tissues, reducing their fertility and sometimes can have a lethal effect on root-knot nematodes and other parasitic nematodes. In spite their wide host range, their field of action is very specific, and therefore, within a mixed population

of phytoparasites, some of them can escape from *Pasteuria* nematicide action. Furthermore, they are not able to reproduce into the soil in the absence of nematodes (Barres *et al*. 2006).

- ***Pochonia chlamydosporia* (sin= *Verticillium chlamidosporum*).** Antagonist fungus of nematodes, parasites different species of *Globodera, Heterodera* and *Meloidogyne*. When their hosts are absent, it can survive as a saprophyte on organic remains. *P. chlamydosporia* needs oxygenated soils and nutrient availability, therefore in poor soils nutrients must be added or the level of inoculum must be increased. At high temperatures (30 ºC) the development of the fungus is slower than the juveniles inside the nematode egg, so that the nematodes can escape from the antagonist (Leij de *et al*. 1993). The effectiveness of *P. chlamydosporia,* as a biological control agent of root-knot nematodes, is affected by the amount of fungus in the rhizosphera, the size of the galls where the female's nematode are developed, as well as by the developmental rate of nematode eggs. This fungus is less effective to control the nematodes in very infected soils because the root galls produced by *Meloidogyne* are large and many eggs can escape from the parasitic fungus.
- **Plant growth promoting bacteria** (PGPR). These are rhizobacteria, i. e., bacteria which develop in the rhizosphere, which are antagonists of soil pathogens or, when colonising roots, establish "biological barriers" to avoid their invasion by nematodes and other pathogens. Another beneficial effect frequently observed is the stimulation of growth, in plants which are in contact with the bacteria, and for this reason, they are called **plant growth promoting rhizobacteria** (*Plant Growth Promoting Rhizobacteria*, PGPR) (Suslow 1982). There are several commercial rhizobacterial products that have been successfully used, generally as seed coverings or coatings, so that when the plant germinates the bacteria colonises the roots, and protects them from the first growth stages, which is usually the most critical period (MBTOC 1995).
- ***Bacillus subtilis*.** It is a Gram-negative endospore-forming bacteria, which gives them resistance to adverse conditions. Its action can enhance plant growth by eliminating non parasitic pathogens from roots, or through the production of substances biologically active (Broadbent *et al.* 1977).

- ***Muscodor albus*.** It is an endophytic fungus which produces a mixture of volatile organic compounds that are lethal to a wide variety of human and plant pathogenic fungi and bacteria (Strobel *et al.* 2001), amongst them, those of soil origin (Riga *et al*. 2008).
- **Mononchids.** A family of predatory nematodes, although their presence are not very common in the conventional crops due to the action of agrochemicals on the soil (Thorne 1927, 1961).
- However, nowadays, the commercial biological control agents are not considered suitable alternatives for the control of nematodes. In accordance with the current experience, the substitution of synthetic nematicides by biological control agents in the short term is not foreseeable. However these organisms can have a role within integrated or ecological control schemes, in developed as well as in developing countries. In general, the biological control with nematicide or nematostatic agents is less consistent and effective than that of synthetic nematicides, and its action is slower (Barres *et al*. 2006).

3.8. Soil biodisinfection and management of organic matter

The addition of organic matter to soil to increase fertility and manage the pathogens is a common practice, almost as old as agriculture, with beneficial effects on physical, chemical and biological parameters. A wide range of materials can be used as organic amendments in the management of phytoparasitic nematodes, phytopathogenic fungi and weeds. In soils infected with phytoparasitic nematodes, the addition of organic matters has shown to be a satisfactory control method for some of them, with effectiveness depending on the chemical composition and the physical properties of the material, which determine the type of organisms involved in their decomposition on the soil and the products that will be generated. To avoid the phytotoxic effects on the crop without losing the biocide activity, Rodríguez-Kábana (1986, 1996) recommends that the organic amendments have a relation C/N between 8-20.

The nematicidal effect of organic matter is produced through different mechanisms. The studies carried out show that the nematicide effect of the amendments can come from the release of toxic compounds, as well as the function that organic matter has on the soil, because it is a substrate that favours the development of microfauna and microflora, and can even introduce antagonist microorganisms. The main problem in the use of organic matter is the variability in the composition of the materials used (Stirling 1991). Another negative aspect of the organic amendments is that some of them can cause accumulation of harmful compounds or increase the inoculum of some soil pathogens (Cook and Baker 1983, Rodríguez-Kábana 1996). The use of organic matter, combined with other alternatives (such as, for example, solarization) can increase its effectiveness, and lower amounts of organic matter can be used without losing effectiveness and reducing costs (Bello *et al.* 2000, 2001, 2003).

Soil biodisinfection differs from the use of organic amendments by the special characteristics that the materials used as biofumigants must have, by the doses and by the method of application. Firstly, organic matter can have a biofumigant function if it is in the decomposition process, which does not occur with organic matter that is usually added as fertilizer (Bello *et al.* 2003), and which is a stabilized organic matter (composts or "aged" manures). Furthermore, the method of application must enhance the processes of gas production during decomposition of organic matter and its retention. This must be carried out during at least two weeks, because in most cases the effect of the gases is biostatic, which requires extending through time its action on the pathogens (García Álvarez *et al*. 2004).

Soil biodisinfection and biofumigation is based on the procedures or volatile substances resulting from the decomposition of organic amendments, and agroindustrial residues as fumigants, for the control of vegetal pathogen organisms (Kirkegaard *et al.* 1993, Bello 1998, Bello *et al*. 2000, 2001, Díez Rojo *et al.* 2008, 2009, 2011, González López *et al.* 2011). Biofumigation with solarization (biosolarization) made repeatedly not only does not have negative effects on the soil, but also, compared with soils without organic matter supply, improves the nutritional state of soil, increases the contents of organic matter, revitalizes the microbial activity and the biogeochemical cycles (Ros *et al.* 2002). It is especially effective when it is included within an integrated management programme of the horticultural systems instead of being applied separately (Bello 1998, León de *et al.* 2002). Biofumigation permits the use of crop remains contaminated by fungi, bacteria or virus that cause disease in the crops. The use of pepper remains with *Phytophthora capsici* or tomato spotted wilt virus (TSWV) mixed with fresh sheep manure and chicken manure did not affect the crop (Guerrero *et al*. 2004, Arriaga *et al*. 2011). Pepper crop residues combined with nitrogene-rich organic matter and covered by plastic as an alternative were also able to reduce *M. incognita* populations (Piedra Buena *et al*. 2007). In regards to tomatoes, Zanón *et al.* (2004, 2011) made a trial in the laboratory with plants infected with the ToMV virus and the bacteria *C. michiganensis,* concluding that if tomato remains are used in biofumigation, these do not represent a risk as an inoculum source for the next crop considering the thermal conditions of the greenhouse.

The use of organic wastes, such as biofumigant material or raw material for compost, are not only alternatives that have lower cost and environmental impact than when they are used as substitutes for fossil fuels or other industrial uses, but also give value to these materials, because they would not be "waste" but "subproducts" of the system, capable of supplying improvements in soil fertility and the control of pathogens (Piedra Buena 2004, Arriaga *et al*. 2011). Biofumigation does not have negative effects on the environment or on consumer's health, and it does not show limitations to being used in integrated production and even in organic agriculture (León de *et al*. 2002), although the content of nitrogen of the materials used must be taken into account.

3.9. Cultural practices

The control techniques of phytoparasitic nematodes associated with cultural practices include the "trap crops", rotations, crop associations, fallow and management of weeds, pruning and elimination of diseased roots, the election of the planting time, deep ploughing, use of covers and management of plant nutrition, hygiene and cleaning sanitary measures, the water management and irrigation, as well as the cultivation in substrates and without soil, make possible the regulation of nematode populations through the knowledge of their agroecological characteristics and through an adapted management to the favourable conditions in each case. Their effectiveness changes according to the crop system and the environmental conditions of the area, therefore, it is necessary to carry out adaptations at a local level. It can be stated that a crop system can be designed for the management of most of the disease problems (MBTOC 1995). The agronomic techniques of management of moisture, temperature, pH and other soil parameters can contribute favourably to the control of these pathogens in some of their sensitive stages to specific conditions of moisture, temperature, pH or other parameters. The problem is that for all the cases there is not enough concrete knowledge of those required conditions are (Torres *et al.* 2007).

- **Crop rotation.** This alternative is especially valid for the control of cyst nematodes like *Heterodera* and *Globodera* which are specific nematodes (Cooke 1993, Dimov 1997). Crop rotation is usually of a limited value for nematodes with a broad range of hosts such as *Meloidogyne* (Trudgill 1997). It is necessary to know the behaviour in relation with the hosts of the different species of nematode knot of *Meloidogyne* genus, in order to plan crop rotations, for example, populations of *M. javanica* do not parasitize our pepper cultivars (Robertson *et al*. 2006), these populations come from the most representative horticultural crops of Spain. However, it is possible to increase the system suppressiveness including crops that inhibit their development (MBTOC 2007). Some plants are bad hosts, such as cereals (sweetcorn, sorghum), forage (*Crotalaria* spp., *Eragrostis curvula*), crucifers (cabbage, cauliflower), and other crops like sesame, tagetes, garlic, onion, strawberry, peanut (at least for *M. incognita* and *M. javanica*), parsley, cassava, radish and other local crops (Atherton and Rudich 1986). Furthermore, brassicas produce methyl-isothiocyanate and related compounds that have nematicidal and fungicidal activities. Sesame and some *Tagetes* species have also shown nematicidal effects, and they can be planted as sole suppressor crops or between the rows of a main crop as **associated crops**.
- **Associated crops and plant extracts.** Some plants contain allelopathic compounds, that is to say, substances capable of inhibiting or being toxic for the development of other plants, pathogens or nematodes. These vegetal compounds can reach the soil when incorporating such plants as organic amendment, vegetal extracts, or be released in the soil while the plant follows its crop cycle, such quality can be useful for including these plants in crop rotations or associations (MBTOC 2007). The most studied plants have been the neem tree (*Azadirachta indica*), sesame (*Sesamum orientale*), different species of *Tagetes*, castor-oil plant (*Ricinus communis*) and mustard (*Brassica campestris*).

- **Fallowing.** Its purpose is decreasing the populations when the nematode does not find an appropriate host (MBTOC 2007). The main limitation is due to the presence of adventitious plants that act as host plants, which leads to this alternative losing its effectiveness. The adventitious plants must be removed either immediately, so the nematode will not find an appropriate host, or once *Meloidogyne* has already begun to parasitize them, when egg masses have not appeared yet, using the plants as a "trap" for the nematode. Its use is restricted to greenhouses where the crop area is limited (Piedra Buena 2004). However, mixed techniques of fallowing and biofumigation have been described, whose overall effect in vineyards is highly positive (Bello *et al.* 2004). The following practices are favourable actions for the control of nematodes: the destruction or elimination of susceptible or already parasitized roots by nematodes, stopping their reproduction, reducing their populations and eliminating, if appropriate, the aerial part of the plant.
- **Planting time.** Planting time must coincide with periods when environmental conditions are unfavourable for the activity of nematodes, such as temperature lower than 15 ºC in the case of thermophilic species, as well as when population density is low, in order to prevent damage in the crops. This practice may have limitations in those crops or areas where production period is clearly-defined, due to weather conditions or marketing conditions (MBTOC 1995).
- **Ploughing.** Deep ploughing can reduce nematode populations because it moves them to deep soil layers where roots do not reach, likewise it stimulates the soil antagonist microflora and helps to reduce soil moisture (MBTOC 1995).
- **Water management.** In some areas where water and soil are available and they are not limiting factors, periods of flooding can be carried out as a management measure of nematode populations. The effect of this practice comes from the anaerobiosis it causes, which acts on nematodes directly, decreasing the available oxygen for their breathing, or indirectly, due to the production of metabolites by anaerobic microorganisms, which are toxic to many soil pathogens (Cook and Baker 1983). It is particularly effective when organic matter is incorporated into soil prior to

flooding (MBTOC 1995). Furthermore, it must be highlighted that rain and irrigation water favour the spread and dispersal of nematodes. An adequate management of irrigation may help to avoid some cases spreading. The localised irrigation techniques can prevent its spread (Barres *et al*. 2006). The impact on the propagation of nematodes has been highlighted when some vineyard areas that had been traditionally cultivated in dry land have changed to irrigation in the Iberian Peninsula, and such phenomenon must be taken into account in the restructuring to other crops (Bello *et al.*1996).

- **Cover crops.** They are non-commercial crops that are cut at a certain level of maturity, and turned back into the soil as green or dry residues, which permit the regulation of soil temperature, influencing on the duration of the nematode cycle. On the other hand, its decomposition stimulates the activity of antagonist microorganisms of soil pathogens (MBTOC 1995). Also other materials can be used as covers, such as rice husks or sawdust coming from the forest industry or the own rests of the crop.
- **Trap crops.** This technique can contribute favourably to the control of populations of sedentary endoparasitic nematodes, such as the root-knot nematodes of the genus *Meloidogyne* or the cysts producers of the genus *Heterodera*. Their use consists of cultivating plants which are fast crops, but more importantly with a high root development, and removes them at the right moment, before adult nematodes lay the eggs, that is to say, complete a life cycle. It is essential in this technique to destroy the roots of the trap crop, because when terminating the crop, the development of nematodes is also stopped. **The trap crops would not be effective in the case of migratory nematodes such as ectoparasites or mobile forms of endoparasites,** such as juvenile stages, since they move continuously from some roots to others, and this does not prevent them from feeding and developing. It would be advisable to develop protocols or recommendations, which summarise the most adequate modes of action to achieve the goals of the trap crops in the management of nematodes (Barres *et al*. 2006).

- **Soil fertilization and plant nutrition.** The resistance of plants to pathogens, when fertilization is made for an adequate nutrition of the plant is well-known in agriculture. On the other hand, it must be taken into account that excessive nitrogenised fertilization can have negative effects on phytoparasitic nematodes and, especially if it is used excessively, can have negative repercussions on the capacity of soil self-regulation because it also reduces the saprophagous and predatory nematodes. Likewise a negative effect has been observed on symbiosis *Rhizobium*-leguminous, observing a decrease of nodulation caused by this bacteria in soils treated with high doses of nitrogen (Bello *et al.* 1994). Furthermore, an appropriate fertilization can stimulate antagonist microorganisms or increase the host resistance **(induced resistance)** through any other mechanisms. Fertilization affects plants as well as pathogens, an example is the harmful effect caused by the introduction of urea or ammoniacal nitrogen sources on the phytoparasitic nematodes (Spiegel and Netzer 1984). This effect can be due to changes in the activity of the soil microorganisms or the gases released by the biological decomposition of these fertilizers on the soil (MBTOC 1995).
- **Substrates.** Substrates are an often used alternative as crop means, especially in some forms of production and crops where the control of pathogens is not possible or effective. The disadvantages of their use are the problems that may be caused by their residue management and prevention of spills, whether own substrates as the nutritive solutions (Barres *et al*. 2006). Some substrates (hardwood bark, composted or not) may have a suppressive effect on soil pathogens, especially fungi such as *Phytophthora* spp., *Pythium* spp., *Rhizoctonia solani* and several *formae specialis* of *Fusarium oxysporum*. Their use is usually limited to potted ornamental plants, but they have the potential to be used as sources for microbial antagonist to induce suppressiveness (Diánez *et al.* 2003).
- **Integrated management.** Integrated management consists of the use of monitoring techniques of pests and diseases, as well as the combination of management alternatives which are environmentally friendly and viable from the economic point of view (Torres *et al.* 2007, Collange *et al.* 2011). The treatment programmes include different biological, cultural, physical, chemical and

mechanical methods, but must be based on ecological criteria. In the systems in which MB was used, it is necessary to substitute it for integrated management systems with ecological criteria, due to the advantages that these systems have and also because of the fact that there is not a single alternative which has the same effectiveness as this agrochemical (Bello *et al.* 1996, Rodríguez-Kábana 1996).

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Chapter 16

Non-chemical management of bacteriosis and virosis

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1. Introduction

Soil disinfection is considered a type of intervention in agrarian systems whose main purpose is to improve the quality of products obtained. In reference to the importance of soil in crop systems, the optimisation of management is vital for the reduction of environmental impact of agrarian practices, pollution caused by agrochemical products being decreased and good management of agrarian residues being obtained.

Soil disinfection and substrates can be obtained through the application of different techniques, but the selection of the disinfection methods must be done in accordance with the conditions of each crop system. The different disinfecting treatments can have a total biocide action, be biostatic or have little biocide activity.

Soil disinfection techniques can be classified as chemical and non-chemical, combining them being possible with the purpose of advancing and improving, to the maximum extent, the positive effects offered by them. In certain cases, the combination of chemical techniques with other control methods guarantees a good result (Rodríguez-Kábana, 1998). Nevertheless, the most common practice is to use chemical products, which is mainly due to how easy it is to apply them (Barres, 2006).

As is well known, the increase of social and legislative pressure to restrict the use of chemical fumigants has created an interest in assessing alternatives for the management of soil diseases (Chellemi *et al*.,1994), where different non-chemical disinfection techniques play a highlighted role.

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In this chapter, the following two non-chemical disinfection techniques are dealt with due to their importance: **solarization** and **biofumigation**, and also the combination possibilities between them.

Solarization was described for the first time by Katan *et al*. (1976) in Israel where it was observed that when soil was covered by transparent polythene sheets (0.03 mm thick), during hot seasons, its temperature increased due to the sun heating it. Thus, an important reduction in populations of some pathogens (mainly fungi) was achieved which led to the disappearance of plants affected by diseases associated with these pathogens. This method of disease control is similar to artificial heating by steam or other treatments, which reaches temperatures between 60º y 100 ºC, but with important biological and technological differences.

Solarization is superior to chemical fumigation as it is cheaper and safer, does not cause toxicity or pesticide residues and does not require sophisticated machinery. Therefore, it can be defined as a simple method that does not imply risks and toxic materials (Mansoori and Jaliani, 1996).

In certain zones, solarization can reduce the number of phytopathogen microorganisms of the soil prior to the stage when they serve as limiting factors for plant growth (Stapleton and DeVay, 1984). Other microorganisms with positive effects, like bacteria, known as *Gram possitive Bacillus* spp., can survive soil solarization, showing a positive effect in plant growth (Stapleton and DeVay, 1984). We must not forget about the important biological changes that are associated with the destruction of important mesophilic microorganisms, which create a partial "biological vacuum" where the substrate and the nutrients are available for a re-colonization once the treatment has finished. Many of the plant parasites and pathogens that live in the soil are not able to compete with other microorganisms adapted to survive in the soil, including many antagonists of plant pests which re-colonize the substrate easier, surviving solarization. This group includes *Bacillus* and *Pseudomonas* spp. bacteria, fungus as *Trichoderma* and some nematodes (Stapleton, 2000).

The term **biofumigation** was proposed by J.A. Kirkegaard *et al* in 1993 to refer to the suppressive effects associated with the release of isothiocyanates during hydrolysis of glucosinolates, which appeared in brassicas. Considering this definition, it can be interesting to control pests and soil diseases (Matthiessen and Kirkegaard, 2006).

Since first being defined, "biofumigant" adjective has become part of the vocabulary used for pest control, and this concept is now applied to the beneficial effect of volatile compounds released in soil from organic material and agro-industrial residue decomposition (Bello, 1998; Bello *et al*., 2002; Stapleton, 2000).

Thus, biofumigation is considered as a non-chemical alternative to methyl bromide (MBOTC, 1997) that regulates pathogen presence in soil through degradation processes of organic matter (Bello *et al*., 1997 a; b). The resulting gases are the result of biodecomposition of this organic matter through the bioenhancement effect of soil organisms or those organisms that are associated to organic amendments and whose negative effects, regarding the environment and health, are not known (Bello et *al.,* 2000).

Bello *et al*. (2002) describe biofumigation as an easy technique for farmers and technicians that, as it was formerly commented, it only differs from organic amendments in the choice of the biofumigant that should be partially decomposed, and also in the application method. This method must consider the need to retain, for at least two weeks, the resulting gases as its effect, in most of the cases, is not biocide, but biostatic. For this reason, it is required to extend its action time on pathogens. It has been shown that any agroindustrial residue or those combinations with a C/N proportion between 8-20 can have a biofumigant effect, thus avoiding phytotoxic effects on crops without losing biocide activity (Rodríguez-Kábana, 1998).

Biofumigation appears as a variant to the addition of organic amendments to soil (Piedra-Buena *et al.,* 2007). The technique differs from the use of these amendments in the characteristics of the used materials, doses and application method (Tello, 2002). An organic material has got a biofumigant function when it is in the initial phases of decomposition; this does not happen with organic matter which is normally added as a fertilizer (García-Álvarez and Bello, 2004) and is handled as established organic matter (compost or mature manure) (Piedra Buena *et al*., 2006).

Combining soil amendment with solarization increases the effectiveness against pathogens and reduces the organic matter quantity applied per hectare (Bello *et al.,* 1998; Gamliel and Stapleton, 1993).

There are numerous works where chemical disinfection techniques are used with solarization and organic matter supply, mainly with biofumigant materials, a combination that can be known as "**biosolarization**" or **"biodisinfection"**. Among the benefits of combining organic amendments and solarization, we find that the temperature needed to control populations of pathogens can be reduced. This last point is an advantage which is really important in those areas where the application of the technique was restricted due to adverse environmental conditions (Keinath, 1996).

1.2. Biofumigation effects: problematic crop residues

Biofumigation has been established as a common practice that allows the use of local resources in the control of pathogens in plants, reducing production costs and transforming agriculture in an alternative way to solve environmental impact problems as created by crop residues and agro-industry (Bello *et al*., 2003). In the last few years, many new works have been made with the purpose of studying the disease control transmitted through the soil with the appropriate management of crop residues (Bailey and Lazarovits, 2003). Crop residues that are considered as no value waste and are catalogued as a source of pollution can be used as biofumigant materials (Piedra-Buena *et al.,* 2007).

Crop residues are often carriers of insects, mites and several pathogens of fungal, viral or bacterial nature. The capacity of some pathogens to survive in vegetable residues is well known, some being infectious for some years, as is the case with the mosaic tomato virus, ToMV (Vilaseca, 2007), or some of the bacteria of *Clavibacter*, *Agrobacterium*, *Erwinia*, *Pseudomonas*, *Ralstonia* and *Pseudomonas* genus (Vidaver and Lambrecht, 2004; Biosca *et al.,* 2003). These plant residues with infected phytopathogenic agents can also act as an inoculum source for adjacent crops (Aguilar, 2002). Removal of plant debris surrounding the greenhouses is considered a preventive method for fighting against pests and diseases of horticultural crops, in phytopathology and also in integrated production systems (Aparicio *et al.,* 1995) as, even when they are at a certain distance, the pathogens appearing in residues can constitute the primary inoculum to cause crop disease.

The purpose of the present study is intended to obtain results about application of biofumigation techniques and its combination with solarization in virosis and bacteriosis control. For that, two of the phytopathogen bacteria whose presence would imply a high risk in crops, mainly horticultural crops, were selected so their yield decrease could be monitored. *Ralstonia solanacearum* and *Clavibacter michiganensis* subsp. *michiganensis* were the selected bacteria. This selection was made considering two criteria: the first one was the inclusion of both bacteria in the A2 list of quarantine pathogens classified by EPPO. Thus, their presence is potentially dangerous, requiring drastic treatments and fast solutions. The second criterion was based on the inefficiency of chemical treatments used in their control, especially the MB, and is a response to the need to search for less harmful alternatives for the environment. In this work, results obtained with *C.m. michiganensis* are shown.

This work also covers the preliminary study of biofumigation/biosolarization influence on one of the most stable viruses, which is easily transmitted mechanically, and stays for a long time in the soil as it is the tomato mosaic virus (*Tomato mosaic virus*, ToMV). This virus is among those that most affected tomato crops before the appearance of resistant varieties. This resistance has made tomato producers forget about the problems that this virus has caused in crops for many years. Even today, it can still be one of the most significant viruses as it continuously appears, producing big losses in this crop. It especially affects autochthonous varieties, as they possess no resistant genes, like “cherry” tomatoes, whose crop and trade has increased in recent years. Recently a new risk has emerged, overcoming resistance in trade varieties due to the detection of a new virus strain (Tm-2^2) (Aramburu and Galipienso 2005) which is again threatening the sanitary future of this vegetable. Crop remains infected with ToMV can remain infectious for long periods of time, even years in the case of dry leaves and crop residues.

In Spain and, mainly in the southeast of the peninsula, tons of horticultural residues are generated, mainly from pepper and tomato crops, which could have biofumigant activity. Its addition in soil for its use in biofumigation/biosolarization would not only pose a solution to environmental pollution, but would allow the removal of one of the main sources of re-infection in new crops if pathogen degradation was achieved.

There are few bibliographic references about the microorganisms studied in this work, this being one of the pioneering studies in this topic.

2. Material and methods

2.1. Biofumigation and biosolarization in controlled conditions

Seedbeds were prepared in order to obtain healthy plants so that they could later be used as a 'negative' control test for infection by the multiplication of virosis and bacteriosis in controlled conditions, and also to proceed afterwards with the transplantation to a substrate submitted to biofumigation and biosolarization treatments. To that effect, tomato seeds of the Marmande variety were used, which had been previously thermo-treated (24 h at 80 ºC) and, once they had been pre-germinated were transplanted in planting trays that contained substrate (a mixture of peat and silica sand). Throughout the trial, the tomato plants were kept in the greenhouse under phytosanitary isolation conditions. Once 14 days had elapsed after sowing, the seedlings had achieved the appropriate size (4 true leaves) to be transplanted (3-4 true leaves) and/or for its inoculation with ToMV or *C.m*. *michiganensis.*

Plants that were later used as a 'negative' control test (healthy plants) stayed under these isolation conditions for approximately a month until they achieved enough vegetable mass to be chopped and mixed in the infertile substrate. Before its chopping and incorporation into the substrate, these tomato plants were analysed to guarantee their good phytosanitary state. To that effect, the serological technique DAS-ELISA was used with the corresponding commercial anti-sera (Loewe Biochemica Sauerlach, Germany Nº 07047S/500 for ToMV and Number. 07063 for *C.m.michiganensis*).

2.1.1. Obtention of vegetable material infected with ToMV and *C.m*. *michiganensis*

In order to obtain vegetable material infected with ToMV an artificial mechanical transmission was carried out on tomato seedlings that had reached a development state of 3-4 true leaves. To obtain enough infected vegetable material for proposed tests a total of 60 healthy seedlings were inoculated in isolation conditions, as was described above. The solution to carry out such an inoculation (transmitting the disease) was prepared on infected and homogenised material with an inoculation buffer (1g/4ml; phosphate buffer Na/K, pH: 7,2 0,01M + Sodium bisulphite 0.5 % + EDTA 0.5 %). Plants were sprinkled with an abrasive component (Carborundum of 600 mesh), the foliate sheet of the seedlings subsequently smoothly rubbed to achieve inoculation. Once the inoculation was completed, they were kept in a greenhouse under the conditions formerly described and, after 15 days, the presence of the virus in the plants was confirmed through serological diagnosis using the DAS-ELISA technique.

In the case of the seedlings with bacteria, they were allowed to grow under semi-controlled conditions in the greenhouse until they got four true leaves. Once they had been prepared, the next step was to adjust bacteria solutions to 10^8 UFC ml $^{-1}$ according to the standard 0.5 of McFarland. With the help of needles and sterile syringes, containing around 30 µl of bacterial solution, micro wounds were applied in the axillaries buds of leaves (up to three wounds for each seedling). A solution drop was then deposited in the damaged area which was covered with sterile cotton and impregnated at the same time with a bacterial solution. Afterwards, it was covered with paraffin, avoiding dryness, and allowing the development of a systemic infection. The inoculated seedlings were kept in a greenhouse under controlled conditions at temperatures between 18-30 ºC. Symptoms' evolution was assessed once 15 days had elapsed after inoculation. The bacteria presence was confirmed through visual observation of the symptoms (wilting and chancre) and through the use of the DAS-ELISA serological technique, using an anti-serum specific for the bacteria that was studied.

2.1.2. Biofumigation and biosolarization trial for bacteriosis and virosis control under controlled conditions

These trials were carried out in plant pots (15 cm diameter and 15 cm height) that contained 500g of substrate prepared by mixing peat and silica thick sand in a 4:1 (v/v) proportion to favour drainage. The rest of the artificially infected tomatoes were added to pots (see section 2.1.1.). Concerning the features of the peat used, this was a crop substrate formed by peat of the Gramoflor (Gramoflor Vertriebs gmbH and Co., Alemania) brand: natural organic amendment, neutral peat called "*bruna di sfagno*", with an organic carbon content of biological origin of 35 %, organic nitrogen 0.3 %, organic substance 60 % and with a PH of 6.0.

The substrate was disinfected before use in an autoclave for 1 hour at 121 ºC before being distributed in the different pots.

These plants, which had been artificially infected with pathogens under study, were chopped and later mixed with the substrate. Three doses of different vegetable material were used: 5g, 10g and 15g for each 500g of substrate and with four replications per trial. In the same way, negative controls were prepared, although healthy material was used, or only the substrate without any addition of vegetable material, this being considered as a 0 dose.

These doses were chosen in relation to the biomass of the tomato plants. Thus, it the trial was moved to the real field, the 5g, 10g and 15g for each 500g of substrate would respectively correspond to 25, 50 and 100 t of vegetable material per hectare, adjusting the amounts to the ones indicated by Bello *et al*. (2003).

Seven groups of plant pots were designed, each one formed by 56 pots, which coincided with the weeks of thermal treatment they were going to be submitted to (from 0 until 6). In every trial, half of the pots (12 pots containing infected vegetable material, 12 pots with healthy vegetable material and 4 pots without added vegetable material) were locked in plastic bags under hermetic conditions to avoid the release of volatile substances during the decomposition process of the organic matter. These bags were made of transparent thermic polyethylene (300 gauge thick, 25 x 40 cm). Pots disposed in bags were identified with a B (bagged pots), while those that remained opened were identified as NB (non-bagged pots). The preparation scheme and distribution of plots is explained in figure 1.

Tested temperatures that were used to study the survival of pathogen agents in pot batches were 25 and 45 ºC (Basu, 1970). These temperatures were selected because an average temperature of 25 ºC is easily reached under field conditions due to cli-

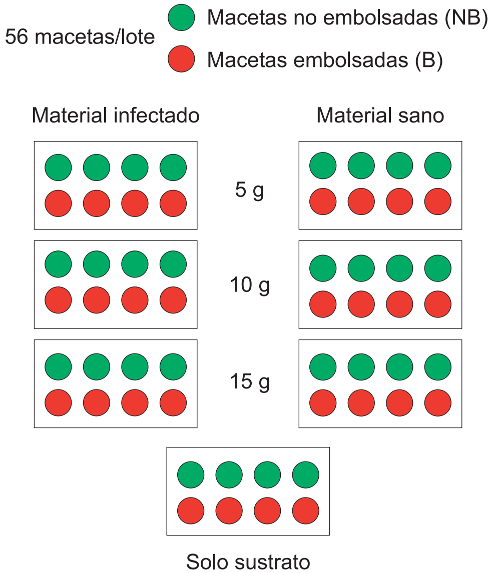


Figure 1. Preparation of the batches in pots to be subject to thermal treatments

mate conditions in Spain. Also, the applied biofumigation technique in soil can be combined with other techniques, like solarization, covering the soil surface with a plastic sheet and, in that case, soil temperatures under soil conditions could reach, and even exceed 45 ºC.

Once all pots had been prepared (56 for each batch or "treatment week"), they were placed in heaters at the experimental temperatures. Once treatment periods (0 to 7 weeks) were finished, the batch was taken out of the heater and all the pots were transferred to the greenhouse where a two-week cv, Money-Maker healthy tomato seedling was transplanted per each pot. These seedlings were obtained in disinfected substrate to be used as a biological trap throughout the trial. After this, the pots were watered until saturation.

Once they were in the greenhouse, and for the remainder of the trial, the plants remained under controlled conditions of humidity and temperature (18-30 ºC). The treatment effect and the possible transmission of pathogen agents under study were assessed once a period of 30-40 days elapsed from the transplant taking place. To that effect, all the plants with, and without disease symptoms, were analysed through the DAS-ELISA serological technique, as has been described in the former section, where specific anti-sera were used. When the tests showed negative results for all the plants concerning the corresponding pathogen presence, it was considered that the pathogen had not infected the plants and that, therefore, the populations were reduced, at least, under the detection limit of the used diagnosis technique. Nevertheless, low levels of pathogens could still remain in association with the substrate (Noble and Roberts, 2004).

2.2. Statistic analysis

The incidence of the disease, expressed as a percentage of plants with a positive result to DAS-ELISA technique, was evaluated through the factorial analysis ANOVA using the Statgraphics Plus software 4.1. (Manugistics Inc., Rockville, MD,USA) program. The data comparing pots containing infected remains were analysed with consideration of fixed factors: doses (5, 10 and 15g), duration of weeks of thermal treatment (0 until 7 weeks, 25 and 45 ºC) and pots' treatment (bagged or non-bagged).

The significant differences were analysed by comparing the measurements through the LSD test ("*Least Significance Difference*") of Fisher (P< 0,05). Regression analysis was carried out through the EXCEL (Microsoft Office, 2003) program.

2.3. Introduction and recovering of bacterial strains in artificially infested substrates and determination pathogens in association with vegetable tissues

These trials were based on studies carried out by Trevors and Finnen (1990) and by Basu (1970) with modifications in the methodology that are specified in Figure 2.

Counts of different observed colonies were carried out in the plates, identifying *C.m. michiganensis* bacteria by visual assessment of morphology (shape, size, colouring, borders, etc.) and through serological analysis of pure cultures. When the result was positive, inoculation in tobacco leaves was used as a test, showing the pathogenicity after thermal treatments. Some of the selected colonies in this trial were identified through the 16S ribosomic gene sequencing amplified with pA-pH primers (Edwards *et al.*, 1989). The bacteria longevity was also studied in direct relation with the vegetable remains (Zanón and Jordá, 2008).

Figure 2a. Scheme of the introduction and weekly recovery of bacterial strains in substrate infected artificially (AFE: Physiological sterile water)

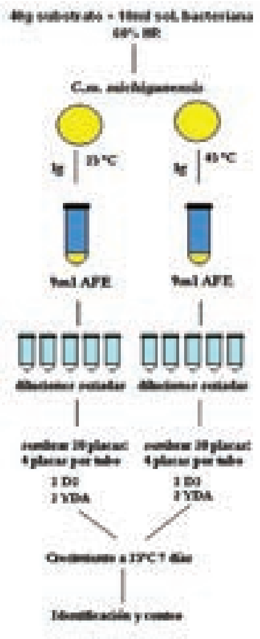


Figure 2. Scheme of the survival of pathogens associated with vegetal tissues

3. Results and discussion

3.1. Effects of tested techniques against *C.m. michiganensis*

Once they had been treated at a temperature of 25 ºC, the percentage of plants showing a positive result to *C.m.michiganensis* presence, after being analysed by the serological technique known as DAS-ELISA when the corresponding weeks of treatment in pots (bagged or non-bagged) were finished, are specified in Tables 1 and 2. “Week 0” or “Week without any type of thermal treatment ” was considered as the maximum starting infection level.

Table 1. Incidence of the disease, expressed in percentages of plants showing positive result by DAS-ELISA to the presence of C. m. michiganensis after the weeks of treatment at 25 ºC

	Incidence of the disease 25 ºC (%)	
	Type of potsa	
Week	NB	B
0[b]	50,0	58,3
1	8,3	16,6
2	25,0	16,6
3	25,0	8,3
4	0,0	0,0
5	8,3	0,0
6	8,3	0,0

[a] NB: Non bagged pots; B: hermetically bagged pots.
[b] Week 0 without thermal treatment: maximum level of infection.

Considering the results obtained, it is inferred that, after four weeks of treatment at 25 ºC, the bacteria is not detected on plant growth in pots containing infested tomato remains which had been bagged during the treatments. However, after six weeks of treatment at 25 ºC in non-bagged pots, the presence of the bacteria was still detected in the plants, therefore, this is not an effective method.

The main difference between pots is found in the bagged pots, where the release of the substances generated during the decomposition of the vegetal material was not permitted, retaining the gases with possible bactericide effects. The fact that after the first week of treatment the infection percentage was higher in the plants growth in bagged pots is related with the favourable moisture conditions, because we must not forget that the substrate is taken to field capacity once the vegetal material has been added and before the thermal treatments have been applied. This condition, together with temperature, is favourable for bacterial development. Therefore, the effects associated with biofumigation in relation to the decrease of the disease incidence are observed after two weeks of treatment at 25 ºC.

The healthy transplanted plants that showed systemic infection symptoms after 6 weeks of treatment at 25 ºC imply the presence and importance of the bacterial inoculum which is associated with soil as well as harvest remains.

The variance analysis for the incidence of the disease showed the weeks of treatment as the doses of the infested material incorporated into pots were significant factors, with $P<0,05$, but not with its interactions (Table 2).

Table 2. Results obtained from the variance analysis of the average values of the incidence of the disease for the factors: doses of infected material, number of weeks of thermal treatment and type of pots (open or close), as well as for their interactions, for the treatments at 25 ºC

Effects	G.L.	Values of P[a]
doses	2	0,042
weeks	6	0,002
pots	1	0,509
Interactions		
doses*weeks	12	0,132
doses*pots	2	0,641
weeks*pots	6	0,823

[a] Values of $P<0,05$ imply significant differences.

The results obtained through the LSD test of Fisher ($P< 0,05$) for the different factors considered can be observed in table 3.

Table 3. Results obtained through the carrying out of the Fisher test (LSD with P<0,05) for the different factors considered in the treatments at 25 ºC: doses, type of pots (NB=non bagged, B=bagged) and weeks of treatment, 40 days after transplanting. The averages represent the incidence of the disease in accordance with the percentage of plants affected by C.m.michiganensis

Temp.	Pots	LS averageZ	Doses (g)	LS average	Week	LS average
25 ºC	BX	14.28 a[y]	10[x]	10.71 a	4	0 a
	NB	17.85 a	5	10.71 a	6	4.46 a
			15	26.78 b	5	4.46 a
					1	12.5 a
					3	16.66 a
					2	20.83 a
					0	54.16 b

[X] Four replications per dose and treatment.
[Y] The values of each column followed by the same letter do not differ significantly in accordance with the LSD method.
[Z] Incidence of the infection expressed as percentage of plants affected by the bacteria 40 days after transplanting using the DAS-ELISA technique.

Even so, it must be highlighted the non existence of significant differences at statistical level, which is due to an analysis of numerical comparisons equivalent to differences between the number of pots with disease incidence. Yet, at a practical level, the disappearance of the disease after four weeks of treatment at 25 ºC in the case of the bagged pots provides very interesting results if the presence/absence of the bacteria is taken into account in association with the infested vegetal remains which come into contact with healthy plants and its capacity to infect them. The results obtained under controlled conditions must be considered through the design of a model which reduces the management of material which would be required in field conditions.

The results obtained also show the difference which would be produced by covering the soil (or to compact the upper layers) when applying the biofumigant materials, and such action is recommended by several authors (Tello and Bello, 2002; Bello *et al*., 2002) who favour, in this case, the effects associated with the decomposition of the tomato remains like those reported by Kim and Kil (2008) or by Ríos *et al*. (2008).

On the other hand, there are significant differences with respect to the doses of infested vegetal material which was mixed with the substrate, so that higher doses brought about higher infection.

Undoubtedly, one of the most important results is deduced from the differences observed when comparing the duration of the thermal treatment because the maximum level of infection obtained for the week 0 or "without treatment" decreases with the treatments from the first week. Although significant differences are not observed between the treatment weeks, the incidence of the disease decreases as they advance, so that the lowest incidence is obtained after the fifth and sixth week of exposure at 25 ºC. As anomalous data, the non infection observed after four weeks of treatment is considered. But the fact that new plants appear affected again in later weeks implies that the bacteria was never controlled at 25 ºC after 6 weeks of treatment. If we interpret the statistical analysis, significant differences are not considered between the bagged and non-bagged pots.

With respect to the treatments at 45 ºC, table 4 shows that the plant percentages with positive results referred to the presence of *C.m.michiganensis*. Again, it is observed that the effect of controlling the disease, from the detection techniques used, is quicker in the pots that remained in plastic bags hermetically and retained the volatile substances given off by the decomposition of the vegetal material added, than in the case of the pots which were open. In this case, two weeks of treatment at 45 ºC for bagged pots and four weeks of treatment at 45 ºC for the pots which remained open are enough to control the disease caused by *C.m.michiganensis*.

Table 4. Incidence of the disease, expressed in percentage of plants showing positive result by DAS-ELISA to the presence of C. m. michiganensis after the weeks of treatment at 45 ºC

	Incidence of the disease 45 ºC (%)	
	Type of pots[a]	
Week	NB	B
0[b]	83,0	75,0
1	0,0	8,3
2	16,6	0,0
3	25,0	0,0
4	0,0	0,0
5	0,0	0,0

[a] NB: non bagged pots; B: hermetically bagged pots.
[b] Week 0 without thermal treatment: maximum level of infection.

Table 5. Results obtained from the variance analysis of the average values of the disease incidence for the factors: doses of infected material, number of weeks of thermal treatment and type of pots (open or close), as well as for their interactions for the treatments at 45 ºC

Effects	G.L.	Values of Pa
doses	2	0,016
weeks	5	0,000
pots	1	0,064
Interactions		
doses*weeks	10	0,109
doses*pots	2	0,339
weeks*pots	5	0,129

[a] Values of P<0,05 imply significant differences.

The variance analysis for the incidence of the disease showed, as in the treatments at 25 ºC, that the weeks of treatment as the doses of infested material were added into the plots resulted in significant factors with P<0,05, but not in any of the interactions (Table 5). In table 6, the results obtained through the LSD test of Fisher (P<0,05) for the different factors considered are shown.

Table 6. Results obtained through the carrying out of the Fisher test (LSD with P<0,05) for the different factors considered in the treatments at 45 ºC: doses, type of pots (NB=non bagged, B=bagged) and weeks of treatment, 40 days after transplanting. The averages represent the incidence of the disease in accordance with the percentage of plants affected by *C. m. michiganensis*

Temp.	Pots	Average LS Z	Doses (g)	Average LS	Week	Average LS
45 ºC	BX	13.88 ay	5x	8.33 a	5	0 a
	NB	20.83 a	15	20.83 b	4	0 a
			10	22.91 b	1	4.16 a
					2	8.33 a
					3	12.5 a
					0	79.16 b

[X] Four replications per dose and treatment
[Y] The values of each column followed by the same letter do not differ significantly according to the LSD method.
[Z] Incidence of the infection expressed as percentage of plants affected by the bacteria 40 days after transplanting using the DAS-ELISA technique

Likewise in the case of treatments at 25 ºC, the statistical study of the incidence of the disease in relation with the weeks that the treatments lasted, shows significant reductions from the first week at 45 ºC. This result is consistent with previous studies concluding that the high soil temperature is the factor which causes the most adverse effect on the inoculum of *C.m.michiganensis* (Gleason *et al*., 1991; Antoniou *et al.,* 1995; Basu, 1970).

The number of diseased plants is reduced until arriving at weeks 4 and 5 where there are no diseased plants. Again, although the numerical studies do not show statistically significant differences between the duration of the treatments, at a practical level, it is interesting, because there are clear differences between the absence or presence of diseased plants.

After four weeks of treatment at 45 ºC, the incidence of the disease caused by *C.m.michiganensis* in plants and transmitted through remains of vegetal material infested and added to the substrate is controlled.

After the treatments at 25 ºC (table 7), the populations of the pathogen bacteria in the general growth medium YDA decreased sharply and they were not identified until the second week of treatment. However, in the case of the selective medium D2, a gradual decrease of the population density of *C.m.michiganensis* was observed until the third week, because after four weeks of treatment at 25 ºC its growth in the plates was not detected.

Table 7. Average of the count of colonies of C.m.michiganensis coming from the substrate treated after a period of 6 weeks at 25 ºC

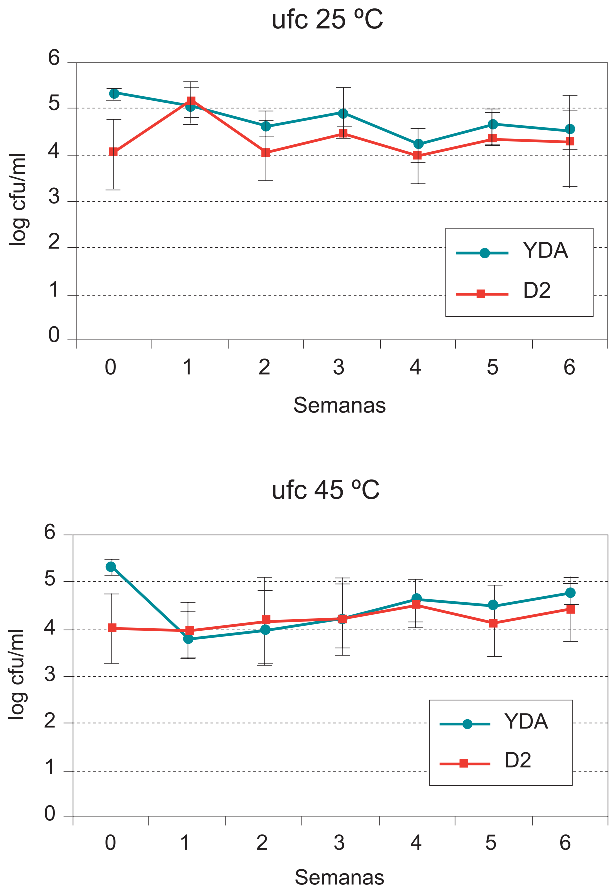
Nutritive medium	Log cfu g^{-1} substrate [a] Week. Treatment at 25 ºC						
	0	1	2	3	4	5	6
YDA	5,316	0,000	4,390	0,000	0	0	0
D2	4,748	5,819	3,867	3,238	0	0	0

[a] Average of the count of ufc g-1 of the substrate in Petri plates from the method of seriated dilutions, with 5 dilutions and 2 replications per tested nutritive medium (5 x 2 x 2).

The identification and count of the colonies was made at first sight, and the pure cultures were obtained and analysed by DAS-ELISA, permitting the identification of the corresponding colonies of *C.m.michiganensis* due to their yellow colour, in the two growth media tested.

The bacterial solutions prepared from pure culture with positive result to the ELISA technique and inoculated in tobacco leaves showed positive phytopathogenicity reactions, therefore it is deduced that after three weeks of treatment at 25 °C, the *C.m.michiganensis* cells introduced in the substrate as free cells through the direct addition of the bacterial solution to the substrate remain infective.

Figure 3. Growth of the bacterial colonies different to C.m.michiganensis in the growth media after the thermal treatments at 25 °C and 45 °C



After the treatments at 45 °C colonies of *C.m.michiganensis* were never detected in the plates (table 7)

A weekly monitoring of the different types of colonies which were observed in the plates was carried out, and the effect of the thermal treatments on them was also assessed. Consequently, the number of other colonies which grew on the selective medium D2 after the treatments, as was expected, was lower than those observed in the general growth medium YDA (figure 3), observing that after the weeks of treatment at both temperatures, the levels of presence of some bacteria are maintained in high population densities.

Some of the pure cultures of these bacteria were identified as *Paenibacillus* sp. and *Brevibacillus* sp. by 16S ribosomal gene sequencing (Edwards *et al*., 1989), and whose sequences were deposited in the GenBank NCBI (Accessions No. EU857426 and No. EU857427, respectively).

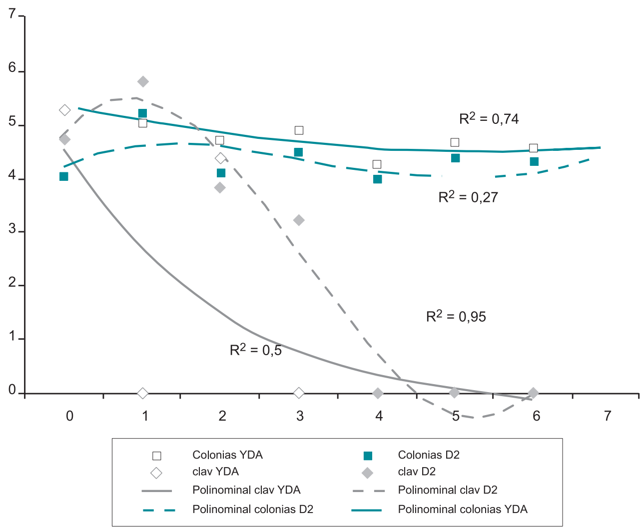
These bacteria have been reported in different studies because they show antibiotic activity (antibiosis) against some phytopathogen organisms.

The effect of the thermal treatments on the population dynamics of *C.m.michiganensis* and the rest of the colonies assessed at 25 ºC (figure 4) implies differences between these colonies, especially with respect to its behaviour depending on the available nutritive medium. The regression analysis for these results (following a polynomial model), show a sharp decrease in the pathogen colonies in both nutritive mediums, while the populations of saprophyte colonies remained constant after the weeks of treatment at 25 ºC

The results of spreading in plates the bacterial solutions obtained from these vegetal residues and, following the method of seriated dilutions, showed antibiosis reactions. The bacteria involved in these reactions were identified through sequencing of the 16S ribosomal gene (Edwards *et al*., 1989) as different strains of *Bacillus subtilis* (GenBank Accesión No. EU857428) and showed a bacteriostatic effect and not bactericide; as bacteria growth is slowed down they remain intact, and though they are not destroyed, they do not advance.

According to the results shown, and supported by the findings of Trevors and Finnen (1990), the survival of bacteria on the soil is very difficult to establish, so that the results obtained have to be considered as approximations, providing valuable ecological information for an appropriate agrarian management.

Figure 4. Effect of the thermal treatment at 25 ºC in the population dynamics of C.m.michiganensis and other colonies of bacteria in the nutritive media tested (YDA and D2)



3.2. Effect of the techniques tested against ToMV

After the application of the biofumigation and biosolarization trials presented, plant infection by ToMV was detected after 4 weeks of treatment for pots that remained bagged hermetically at 25 as well as at 45 ºC and with the three doses of vegetal material tested, and a more exhaustive study of the effects observed in the trial conditions is still pending. Currently, trials corresponding to 5 and 6 weeks of thermal treatments are being carried out, where the significance of moisture conditions can be highlighted, because in the case of non-bagged pots at 45 ºC, the virus has not been detected in association with the substrate nor with the vegetal material.

4. General discussion

The results obtained in this work show the effect that the combination of solarization and biofumigation techniques can have on the control of bacterial diseases transmitted by the phytopathogen agents tested.

Starting from the bioassay in pots, the effect of temperatures above 40 ºC on the eradication of pathogen bacteria associated with substrate is shown, corroborating the results obtained by Pullman *et al.* (1981a; 1981b). In this case, it is advisable to point out that the combination of the detection methods recommended by Noble and Roberts (2004) and Graham and Lloyd (1978) have been used, such authors use, respectively, the serological technique of ELISA, because it is the most common used technique to detect pathogens in soils and composts, while the latter support the use of healthy tomato plants as a biological trap, which easily detects the presence of pathogen bacteria capable of invading the plants by direct contact with the root system, also providing information about its capacity to be established and multiplied in the plant.

The infections associated with the bacterial inoculum which is able to survive in soils and substrates, freely or associated with crop remains, present problems which cause severe symptoms responsible for important losses in the yield. Considering the results obtained, also the importance of the high infective capacity inoculum is shown and converts it in focus of infection; and the transmission of bacterial diseases from the crop remains has been reported by many authors (McCarter, 1976; Graham and Lloyd, 1979; Leben, 1981; Nesmith and Jenkins, 1983; Noble and Roberts, 2004; Ji *et al*., 2005). This is shown when considering the high levels of infection reached by the plants that grew in pots amended with high infective crop remains and that were not subject to any type of treatment (corresponding to the weeks 0).

Considering the levels of infection mentioned before, referring to the presence of pathogens in the substrates associated with vegetal material, positive effects are observed after the treatments at 25 ºC, for the different doses of added remains, this technique is considered as biofumigation. These effects shall mean a decrease in the infection level of the bacterial disease in the seedlings grown in treated substrates, but, when managing quarantine bacteria, they cannot be considered as favourable results, because a decrease does not mean the eradication of the pathogen, with the potential risk that the bacterial presence shall represent in the field. Even so, interesting information is obtained about the behaviour of pathogens under trial conditions, which can be considered in cases of an infection focus being detected, in addition to the results obtained by the rest of authors.

Furthermore, the behaviour of saprophytic bacteria present in the substrates after the different thermal treatments has been studied, and antibiosis reactions were observed, although they have only been obtained under *in vitro* conditions, and with positive results against the bacteria *C.m.michiganensis*. Also, the isolation of different bacteria used for the biological control of some pathogens is especially interesting, mainly in the search for alternatives for the chemical control.

All the results support the effectiveness of adding the harvest remains to the soil (but buried) with high levels of moisture, so that a double benefit is obtained: the use of the volatile substances released from their decomposition as well as the elimination of vegetal remains that are left after the harvest and can become an important infection source. Furthermore, the addition of these remains to the soil, shall mean an extra supply of organic matter, which shall permit a decrease of the manure additions, and such supply favours the level of nutrients in the soil as well as the soil characteristics, mainly of its structure.

As a general conclusion, it can be stated, that the combination of biofumigation techniques, using tomato crop remains together with solarization, which permits the reaching of high temperatures in soil, can be tested in the field as an alternative to the use of methyl bromide in

Spain for the control of diseases caused by *C.m.michiganensis;* and with favourable preliminary results for the control of the ToMV virus (based on the trials made by Vilaseca *et al.,* 2006). This effect is also favoured by the weather conditions of the country, this combination being associated with a yield increase and improvement of crop productivity.

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Chapter 17

Grafting horticultural plants as technique for the control of soil pathogens

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1. Concepts about grafting onto herbaceous species

1.1 Introduction and historic evolution of herbaceous grafting

Herbaceous grafting is a current crop technique in horticulture. It comes from the East. As Janick (1986) reported, grafting onto herbaceous species was already described in the A.D. fifth century in China. In the seventeenth century in Korea, Hong (1643-1715) wrote a treatise where he reported a grafting technique whereby he joined four rootstocks to only one gourd stem, in two stages, removing some of the fruits, leaving only one or two per plant, in order to obtain big size gourds which would serve for storing rice grains (Janick, 1986; Lee and Oda, 2003).

As early as the twentieth century, other historical references about the use of grafting in cucurbits highlight their interest. During the twenties, the bottle gourd (*Lagenaria siceraria* Standl.) was used as watermelon rootstocks (*Citrullus lanatus* Matsum et Nakai) to fight against the decrease in yield due to the incidence of soil pathogens associated with the repetition of crops in rotation (Lee, 1994). In 1947 grafting cucumber and melon plants onto *Cucurbita ficifolia* was first carried out in Holland. In France, the first reference about melon grafting reported the use of *Benincasa cerifera* as rootstock and dates from 1959.

The development and use of plastics in agriculture led to an increase of yield in plants in specialised nurseries and permitted the production and distribution of grafted plants in countries such as Korea or Japan. In the sixties, grafting was introduced in the commercial production of cucumber and tomato crops in these countries. At the end of the eighties and beginning of the nineties of the last century, this technique was extended to horticultural species like watermelon, cucumber, melon, tomato and aubergine (Oda, 1993).

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The development of this technology in Spain began in Valencia at the end of the seventies and it was developed in Valencia and Almería through the eighties. Currently, in Almería, more than 95 % of the cultivated watermelon area is grafted (Camacho and Fernández-Rodríguez, 2000), and the number of hectares that use this crop technique is increasing more and more in order to guarantee the production of species like tomato, melon or cucumber.

In the following table, a summary of the data obtained about the percentage of grafted plants produced under protected horticultural conditions in different countries is shown, according to the data obtained by Traka-Mavrona *et al.*, 2000. Oda, 1993. Lee and Oda, 2003. Miguel *et al.,* 2007. Diánez *et al.,* 2008. Huitrón and Camacho, 2008. As well as the non published data by the United Nations Industrial Development Organization, to prepare “Alternative to Methyl Bromide” projects which this United Nations agency is developing in different developing countries, under the Montreal Protocol.

Table 1. Percentage of grafted plants in protected horticulture by countries

Country	Watermelon	Melon	Cucumber	Tomato	Pepper	Aubergine
Japan	93-98 %	30-42 %	72-96 %	32-48 %	*	50-94 %
Korea	98 %	83-95 %	95 %	5 %	5 %	2 %
Greece	100 %	40-50 %	5-10 %	2-3 %	*	*
Spain	98 %	3 %	I	10 %	I	I
Italy	30 %	*	*	*	*	*
Cyprus	80 %	*	*	*	*	*
France	*	I	3 %	30 %	*	*
Holland	*	*	I	50 %	*	*
Israel	40-70 %	*	*	*	*	*
Egypt	I	I	*	*	I	*
Jordan	25 %	*	*	*	*	*
Tunisia	*	I	*	*	*	*
Morocco	25-50 %	*	*	*	*	*
Mexico	I	I	*	I	*	*
Guatemala	I	*	*	*	*	*
Honduras	20 %	I	*	*	*	*

I--- It begins the technique at commercial level. Without comparative data
*-- Non available data.

Photo 1. Grafted watermelon in Colima – Mexico

In Japan, 500 million plants are grafted yearly. A 10 % (40 % in watermelon) of these plants are grafted mechanically (Hassell *et al.,* 2008). In the USA, 40 million tomato plants are grafted for hydroponic crops and scarcely 400000 watermelon plants. Currently, in Mexico, there are 1000 watermelon hectares with grafted plants outdoors, to avoid Fol race 3, in addition to 1000 watermelon hectares and 100 melon hectares (Davis *et al.,* 2008).

1.2 The purpose of grafting

In horticulture, the main purpose of grafting is to create the possibility of cultivating plants that are sensitive to some diseases in a contaminated soil. The system consists of using a rootstock which belongs to other varieties, species, and even other genus of the same crop family. The rootstock must be resistant to the disease that we want to avoid (Louvet, 1974).

The rootstock remains healthy which can provide the nutritive solution to the plant, at the same time it is developed, it also provides the necessary photoassimilates which are prepared in the aerial part (cultivar). When using this technology to prevent soil pathogens, the rootstock root system is left alone and also the aerial part corresponding to the variety. The rootstock-variety interaction modifies, in most cases, the behaviour of the same as a consequence of different reactions such as incompatibility, change in the tolerance to some factors of climate and soil, growth habits, flowering, fruit size, content of soluble solids of the same, flesh firmness, etc.

As it has been previously mentioned, the main use of grafting in horticulture is the control of diseases and soil “fatigue” (Yu-Jin Quan 2000, 2001); but this technology can be used with other purposes, such as to prolong the crop cycle and other procedures in disciplines such as: vegetal propagation, vegetal enhancement, phytopathology, vegetal physiology and special phytotechnics.

In the crop of herbaceous species through grafting, the main useful characteristics of the rootstock are intended to be used, whose results are related with:

- Increase of vigour and possibility of cropping a longer cycle (Louvet, 1974; Choi *et al*., 1980; Ogbuji, 1981; Buitelaar, 1987; Vergniaud, 1990; Miguel *et al*., 2007).
- Increase of yield (Miguel, 1993; Miguel *et al.,* 2007; Alexandre *et al.,* 1997b; Gamayo and Aguilar, 1998; Sánchez, 2000; Trionfetti *et al.,* 2002; Davis *et al.,* 2008).
- Resistance and/or tolerance to diseases and soil pests (Louvet and Peyriere, 1962; Buitelaar, 1987; Messiaen *et al.,* 1991; Gómez, 1993; Miguel *et al.,* 2007; Cohen *et al.,* 2000; Trionfetti *et al.,* 2002; Lee and Oda, 2003; González, *et. al.;* 2008; King *et al.,* 2008; Davis *et al.,* 2008; Kubota *et al.,* 2008).
- Tolerance to abiotic stresses. For example, grafted melon on RS-841 (*C. maxima x C. moschata*) better tolerates low soil temperatures, allowing earlier plantations (Buitelaar, 1987; Vergniaud, 1990; Yu-XianChang *et al.,* 1997; Lee, 2003). Some rootstocks allow the enhancement of tolerance to tomato salinity (Estan *et al.,* 2005) or melon (Colla *et al.,* 2006) or to flooding (Liao-ChungTa; Lin-ChinHo 1996).

- Increase in the calibre of the fruit (Miguel, 1993; Miguel *et al.,* 2007; Camacho, 1999; Sánchez, 2000; Traka-Mavrona *et al.,* 2000; Trionfetti *et al.,* 2002).
- Modification of the attributes of fruit quality (Choi *et al.,* 1980; Lee, 1989; Davis *et al*., 2008).
- Combination of materials of ornamental value as it occurs with cactus of *Gymnocalicium mihanovichii* species var. friedichii Werd. Hort, whose world demand is estimated to be at around 10 million plants annually (Lee and Oda, 2003).

1.3. Graft physiology

The changes produced when grafting a vegetal species, in relation with aspects such as growth and development are the following:

a) Graft union

In herbaceous species, Andrews and Márquez (1993) studied the structural changes produced when grafting tasks were made: in the grafted area, the broken cells collapse and a necrotic layer is developed which will later disappear. Then, the living cells of the rootstock and the variety are developed on the necrosed area. Through the cell division a callus made of parenchymatous cells is formed, which causes the break and invasion of the entire necrotic layer. In the process, the resistance to the traction of the grafting point increases due to the physical union between both vegetal materials (rootstock-variety). From the parenchymatous cells, a new cambium is generated, and a xylem as well as a secondary phloem is differentiated, which allow the vascular connection between the grafted materials.

The union of rootstock and variety is formed by cells which have been developed after grafting has been made. These cells are produced by the two vegetal materials which we graft, and they keep their identity so that they do not mix their cellular contents.

Two stages can be distinguished in the union process. The first is a compatibility reaction, the most characteristic process of which is an active cell division in the adjacent tissues, causing an increase of tracheids. The second stage completes the union, because the vascular continuity by differentiation of the tracheids is restored, such tracheids having been formed in the previous stage in vascular elements (Lindsay *et al.,* 1974). In time, the differentiation of both stages can be 3-4 days each.

In some horticultural species, especially in cucurbits, the stem shows an internal cavity in the hypocotyl and epicotyl and, in most of the cases, it also shows six vascular bundles in a well-defined position. The complete union of these six vascular bundles is sometimes impossible, depending on the grafting technique we use, but the final purpose is the achievement of a quick and complete vascular union (Oda *et al.,* 1993).

b) Graft compatibility

Compatibility is defined as the capability that two different plants have to join and develop satisfactorily throughout the whole cycle as a composite plant (Miguel, 1993).

There is not a clear distinction between a compatible or incompatible graft, because it can include species with a close morphological and physiological relation and, therefore, they join easily and also species that are totally incompatible; but between both of them, an intermediate adjustment could be fixed, there are some plants that join, but as time goes by, show disorders, whether in the union or in the growth habit (Hartmann and Kester, 1991). In general, compatibility is related with taxonomic affinity, but it has significant exceptions. Usually, the incompatibility symptoms do not appear until the plant is well developed. The most obvious are (Hartmann and Kester, 1991):

- High percentage of failures in the graft.
- Excessive development of the union (*miriñaque*) over or under it.
- Yellowing, curling and lack of growth of foliage.
- Premature death of plants.
- Marked differences in the growth rate between rootstock and variety.
- Breaking of the grafting union.

Even if some of these symptoms appear, it does not necessary mean that the union is incompatible, but it can be a consequence of unfavourable environmental conditions, presence of diseases or a bad grafting technique.

The physiological incompatibility can be due to, amongst other things, the lack of cell recognition, response to the injury, the role of phytoregulators, if any, or incompatible toxins (Lee and Oda, 2003).

In cucurbits, it seems that there is a cell recognition mechanism where substances such as phytohormones, are involved. Such substances are released by the injured tissues and affect the cambium activity in the grafting area. Thus, in the Cucumis-Cucumis union the development of phloem in the grafting area is higher than the combination Cucumis-Cucurbita (Tiedermann, 1989).

In any case, it is especially important to highlight how incompatibility can change depending on the grafting techniques and the crop environments, as has been reported by Lee (1994).

Furthermore, the type of cultivar within a specific species, as it occurs in melon, can mean that a rootstock is compatible with some types and incompatible with others.

2. The control of soil pathogens with the use of grafting

Grafting is an eco-compatible technique, which does not generate residues and create jobs wherever it is carried out. This technique is justified from the phytosanitary point of view and according to the bibliography and trials made, in solaneaceous plants, (tomato, pepper and aubergine) and in cucurbits (watermelon, melon and cucumber), the grafting technique controls the incidence and development of the diseases listed: (Now, when we refer to solanaceaus plants, as later when we will refer to cucurbits, the section shall begin with a series of bibliographic references that justify the use of the grafting technique as a defense of this species against soil diseases).

2.1. Solanaceous plants

(Lacasa *et al.,* 2008; García Jiménez *et al.,* 2007; Tello *et al.,* 1998; Besri M, 2004; Black *et al.,* 2003; Miguel A, 2002; Romano and Paratore, 2001; Camacho and Fernández, 2000; Bradley J. 1968).

2.1.1. Tomato

a) Fusarium wilt: caused by *Fusarium oxysporum* f. sp. *lycopersici*

This fungus, which can survive in the soil, causes a vascular disease. It penetrates into the plant through the roots and is spread quickly through the xylem, producing a brown colouring in the conductive vessels, from the roots to the leaf petioles. The initial symptoms are the nerves thinning and the flagging aspect of petioles. The lower leaves turn yellow (sometimes only half of the leaf) and later they wilt and dry, although remain attached to the plant. This disease does not affect the whole plant equally, and diseased branches can appear while others remain healthy. The main roots and the stem base show vascular necrosis (García-Jiménez, 2007). Plant growth stops, fruits ripe prematurely and the plant can even die. There are three identified races of this pathogen and there are several commercial varieties with resistance to one or two of them, which are the more common.

Photo 2. Fusarium in tomato ***cv*** Pitenza

b) Fusarium wilt: caused by *Fusarium oxysporum* f.sp. *radicis lycopersici*

It is significant mainly in greenhouses and crops without soil. It causes the rot of the cortical parenchyma of roots and progress through the conductive vessels of the same, up to the base of the stem. In the neck of the affected plants a necrotic chancre appears which is spread pointed toward the top (Tello and Lacasa, 1988). A general wilt and yellowing of leaves is produced, which begins in the base and spreads towards the tip (García-Jiménez 2007). There are some commercial varieties with resistance to this pathogen (Messiaen *et al.,* 1991).

Photo 3. Grafted tomato affected by Fusarium due to franqueamiento (emission of adventitious roots by the harvested cultivar) of the variety.

c) Corky root: caused by the fungus *Pyrenochaeta lycopersici*

It attacks the root system and, sometimes, the stem base. It produces an early loss of the smaller roots and the main roots become severely corky, with cracks along their length. As a consequence of this, plants show little development, wilt and a drying of the basal leaves. Yield is strongly reduced. There are some varieties with a certain tolerance, but a real protection is only given by grafting onto some rootstocks (García-Jiménez 2007).

d) Pepino mosaic virus, (PepMV)

It seems to be responsible for the alteration known as "tomato collapse". In the fruits of the plants affected by this disease, some mottles are appreciated and mosaics of different green tonalities can be observed in the leaves. The wilting and death of the plants, which is known as "Collapse" or "Sudden death", has only been detected when the virus is associated in the aerial part of the plant and *Olpidium brassicae* in the roots under specific environmental conditions, and it has less incidence and is even not found in greenhouses under a controlled temperature. The solution to this problem has been found when grafting is used on very vigorous rootstocks. As occurs with other diseases, *franqueamiento* (emission of adventitious roots by the harvested cultivar) of the grafted plant means that graft does not take effect. Resistant varieties to "collapse" are not known (Jordá M.C, 2007; Lacasa et al 2008).

Photo 4. Beginning of collapse in tomato

2.1.2. Pepper

a) Tristeza: disease caused by the *Phytophtora capsici* fungus

The attack of this fungus on the pepper plant can appear in any vegetal state, although the most critical phenological state is at the beginning of fructification. At the beginning it is almost imperceptible because the plant does not show symptoms, but later dark spots appear on the plant's neck, and when these spots affect the entire neck a vascular disorder is produced which blocks the sap circulation. In the latter stages, the plant shows wilting without defoliation and early ripening of fruits (García-Jiménez, 2007).

2.1.3. Tomato, pepper and aubergine

a) Verticillium wilt: disease caused by *Verticillium dahliae*

The primary symptoms that can be seen are interveinal chlorosis in the basal leaves, and then yellowing in the tip of leaflets which will dry up. Likewise, this occurs with Fusarium wilt, described for tomatoes, and at midday hours a plant dehydration of the aerial part is observed, recovering at night, until the permanent wilt is produced. These symptoms usually only appear in a part of the plant, but later spread to the whole plant. The vascular system presents a red-brown colour which advances from the base to the tip of the plant. The attack is produced with mild temperatures of 20-25 ºC. This fungus can survive in the soil for several years and also has a wide host range, including cultivated plants as well as weeds. Constant mono-cropping causes aggressive attacks to the plants (García-Jiménez, 2007). In the tomato, the Ve gen provides a good resistance but in the case of the aubergine, no resistant variety is known.

b) Bacterial wilt: caused by *Ralstonia solanacearum*

It causes a systemic disease, which is the main bacterial problem of these crops in mild and tropical areas, but it is not very widespread in Europe, where it is considered as a quarantine pathogen. In the last ten years, foci of this bacteriosis have been described in most of the European countries, including Spain (López, M.M., 2007). At first, unilateral wilting of leaves is identified and roots appear in the stem. The plant dies very soon. The vascular system turns brown. If transversal cuts are made in the stem, a milky exudate can be seen which does not appear if the vascular disease is caused by fungi.

R. solanacearum can survive for many years in the soil and also in weeds, aquatic plants and watercourses (López and Biosca, 2005). Some tomato varieties are resistant. *Ralstonia* has few control alternatives. Methyl bromide is not effective and neither is the combination of 1,3 Dichloropropene + Chloropicrine, however, grafting is effective (Grimault and Prior, 1994, quoted by King *et al.,* 2008).

2.2. Cucurbits

(García Jiménez *et al.,* 2007; Fernández-Rodríguez *et al.,* 2002; Hirai *et al.,* 2002; Trionfetti *et al.,* 2002; Cohen *et al.,* 2000; Miguel *et al.,* 2007; Eldelstein *et al.,*1999; Baixauli *et al.,* 1999; Alexandre *et al.,* 1997a; 1997b; Morra, 1997; Pivonia *et al.,* 1996; Gómez, 1993; Messiaen *et al.,* 1991; García Jiménez *et al.,*1990, 1991; Vergniaud, 1990; Yoshida *et al.,* 1987; Buitelaar, 1987; Alabouvette *et al.,* 1974; Louvet, 1974; Chavagnat *et al.,* 1972; Suzuki, 1972; Louvet y Peyriere, 1962; Gronewegen, 1953).

2.2.1. Watermelon

a) Fusarium wilt: caused by *Fusarium oxysporum* f. sp. *niveum*. (FON)

This fungus causes the massive death of plants in most of the producing areas of the world. Three races of the pathogen are known. The FON penetrates the roots and is localised in the woody vessels of the plant. Due to the blocking and necrosis, it is difficult to transport water and nutrients, thus leaves and sprouts wilt. At the beginning, only yellowing and side wilt are produced in the plant, sometimes in only one sprout. Lesions in the conductive vessels appear as spots that spread from the shoot to the tip. Then, the disease is spread to the other shoots, infesting the whole plant which finally dies. In the roots, brown spots are appreciated throughout the vascular bundles and reddish gummy secretions appear next to the plant's neck. In soils highly infested by the pathogen, plants can wilt and die before reaching the adult stage. The fungus can survive in the soil for more than ten years without cultivating watermelon, as resistant organs (chlamydospores) or colonizing roots of adventitious plants or of other crops to which it does not cause damage (García-Jiménez, 2007).

Photo 5. Trials of grafted watermelon against non grafted watermelon in plots infested by MNSV and Olpidium bornovanus. Close-up Photos of non-grafted watermelons

2.2.2. Melon

a) Fusarium wilt in melon: causal pathogen is *Fusarium oxysporum* f. sp. *melonis*.

This is considered as being the main soil pathogen of melon in many countries. Likewise with the case of watermelon, this Fusarium is vascular. To date, four races of this pathogen are known, 0, 1, 2 and 1-2. In 2000 Gómez and Tello, reported for the first time the presence of the physiological race 1-2 in Spain, and the detection of isolates belonging to races 0 and 1 of the pathogen associated to symptoms of "Wilt type", which meant a world first. The syndrome of the disease includes two types of symptoms: "Type Yellows": which is characterised by venation yellowing followed by a generalised limb yellowing, together with necrotic lesions in stems and petioles, gummy exudations and subsequent plant death. "Type Wilt": which is characterised by a plant wilt, not yellowing, which is developed from the tip of the stems to the base of the plant (Mas and Risser, 1966). There are some commercial varieties of melon with resistance to one or several of them (González-Torres *et al.,* 1994).

Photo 6. Trials of grafted melon against non grafted in plots infested by Fom. The non grafted plots can be observed by the great number of dead plants and the differences in the development

Photo 7. Detail of Fom in the conductive bundles of melon

2.2.3. Cucumber

a) Fusarium wilt of cucumber: Caused by *Fusarium oxysporum* f. sp. *cucumerinum*

This disease appears in all the development states of the cucumber, even in seedlings. First, symptoms appear in the basal leaves, which begin to wilt. Also it is very common to find leaf yellowing, wilt and progressive drying up of limbs. The vessels show a brown colour. The adjacent tissues to the same undergo necrosis and gummy exudates are appreciated outside with different tonalities which vary from pinkish to reddish, and when disease affects the whole vascular system, it causes its death. (García-Jiménez, 2007).

b) Root and stem base rot: caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum* fungus

The first symptoms in the Mediterranean basin are observed in autumn. A rot of the neck, usually on only one side of the stem, is developed, which varies from pale green to amber or brown. As development of the rot increases, a white fungal growth is appreciated in the affected tissues. The plants that show these symptoms reduce their growth, wilt and die a few weeks after the symptoms have appeared (García-Jiménez, 2007).

c) Cucumber Leaf Spot Virus: disease caused by Cucumber Leaf Spot Virus (CLSV)

Its behaviour is similar to that presented by the necrotic spot virus, which we shall deal with later. Chlorotic spots, whose central part are brown, are observed on the leaves of the plants affected by this disease, followed by a process of necrosis. In general, it causes plant dwarfism and flowering delay. It is transmitted by seed and through the *Olpidium bornovanus* fungus (Jordá, M.C 2007).

2.2.4. Watermelon and melon

a) Collapse or sudden death of melon and watermelon crops

There are several pathogens involved in processes that end with wilting and plant death in melon and watermelon crops. Under the ambiguous terms of “collapse” or “sudden death” a series of conditions are included, to which the final destination leads to the sudden death of the plant, generally in advanced development states, this death being caused by a strong imbalance between the hydric needs of the aerial part and the actual amount of water it receives.

In the list of soil diseases made by Gómez in Almería between the years 1987 and 1992, it was decided that the main cause responsible for the death of melon plantations were two pathogens of telluric origin: the Fusarium wilt (causal agent *Fusarium oxysporum* f. sp. *melonis*) being the most frequent, with a presence of 45,1 % in the sampled greenhouses, followed by the melon necrotic spot virus MNSV with a presence of 34,3 % (Gómez, 1993).

Melon necrotic spot virus (MNSV). The association of sudden death to the melon necrotic spot virus (MNSV), has been described in several works (Gómez and Velasco, 1991; Gómez *et al.,* 1993a, b). Its main vector is the *Olpidium bornovanus* fungus, which has been found in a high percentage of greenhouses in Almería. Although this disease was first associated with protected melon crop, it now also affects crops of outdoor melon, watermelon, cucumber and other cucurbits (Jordá,M.C, 2007).

Firstly, small chlorotic spots of approximately 1-2 mm diameter appear in the leaves which become necrotic spots and can even pierce them. The appearance of necrosis is very typical in the leaf venation, forming a grille. It causes stretch marks on the plant stem and neck and, finally, a sudden wilt and subsequent death. A rough skin, spotted with woody marks and internal mottle is appreciated in fruits. It is also transmitted by seed.

b) Monosporascus cannonballus, Acremonium cucurbitacearum

With the same effect, of “collapse or sudden death” in the Valencian Community, as well as in other areas of melon crops (Texas, Arizona, California, Korea, Israel) this is attributed to fungi of Monosporascus genus (Lobo, 1990; García-Jiménez *et al.,* 2007; Beltrán *et al.,* 2008)), possibly associated with others of the Acremonium genus. These fungi are the

Photo 8. Root of gourd infected by *Rhizoctonia* sp.

most significant and have the highest incidence in Spain, and it is very common that they appear together in affected plots. *M. cannonballus* has been described in many countries, mainly in arid and hot areas. The typical symptoms of Monosporascus are round black bulges, which are visible to the naked eye, which correspond to fungus perithecia. In the observations made in Spain, these black bulges appear only in almost dead plants. Before this, a decline of the plant is produced, a gradual advance that affects the youngest leaves and branches, thus the affected plants decline and die prematurely (García Jiménez 2007). All the cucurbits are susceptible to Monosporacus, but while the fungus is easily isolated from the melon and watermelon roots, it is difficult to find it in Cucurbits (Beltrán *et al.,* 2008). Other wild plants of the same or different families can serve as its hosts, although only melon and watermelon are affected by the fungus. Ascospores are considered as the primary inoculum. Solarization is not effective for its control, because this is a thermophile organism (Martyn 2007).

A. cucurbitacearum was originally described in Spain and later it has been detected in the United States (California, Oklahoma and Texas) and Italy. This is a specific pathogen of cucurbits to which it can affect in different degrees, depending on the species (Armengol *et al.,* 1998). It acts causing necrosis of small roots and roots browning in an uninterrupted process which begins in the first development stages of the plant (Alfaro-García *et al.,* 1996).

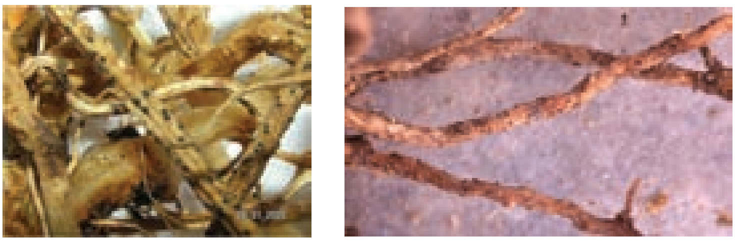

Photo 9. Root of gourd infects primeramente for *Rhizoctonia* sp., then *Monosporascus cannoballus* as saprofito

2.3. Solanaceae and Cucurbits

2.3.1. Tomato, pepper, aubergine, watermelon, melon and cucumber

a) Nematodes: Mainly in Spain, of the ***Meloidogyne*** genus

They are translucent and microscopic worms, with different shapes and sizes, some of them are elongated and cylindrical and others are pear or lemon shaped. In general, they are in the soil and affect the root of several vegetal species. Taking into account the different genera of nematodes described, Meloidogyne is practically the only one that affects cucurbits (Verdejo and Sorribas, 1994).

In the field, plants affected by nematodes usually appear grouped, forming small areas where the plants can die in the first development stages or present a stunted development with a tendency to wilt easily due to hydric imbalances. When these plants are pulled up, some bulges with an irregular shape and size can be appreciated on the roots, known as galls or knots, which appear due to hypertrophy and hyperplasia of the tissues in the feeding area of nematodes. In advanced states of attack, browning and rot of the affected areas is produced. The usual means of transmission of this disease are irrigation water and farming implements. Many tomato varieties and hybrids have a gene, the Mi; which provides resistance to *Meloidogyne incognita*, *M.arenaria* and *M. javanica*, but this resistance is not effective when the soil temperature exceeds 29 ºC. (Kubota *et al.,* 2008). Also, races of Meloidogyne have been identified which are able to exceed the resistance of the Mi gen. The Mi 3 gen is possibly resistant to temperatures higher than 30 ºC (King *et al.,* 2008). Nematodes are not a problem in non grafted watermelon crops, because this species shows a certain resistance. However, in watermelons grafted onto pumpkin, they sometimes cause significant damages.

The nematode attacks in peppers are not so obvious as in tomato or pumpkin crops, however this is one of the most important phytopathological problems when crops are repeated frequently. There are pepper varieties and rootstocks with resistance to nematodes, but this resistance is easily overcome if they are cultivated repeatedly, without soil disinfection.

b) Soil fatigue

The repetition of the same crop gives rise to a decrease in yields and a lack of plant vigour, even when there is no evident pathological cause. This phenomenon is known as “soil fatigue”, and it is more appreciated in some species, probably due to the root exudate of toxic substances for the same species. In addition to crop rotation and the soil disinfection, another method to avoid this effect is grafting onto less sensitive species (Yu-JingQuan 2001).

There are big differences in the autotoxic potential of the different cucurbits, watermelon, melon and cucumber being more sensitive and *Cucurbita moschata*, *Lagenaria leucantha* and *Luffa cilíndrica* being less so. (Yu-JingQuan, 2000).

Amongst the Solanaceae, the pepper is probably the crop most sensitive to “soil fatigue”, but in this case grafting cannot be as effective as in cucurbits, because necessarily it has to be grafted onto its own species.

3. Resistance to soil borne diseases

The resistance to Fusarium is located in the set root-hypocotyl. When there is a vascular pathogen in the soil, this can contaminate the grafted plant if the plant has emitted adventitious roots. In other cases, the resistance is due to the synthesis of several substances that produce tolerance to Fusarium and once they have been synthesized in the rootstock roots, are translocated to the variety, via xylem (Biles *et al.,* 1989); this fact could justify that plants with two root systems (whether by *franqueamiento* or by their own grafting) sometimes offer a resistance comparable with that of the grafted plant that has only the root system of the rootstock. Over the graft, the conductive vessels coming from the variety’s root undergo necrosis, but the continuity of those of the rootstock is enough to guarantee appropriate water and nutrient supply for the plant’s needs. When the two root systems are left or the *franqueamiento* of the variety has been produced, even though grafting provides resistance to a pathogen another pathogen can penetrate to which rootstock does not guarantee resistance over grafting. This is the case of the grafted watermelon to which the variety root has not been cut (Miguel et al 2007); the resistance to Fusarium wilt remains, but immunity against pathogens like MNSV is not guaranteed. The activity of the substances related with resistance to diseases can vary during the different development stages of the grafted plants (Padgett and Morrison, 1990).

The tolerance to Monosporascus of the hybrids *C. maxima x C. moschata* is due to their roots not stimulating the germination of fungus spores (Beltrán *et al.,* 2008). It seems that resistance is due to a lack of recognition between host and pathogen. The population of ascospores in soil cultivated with grafted watermelon, is kept or decreases.

The resistance to *Ralstonia* seems to be due to the difficulty of spreading the bacteria in the lower part of the stem of the resistant plant (Grimault,-and Prior, 1994).

In other cases, as occurs with the pepino virus (PepMV), in tomato, the resistance or tolerance, is due to a higher vigour of the rootstock (higher rate of sap flow) (Escudero *et al.,* 2003).

4. Rootstocks for solanaceae

4.1. Tomato and Aubergine

The compatibility between different species of Solanaceae is reflected in the following table:

Table 2. Compatibility between Solanaceae species

Species	Tomato	Pepper	Aubergine	Nicotiana xanthi	**Datura stramonium**	**Solanum torvum**	**S.integri-folium**	**S.Stramoni florum**	**S.Sessi florum**
Tomato	++++	+	++++	+++	+++	++	+++	+++	+
Aubergine	++++	+	++++	++	+++	++++	++++	++	+
Pepper	+	++++	+	+	+	+	+	+	+

Table 3. Tomato and aubergine rootstocks commercialised in Spain

	K	V	F 0.1	Fr	N	Ps.	Tm
L.esculentum x L. hirsutum							
Aegis	R	R	R	R	R		R
AR 9704	R	R	R	R	R		R
Beaufort (De Ruiter)	R	R	R	R	R		R
Maxifort (De Ruiter)	R	R	R	R	R		R
Multifort (De Ruiter)	R	R	R0,1,2	R	R		R
Unifort (De Ruiter)	R	R	R	R	R		R
Brigeor (Gautier)	R	R	R	R	R		R
King Kong (Rijk Zwaan)	R	R	R	R	R		R
Big Power (Rijk Zwaan)	R	R	R	R	R		R
Emperador (Rijk Zwaan)	R	R	R	R	R		R
Jedi (Rijk Zwaan)	R	R	R	R	R		R
Eldorado (Enza Zaden)	R	R	R	R	R		R
Triton (Western Seeds)	R	R	R	R	R		R
Monstro (Western Seeds)	R	R	R	R	R		R
He-Man (Syngenta)	R	R	R	R	R		R
He-Wolf (Syngenta)	R	R	R	R	R		R
AR 97009 (R. Arnedo)	R	R	R	R	R		R
Huron (Intersemillas)	R	R	R	R	R		R
Javato (Intersemillas)	R	R	R	R	R		R
Jedi	R	R	R	R	R		R
(L. esculentum x L.hirsutun) x L. esculentum							
Resistar (Hazera)	++	R	R	R	R		R
L. esculentum x L. pimpinellifolium							
Spirit (Nunhems)	?	R	R	R	R		R
L. esculentum							
TM 00089 (Sakata)		R	R	R	R	++	
Suketto (Agriset)		R	R	R	R	++	R
Monstro (Western Seed)							
Solanum melongena							
Java (Takii)		R					
Red Scorpion (Takii)							
Solanum torvum							
Torvum vigor (Ramiro Arnedo)		R	R	?	R	R	

Up to a few years ago, tomato grafting in Spain was not very widespread. Most of the hybrids have a range of resistances to soil-borne diseases (V,F,N), which made grafting unnecessary. The spectacular spread of grafting has been due to the importance that “collapse” has achieved. The plants grafted onto interspecific hybrids (*L. esculentum x L. hirsutum*) are more vigorous than non grafted tomato plants, and have born the effects of the same, suffering hardly any damage while the non grafted plants have been devastated. The vigour of the grafted plants also permits the use of a lower planting density, as well as better standing the adverse climatic conditions, mainly the cold.

Interspecific hybrids of *Lycopersicum esculentum x L.hirsutum* are mainly used for tomato. Some interspecific hybrids are made up of L. pimpinellifolium lines, which are resistant or tolerant to *Ralstonia* (Obrero *et al.,*1971).

Also *Lycopersicum esculentum* is used, although they are not as vigorous as the interspecific hybrids, but they have a certain tolerance to bacteriosis and can be used in tomatoes in countries where this type of disease is significant.

In aubergines, rootstocks of the types mentioned before are used. They are recommended in not very fertile soils or soils contaminated by *Pyrenochaeta*. In very fertile soils which are contaminated by *Pyrenochaeta* it is preferable to use *S. torvum* (Ginoux *et al.,* 1991). This rootstock has been used as Solanaceae rootstock in Japan (Kubota *et al.,* 2008). It is resistant to *Fusarium, Verticillium*, nematodes and *Ralstonia solanacearum*. The resistance to nematodes is maintained at high soil temperatures. Another rootstock used for this crop is *Solanum sysimbrifolium,* (Porcelli *et al.,*1990). This rootstock is tolerant to *Ralstonia* and nematodes, although it is not as resistant to *Verticillium* (Bletsos *et al.,* 2003).

The *Solanum melongena* rootstocks are exclusively used for aubergines to which they give more vigour.

4.2. Pepper

Pepper is only compatible with other Capsicum. It displays a bad affinity with other Solanaceae and even with some taxa of its same species. The current pepper rootstocks have a good behaviour regarding “soil fatigue” and root asphyxia, showing a good vigour.

Table 4. Rootstocks to be used in pepper crops specifying resistances

Rootstocks	Vegetal Material	Company	TMV	ToMV	PVY	BPeMV	Pc	Ma	Mi	Mj
Atlante	¿?	Ramiro Arnedo	R, 0							
Brutus	F1	Gautier		R, 0,1	R, 0, 2		IR	R	R	R
Tresor	F1	Nunhems	R	R	IR, 0, 1	R				
WS 2004	F1	Western		R,0			R		IR	

5. Rootstocks for cucurbits

5.1. Watermelon

The rootstocks commonly used belong to one of the following groups:

- **Cucurbita Hybrids. (*C. maxima x C. moschata*).** They are the most used. These rootstocks are also tolerant to Monosporascus, to melon necrotic spot virus MNSV, to *Verticillium*, *Pythium* and nematodes, although they are affected by the last ones under high inoculum density conditions. The interspecific hybrids transmit much vigour to the watermelon grafted onto them.
- **Cucurbita sp.** Also, other Cucurbita species and varieties can be used as watermelon rootstocks, like winter squash (*C. moschata*) as well as other *C. máxima* varieties. All of them are resistant to Fon, but its affinity with watermelon must be checked prior to use, because not all varieties and lines are compatible with it.
- ***Lagenaria siceraria***. Not very commonly used in Spain, although probably the most used watermelon rootstock in Eastern countries. It is resistant to Fon, although susceptible to *F.oxysporum* f.sp *lagenariae* (resistant lines are being selected) and *Monosporascus*. It is less affected by nematodes than the interspecific hybrids. Usually, this roostock is less vigorous than Cucurbita hybrids and less productive. Fruits have a smaller size. In both cases, differences are small.

- ***Citrullus lanatus***. These are some lines of *Citrullus lanatus* or *Citrullus citroides* or hybrids between both of them (Heo 2000, quoted by Lee 2003). They are resistant to the three known races of Fon. Their main advantage is that they are more resistant to nematodes (Meloidogyne) than the other rootstocks. They are not resistant to Monosporascus and to MNSV. The watermelon plants grafted on to this rootstock give better quality fruits than those grafted onto Cucurbita.

Table 5. Resistance of the different rootstocks used in cucurbits

	Resistance of the different rootstocks						
	Fon	**Fom**	**Phom**	**Mon**	**V.d**	**MNSV**	**Nem**
Cucurbita híbrida	+++	+++	++	+++	+++	+++	+
Lagenaria siceraria	+++		?	?	-	?	+
Citrullus sp.	+++		-	-	-	-	+++
Cucurbita moschata	+++	+++	?	?	?	?	++
Cucumis melo		+++	-	-	-	-	-

Table 6. Rootstocks for cucurbits

Rootstocks	Company	Species	Recommended crops by company
Azman RZ	Rijk		
Zwaan	C.maxima x C. moschata	Cucumber	
Watermelon			
Brava	Séminis		
Petoseed	“	Watermelon	
Carnivor	Syngenta	“	----
Ercole	Nunhens	“	----
F-33	Fitó	“	Watermelon
F-90	Fitó	“	Watermelon
Ferro RZ	Rijk		
Zwaan	“	Melon	
Watermelon			
Hércules	Ramiro		
Arnedo	“	Watermelon	
Melon			
Patrón	Clause	“	Watermelon
RS-841	Séminis		
Royal Sluis	“	Watermelon	
Melon			
Shintosa			
camelforce	Nunhems	“	Melon
Shintoza	Intersemillas	“	Watermelon
Squash nº 3	Sakata	“	Watermelon
Strong Tosa	Syngenta	“	Melon
Watermelon			
Titán	Ramiro Arnedo	“	Watermelon
Ulises	Ramiro		
Arnedo	“	Watermelon	
Melon			
Accent	Nunhems	Cucumis melo	Melon
Robusta	Intersemillas	Citrullus sp	Watermelon
T-158	Takii	¿	Watermelon

5.2. Melon

The most widespread rootstocks are the Cucurbit hybrids. With varieties of the Galia and Cantaloup melon types and also alficoz cucumber (*C. melo* var *flexuosus*), the affinity of these rootstocks is usually good, although with other types of melon, (yellow, honey and Spanish green), sometimes, a bulge or rot is produced in the higher part of the graft, which ends up with the death of a stem or the whole plant. These rootstocks provide good vigour and, usually, a yield increase, although it is not shown as clear as in the case of watermelon.

Cucumis melo. When the Fusarium (Fom) is the problem of melon, rootstock of this species can be used, as it is resistant to the Fom races 0, 1 and 2 and tolerant to the race 1-2. Although there are melon varieties with resistance to MNSV, this is not comparable with that of Cucurbita hibrida.

Other cucurbits are mentioned as possible melon rootstocks, such as *C. ficifolius, C. metuliferus, C.zeyheri,* and *C.anguria* (Buzi *et al*., 2004). *C. metuliferus*, resistance or tolerance to *Meloidogyne incognita* is especially interesting (Sigüenza *et al.,* 2005).

Photo 10. Incompatibility of the “Spanish green” melon type onto hybrid of Cucurbita

Photo 11. Incompatibility of the “honey” melon type onto the hybrid of Cucurbita

5.3. Cucumber

Cucumber grafting is spreading quickly. Interspecific hybrids of *C. máxima* x *C. moschata* and *C. ficifolia* are used as rootstocks, which are similar to those used in watermelon and melon crops. These rootstocks are resistant to *F.o. radicis-cucumerinum* (Pavlou, 2002).

Grafting on *C. ficifolia* permits farming in soil contaminated by *Phomopsis sclerotioides*, while non-grafted plants are severely affected (Dufour and Taillens, 1994).

Sycios angulatus behaves similarly to the interspecific hybrids (*C.maxima x C. moschata*) or to *Cucurbita ficifolia* (Lee *et al.,* 1994) and also, is resistant to nematodes.

6. Grafting, transplanting and planting density

When a grafted plant is transplanted, one must be careful not to cover the grafting area, as well as making sure there is a good contact between root ball and soil, to avoid the risk of *franqueamiento*. In early plantations outdoors, it is recommended to use small tunnels to keep high relative humidity and to avoid graft breakage due to wind (Miguel, 1994). When planting on padded soils in warm seasons, special attention must be paid to the size of the hole made on the plastic, with the purpose of avoiding a chimney effect if the size of this is reduced too much (Koren, 2003), (Ricárdez *et al.*, 2006). Some days after transplanting, the new sprouts of rootstock that may have been produced shall be removed.

The main problem of using grafted plants is their cost. The adjustment of the planting density is essential for an optimisation of this crop technique. Trials made by the Research Group AGR 200 of the University of Almería, within the framework of the Methyl Bromide Alternatives project promoted by ONUDI throughout the past four years in different countries from North and Central America, have concluded that densities of 50-60 %, compared with those made in different places using non grafted plants, have increased yield and kept quality in different watermelon, melon and tomato cultivars. Miguel (1993) assesses, for grafted melons and watermelons, a decrease of 30-40 % in density compared with non grafted plants and a 20 % specifically for Spanish melons. In this trial, the grafted plant, in any of its densities, showed a significantly higher yield and bigger size fruits than non grafted plants.

7. Conclusion

Grafting on resistant rootstocks is a resource that permits, in many occasions, to face up to soil pathogens in an effective and ecological way, neither contaminating the product nor the environment. Grafting must not be used solely; but combined with other techniques intended for the same purpose, following a good agronomic practice. When grafting is used, it is convenient to use additional strategies to reduce the level of inoculum in the soil (Davis *et al.,* 2008).

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Chapter 18

The microbial antagonists in the management of mycosis in the aerial part of the plant

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The use of phytosanitary products for the control of diseases in plants is a key factor in intensive agriculture, in spite of the tendency to reduce its application due to different reasons such as the decrease of the levels of residues in the harvested products, scarce effectiveness of phytosanitaries against pathogens, and of course, less incidence in the environment. Therefore, the application of biological control agents (BCA) can be considered as an alternative or a complement to chemical control.

One of the main problems resulting from the application of microbial antagonists is that, as living microorganisms and as such, they are damaged by the application of pesticides and, for this reason, the effectiveness of the process is called into question.

In nature, plants are continuously interacting with microorganism populations. For example, populations of the order of $4{,}5\cdot10^{6}$ microorganisms in 1 g of rhizospheric soil or 10^{7} microorganisms living epiphytically per leaf gram (Lindow and Brandl, 2003).

In general, microorganisms benefit plants, and only a minimum proportion of microorganisms have a negative effect, causing disease. In nature, it is usual that plants are healthy due to a mechanism of self-regulation in populations. This self-regulation is known as biological control (Mondino and Vero, 2006).

Knowledge of the interactions between the own microorganisms and those of plants can permit us to design strategies to slow down or control the development of disease.

The selection of isolates that have shown microbial antagonism, and have even given rise to a higher plant development rate, has permitted the development of commercial formulations whose active ingredients

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are fungi, yeasts, bacteria or a mixture of components. In table 1 some biological control organisms are shown for the control of diseases registered as Other Phytosanitary Defense Means in accordance with the Order APA/1470/2007 of 24th May. In Photo 1, different microbial antagonisms are shown as results of trials for the search of these BCA.

Wilson and Wisniewski (1994) give a wider definition of biological control (BC), which is not only to reduce the use of antagonist microorganisms, indicating that BC is the only way of control that does not imply the use of chemical synthesis pesticides, therefore including the use of natural substances, as the vegetal extracts and the induction of resistance in plants by different mechanisms.

Table 1. Example of some of the formulations made of microorganisms

Brand name	Identification of the means of defense	Registration No.
NITROBACTER	Microorganism nitrogen-fixer: *Azotobacter vinelandii* 10(8) UFC/ml; Soluble liquid	OMDF 0023
MICOCEL	Hydrolyzed yeast extract *Sacharomyces cerevisiae*	OMDF 0021
PROMYC	Ectomycorrhizic fungi	OMDF 0033
GLOMYGEL	Endomycorrhizic fungi	OMDF 0034
ALGACAN BASE	Selection of brown algae.	OMDF 0007
ALGACAN TOMATE	Selection of brown and red algae and microalgae.	OMDF 0010
CITOQUIN MIX	Extracto natural de algas Ascophyllum nodosum	OMDF 0030

Active Substances included in the Annex I of the Council Directive 91/414/EEC (297)

Active Substance	Function	Incorporation
Ampelomyces quisqualis	Fungicide	1/4/05-31/3/15
Bacillus subtilis Strain: QST 713 = AQ 713	Fungicide	1/2/07-31/1/17
Coniothyrium minitans Strain: CON/M/91-08 (DSM 9660)	Fungicide	1/1/04-31/12/13
Gliocladium catenulatum strain: j1446	Fungicide	1/4/05-31/3/15
Paecilomyces lilacinus	Nematicide	08/44 1/8/08-31/7/18

Source: http://www.mapa.es/agricultura/pags/fitos/registro/fichas.

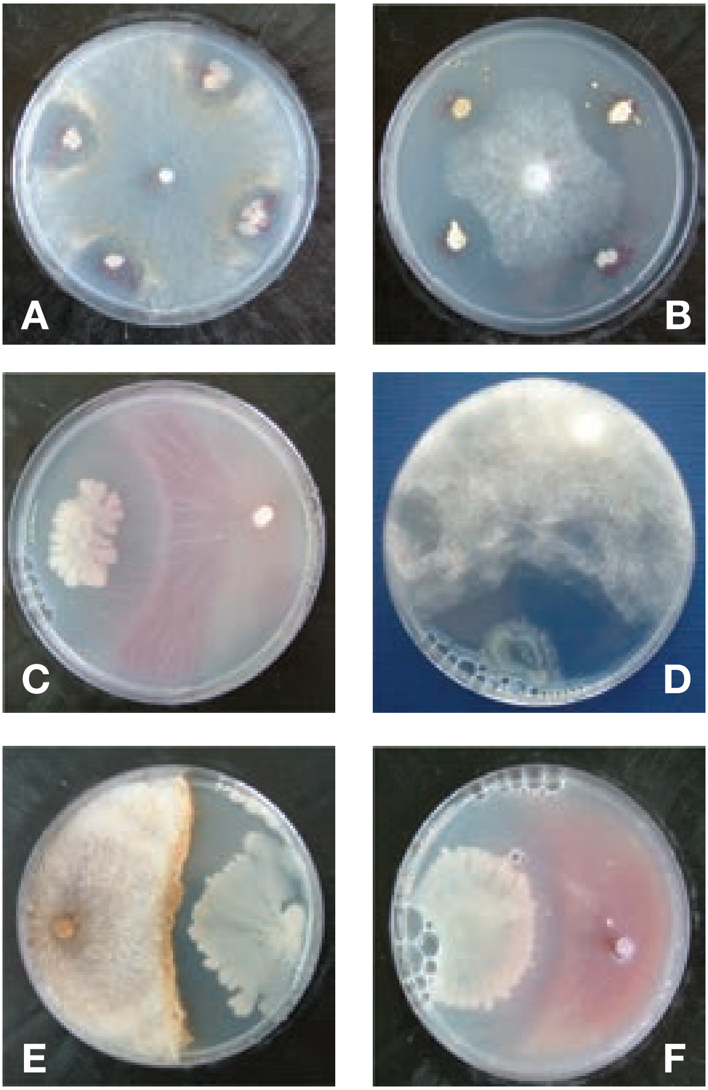

Photo 1. Tests of *in vitro* antagonisms between bacteria rizosféricas and fungi fitopatógenos: a) *R. solani*; b) *P. parasitica*; c) *F. oxysporum f. sp. lycopersici* raza 0; d) *P. aphanidermatum*; e) *R. Solani*; f) *F. oxysporum f. sp. lycopersici* raza 1

When biological control agents are applied, we must take into account the type of microorganisms that are present naturally in the plant. This would allow us a higher effectiveness in the control of pathogens. The microorganisms present in a tomato or pepper plant, in the aerial as well as in the root, are very different in many cases, for example, from those presented by a green bean plant; even more so if we consider the weather of the area, the crop conditions, etc. In reality, the application of products is often considered without taking into account this fact. The use of isolate microorganisms from places similar to those where they are going to be applied, means a better adaptation of the same, giving rise to a more efficient biocontrol.

A natural biocontrol example is suppressive soils. The term **soil or suppressive substrate** is applied to those in which diseases caused by some pathogens are not shown or appear minimally, in spite of phytopathogens being present naturally or having been introduced artificially, cultivating a susceptible host and a favourable aerial environment (Baker and Cook, 1974; Schroth and Hanncock, 1981).

The detection of the phenomenon appears when the incidence or severity of a disease is lower to that expected considering the existing environmental conditions or the soils which surround the area (Cook and Baker, 1983). In order to measure it, it is important to isolate the soil or substrate effect from other possible variation sources: like inoculum density, cultivar, weather conditions or the cultural management (Couteaudier *et al.,* 1987; Rouxel *et al.,* 1991).

A classic example of natural suppressiveness in soils is suppressiveness against the vascular wilts caused by *Fusarium oxysporum.* This phenomenon was recognised for the first time in cotton crops in the nineteenth century by Atkinson (Atkinson, 1892; Weller *et al*., 2002) and was later described for other soils and crops worldwide (Smith and Zinder, 1971; Toussoun, 1975; Scher and Baker, 1980; Alabouvette, 1986; Hopkins *et al*., 1987; Sneh *et al*., 1987; Peng *et al*., 1999; Domínguez *et al*., 2002). Suppressiveness to vascular wilt caused by *F. oxysporum* in these soils limits or reduces the severity of the disease in many vegetal species (Cook and Baker, 1983; Albouvette, 1990). This suppressivenes is generally of natural origin, although in some cases can be induced through cultural practices such as melon and watermelon monocropping (Sneh *et al*., 1984; Larkin *et al.,* 1993). The nature of the suppressiveness in these soils is microbiological, because soil suppressiveness is lost when they are subject to treatments with humid heat, methyl bromide or gamma radiation (Alabouvette *et al*., 1977; Scher and Baker, 1980; Alabouvette, 1986) and is restored when a conductive soil, which has been previously sterilised with heat, is mixed with a small part of the original suppressive soil (Scher and Baker, 1980; Alabouvette, 1986).

Amongst the microorganisms described as responsible for the suppressiveness to Fusarium wilt, we can find bacteria as well as fungi, although the main mechanisms are the competition for the iron and carbon sources in the rhizosphere. Bacteria like *Alcaligenes* sp. (Yuen *et al.*, 1986) or *Pseudomonas* spp. (Kloepper *et al.*, 1980; Scher and Baker, 1982; Lemanceau and Alabouvette, 1993) inhibit *Fusarium oxysporum* through the competition for iron by means of the production of siderophores and fungi such as *Trichoderma harzianum* (Sivan and Chet, 1989) or non-pathogen strains of *Fusarium oxysporum* which compete for carbon sources (Rouxel *et al*., 1979; Alabouvette *et al*, 1984; Albouvette, 1986; Larkin *et al*, 1996; Larkin and Fravel 1998, 1999).

The suppressiveness of diseases can be achieved through the management of the physical-chemical and microbiological environment, using cultural practices such as the use of soil amendments, crop rotations, use of fumigants or solarization (Whipps, 2000).

The induction to suppressiveness can be achieved in some cases through monocropping the same susceptible host with a cultivar that has incomplete resistance (Shipton, 1977; Hopkins *et al.,* 1987). We find such an example in cereal take-all caused by *Gaeumannomyces* (formerly *Ophiobulus*) *graminis var. tritici*, where the disease can be controlled by the combination of crop rotation and farm works and practices that reduce the inoculum potential of the pathogen in the soil (Weller *et al.,* 2002). The development of suppressiveness to cereal take-all is produced when three elements are combined: wheat monocrop or another cereal susceptible to the disease, the presence of the causal agent of the disease, and the severe appearance of the disease at least once (Shipton, 1975; Hornby, 1979, 1998; Cook and Weller, 1987). The advantage that represents induced suppressiveness through monocropping in the extensive cereal production systems is obvious. This advantage is not so obvious in the intensive horticultural and ornamental systems. Due to suppressiveness has a temporary character, and tends to disappear in the lack of a host, meaning a lack of interest from nurseries and in the production of plants in pots, because substrate is usually consumed within the productive cycle. But even for the horticultural and flowering production "without soil", where substrates are generally used again, little interest is shown in suppressiveness, because some disease intensity must be born during some productive cycles; although, this is low in the case of cultivars with partial resistance, like the induced suppressiveness against vascular Fusarium watermelon wilt, when the crimson sweet cultivar is used in monocropping (Larkin *et al.,* 1993 a) or in the case of monocropping of resistant varieties of melon against vascular Fusarium melon wilt (Sneh *et al*., 1984). However, in most of the known examples (using susceptible cultivars) suppressiveness would be so high that several replantings would be required, as in the case of the decline of radish seedlings induced by *Rhizoctonia solani* (Henis *et al.,* 1978), to induce such suppressiveness. This approach is not very realistic in current intensive horticulture, which is characterised by the use of expensive factors of production that require a quick and high profitability of the production process. In short, the previous presence of the active phytopathogen is compulsory in the suppressiveness induced by monocropping (Baker and Chet, 1982), therefore, it is not applicable in crops on substrates (Migheli and Aloi, 1992)

Suppressiveness can be induced by inoculation or introduction of some selected antagonists for soils or substrates (Baker and Chet, 1982; Lewis and Papavizas, 1991; Becker and Schwinn, 1993; Campbell, 1994). During the last 30 years, a great number of soil microorganisms have been described as being biological control agents of plant diseases caused by soil pathogens. Different control strategies have been developed, based on the introduction of them alone or mixed with these biological control agents. Unfortunately, this approach to the control of diseases has not been generalised due to different reasons. Some of the agents introduced control only one of the several important diseases of a crop, others provide only a partial control of the disease or simply its survival in the means is not long enough to have a significant effect on the disease control (Weller, 1988; Hoitink and Boehm, 1999). The factors that control the capability of the biological control agents to be established in the roots have been widely studied:

Rhizosphere competence is one of these factors (Harman, 1992). The study of competence of these microorganisms in the rhizosphere is justified, at least, from a theoretical point of view, because the root is the main organ to be protected in the soil, in addition to one of the main nutrient sources (root exudates, mucilagous substances, cell remains, etc.) (Hoitink and Boehm, 1999).

The supply of nutrients together with the biological control agents, which permit bearing the biological activity of these agents without the pathogen activity stimulus (Steinmetz and Schönbeck, 1994; Lewis *et al*., 1998). The addition of organic amendments, like green manure, aged manure or compost supply the feeding source that permits the activity of the microorganisms responsible for the biological control, when they are added sufficiently in advance to planting (Baker and Cook, 1974; Cohen *et al*., 1988; Hoitink *et al*., 1997). The use of these organic amendments can be very effective in the control of diseases caused by many soil phytopathogens, including species of the genera *Pythium, Phytophthora, Fusarium spp.* a *Rhizoctonia solani.* Soil microorganisms stimulated by nutritional supply contribute to suppressive activity in these soils, through four main mechanisms of biological control: competition, antibiosis, parasitism/predation and induced systemic resistance (Lockwood, 1988). Unfortunately, the use of organic matter in the agricultural production systems has moved to a secondary role. The advances occurred in agriculture from the first years of the twentieth century, with the use of inorganic fertilizers and synthetic fungicides, the development of varieties resistant to diseases and the enhancement of cultural techniques, which permitted the farmers to break the existing union between the supply of organic amendments and soil fertility. As a consequence of this breaking with habit, products such as manure, which was considered as a valuable subproduct, have become a solid residue; "residue" which, generally causes problems related with its elimination, because of water pollution or making easier the spread of animal pathogens (Bruns, 1996). As a consequence of this situation, the mineralization of organic matter in soils has been produced throughout time, and also worsened the structure leading to the development of many diseases caused by soil pathogens, sometimes to epidemic proportions. This situation appears in those crop systems where there are no other effective alternatives, than the genetic resistance, the chemical control or appropriate cultural practices; an illustrative example of this situation is root rot caused by *Phytophthora* in avocado (Baker and Cook, 1974). A similar situation occurs in the crops in pots or containers where peat is the only organic component, alone or mixed with other inert substrates; most of the peats, as occurs in highly mineralized soils, have reduced energetic reserves and are not suppressive against diseases (Hoitink *et al.*, 1991).

The biological control of diseases, made by these biocontrol agents, is produced through different mechanisms of action which generally occur simultaneously. Between the modes of action described we can find: the inhibition of pathogen by antimicrobial compounds (antibiosis); competition for iron through the production of siderophores; competition for the space to be colonized and the nutrients provided by seeds and roots; the induction of mechanisms of resistance of plants; the inactivation of germination factors of the pathogens present in the exudates of seeds and roots; the degradation of pathogenicity factors of pathogens, such as toxins; parasitism that involves the production of extracellular enzymes (such as chitinases or β 1-3 glucanase) which degrade and break the cell walls of pathogens (Keel and Défago, 1997; Whipps, 1997).

There are many bibliographical references to bacteria, fungi, yeasts and viruses as being biological control agents. The interaction between the components of the disease triangle (pathogen-susceptible plant-environment) and the own antagonist is usually very specific. Logically, this has an advantage, because the BCA acts in an inhibitory way against the pathogen, however, this leads to each type of commercial formulation having to be specific for a particular pathogen, and a specific product for each pathosystem having to be designed. In this sense, there are commercial formulations which are wrongly known as “vaccines” that contain attenuated pathogens, that is to say, they are not going to cause disease, but they are going to generate a specific defensive response against that pathogen in the plant, reducing the level of damage in cases of incidence. Other products contain elicitors that generate the same type of response in the plant.

One of the first cases of antagonist bacteria studied was *Agrobacterium radiobacter*, for the control of crown gall caused by *Agrobacterium tumefaciens*. The control was made through antibiosis by different types of antibiotics that were coded by genes present in plasmids. This led to the preparation of formulations whose active ingredient was the *Agrobacterium radiobacter* K84 bacteria. But the transfer of the plasmids of the antagonist strains to *Agrobacterium tumefaciens,* gave rise to resistant strains. From then on, a new strain of *Agrobacterium radiobacter* was obtained and designed as K1026. This strain was genetically modified, and was not capable of transferring the plasmid to the pathogen bacteria (Reader et al., 2005). This strain was used as an active ingredient and was the first genetically modified microorganism used in the environment and the first case of resistance to a BCA.

Likewise, there are many examples of rhizobacteria associated with the control of pathogens, not only root but also aerial. These rhizobacteria generate mechanisms of systemic resistance in the plant. These examples, which are capable of promoting the plant growth and are also associated with the control of phytopathogens, include bacteria of the genera *Bacillus* (Bai et al., 2002), *Pseudomonas* (Siddiqui and Shaukat, 2003; Hass and Defago, 2005; Diánez, 2005) and *Streptomyces* (Sabaratnam and Traquair, 2002; Cao et al., 2002). Some examples of these formulations are included in table 1.

Likewise, non-rhizospheric bacteria of the *Bacillus* genus is associated with the control of *Gibberella zeae* in wheat (Khan et al., 2001), *Pseudomonas fluorescens* A506, as biological control agent of *Erwinia amylovora*, responsible for fireblight (Anderson et al., 2004) and El-Hendawy et al., 2005, detected that foliar applications of the bacteria *Rahnella aquatilis*, could control the symptoms caused by *Xanthomonas campestres* pv*. vesicatoria* in tomato.

There are some viruses that can be also considered as BCA. Bacteriophages have been detected which are capable of causing lysis of *Xanthomonas campestris* pv *pruni* (Randhawa and Civerolo, 1986) or *Erwinia amylovora,* a causing agent of fireblight (Gill et al., 2003). The problem of bacteriophages is their low survival rate (no more than 48 hours) if their host is lacking. Unlike bacteriophages, the virus that affects fungi, mycovirus, are found generally in the cytoplasm or inside the mytochondria, and do not cause lysis of the parasitized fungus. To be transmitted from one fungus to another it is necessary that they come into contact, prompting anastomosis between the donor and the receiving fungus, and therefore they must belong to the same compatibility group (Mondino and Vero, 2006). In general, the presence of a mycovirus confers hypovirulence to the fungus. The best known case is *Cryphonectria parasitica* hypovirulence which causes rot in chestnut trees; also hypovirulent strains have been detected in *Botrytis cinerea* (Vilches and Castillo, 1996), *Sclerotinia sclerotiorum* (Xie et al., 2006) *and Rhizoctonia solani* (Dilip et al., 1998). The use of these hypovirulent fungi can be a biological control method, if not many of these strains are usually less competitive than the own pathogens, therefore, they are not capable of colonising and being established with the same speed.

Yeasts have been also very studied as biocontrollers in postharvest diseases, grain storage and aerial diseases. In this way, *Cryptococcus nodaensis* and *Cryptococcus* sp. are capable of controlling the *Fusarium* attack during wheat flowering. The levels of protection, by spraying these antagonists, reached up to 60 % (Mondino and Vero, 2006).

Maybe, the most studied biological control agents are the filamentous fungi, and also the more commercial formulations that have been developed from them. Amongst them, we must highlight the cases of *Ampelomyces quisqualis*, mycoparasite fungus of oidium, which has a curative behaviour for different species of *Trichoderma* and *Gliocladium.*

The fungi of the *Trichoderma* genus have been studied as promoting agents of plant growth, as BCA of aerial diseases and as BCA of soil pathogens. Their high capability of colonization and the different mechanisms used by these fungi, make them an effective element of biological control. *Trichoderma* colonizes the plant roots favouring the aerial and root development and prevents the attack of root phytopathogens. Furthermore, this root colonization generates systemic resistance controlling at the same time aerial pathogens (Yedidia et al., 2003). Other works show the capability of the fungus to destroy resistance and phytopathogen fungi propagation structures, such as the *Sclerotinia cepivorum* sclerotia by *T. vride* (Clarkson et al, 2003). Also it has been used to cover seeds, to avoid the decline of seedlings and to increase the protection of the same (Ezziyyani et al., 2004). Likewise, it has been detected inside the plant roots, increasing their effectiveness in the control of root pathogens (Avila Miranda et al, 2006).

As BCA of aerial diseases, it has been described as effective against *Botrytis cinerea* in vine, (Elad et al., 2000), *Fulvia fulva* in tomato (Elad et al., 2000), or Fusarium wilt in wheat (Pereyra et al., 2005).

Nowadays, there are many commercial formulations, whose active principle is different strains of *Trichoderma*, for the control of different plant pathogen (see table 1).

The biological control of a pathogen of the aerial area is carried out by the introduction and establishment of the antagonists in the plant surface. The antagonist applied, must be multiplied and colonize the plant surface in order for it to act as BCA (Bettiol, 1991). The antagonist colonization must be prior to the pathogen establishment, so that it can inhibit infection or, at least, reduce the multiplication or sporulation and disease dissemination.

The number of applications necessary for biocontrol to occur depends on the pathosystem where the antagonist shall be established. It is worthless to select a good antagonist for a specific pathogen, if later a bad or inappropriate application is made. For this reason, it is important to know where, when and how many applications are necessary for an effective biocontrol. There are many answers to these possible questions. Obviously it depends on the antagonist and its mode of action, because these can be applied on leaves, fruits, roots, etc. or directly in the soil. With respect to “when”, BCA can be applied before planting, during the different development stages of the plant, or even, in postharvest. The mode of application is dependent on the commercial formulation, and of course, on the place where it must be applied.

The answers are related with the type of pathogen we want to control, the type of plant and the BCA used, as well as the mechanisms of action of the same. It is obvious that an antagonist parasitizes its host if it comes into contact with its structures. On the contrary, if its mechanism of action is inducing resistance to the plant, this must be applied before the pathogen enters. For example, fruits can be sprayed with the biological formulation in postharvest, roots can remain submerged in a solution with the antagonist, seeds can be added with the biological control agent or the cuts made in the plant, can remain impregnated with a paste which contains the BCA.

Generally, biological control lacks in curative capability, therefore, it must be applied preventively. Logically, there are some exceptions, like the oidium control (ectoparasitic fungi) by mycoparasites, where the control can be made through foliar applications.

We must not forget that the farmer must familiarize himself with this control method, and therefore its applications must be adapted to those used when chemical phytosanitaries are applied. Unlike biological control of pests, the establishment of BCA cannot be determined; it is not possible to assess it, except for analytical methods, and for this reason, on many occasions the farmer gives it up, for fear of the disease developing.

Within the mechanisms that antagonists exercise on the own plant, one of the most studied, as we have mentioned before, is induced resistance. The knowledge of genetic and molecular basis which control these mechanisms of defence that plants exercise, ensures that equivalence can be established with the immune system of animals. These systems are systemic, and therefore, they activate not only in the tissue where the pathogen has been detected or been recognised. This property can be exploited in agriculture and several studies are being carried out whose main goal is the development of agrochemical products, which contain activators of that resistance. These studies go beyond and other studies are being carried out based on the use of antimicrobial peptides to enhance resistance in transgenic plants. The restrictions that regulate the use of genetically modified organisms, avoid the commercialisation of these plants resistant to pathogens, although these techniques offer future chances in the biological control of pathogens.

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