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Title:

Developing frameworks for identifying the biological control agents of *Drosophila suzukii* in Lombardy, Italy

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"If all insects on Earth disappeared, within 50 years all life on Earth would end. If all human beings disappeared from the Earth, within 50 years all forms of life would flourish."
— Jonas Salk, Biologist

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Abstract

Developing frameworks for identifying the biological control agents of *Drosophila Suzukii* in Lombardy, Italy

Many invasive pests are arthropods that every year reach, colonize and spread into new areas from their native countries. Invasive species are the second largest threat to biodiversity after habitat loss as they compete with natives for food and spaces. *Drosophila suzukii* (Matsumuta) (Diptera: Drosophilidae) -spotted wing drosophila (SWD)- a hazardous quarantine pest native to Eastern and southeastern Asia infested simultaneously during 2008, for the first time in the European continent, Italy and Spain. Huge monetary losses, due to direct damage costs, market loss, management techniques, and rejection of exports for altered processing practices were immediately associated to *D. suzukii* arrival in Italy.

As oviposition of *D. suzukii* begins at ripening very close to harvest, finding the most promising natural enemy, alternative to chemicals, is an important tool in the control of this fruit fly. The aim of the present research was to deepen the knowledge on pest-parasitoid relationship in Lombardy inventoring the natural native enemies present in the area in association to the new pest. Six parasitoids have been found in association to drosophilids in Lombardy: *Pachycrepoideus vindemmiae* (Rondani, 1875) and *Spalangia erythromera* Forster, 1850 (Hym.: Pteromalidae), *Leptopilina boulardi* (Barbotin, Carton and Kelner-Pillaut, 1979) and *Leptopilina heterotoma* (Thomson, 1862) **(**Hym.: Figitidae), *Trichopria drosophilae* (Perkins, 1910) (Hym.: Diapriidae) and *Asobara tabida* Nees von Esenbeck, 1834 (Hym.: Braconidae)**.** Among the six parasitoids found in field monitoring, the attention was focused on the pupal parasitoid *T. drosophilae* (Hymenoptera: Diapriidae). Laboratory tests were made and information on the performances of *T. drosophilae* in relation to host prey (*D. suzukii* vs *D. melanogaster*) and to *D. suzukii* was acquired. *T. drosophilae* revealed to be a good candidate for mass rearing and biological control methods.

Information on the coexinstence of *D. suzukii* with other exotic drosophilids were also acquired. The presence in Lombardy of two other exotic drosophilids was ascertained: *Chymomyza amoena* (Loew, 1862) and *Zaprionus tuberculatus* (Malloch, 1932). This was the first report for Lombardy region as *C. amoena* was first detected in Italy in Veneto in 1999 (Bächli *et al*. 1999) and *Z. tuberculatus* was first detected in Trentino in 2013 (Raspi *et al*. 2014). In the present study, the population, seasonal activity, favorable habitat and hosts of these two exotic pests were also studied.

Rationale and aims

Drosophila suzukii (Matsumuta) is spreading fast all around the world, and making lots of damage in cultivated yards and gardens. Chemical products which are generally fast-acting and limit the damage done to crops are increasing consequently leading to side effects. Several organic chemical pesticides available within attacked areas (e.g., Spinosad) have shown under laboratory conditions excellent potential. However, organic fruit growers still have limited options as few products, appropriate for organic use, have been found to be effective against *D. suzukii*. Equally, the use of chemical insecticides can be very disruptive to natural enemies already being used in integrated pest management strategies within cropping ecosystems. Therefore, there is the need to screen for potential biological control as alternatives the use of chemicals agents for *D. suzukii*.

The current work aims primarily at the research of natural enemies to be implied against *D. suzuki*. Chapter 3 refers to field researches in Lombardy organized with the main purpose to list the diversity of natural enemies found in relation to drosophilidae. List, species abundance and phenology throughout the year are given.

Chapter 4 refers to a laboratory study aimed at deepening information about one of the species detected (*Trichopria drosophilae* Perkins) that seemed most promising as biological control agents of *D. suzukii*. As few is the information in literature on this parasitoid, tests were executed at different temperatures on both *Drosophila melanogaster* Meigen and *D. suzukii*. Biological performances, optimum temperatures and thermal limits of *T. drosophilae* and also the most adapt host were looked for.

As it is important to find field relationship between exotic species and authoctonous ones, chapter 5 reports information on the possible presence of two other exotic drosophilids (*Chymomyza amoena* (Loew) and *Zaprionus tuberculatus* Malloch) in the same areas where *D. suzukii* has been already detected. Notes on dynamics of these species in Lombardy are also provided.

1. *Drosophila suzukii* (Matsumura, 1931)

1.1 Taxonomy and Origin

Drosophilidae is a large, cosmopolitan family that encompas approximately 80 genera and more than 4,000 species (Bächli 2016) which are distributed in the subfamilies Steganinae and Drosophilinae (Baechli *et al*. 2004, Gottschalk *et al*. 2008). Two monotypical genera, *Apacrochaeta* Duda 1927 and *Sphyrnoceps* de Meijere 1916, have not been included in any subfamily (Baechli *et al*. 2004, Gottschalk *et al*. 2008). In Europe, Steganinae is represented by seven, and Drosophilinae by 10 genera (Baechli *et al*. 2004). The genus *Drosophila* Fallén 1823 from Drosophilinae is a large group belongs to the tribe Drosophilini, subtribe Drosophilina and infratribe Drosophiliti which contains over 2,000 species arranged into 13 subgenera all over the world of which three are present in Europe (O'Grady and Markow 2009, Baechli *et al*. 2004). Overall, 1,189 species are classified in *Sophophora* subgenus in which *Drosophila suzukii* (Matsumura, 1931) is included (Bächli 2016).

The invasive *D. suzukii* is native to the temperate East Palearctic zoogeographical regions from eastern and south-eastern Asia. It was detected and invaded simultaneously in North America and Europe from 2008 (Chabert *et al*. 2012, Hauser 2011, Cini *et al*. 2012). In Italy, this hazardous quarantine pest was reported for the first time in 2009 from Trentino - Northern Italy- by Grassi *et al*. (2009). In 2011, during the double check of the contents of Malaise traps deployed in 2008 in Tuscany (San Giuliano Terme, Pisa, Italy) Raspi *et al*. (2011) have found some *D. suzukii* adults so Italian invasion needs to be dated anticipatedly. Following these detections, the insect rapidly spread all over Italy (Süss and Costanzi 2010, Franchi and Barani 2011, Pansa *et al*. 2011, Griffo *et al*. 2012, Tiso 2013).

1.2 Morphology

The adult males and females of *D. suzukii* are small flies approximately 2.0– 4.0 mm long with a wing span of 6 – 8 mm. Males are usually slightly smaller (2.0–3.5 mm) than females (2.5–4.0 mm) (Kanzawa 1939). The head is entirely yellow; compound eyes are red to red orange covered with short and dense pilosity. Antennae are 3-segmented with all segments yellow, and the terminal segment (flagellum) with a dorsal plumose arista typically forked at the tip through the main stem and the last lateral hair. The fork-type in the antennae is one of the key characters in separating Drosophilidae from other Diptera families with plumose arista. In females, wings are completely transparent but in males there is a single dark spot on edge near the tip (Figure 1-1 a, b & c). Notably, small and young males of *D. suzukii* sometimes lack their wing spot, which could lead to misidentifications (Hauser 2011). It should also be noted that the spots are not visible when the male first emerges as they start to appear from 10 hours to two days depending on the temperature (Beers *et al*. 2010). However, there is another difference that allows to easily recognize *D. suzukii* males: front legs in first and second tarsomere pose sex combs with sharply tapered and pointed teeth, both parallel to the length of the foot, with 3 to 6 "teeth" each, while in females and other drosophilids lack these teeth (Kopp 2011) (Figure 1-2). In *D. suzukii* females, ovipositor has strong saw-like teeth darker than rest of ovipositor (Figure 1-1 d). In both sexes at the end of abdominal segments, there are completely unbroken dark bands; but last segment can be all dark brown; no other stripes, spots or patterns are on the body (Radonjić and Hrnčić 2015) (Figure 1-1 a & b).

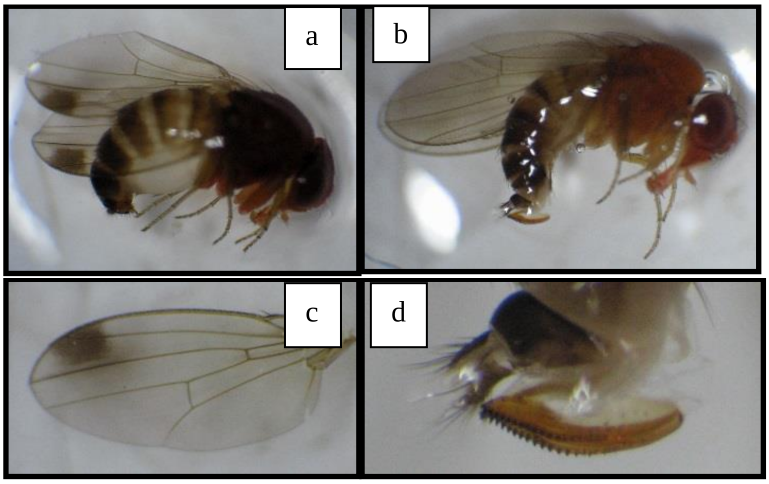

Figure 1-1 (a) Male; and (b) Female of* D. suzukii*; (c) Male wing; (d) ovipositor of female in lateral view.

***Figure 1-2 Sex combs on the foretarsi of male* D. suzukii**

Eggs of *D. suzukii* are oval, milky-white on average 0.62 × 0.18 mm. At the anterior end, two long filaments (0.7 mm long), extensions of the chorion, extend from the dorsal surface and they have two white respiratory filaments. The presence of these filaments protruding into the air is necessary as they allow gas exchange for the still-developing egg laid under the skin of the fruit in the mesocarp (Kanzawa 1939, Bolda *et al*. 2010). Figure 1-3 shows these filaments as well as the micropyle (channel into the ovum) through which sperm travel during egg fertilization.

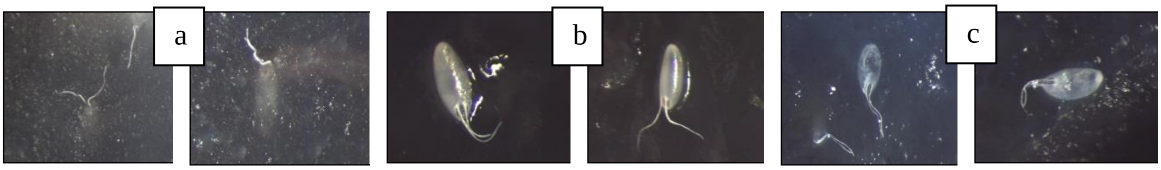

Figure 1-3* D. suzukii *eggs: (a) Eggs laid below the surface and respiratory filaments are out of diet; (b) Eggs left on the surface of diet (micropyle -sperm entry); (c) Hatched eggs.

The apodous larva of *D. suzukii* is amphipneustic having anterior (prothoracic) spiracles not adjacent, and posterior spiracles on two short processes, which are black in color. The cylindrical body has a soft transparent exoskeleton, milky-white with black cephalopharyngeal sclerites and also interior organs clearly visible in transparency. Larval development occurs through three instars before pupation (Thyssen 2010, Walsh *et al*. 2011) (Figure 1-4).

***Figure 1-4 Larva of* D. suzukii**

***Figure 1-5 Pupa of* D. suzukii**

The pupa is exarata, at first white and then it turns reddish-brown. It is surrounded by the last instar larval cuticle that becomes the puparium or pupal case to enclose the metamorphosing fly until it ecloses (Figure 1-5 & Figure 1-6). Puparium possess two distinctive pairs of horn-shaped protrusions at both ends, which are its respiratory organs; each anterior protrusion bears seven to eight radially arranged branches that is characteristic of species (Figure 1-7). Pupation may occur inside or outside the fruits (Kanzawa 1935).

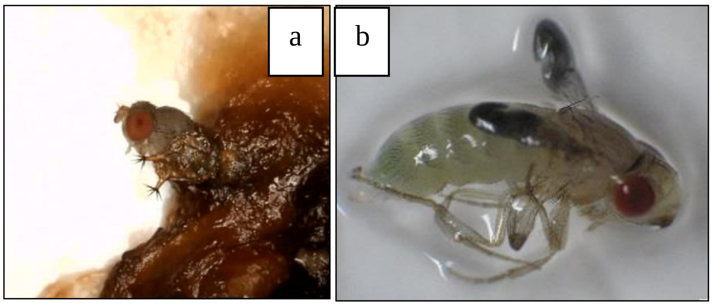

Figure 1-6 (a)* D. suzukii *adult emerging (photography: Daniela Lupi), (b)* D. suzukii *adult eclosion (puparium has been just removed from around pupa and wings become black)

Figure 1-7 Respiratory tubes of the anterior spiracles with seven radially branches in* D. suzukii *pupa

1.3 Life cycle

Multiple generations per year occur. Observation across a wide geographical range in Asia indicated that the number of generations depends on the climatic conditions of the area and the year examined and could range from 3 to 13 (Kanzawa 1939). Kanzawa (1939) reported that in Japan, it completes 10-13 generations in one year, while according to the degree-day model developed by Coop (2010), it is to complete 3 to 9 generations per year in the West United States, Canada and also northern Italy. *D. suzukii* overwinters as adult (Dalton *et al*. 2011, Walsh *et al*. 2011, Kaçar *et al*. 2016). Flies emerge in spring but some adults may be active during warm winter days (Kaçar *et al*. 2016, Rossi Stacconi *et al*. 2016). Initial spring activity for *D. suzukii* is predicted to begin at 250 Degree-Days, 50% egg laying on 490 DD, and an accumulation of 744 DD for development from egg to adult in the first generation (Walsh *et al.* 2011, Wiman *et al*. 2016).

Kanzawa (1939) studied the developmental times at two constant temperatures (15°C and 25°C) (Table 1-1). *D. suzukii* eggs may hatch after 2 hours (at 25°C) till 3 days (at 15°C). At 25° C, larval development occurs in 6 days (2 days per instar). The pupal stage lasts for 4 -9 days till adults' emergence (Walsh *et al*. 2011); so, it takes 9 days in mean to complete one generation at 25℃. Usually male adults emerge before females to reduce the risk of virginity in offspring. Adults can survive for 20-30 days at 25°C (Trottin *et al*. 2014).

Table 1-1 Development time of different life stages of* D. suzukii *at 15 and 25˚C (Kanzawa 1939)

Life stage	Average time at 15℃	Average time at 25℃
egg	1 d 20 hr (44 hrs)	0 d 13 hr (13 hrs)
1st instar	3 d 4 hr (76 hrs)	1 d 20 hr (27 hrs)
2nd instar	2 d 19 hr (67 hrs)	0 d 23 hr (23 hrs)
3rd instar	5 d 6 hr (126 hrs)	2 d 7 hr (55 hrs)
Pupal period	10 d 13 hr (253 hrs)	4 d 13 hr (109 hrs)
Total larval period	11 d 2 hr (266 hrs)	4 d 11 hr (107 hrs)
Oviposition to adult emergence	22 d 17 hr (545 hrs)	9 d 15 hr (231 hrs)

1.4 Invasion patterns and impacts

D. suzukii is a polyphagous pest of 15 families of small and stone fruits (Kanzawa 1939, Grassi *et al*. 2012, Walsh *et al*. 2011). The most impacted crops include raspberry, blackberry, blueberry and cherry (Lee *et al*. 2011a, Asplen *et al.* 2015). Raspberry is particularly at risk for its flavors, and soft epicarp making attraction and oviposition relatively easy for *D. suzukii* (Lee *et al*. 2011b, Bellamy *et al*. 2013, Burrack *et al*. 2015, Abraham *et al*. 2015).

Usually most of the damages are due to larval feeding on fruit pulp which causes fruit turns brown and soft. Both oviposition scars and larval feeding activity provide access to secondary infections of pathogens such as fungi, yeasts and bacteria, or to other drosophilids which would not be able to penetrate into healthy fruits.

1.5 Management strategies

The current state of *D. suzukii* management framework in fruit production areas relies on IPM systems; and long-term restructuring of the programs includes physical, cultural, biological and chemical control methods (Cini *et al*. 2012, Asplen *et al*. 2015, Haye *et al*. 2016).

Degree-day tools created can also be very useful to integrate various approaches (Coop and Dreves 2013).

1.5.1 Monitoring. It is actually very difficult to correlate trap catches with actual population density, and almost impossible to calculate thresholds for management activities. Physiological status, fruit competition, bait efficacy and many other variables may influence trap efficiency. However, trap catches are a good way to detect the presence of *D. suzukii* in field and flight period asa dult trapping can be indicative of pest pressure (Tochen *et al*. 2014). Bucket-style traps or quart containers used for monitoring other *Drosophila* flies can be effectively used for monitoring *D. suzukii*. Mixtures of yeast, sugar, and water; fruit purees, distillates from apple cider vinegar or wine; ethanol, acetic acid, and phenylethanol in 1: 22: 5 ratios are potent baits for monitoring *D. suzukii* populations. Addition of a small drop of dish soap as a surfactant or placement of a sticky card within the traps improves trap efficiency by retaining the flies which have already entered the traps. *D. suzukii* monitoring can last all the or can start just before fruits begin ripen (Diepenbrock *et al*. 2016) depending on the purpose of the research. Trap location is also important, as they appear to perform best when deployed under cool and shady areas in the field (Walsh *et al.* 2011).

1.5.2 Physical control. Physical exclusion has significant potential for use under protected culture such as tunnels (Lee *et al*. 2011b). Insect proof nets has shown promise for reducing *D. suzukii* infestation in small-scale plantings of blueberries and raspberries in North America (Link 2014, Cormier *et al*. 2015, Rogers *et al*. 2016) and of blueberries in Europe (Kawase *et al*. 2007, Grassi and Pallaoro 2012).

1.5.3 Cultural control. Since *D. suzukii* is known to use noncrop hosts growing up at the borders of crop fields, immigration into fields is a major source of ovipositing flies during the growing season (Klick *et al*. 2015, Lee *et al*. 2015, Pelton *et al*. 2016). Therefore, managing pest on wild host plants is necessary. Moreover, ripe fruits should be picked frequently to minimize population build-up. All damaged fruits on the ground should be removed and destroyed, either by burial or disposal in closed containers (Walsh *et al*. 2011). Also, the selection of more resistant cultivars with fruits with firmer skin could prevent *D. suzukii* oviposition (Kinjo *et al*. 2013).

1.5.4 Chemical control. Fruits appear to be susceptible to *D. suzukii* from light straw color till harvest time and beyond. Bruck *et al.* (2011) showed that organophosphorus and pyrethroid insecticides will provide a weak of control (5- 14 days). Three insecticides with favorable characteristics include lambda-cyhalothrin, spinosad and Malathion, which allow producers to incorporate the principles of efficacy, fruit susceptibility, and resistance management (Haviland and Beers 2012). It is suggested to rotate classes of insecticides to delay insecticide resistance development, and prevent phytotoxicity to some cultivars.

1.5.5 Biological control agents

1.5.5.1 Entomopathogenic fungi. Two entomopathogenic fungi, *Isaria fumosorosea* Wize and *Metarhizium anisopliae* (Metschn.), were considered as potential biological control agents of *D. suzukii* (Naranjo-Lazaro *et al*. 2014, Cuthbertson and Audsley 2016). The results showed that the percentage of the mortality of the adult *D. suzukii* was between 40% and 85% using different strains of fungal *I. fumosorosea*, and between 12% and 40% reduction by different strains of *M. anisopliae* (Naranjo-Lazaro *et al*. 2014, Cuthbertson and Audsley 2016). Totally, entomopathogenic fungi could be used as biological control agents but they may not be enough to control fly populations as individual agents (Cuthbertson and Audsley 2016).

1.5.5.2 Enthomopathogenic nematodes. The entomopathogenic nematodes, *Steinernema feltiae* (Filipjev), *Steinernema carpocapsae* (Weiser), *Steinernema kraussei* (Steiner) and *Heterorhabditis bacterophora* (Poinar) were used against *D. suzukii*. Cuthbertson and Audsely (2016) reported that all these nematode species had an impact on survival of both larvae and pupae of *D. suzukii* with a mortality more than 50%.

Demonstrating that nematode species perform much better against larvae and pupae when they are applied as a soil drench, they do not work well on being applied to fruit. Using the nematodes as an alternative to complete reliance on IPM is recommended (Cuthbertson and Audsely 2016).

1.5.5.3 Invertebrate predators. Various predators were found in association to *D. suzukii* larvae in fruits. The predation rate of some predators including *Orius majusculus* Reuter, *Orius laevigatus* (Fieber), *Anthocoris nemoralis* (Fabricius) (Heteroptera: Anthocoridae), *Atheta coriaria* Kraatz (Coleoptera: Staphyliniidae) and *Hypoaspis miles* Berlese (Acari: Laelapidae) were evaluated by Malagnini *et al*. (2014) and Cuthbertson *et al*. (2014a). *Orius laevigatus* did not display any predatory activity against *D. suzukii* but *O. majusculus*, *A. coriaria* and *H. miles* had shown slight predatory activity (Malagnini *et al*. 2014, Cuthbertson *et al*. 2014). Only *A. nemoralis* caused 45% mortality on *D. suzukii* preferring male adults (Cuthbertson *et al*. 2014). According to Cuthbertson *et al*. (2014a) none of these invertebrate predators are able to control *D. suzukii* individually but in the open field they could play a role in helping to reduce population, and conservation of their population is encouraged.

1.5.5.4 Parasitic wasps

A number of hymenopteran parasitoids have been reported in association with *D. suzukii* in its native area. In particular, species of the genera *Ganaspis* and *Leptopilina* (Hymenoptera: Figitidae) and *Trichopria* (Hymenoptera: Diapriidae) are reported as parasitoids of *D. suzukii* in Japan (Cini *et al.* 2012). *Ganaspis* species showed the highest rates of *D. suzukii* parasitism. *Ganaspis* species lay eggs in larvae that are feeding in fruits, and exhibit a high level of specificity for *D. suzukii*. By contrast, *Leptopilina japonica* and *Asobara japonica* (Hymenoptera: Braconidae) were only able to attack *D. suzukii* larvae and pupae in fallen decaying fruits, and also attack a wide range of drosophilid hosts (Mitsui *et al*. 2007, Ideo *et al*. 2008, Mitsui and Kimura 2010, Novkovic *et al*. 2011, Kasuya *et al*. 2013).

Nearly 50 parasitoid wasp species, belonging to four families (Braconidae, Diapriidae, Figitidae and Pteromalidae) distributed at least in 16 genera are known to develop on *Drosophila* spp. (Carton *et al*. 1986). Among them, some species have been found also in association to *D. suzukii*.

Two larval parasitoids, *L. heterotoma* and *L. boulardi* due to their high population all around the world have been studied in different researches. Several populations of these two larval parasitoids have shown different parasitism rate on *D. suzukii* according to geographical population and genetics (Kraaijeveld and Godfray 1999). In France, one population of *L. heterotoma* had a peak parasitism rate of 95% while *L. boulardi* was able to parasitize 67% of exposed hosts in laboratory conditions (Chabert *et al*. 2012). On the contrary, in northern Italy there is a strain of *L. heterotoma* able to overcome the immunological response of *D. suzukii* and hence to complete development in the host (Rossi Stacconi *et al.* 2015) while other populations of both *L. boulardi* and *L. heterotoma* proved that they were unable to parasitize *D. suzukii* (Mazzetto *et al.* 2016).

Furthermore, another larval parasitoid *Asobara tabida* (Hymenoptera: Braconidae) emergence has been reported from field sampled Japanese *D. suzukii* populations in its native range (Mitsui *et al.* 2007). This Asian *Drosophila* parasitoid which is found in North America and Europe does not show a promise biological control of *D. suzukii* (Chabert *et al*. 2012, Kacsoh and Schlenke 2012). *A. brevicauda*, *A. japonica* and *A. leveri* were also reported in association with *D. suzukii* in South Korea and China. *A. brevicauda* can develop only on *D. suzukii,* while *A. japonica* and *A. leveri* could parasitise both *D. suzukii* and other drosophilids. As these three *Asobara* species may have the potential for using in classical biological control and may contribute to the suppression of *D. suzukii* in the newly invaded regions more researches are needed (Daane *et al*. 2016, Guerrieri *et al.* 2016, Mitsui and Kimura 2010).

On the other hand, two cosmopolitan pupal parasitoids have been reported to attack and develop from *D. suzukii* in North America and Europe: *Pachycrepoideus vindemmiae* (Rondani) and *Trichopria drosophilae* Perkins (Chabert *et al.* 2012, Gabarra *et al.* 2015, Rossi Stacconi *et al.* 2013 and 2015).

P. vindemmiae has a wide host range, as it has been reported in association to over 60 fly species worldwide (Carton *et al*. 1986, Wang and Messing 2004). In the laboratory, its parasitization efficacy was confirmed with parasitism up to 60% on infested raspberry by *D. suzukii* (Chabert *et al*. 2012, Rossi Stacconi *et al.* 2013, Gabarra *et al*. 2015). However, field-parasitism levels are typically lower in crop systems (Miller *et al*. 2015).

T. drosophilae is more specialized on frugivorous *Drosophila* spp., occupying a worldwide geographic range including Europe, Africa, North America, and Australia (Carton *et al*. 1986) have shown more effectiveness on *D. suzukii* compare to other parasitoids (Chabert *et al*. 2012, Rossi Stacconi *et al*. 2013). Recently, in a research in two heavily infested commercial strawberry fields in Northeastern Spain, up to 10.7% parasitism on *D. suzukii* by *T. drosophilae* was reported, and the parasitism rate in laboratory was achieved 68% (Gabarra *et al*. 2015).

***Pachycrepoideus vindemmiae* (Rondani, 1875) (Hymenoptera, Chalcidoidea, Pteromalidae) (**Figure 1-8**).** This parasitoid is a cosmopolitan idiobiont ectoparasitoid (Alphen and Thunnissen 1984) attacking pupae of 13 families of Diptera including Anthomyiidae, Calliphoridae, Fanniidae, Lonchaeidae, Muscidae, Phoridae, Piophilidae, Sarcophagidae, Stratiomyidae, Tachinidae, Tephritidae, Cecidomyidae and Drosophilidae (Wang and Messing 2004, Noyes 2002). *P. vindemmiae* accepts pupae as soon as the space between the pupae and puparium wall is formed while it rejects puparia containing adult flies about to emerge (Nøstvik 1954). This species is either a primary parasitoid or a secondary parasitoid when the female lays egg on hosts previously infested by its conspecific or heterospecific (Chen *et al*. 2015, Wang *et al*. 2016a). It can be a solitary parasitoid or can show gregarious characteristics when reared on larger hosts (Crandell 1939).

P. vindemmiae larva has six instars. Each instar is similar in appearance; all are conical in shape with the prothoracic segment larger than the remaining segments narrowing posteriorly (Wang *et al*. 2016a). *P. vindemmiae* larvae possess small mandibles, lack appendage and remain largely immobile on the host surface in all instars (Tormos *et al*. 2009). Significantly, more male offspring emerge earlier, especially on the first days during emergence, sex ratio is male biased (Hu *et al*. 2012).

Figure 1-8 **P. vindemmiae:** ***male (on the left) female (on the right)***

***Spalangia erythromera* Forster, 1850 (Hymenoptera, Chalcidoidea, Pteromalidae) (**Figure 1-9**).** *S. erythromera* is a pupal ectoparasitoid, and lays its eggs in the space between the pupa and the puparium (Carton *et al*. 1986). This parasitoid is common throughout Western Europe (Noyes 2003) and in the Nearctic region extending from Alaska to at least southern USA and possibly into South America (Gibson 2009). The horn fly, *Haematobia irritans* (Linnaeus), is the only confirmed New World host record based on voucher specimens of Depner (1968). Davis *et al.* (1996) reported it as the parasitoid of *Drosophila buskii* Coquillett, *Drosophila kuntzei* Duda, *Drosophila melanogaster* Meigen, *Drosophila phalerata* Meigen and *Drosophila subobscura* Collin. Noyes (2003) also listed *Muscina* sp. (Muscidae) and several other hosts in Anthomyiidae, Lonchaeidae, Phoridae and Sepsidae in Europe. According to Knoll *et al*. (2017), *S. erythromera* was able to reduce 62.3% of *D. suzukii* emergence under the laboratory conditions although it produced a few offspring on this host.

***Figure 1-9 Female of* S. erythromera**

Leptopilina boulardi **(Barbotin, Carton and Kelner-Pillaut, 1979) and *Leptopilina heterotoma* (Thomson, 1862) (Hemenoptera, Cynipoidea, Figitidae) (**Figure 1-10**).** *L. boulardi* and *L. heterotoma* are both cosmopolitan endoparasitoid of early instar dipterous larvae, acting as koinobiont. The first larval instar is highly specialized (known as the 'eucoiliform' type larva), having the mouth with reduced mandibles adapted for fluid feeding, three pairs of long, fleshy thoracic processes, and a long tail which are lost in the following instars (Hanson and Gauld 2003, Keilin and Baume-Pluvinel 1913, Huzimatu 1940). As the first instar larva grows, its body becomes proportionately larger in relation to its caudal appendage (Jenni 1951, Wishart and Monteith 1954).

L. heterotoma and *L. boulardi* are able to attack the larvae of *D. suzukii* but this parasitisazion is not successful as the pest is able to bypass this attack encapsulating the eggs into haemocoel (Poyet *et al.* 2013, Sorrentino *et al*. 2002 and Jung *et al*. 2005). An exception is a population of *L. heterotoma* found in Northeast Italy that is able to develop successfully in *D. suzukii* (Rossi Stacconi *et al*. 2015). The two main mechanisms adopted by parasitoids to avoid the encapsulation process by their hosts include the presence of a non-reactive coating on their eggs, or a suppression of the host immune system by injecting venom into hosts along with their eggs (Kacsoh and Schlenke 2012, Lee *et al*. 2009). For both strategies, genetic intra-specific variations are present, and a diverse immuno-suppressive effect has been observed among different populations (Dubuffet *et al.* 2007, Dupas and Carton 1999, Dupas *et al*. 1996).

Figure 1-10 **L. heterotoma:** ***female (above) and male (below)***

Asobara tabida **Nees von Esenbeck, 1834 (Hymenoptera, Ichneumonoidea, Braconidae**) (Figure 1-11). *A. tabida* is a larval koinobiont and endoparasitoid of Drosophilidae larvae breeding on fermenting fruits (Chabert *et al*. 2012, Vet and Bakker 1985, Janssen *et al*. 1988, Ideo *et al*. 2008). Its geographic range includes the northwest of America (Hoang 2002), Japan (Mitsui *et al*. 2007) and Europe (Carton *et al*. 1986). In Japan *A. tabida* has been found emerging from *D. suzukii* pupae collected in the field (*Mitsui et al.* 2007). Several laboratory experiments have shown that European population of *A. tabida* could oviposit in *D. suzukii* but did not survive on this host (Chabert et al. 2012, Kacsoh and Schlenke 2012). A possible explanation is that local European populations of *A. tabida* have not yet adapted to this exotic host (Chabert *et al*. 2012). It is also possible that geographic variability occurs in the flies' immune resistance against parasitoids or in parasitoid species for their preference or effectiveness against flies (Rossi Stacconi *et al*. 2015). Oviposition by *A. tabida* in larvae of alien *Drosophila* species may therefore only occur when native host species are scarce and may thus help the parasitoid to persist during the period when the preferred hosts cannot be found (van Alphen and Janssen 1981, Carton *et al*. 1986, Janssen *et al*. 1988, Kraaijeveld and van der Wel 1994, Kraaijeveld *et al.*1995).

Figure 1-11* A. tabida: *male (left) and female (right)

***Trichopria drosophilae* (Perkins, 1910) (Hymenoptera, Diaproidea, Diapriidae) (**Figure 1-12**).** *T. drosophilae* belongs to the subfamily of Diapriinae, which is mainly associated to Diptera Ciclorrapha. It is an idiobiont endoparasitoid which attacks and develops in puparia of the common fruit fly, *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) and other frugivorus Drosophilidae (Roberts 1935, Knutson and Berg 1963, Carton *et al*. 1986, Fleury *et al.* 2009). *Trichopria* lays its eggs in the hemocoel of the host. Unconsumed residues of the host and the meconium stay in the back part of the puparium, while emergence occurs through a hole gnawed through the operculum localized in the frontal part (Carton 1986) (Figure 1-13).

There is, however, limited information on the biology of *T. drosophilae* in general and its association with *D. suzukii* in particular.

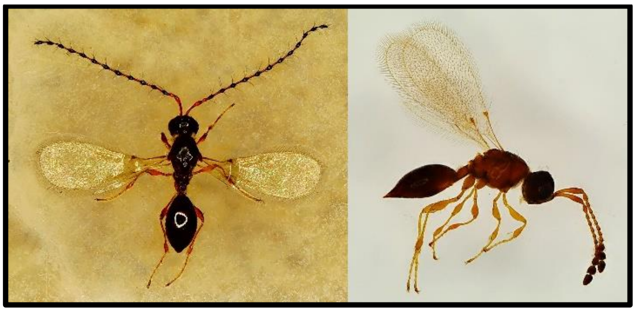

Figure 1-12* T. drosophilae: *male (left) female (right)

Figure 1-13 Hole gnawed by* T. drosophilae *and exit from* D. suzukii *pupae

2. Exotic drosophilids coexisting with *D. suzukii* in Italy

2.1 *Chymomyza amoena* (Loew, 1862)

Chymomyza Czerny, 1903 with 60 described species (Bächli 2016; Markow and O˚Grady 2006b) belongs to the family Drosophilidae, subfamily Drosophilinae, tribe Drosophilini, subtribe Drosophilina and infratribe Drosophiliti. The species *C. amoena* is endemic to eastern North America (Band 1988a) and was recorded in Europe for the first time from the Czech Republic in 1975 (Màca 1985). Subsequently in 1999 one specimen of *C. amoena* had been collected from Veneto -Northeastern Italy- and reported by Bächli *et al*. (1999). Nowadays, it has spread widely in Eastern and Central Europe (Baechli *et al.* 2004). Its success on the European Continent might be due to finding an open ecological niche, to superior competitive ability (Máca and Bächli 1994), ecological versatility, and cold hardiness (Band 1996).

C. amoena is considered a forest species (Band *et al*. 1998, Band 1996) although the adults are collected in gardens and in woodland areas (Sabath 1974, Burla and Bächli 1992). As females are not able to oviposit in undamaged fruits, it is considered a secondary invader of domestic and wild apples (*Malus domestica*, *M. coronario*), pears (*M. pyramidus*), plums (*Prunus domestica*), wild cherries, hazelnuts, chestnuts, and acorns (Band 1988a, 1988b & 1988c, Band 1995a & 1995b, Band 1996, Band and Band 1980, Band and Band 1984, Band *et al.* 2005, Burla and Bächli 1992). *C. amoena* are yellowish flies with pale yellowish legs. In both genders, wings have two broad, transversal, distinct dark brown bands and a dark spot along R1 (Bächli *et al*. 2004) (Figure 2-1).

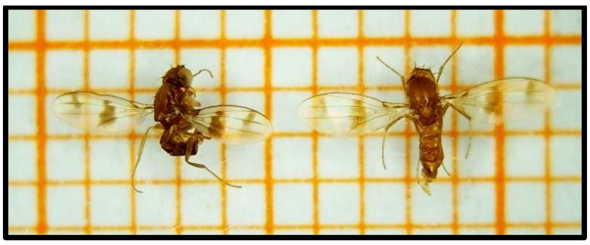

Figure 2-1 **C. amoena:** ***male (left) and female (right)***

The eggs from *Chymomyza* species are distinct. There are approximately eight short curved filaments regularly decreasing in size from posterior to anterior (Figure 2-2) (Throckmorton 1962).

Figure 2-2 **Chymomyza** ***sp. egg (ex Throckmorton 1962)***

Figure 2-3 Anterior pupal spiracles in **C. amoena** ***(ex Throckmorton 1962)***

The larvae of this species like other drosophilid flies are amphipneustic type. Anterior spiracles are not adjacent, and posterior spiracles are positioned on two short black processes (Thyssen 2010).

The pupae of *C. amoena* are exarate and the puparium spiracle is basically as in obscura group species, except that the rim of the spiracle is extended slightly as an antibasal lip (Figure 2-3) (Throckmorton 1962).

C. amoena is multivoltine (Band 1988a). Band (1988a & 1988b) reported that this species is polymorphic for developmental time that can vary from 30 up to 64 days during the breeding season. They oviposit eggs in clusters of 10 eggs (Band 1989). *C. amoena* larvae appear to be among the dipteran species that have no obligate diapause (Band and Band 1980, Tauber and Tauber 1982). Overwintering mechanisms employed by *C. amoena* larvae appear to depend upon larval feeding site (Band and Band 1982). According to Band and Band (1983), apples, ornamental crabapples, black walnuts and endemic crabapples are autumn hosts of this exotic drosophilid in overwintering niches. Population is also polymorphic for cool resistance. As *C. amoena* larvae in summer were found to be either potentially freeze or supercool tolerant. It shows a shifting polymorphism between 50% FT (freeze tolerant, larvae in winter supercooled to avoid freezing) and 50% FS (freeze sensitive) types in a severe winter but in mild winters many larvae remain FS (Duman *et al*. 1982).

Although *C. amoena* oviposit a mean of 10 eggs per cluster in fruits, the emergence and survival is low. According to Band (1989), there is also a winter mortality of over fifty percent. Although *C. amoena* is considered as a low density and furtive species (Band 1989, Band and Band 1986) in autumn 1984 a heavy invasion of black walnut husks, *Juglans nigra* happened in Michigan State (Band and Band 1986). Since *C. amoena* is a low density species, there is no work and report of parasitization on it.

2.2 *Zaprionus tuberculatus* Malloch, 1932

Zaprionus Coquillett, 1901 with 61 species is classified under two subgenera: *Anaprionus* (12 species), and *Zaprionus* sensu stricto (49 species) (Bächli 2016). This drosophilid belongs to the subfamily Drosophilinae, tribe Drosophilini, subtribe Drosophilina and infratribe Drosophiliti.

Recently, three distantly related Afrotropical species of the genus *Zaprionus* (*Z. indianus*, *Z. tuberculatus* and *Z. ghesquierei*) became invasive and have been detected in the Palearctic region (Chassagnard and Kraaijeveld 1991). In Europe Continent, *Z. indianus* Gupta 1970 commonly known as "Fig fly" native to Africa, the Middle East, and southern Eurasia (Chassagnard and Kraaijeveld 1991, Bächli 2016) occurred in Italy-Venice in 1988 (Bächli 2016), and later in 2014, *Z. tuberculatus* commonly known as "Vinegar fly" or "Pomace fly" native to the Afrotropical region and the islands of the Indian Ocean (Chassagnard and Tsacas 1993) was collected and reported from Italy- Trentino (Raspi *et al* 2014).

Z. indianus is a generalist that breeds on fallen fruit and fruit on the tree (van der Linde *et al*. 2006). It can utilize over 80 host plants (Yassin and David 2010). This species has been recorded as a pest on oranges, peaches, and figs in Brazil (Santos *et al*. 2003), and might therefore become a pest in Italy. If it can spread in the country, control will be difficult because its breeding substrates are not limited to cultivated fruits (Santos *et al*. 2003, Steck 2005), and because of its ability to lay eggs on fruits that are still on the plant (Tidon *et al*. 2003, Steck 2005).

Adaptation of *Z. tuberculatus* to cooler climates was unpredictable as its native habitat is Uganda (Buruga 1976). The occurance of this species at highest latitudes indicates plasticity in tolerance to environmental conditions and potential ability to survive in North Italy. It is hypothesized that this species can spend colder months in hibernation, and when the temperature is raising, and plants are flowering and fruiting, the pest can start the activity.

Adults in *Zaprionus* genus show slight sexual dimorphism. Distinctive bright white or silvery stripes extend longitudinally from the fronto-orbital plates down the mesonotum to the scutellum. The adults of *Z. tuberculatus* are about 3.5 mm with yellow head with red eyes, yellowish brown thorax with three whitish stripes, one in the middle and two extending longitudinally from the fronto-orbital plates down the mesonotum to the scutellum bordered by dark brown stripes, and two other whitish stripes in two sides of notum (Markow and O'Grady 2006b). The abdomen and legs are yellow too (Figure 2-4 and Figure 2-5).

Profemures in *Z. tuberculatus*, as well as in several other congenerics poses a single enlarged ventral tubercles (Markow & O'Grady 2006b). This species is characteristic because the tubercule has a strong seta in apical part followed by a smaller adjacent one (Baechli *et al*. 2004, Markow and O'Grady 2006b) (Figure 2-6).

Figure 2-4* Z. tuberculatus: *male (left) and female (right)

The eggs have four filaments like other African *Zaprionus* spp. (Figure 2-7). The larvae are amphipneustic (Okada 1968). In all *Zaprionus* species, the puparia are reddish brown (Yassin and David 2010) with clubbed type branches in the anterior spiracles (Okada 1968). The number of these branches varies from 11 to 21 (Yassin and David 2010).

***Figure 2-5 dorsal and lateral view of* Z. tuberculatus**

***Figure 2-6 profemore in* Z. tuberculatus**

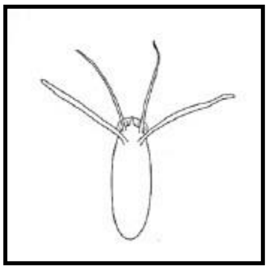

Figure 2-7* Z. tuberculatus *egg (ex Tsacas* et al. *1977)

In *Z. tuberculatus*, the life cycle takes more than five weeks for adult emergence (Buruga 1976). Unfortunately, there is few information about life cycle and overwintering of *Z. tuberculatus* in the literatures, and it needs more works and researches.

Z. tuberculatus is a polyphagous species found in anthropic habitats as well as in the wild and had been detected in the fruits of 49 plants species of tropical fruit, and the flowers from several families (Brake and Bächli 2008, Lachaise and Tsacas 1983, Makow and O'Grady 2006, Okada and Carson 1983, Yassin *et al*. 2012, Buruga 1976). The female Zaprionids during feeding on several plant families lays eggs in the flowers. These eggs develop in a few days to form active feeder larvae so that the flower withers and rots (Buruga 1976).

Even if this species is widespread very little is known about its biology and ecology and also about agricultural damages deriving from its presence with special emphasis to soft fruit cultivations (Raspi *et al*. 2014).

Literatures report no information about *Z. tuberculatus* parasitoids, but Marchiori *et al.* (2003) found different species attacking the congeneric *Z. indianus*: *P. vindemmiae* (Hymenoptera: Pteromalidae), *Spalangia endius* Walker (Hymenoptera: Pteromalidae) and *L. boulardi* (Hymenoptera: Figitidae). It is feasible to find the species in association also to *Z. tuberculatus*.

3. Field survey on parasitoids of *Drosophila* spp. in North Italy*

3.1 Material and methods

Field monitoring of parasitoids of *Drosophila* spp.

Surveyed sites

Field surveys were carried out in three sites (Site) in Lombardy region during 2014. Following are the fruiting plants (and sites) selected: rows of raspberry patches in Arcagna and Minoprio and, a blueberry plantation in Minoprio. Locations and characteristics of the surveyed sites are given in Table 3-1.

Table 3-1 Locations and characteristics of the sites where field surveys were carried out in 2014

Region	Site	Position	Crop/vegetation
Lombardy	Arcagna	45º20′17″ N 9º27′07″ E 79 m a.l.s.	Raspberry
	Minoprio	45º43′37″ N 9º05′09″ E 334 m a.l.s.	Raspberry and Blueberry

The population levels of *D. suzukii* at each site were evaluated by placing a trap baited with 250 mL of apple cider vinegar (ACV) (5% acidity). The traps were replaced weekly throughout the trial period (July–October). Removed traps were taken to the laboratory, where the ACV was filtered with a funnel (diameter 26 cm) lined with a fine mesh net to retain all insects. Using a brush, all of the Drosophilidae were collected, counted, and preserved in glass tubes (8 × 60 mm) filled with 70 % (v/v) ethanol. Finally, with a stereomicroscope and identification key (Vlach 2010), all *D. suzukii* flies were separated from the other Drosophilidae, and the numbers of male and female *D. suzukii* were recorded.

Field collection and laboratory observation of parasitoids

The presence and abundance of the parasitoids of *Drosophila* spp. were assessed following a modified protocol used by Fleury *et al.* (2004). Two open traps (Block) were placed in three different positions along the diagonal of each site from July to October 2014. The open traps consisted of a delta trap, on the bottom of which were placed two plastic dishes (diameter 90 mm), one with split banana and one with healthy blueberries (fruit). Split banana was chosen because it was largely used in similar studies (Novkovic´ *et al*. 2012, Fleury *et al*. 2004, Chabert *et al*. 2012), while blueberries were chosen both as a favorite host of *D. suzukii* (Kinjo *et al*. 2013, Mazzetto *et al*. 2015) and because they were already used to capture parasitoids (Rossi Stacconi *et al*. 2013). Each dish represented a statistical unit. To allow oviposition of both larval and pupal parasitoids, the dishes were exposed to natural field colonization for 7 or 14 days. The fruit in six of the dishes was changed every 7 days and the fruit in the other six dishes was changed every 14 days. Overall, four treatments, each with three repetitions, were compared in each site: (1) dishes with banana exposed for 7 days; (2) dishes with blueberries exposed for 7 days; (3) dishes with banana exposed for 14 days; (4) dishes with blueberries exposed for 14 days.

After field exposure, the dishes were transferred to the laboratory, where they were arranged to allow the adult emergence of *Drosophila* spp. and of parasitoids. The fruits from each dish were placed in disposable, net-covered cups (height 76 mm, diameter 60 mm, volume 100 mL). The disposable cups were kept at room temperature (about 25 ºC) for 40 days (i.e., the period necessary to obtain parasitoid emergence), and checked every 48 h to observe adult emergence. All *Drosophila* spp. adults were removed, but only the adults emerged in the first 10 days were counted and stored in 70 % (v/v) ethanol inside micro tubes (length 44 mm, diameter 10.8 mm, volume 2 mL) to avoid overlapping generations. Next, they were examined to separate individuals of *D. suzukii* from those of other *Drosophila* species. All parasitoid adults were removed, counted, and stored in 70 % (v/v) ethanol inside micro tubes (length 44 mm, diameter 10.8 mm, volume 2 mL) throughout the 40-day period. The adults were then examined, separated, and identified using specific keys (Bouček 1963, Graham 1969, Forshage and Nordlander 2008, Vlach 2010). Some individuals of each species of the different areas were sent to the respective specialist to assure a correct specific identification.

Statistical analyses

To test differences on emerged adults of parasitoid species from different fruits in each site all data were examined. Levene's test for homogeneity of variance was previously applied to all data. One-way analysis of variance (ANOVA), followed by Tukey-Kramer's HSD test (Tukey 1949) was applied in case of homoscedasticity. In the case of heteroscedasticity ($p<0.05$), non-parametric test, Kruskal-Wallis, was used. Statistical analyses were performed through SPSS Statistics 22 (IBM Corp. Released 2013, Armonk, NY).

3.2 Results

Field monitoring of parasitoids of *Drosophila* spp.

Field monitoring by traps baited with ACV confirmed the presence and abundance of *D. suzukii* in all surveyed sites. Despite the capture of large numbers of the exotic fly in ACV traps, relatively few (below 0.5 %) *D. suzukii*, as compared to other *Drosophila* spp., emerged from the field-exposed fruit dishes (Table 3-2). Six parasitoid species were obtained from the field-exposed fruit dishes: *L. boulardi*, *L. heterotoma*, *A. tabida*, *P. vindemmiae*, *T. drosophilae* and *S. erythromera* (Table 3-2). The presence and abundance of these species varied greatly by site. Overall, *L. boulardi*, *L. heterotoma*, *P. vindemmiae* and *T. drosophilae* were more common and generally found during field monitoring. On the contrary, *A. tabida* and *S. erythromera* were found occasionally and in fewer numbers. Considering the captures, the larval parasitoid *L. boulardi* were the most abundant. Adults emerged 15–25 days after fruit dish removal, and in both two surveyed sites the presence of *L. boulardi* was recorded from July to October (Figure 3-1a). Aside from abundance, differences were found across the season. The highest level of parasitism was recorded during the summer (weeks 40–41: 680 wasps). Statistical analysis of dataset 7–14 days showed that the presence and abundance of *L. boulardi* was not significantly affected by fruit used in the dishes (Table 3-3). In general, significantly more adults emerged from dishes with banana while no significant differences were found among the exposure for 7 or 14 days, and sites (Table 3-3 and Table 3-4).

Based on the total number of emerged adults, the second parasitoid was pupal parasitoid *P. vindemmiae* (Table 3-2). In this instance, adults emerged, albeit in variable amounts, 20-30 days after fruit dish removal following field exposure in both sites from mid-July to mid-October. This pupal parasitoid was most abundant in Arcagna. In both sites, two main peaks of emergence were observed from fruits exposed during July (weeks 32–33: 357 wasps) and late summer (weeks 38–39: 572 wasps). Thereafter, the number of parasitoids decreased to fewer than 30 wasps in October (Figure 3-1c). Statistical analysis of dataset 7-14 days demonstrated that the presence and abundance of *P. vindemmiae* were significantly affected by fruit (Table 3-3 and Table 3-4). Overall, significantly higher numbers of adults emerged from dishes with banana exposed for 14 days. By contrast, no significant effects were found during analysis of dataset 7 days (Table 3-3 and Table 3-4).

Table 3-2 Total numbers of **Drosophila** *spp. and % of* **D. suzukii** *collected by apple cider vinegar (ACV) traps and emerged from fruit dishes, and total numbers of parasitoids emerged from fruit dishes exposed in the field in 2014*

Sites	Time exposure	ACV traps		Fruit dish		Parasitoids (no.)					
		Drosophilidae (no.)	*D. suzukii* (%)	Drosophilidae (no.)	*D. suzukii* (%)	*L. boulardi*	*L. heterotoma*	*P. vindemmiae*	*T. drosophilae*	*A. tabida*	*S. erythromera*
Arcagna	7	709	76.9	9808	0.1	725	7	107	33	22	0
	14			462	4.11	211	8	1133	25	40	0
Minoprio	7	10366	84.05	10049	1.72	831	81	139	45	3	4
	14			2664	0.43	273	89	255	26	11	3
Total		11075	83.60	22983	0.01	2040	185	1634	129	76	7

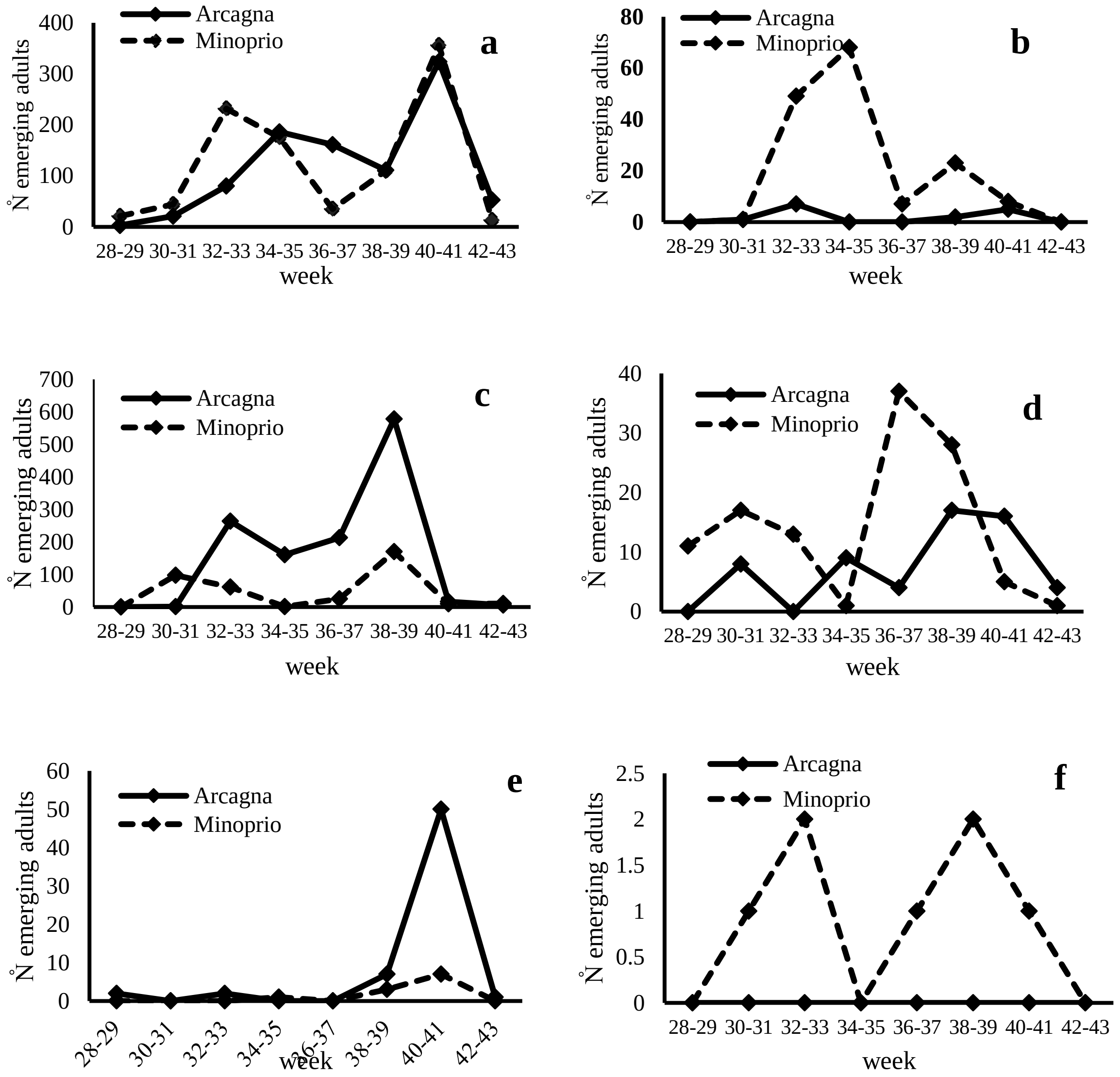

Figure 3-1 Total numbers of **Leptopilina boulardi** ***(a),*** **Leptopilina heterotoma** ***(b),*** **Pachycrepoideus vindemmiae** ***(c) and*** **Trichopria drosophilae** ***(d),*** **Asobara tabida** ***(e), and*** **Spalangia erythromera** ***(f) emerged from banana and blueberries exposed in the field in Arcagna and Minoprio 2014.***

The species that ranked third in abundance was *L. heterotoma* which was observed throughout the survey period except during the first two weeks, although specimen numbers were considerably in low number in Arcagna. Indeed, fewer than 100 adults in total emerged from fruits with the highest number obtained during August in Minoprio (weeks 34–35: 70 wasps) while no *L. heterotoma* were captured from week 34-38 in Arcagna (Figure 3-1b). A high variability in distribution and relative abundance of *L. heterotoma* among two sites was observed, and statistical analysis detected no significant differences for dishes with different fruits and exposed days (Table 3-3 and Table 3-4). Like *L. boulardi*, adults of *L. heterotoma* emerged 15-25 days after fruit dish removal following field exposure during July to October.

The pupal parasitoid *T. drosophilae* was recorded as the fourth dominant parasitoid (Table 3-2). When observed, its adults generally emerged 18-22 days after fruit dish removal. Where its numbers were fewer than *P. vindemmiae*, this pupal parasitoid was consistently collected from July to October, reaching the highest level in September (weeks 36-37: 41 wasps) (Figure 3-1d). Statistical analysis of two sites datasets resulted in no significant effects of sites but there is a significant difference for fruit on the numbers of *T. drosophilae*. Overall, *T. drosophilae* was more attracted to blueberry/kiwi exposed for 14 days (Table 3-3 and Table 3-4).

The remaining two species, *A. tabida* and *S. erythromera*, were occasionally found. Totally, seventy-six *A. tabida* specimens emerged from fruits exposed during September and October (Figure 3-1e and Table 3-2). Seven specimens of pupal parasitoid *S. erythromera* were detected throughout the season only from Minoprio (Figure 3-1f and Table 3-2).

Table 3-3 Mean number of emerged parasitoid adults pooled over the season for site, fruit, exposure and the results of Kruskall-Walis test

Parasitoid	Sites	7 day exposure		14 day exposure	
		Banana	Blueberry/Kiwi	Banana	Blueberry/Kiwi
L. boulardi	Arcagna	13.34 ± 3.24 a	4.56 ± 1.92 a	4.55 ± 1.87 a	6.00 ± 1.56 a
	Minoprio	5.10 ± 1.82 a	7.40 ± 2.32 a	4.21 ± 1.38 a	3.29 ± 0.92 a
L. heterotoma	Arcagna	0.15 ± 0.07 a	0.02 ± 0.02 a	0 ± 0	0.40 ± 0.22 a
	Minoprio	1.01 ± 0.34 a	0.21 ± 0.10 a	1.66 ± 0.95 a	0.68 ± 0.37 a
P. vindemmiae	Arcagna	2.20 ± 0.74 a	0.49 ± 0.20 a	54.55 ± 13.92 b	2.10 ± 1.03 a
	Minoprio	1.92 ± 1.05 a	0.13 ± 0.05 a	6.10 ± 2.33 b	0.63 ± 0.19 a
T. drosophilae	Arcagna	0.22 ± 0.11 a	0.59 ± 0.17 a	0.65 ± 0.25 a	0.60 ± 0.31 a
	Minoprio	0.30 ± 0.20 a	4.01 ± 1.80 b	0.08 ± 0.06 a	1.45 ± 0.532 b
A. tabida	Arcagna	0.27 ± 0.14 ab	0.27 ± 0.10 ab	0 ± 0	2.00 ± 1.34 b
	Minoprio	0.01 ± 0.01 a	0.03 ± 0.03 a	0.18 ± 0.18 a	0.11 ± 0.06 a
S. erythromera	Arcagna	0 ± 0	0.02 ± 0.02 a	0.05 ± 0.05 a	0 ± 0
	Minoprio	0 ± 0	0.06 ± 0.03 ab	0 ± 0	0.11 ±0.05 b

Means of specimens followed by different letters are statistically different

Table 3-4 Mean number of emerged parasitoid adults pooled over the season for sites, fruit and the results of Paired t-test

Parasitoid	Sites	Fruits	
		Banana	Blueberry/Kiwi
L. boulardi	Arcagna	10.46 ± 2.31 a	5.03 ± 1.38 b
	Minoprio	4.78 ± 1.26 a	5.91 ± 1.53 a
L. heterotoma	Arcagna	0.10 ± 0.05 a	0.15 ± 0.08 a
	Minoprio	1.25 ± 0.40 a	0.38 ± 0.15 a
P. vindemmiae	Arcagna	19.36 ± 5.52 a	1.02±0.37 b
	Minoprio	3.44 ± 1.09 a	0.31 ± 0.08 a
T. drosophilae	Arcagna	0.36 ± 0.11 a	0.59 ± 0.15 a
	Minoprio	0.22 ± 0.13 a	3.09 ± 1.17 a
Asobara tabida	Arcagna	0.18 ± 0.09 a	0.84 ± 0.45 a
	Minoprio	0.08 ± 0.07 a	0.06 ± 0.03 a
Spalangia erythromera	Arcagna	0.02 ± 0.02 a	0.02 ± 0.02 a
	Minoprio	0 ± 0	0.08 ± 0.03 a

Means of specimens followed by different letters are statistically different

3.3 Discussion

Our research demonstrated the presence of six parasitoid species related to frugivorous Drosophilidae in North Italy. Overall, the survey revealed the high variability in the abundance and population trend of each parasitoid species across two sites. This variability is probably related to survey site characteristic differences, and consequently, to the trend in Drosophilidae population levels. Different types of trees, bushes, and shrubs, such as mulberries, blueberries, cherry, peach, and fig trees, most of which are favorite hosts of *D. suzukii*, may have provided supplementary or alternative food for *Drosophila* spp. throughout the season, which would account for the lower fly numbers that emerged from fruits exposed in these localities. As would be expected, these lower Drosophilidae numbers mirrored lower amounts of parasitoids emerged from fruits exposed there. The influence of surrounding vegetation on Drosophilidae attraction was also evident from the opposite perspective, that is, when high numbers of other drosophilids were captured in ACV traps. In different sites where monitoring was conducted in blueberry and raspberry plantations with limited presence of other host plants (especially after the harvest), it made the fruits exposed in the field more attractive to drosophilids. The influence of the site on *Drosophila* spp. abundance and composition has been already demonstrated; for example, Ferreira and Tidon (2005) showed that Drosophilidae populations varied according to the level of urbanization.

In both surveyed sites, *D. suzukii* was always present as determined by ACV traps. However, the highly variable rate of trap capture of the exotic flies in comparison with other Drosophilidae highlighted the scarce selectivity of ACV as others have previously observed (Landolt *et al*. 2012b, Cha *et al*. 2014, Iglesias *et al*. 2014, Burrack *et al*. 2015). Despite its high presence, very few adults of the exotic fly emerged from the field-exposed fruits, probably due to competition for food between *D. suzukii* and native drosophilids. Although the competition between *D. suzukii* and other drosophilids has not yet been investigated, studies of food competition have been conducted in a number of congeneric species (Montchamp-Moreau 1983, Fleury *et al*. 2004). Consequent to the low number of *D. suzukii*, most parasitoids found in our survey emerged from native drosophilids (more than 20,000), this compromises the evaluation on the precise association of these parasitoid species to *D. suzukii*. Other drosophilids were not identified because our study was aimed at detecting the parasitoids and evaluating their potential as biological control agents of *D. suzukii*.

However, these data could provide useful information on communities of frugivorous drosophilids and their interactions with natural enemies, not yet investigated in our regions and worthy of further studies.

Among six parasitoid species obtained, *A. tabida* and *S. erythromera* were only occasionally recorded in the sites observed. The association between *Asobara* spp. and their hosts have been studied in other countries, especially in Japan (Mitsui *et al*. 2007, Mitsui and Kimura 2010, Novkovic´ *et al*. 2012, Kohyama and Kimura 2015, Nomano *et al*. 2015). High levels of competition with other parasitoids, host unsuitability, and egg encapsulation in *A. tabida* may be the principal reason for its scarcity in our field studies. Four other species were more abundant in almost all surveyed sites, which showed that they likely play a role in drosophilid population regulation.

The presence of larval parasitoids, *L. boulardi* and *L. heterotoma*, generally observed in Europe (Kraaijeveld and Van Alphen 1994, Fleury *et al*. 2004, 2009, Moiroux *et al*. 2013) was confirmed in Lombardy from our findings. Different resources and environmental factors have been shown to affect the coexistence of *L. boulardi* and *L. heterotoma*. Of these two species, the second one has been considered more generalist and therefore, a better competitor than the first because it can exploit alternative host species (Fleury *et al*. 2004). However, in a survey conducted in Tunisia, *L. boulardi* proved to be a better competitor than *L. heterotoma* (Carton *et al*. 1991). A similar competition could explain the variability in the presence and abundance observed in our survey.

Pachycrepoideus vindemmiae was the main pupal parasitoid found during our field monitoring as observed in France (Fleury *et al*. 2009). This parasitoid is reported to be one of the three most abundant frugivorous *Drosophila* parasitoids in South France (Chabert *et al*. 2012), and its presence has already been assessed in other Italian areas (Nøstvik 1954). *Pachycrepoideus vindemmiae* was collected in both surveyed sites but showed different population dynamics. Population trend differences between the localities might be influenced by the presence of alternative hosts. In fact, *P. vindemmiae* is known to parasitize over 60 fly species, and was shown to dominate interspecific competition (Wang and Messing 2004, Rossi Stacconi *et al*. 2013). Higher numbers of this pupal parasitoid emerged from the fruit exposed for 14 days, in which higher numbers of pupae promoted the attraction of *P. vindemmiae*. This species was also more attracted to banana traps as explained above for *Leptopilina* spp. (Chabert *et al*. 2012, Rossi Stacconi *et al.* 2013).

Although *T. drosophilae* is considered the other main widespread and global pupal parasitoid of drosophilids (Fleury *et al.* 2009, Asplen *et al*. 2015), fewer individuals of this species were recorded in our field survey and in another area of North Italy (Rossi Stacconi *et al*. 2015). Little information existed on this parasitoid (Romani *et al.* 2002 & 2008, Small *et al*. 2012) until introduction of the exotic fly sparked interest. Currently, *T. drosophilae* has proved it can successfully parasitize *D. suzukii* in previous laboratory research (Chabert *et al.* 2012, Gabarra *et al*. 2015).

Our results suggest the possibility of natural enemy mediated apparent competition in communities of *Drosophila* spp. and their parasitoids. As a matter of fact, high numbers of indigenous Drosophilidae emerged in spite of the abundance of parasitoids. Consequently, the possibility that native parasitoids can effectively adapt to and control *D. suzukii,* also due to its higher resistance to some parasitoids, still remains limited. Moreover, although more efficient parasitoids were reported in the area of origin, a specific enemy of *D. suzukii* to release in a classical biological control program is yet to be identified. Therefore, further investigations on indigenous enemies, in particular on *T. drosophilae*, that revealed to be a promising biological control agent, should be carried out to achieve effective control of *D. suzukii* through their rearing and release in the field in augmentative biological programs.

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Appendix 1. Copies of abstracts of papers

- Mazzetto F, Marchetti E, **Amiresmaeili N**, Sacco D, Francati S, Jucker C, Dindo M.L, Lupi D, Tavella L (2016) *Drosophila* parasitoids in northern Italy and their potential to attack the exotic pest *Drosophila suzukii*, Journal of Pest Science, DOI 10.1007/s10340-016-0746-7.

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ORIGINAL PAPER

Drosophila parasitoids in northern Italy and their potential to attack the exotic pest *Drosophila suzukii*

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Abstract *Drosophila suzukii* is an invasive alien pest recently introduced into Europe and North and South America. Several control methods have been tested, and the ability of natural enemies to control this pest has been investigated. This study aimed to identify the main parasitoids of drosophilids in North Italy via field surveys, and to evaluate the ability of some of those species emerged to parasitize *D. suzukii* compared to indigenous *D. melanogaster*. A nine-site survey from July to October 2014 that exposed fruit (banana and blueberry) for 7 and 14 days obtained six parasitoid species, ranked from highest abundance: *Leptopilina boulardi*, *L. heterotoma* (Hymenoptera: Figitidae), *Pachycrepoideus vindemiae* (Hymenoptera: Pteromalidae), *Trichopria* cf. *drosophilae* (Hymenoptera: Diapriidae), *Asobara tabida* (Hymenoptera: Braconidae), and *Spalangia erythromera* (Hymenoptera: Pteromalidae). The presence and abundance of these species varied greatly among the sites and across the season. The field survey results showed a relationship between parasitoids and indigenous *Drosophila* communities and a high host competition. The ability of larval parasitoids *L. boulardi* and *L. heterotoma* and pupal parasitoid *T.* cf. *drosophilae* to parasitize the exotic and indigenous hosts was laboratory tested. Both larval parasitoids failed to develop on *D. suzukii*, but high mortality was recorded in larvae exposed to *L. heterotoma*. On the contrary, *T.* cf. *drosophilae* developed successfully on *D. suzukii*, with no significant differences between the exotic and indigenous hosts. These results beg further investigations of indigenous enemies, particularly *T.* cf. *drosophilae*, for effective biological control of *D. suzukii*.

Keywords Spotted-wing drosophila · Fruit dish traps · No-choice and choice tests · *Leptopilina boulardi* · *Leptopilina heterotoma* · *Trichopria* cf. *drosophilae*

Key message

- Knowledge of the distribution and abundance of parasitoid species related to frugivorous Drosophilidae is key to the implementation of a biological control against *Drosophila suzukii*.
- Six parasitoid species (three larval and three pupal parasitoids) related to frugivorous Drosophilidae were detected in North Italy.
- Laboratory experiments demonstrated the capability of *Trichopria* cf. *drosophilae* to parasitize successfully *D. suzukii*.
- Rearing and release of parasitoids, such as *T.* cf. *drosophilae*, could be implemented for effective control of the exotic fly.

Special Issue: Spotted Wing Drosophila

Communicated by A. Biondi.

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Appendix 2. Copies of abstract of oral communications and posters

- **Amiresmaeili N,** Jucker C, Rocco A, Girgenti P, Colombo, M, Marchetti, E and D Lupi (2015) "*Trichopria drosophilae*: a potentially successful control agent of *Drosophila suzukii"*, XVIII International plant protection congress, 24-27 August 2015, Berlin (Germany). (Oral)
- **Amiresmaeili N**, Colombo M and D Lupi (2015) "Spotted Wing Drosophila: notes on dynamics in Lombardy (Italy) and susceptibility of some small fruits", XX Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology, 23rd-25th September, Perugia (Italy). (Poster)
- **Amiresmaeili N**, Colombo M and D Lupi (2015) "Impact of High Temperature on *Trichopria drosophilae* (Perkins,1910) (Hymenoptera: Diapriidae)", European PhD Network in "Insect Science" - 6th Annual Meeting and SEI-SIPaV Joint Workshop, 11 – 13 November, Florence (Italy). (Oral)
- **Amiresmaeili N**, Jucker C, Zenga EL and D Lupi (2016) "Sulla presenza di *Pachycrepoideus vindemmiae* in un'azienda biologica in Lombardia"**,** XXV Italian National congresso f entomology, Padova (Italy) 20-24th June 2016. (Poster)
- **Amiresmaeili N and D Lupi** (2016) "SWD: invasive pest from the fruit attacks to control", XXI Workshop on the developments in the Italian PhD research on food science technology and biotechnology, Università degli studi di Napoli Federico II, Naples (Italy) 14-16th September 2016. (Oral)
- Jucker C, **Amiresmaeili N.** and Lupi D (2017) "On the precence of Vespoidea in an Italian racecourse" 7th meeting of the IOBC/WPRS working groupLandscape management for functional biodiversity, Dundee (Scotland) 29-31th March 2017. (Under publication)

O BI III-3

Trichopria drosophilae*: a potentially successful control agent of *Drosophila suzukii

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Drosophila suzukii (Matsumura, 1931) (Diptera: Drosophilidae) native to South East Asia is one of the most important invasive pests of unripe fruits which invaded European countries from 2008. Insect damage is due to egg-laying inside unripe cane fruits and stone fruits, high reproductive capability and numerous overlapping generations per year. During 2013 and 2014, different field and laboratory surveys had been developed in order to detect the natural enemies of *D. suzukii* in Lombardy and Emilia Romagna (Northern Italy). Among the parasitoids detected, *Trichopria drosophilae* Perkins, 1910 (Hymenoptera: Diapriidae)

appears to be one of the most promising enemies of this hazardous pest in these areas. Laboratory tests were developed to improve the poor knowledge on *T. drosophilae* biology and the relationship between this parasitoid and drosophilids (*D. suzukii* included). A trial was aimed at evaluating the duration of the pupal development of *D. suzukii* and of the natural host *Drosophila melanogaster* Meigen, 1830. Following this experiment, one-day old pupae of both species were exposed to *T. drosophilae* under no-choice conditions. Furthermore, trials at 25±0.5°C allowed to evaluate the reproductive capability of *T. drosophilae on D. suzukii* and on native drosophilids, while trials at different temperatures (between 0°C and 40°C) allowed to evaluate adult survival in different environmental conditions. The results showed a good adaptability of *T. drosophilae* on *D. suzukii*. One-day old *D. suzukii* and *D. melanogaster* pupae were successfully parasitized by *T. drosophilae* with no significant difference between these two species. Adults were able to survive from 5 to 33°C. Therefore, 34°C were established as the upper thermal limit for adult survival. At lower temperatures, adults were able to survive more than four months but did not reproduce. The information acquired so far is in line with the biology of *D. suzukii* which prefers milder temperature, and confirm the hypothesis that *T. drosophilae* can be a successful control agent of this hazardous pest.

XX Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology
Perugia, September 23rd-25th, 2015

Spotted Wing Drosophila: notes on dynamics in Lombardy (Italy) and susceptibility of some small fruits

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The experimental activity executed during this doctorate thesis was *Drosophila suzukii* (Matsumura, 1931) which is part of a bigger project GEISCA, financed by Italian Ministry for Education, University and Research in PRIN 2010-2011. The first detection of this hazardous quarantine pest (Suss and Costanzi, 2010) was first dated in 2009 by Grassi et al., but backdated to 2008 by Raspi et al in 2011. The result of the experimental activity of the first year of this study refers to field activity, included evaluation of *D. suzukii* flight period and its attractiveness to different soft fruits.

Drosophila suzukii: note sulle dinamiche in Lombardia (Italia) e sulla suscettibilità di alcuni piccoli frutti

L'attività sperimentale eseguita nel primo anno di questa tesi di dottorato è parte di un progetto più ampio denominato GEISCA finanziato dal MIUR nel bando Prin 2010-2011. Durante il primo anno di attività ci si è occupati di *Drosophila suzukii* (Matsumura, 1931) (Dip: Drosophilidae) un insetto da quarantena invasivo (Suss e Costanzi, 2010), presente in Italia dal 2008 (Grassi et a.l, 2009; Raspi et al., 2011), che danneggia fortemente sia i frutti di bosco che frutta con nocciolo. L'attività sperimentale ha riguardato la valutazione del periodo di volo di *D. suzukii* e di altri drosophilidi nativi. Inoltre è stata valutata il tasso attrattività di differenti specie di piccoli frutti e varietà diverse.

Key words: *Drosophila suzukii*, peak flight, attractiveness rate, soft fruits.

1. Introduction

According to this PhD thesis project, this poster reports the main results of two activities related to *Drosophila suzukii*, an exotic hazardous quarantine pest:

(A1) Seasonal flight activity of *D. suzukii* as monitored by baiting trap catches;

(A2) The attractiveness rate of different soft fruits to *D. suzukii*.

2. Materials and Methods

In order to consider the seasonal flight of *D. suzukii* in various cane fruit shrubs, traps with apple cider vinegar were set out in two different sites: Minoprio (45° 43′ 39″ N; 09° 05′ 09″ E; 333 m above sea level) and Arcagna (45° 20′ 23″ N; 09° 27′ 03″ E; 83 m above sea level). To analyze seasonal flight activities of this hazardous pest, one apple cider vinegar trap was set in each locality from July 2013 to May 2015 and checked periodically. Moreover, to evaluate the attractiveness rate of different soft fruits, baited traps were positioned in the vicinity of blueberry- two varieties of Arapaho and Guarany- (in Minoprio), raspberry- varieties of Himbo Top and Erika- (in Minoprio and Arcagna), and blackberry (in Arcagna) during fruit growing period from June to October 2013 and 2014. Traps were sampled weekly and entrapped specimens were transferred to the laboratory of DeFENS at the University of Milan. The specimens were kept in 70% alcohol and sorted at the laboratory. *D. suzukii* specimens were separated, counted and recorded under the stereomicroscope (Wild Heerbrugg M5A, Leica Geosystems Gmbh, Heerbrugg, Switzerland).

3. Results

Because of the selective bait, samples were biased, and different species of drosophilids were captured. According to figure 1, adults of *D. suzukii* were present throughout the trapping seasons with several peaks in September, October and November. During the trapping period, there was a synchronize between *D. suzukii* captured during 2013 and 2014. *D. suzukii* in both years started to increase in abundance from middle-August to reach a peak in early-September and reached a top notch in November. It started rapidly to decrease in January, then captures were very low till the beginning of May.

Impact of high temperature on *Trichopria drosophilae* (Perkins, 1910) (Hymenoptrea: Diapriidae)

Nassim Amiresmaeili[1,2], Mario Colombo[1], Daniela Lupi[1]

Trichopria drosophilae is a parasitoid of *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) which was recently found in Lombardy. *D. suzukii* damages fruit production in summer and early fall; therefore the ability of *T. drosophilae* adults to tolerate higher temperatures was examined through laboratory and field experiments. The resistance in laboratory to temperatures higher than 25°C was examined in climatic chambers under the photoperiod 14:10 Light:Dark. Among the temperatures tested, 37°C appeared as the thermal limit for the survival of *T. drosophilae*, surviving only 3.44±0.03 hours. At 33°C, adults survived for 5.81±0.19 days, but no progeny emerged. At 30°C they survived 18.06±0.88 days, and preimaginal development completed in 19.67±0.10 days. To confirm this information, field experiments were conducted in a blackberry and in a raspberry plantation in Guanzate (CO). *T. drosophilae* (100 males and 100 females) were fortnightly released from June to September 2015 into a cage specifically built to allow the colonization of the field, and reproduction under favorable conditions. Twenty-eight sentinel traps were also positioned in fields within vegetation and fruits, at 10 and 20 meters from central traps, on four sides. Sentinel traps and ripening fruits were weekly collected and conserved in transparent boxes to allow emergence of drosophilids and parasitoids. Field experiments confirmed laboratory trials: high temperature (upper to 37.5°C) caused high mortality, and reduced the fecundity of parasitoid. No *T. drosophilae* emerged from fruit and sentinel traps when the temperature was higher than 31°C, for at least 7 hours. 10

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Sulla presenza di *Pachycrepoideus vindemiae* in un'azienda biologica in Lombardia

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Pachycrepoideus vindemiae (Rondani, 1875) (Hymenoptera: Pteromalidae) è un parassitoide idiobionte che attacca i Ditteri cilorrafi afferenti a 13 famiglie diverse, tra cui anche i Drosofilidi. Questo parassitoide è cosmopolita ed è stato spesso trovato in associazione con la specie esotica invasiva *Drosophila suzukii* Matsumura, 1931 anche in Italia. La valutazione della presenza e dell'azione di *P. vindemiae* in differenti realtà produttive è un punto di partenza per la pianificazione del controllo biologico di *D. suzukii*. Nel presente lavoro si è voluta quindi studiare la fenologia e la distribuzione dello Pteromalide in appezzamenti con coltivazione di piccoli frutti all'interno di un'azienda biologica della Provincia di Como (45°42'41" N 9°00'49" E). Dalla fine di giugno a metà ottobre 2015 sono state equamente ripartite in due appezzamenti, rispettivamente di lampone e mora, 28 trappole-esca contenenti larve mature e pupe di *Drosophila melanogaster* Meigen, 1830 allevate su banana. Le trappole sono state sostituite a cadenza settimanale e successivamente conservate in laboratorio per consentire lo sfarfallamento dei parassitoidi. Parallelamente si è proceduto alla raccolta di frutti di mora e lampone che sono stati in parte mantenuti in appositi contenitori in laboratorio e in parte congelati. Le trappole-esca e i campioni di frutta sono stati controllati periodicamente in laboratorio, fino a 40 giorni dalla raccolta, per garantire il conteggio, la determinazione a livello di specie e la ripartizione nei sessi dei parassitoidi sfarfallati. Dai frutti raccolti in campo, allo stesso tempo, sono stati conteggiati gli sfarfallamenti sia dei limitatori naturali con le stesse modalità descritte per le trappole-esca, che di *D. suzukii* nei 10 giorni successivi al prelievo e i frutti congelati sono stati impiegati per valutare il grado di infestazione contando le larve e le uova del Drosofilide. La presenza di *D. suzukii* è stata inoltre monitorata mediante il posizionamento di trappole innescate con aceto di mele. Il monitoraggio ha evidenziato che *P. vindemiae* è stato il parassitoide più abbondante, sia tra quelli sfarfallati dalle pupe di drosofilidi esposte, sia tra quelli sfarfallati dalla frutta raccolta. In totale sono stati raccolti 1537 adulti di *P. vindemiae* dalle trappole-esca (da lamponi 562; da more 975) e 328 dalla frutta raccolta in campo (da lamponi 247; da more 81). La fenologia della specie mostra una distribuzione unimodale con la presenza massima di *P. vindemiae* nel mese di settembre in entrambi gli appezzamenti. Il rapporto tra i sessi è risultato sbilanciato a favore dei maschi con un valore complessivo di 1,40 maschi per femmina. I dati sulla presenza di *P. vindemiae*, *D. suzukii* e altri Drosofilidi sono stati inoltre confrontati con i dati meteorologici. A differenti set di dati così ottenuti è stata applicata l'analisi delle componenti principali (PCA). L'abbondanza del parassitoide nelle trappole-esca è risultata correlata alla presenza di *D. suzukii* nelle trappole ad aceto. È meno chiara la relazione tra *P. vindemiae* e *D. suzukii* nella frutta raccolta, dove si evince una correlazione con i campioni di mora, ma non con quelli di lampone. Nel periodo di monitoraggio la presenza di *P. vindemiae* e di *D. suzukii* è risultata negativamente correlata alla temperatura media giornaliera.

Attività svolta nell'ambito del Progetto PRIN-2010: "Insetti e globalizzazione: controllo sostenibile di specie esotiche in ecosistemi agro-forestali (GEISCA)".

Spotted Wing Drosophila: invasive pest from the fruit attacks to control

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The current study focused on the quarantine pest *Drosophila suzukii* (Diptera: Drosophilidae) which has invaded Italy since 2008, and made considerable economic damages in small fruits such as raspberry, blackberry and blueberry. In current study, information about insect presence in field (flight period), and the susceptibility of different fruits with similar harvesting period in four areas during 2013-2015 are given. Also, to avoid damaging fruits by *D. suzukii*, a pupal parasitoid, *Trichopria drosophilae* (Hymenoptera: Diapriidae) which was identified as a natural enemy in Lombardy region, and because of its high ability to parasitize the pupae of this pest, mass rearing and augmentation in biological farms was done.

Spotted Wing Drosophila: parassita invasivo dagli attacchi di frutta al controllo

L'attuale studio si è concentrato sulla specie esotica *Drosophila suzukii* (Ditteri: Drosophilidae), che ha invaso l'Italia a partire dal 2008 arrecando notevoli danni economici a piccoli frutti come il lampone, mora e mirtillo. Si riportano informazioni relative alla presenza dell'insetto in quattro aree in tre anni diversi (periodo di volo) e alla suscettibilità di diversi frutti con periodo simile di maturazione. Inoltre per limitare i danni ai frutti un parassitoide pupale, *Trichopria drosophilae* (Hymenoptera: Diapriidae) è stato studiato attraverso l'allevamento massale e il sistema aumentativo (usato in lotta biologica).

Key words: raspberry, blackberry, blueberry, exotic pest, damages on small fruits.

1. Introduction

The cultivation of fruits and vegetables, one of the most essential of human endeavors is always under risk. Unfavorable weather conditions and pest infestation can undermine the diligent efforts of even the most experienced farmers through damaging or destroying, and lowering yields.
As a result of human activities -such as development of international food trade and intercontinental fruit transportation- the biogeographic barriers broke down accelerating the movement of species and causing drastic changes in the composition of biotic communities (Williamson and Fitter, 1996). These exotic species sometimes can become invasive, and cause considerable agricultural losses in the new habitat that they colonized (Pimentel *et al*., 2000).
Drosophila suzukii (Matsumura, 1931) (Diptera: Drosophilidae), native to South East Asia, is one of the most important invasive pests of fruit crops with a very wide host range (kiwis, persimmons, figs, strawberries, apples, sweet cherries, plums, peaches, pears, raspberries, blackberries, blueberries, table and wine grapes). This fruit fly which entered Italy in 2008 has immediately adapted to the climate conditions, and nowadays wide spread in almost all Italian regions (Raspi *et al*., 2011). *Drosophila suzukii* (SWD) is the one of rare *Drosophila* species which lays eggs inside unripe fruits using its serrated ovipositor. Damage of this species is then caused by larvae feeding inside the fruits on the pulp. The damage caused by drosophilid larvae feeding inside fruit is imperceptible at first, subsequently, secondary fungal or bacterial infections may contribute to further fruit deterioration through the oviposition hole. Because of the great damages by SWD and its biological characteristics, it is considered as a quarantine pest. Total monetary loss SWD are due to direct costs but also market loss, management techniques, and rejection of exports for altered processing practices have been extensively reported from different crops (Cini *et al*., 2012). A preliminary study estimated that Trento province (Northern Italy) which devoted a big area -approximately 400 hectares- to small fruit cultivation, faced a loss around 500,000 € in 2010, and 3 million € in 2011 (Cini *et al*., 2012).
Currently strategies to protect fruit from infestation by SWD are dominated by insecticide applications, but the use of natural enemies can be a good remedy to avoid side effects of chemicals. Different parasitoids already found in association with *D. Suzukii* (Mazzetto *et al*., 2016), but the study in different productive areas of their activity is necessary to improve the knowledge on these natural enemies.

Appendix 3. Speeches

- **Amiresmaeili N (**2014) Associazione Italiana Agricoltura Biologica Lombardia, Milan, Italy. "Aspetti Normativi e Scientifici dell'agricoltura Biologica"-"Impiego della lotta biologica e integrata nell'area del Sud-Ovest asiatica (Iran)".

Appendix 4. Co-tutor of students

- Riccardo Carlizzi (bachelor student)
- Paolo Corti (bachelor student)
- Lorenzo Sala (bachelor student)
- Emanuele Luigi Zenga (master student)
- Matteo Zugno (master student)

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All of the laboratory works presented henceforth was conducted in the laboratory of DeFENS, University of Milan. Field works carried out in four localities in Lombardy region, Northern Italy: Montanaso Lombardo (prov. Lodi), Camnago Volta, Guanzate and Vertmate con Minoprio (prov. Como). All projects and associated methods were approved by the University of Milan’s Research Agriculture Board.

Data in Chapter 3 has been included in the article published in the Journal of Pest Science [Mazzetto F, Marchetti E, Amiresmaeili N, Sacco D, Francati S, Jucker C, Dindo ML, Lupi D, Tavella L, doi 10.1007/s10340-016-0746-7].

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