Biology and ecology of *Apanteles taragamae*, a larval parasitoid of the cowpea pod borer *Maruca vitrata*

Elie Ayitondji Dannon

Propositions

1. Like plant leaves, upon herbivory, flowers can produce volatile compounds that attract their natural enemies (*This thesis*).
2. When two solitary koinobiont parasitoids compete intrinsically for the same host, the early acting species does not always prevail (*This thesis*).
3. The response of a parasitoid to herbivore-induced plant volatiles is affected by multiple infestation. Research should thus include herbivory on different plant organs such as flowers and leaves (Based on Lucas-Barbosa et al. (2011) The effects of herbivore-induced plant volatiles on interactions between plants and flower-visiting insect. Phytochemistry DOI 10.1016/j.phytochem.2011.03.013).
4. Niche partitioning by two natural enemies optimizes control of their shared host/prey (Based on Amarasekare P (2000) Coexistence of competing parasitoids on a patchily distributed host: Local vs spatial mechanisms. Ecology 81: 1286-1296).
5. Despite a long research history, the fitness benefit for plants producing herbivore-induced plant volatiles still needs to be determined (Based on Dicke M, Baldwin IT (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry-for-help'. Trends in Plant Science 15: 167-175).
6. Thorough scientific writing including speculations is useful for determining future research themes (Based on Janzen HH (1996) Is the scientific paper obsolete? Canadian Journal of Soil Science 76: 447-451).
7. Open access is a communication tool that can increase the impact of scientific publications.
8. The spongy texture is a highly desirable factor that determines the acceptability of cowpea snacks (cowpea fried paste locally called “Ata”) by consumers in Benin.

Elie Ayitondji Dannon

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Wageningen, 9 September 2011

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Abstract

Maruca vitrata Fabricius is a key insect pest of cowpea in West Africa. Larvae of this moth can cause up to 80% of yield losses. The first classical biological control programme against *M. vitrata* started in 2005 with the introduction of *Apanteles taragamae* Viereck from Taiwan into Benin by the International Institute of Tropical Agriculture (IITA), Benin station. Thorough knowledge on the bioecology of *A. taragamae* is a prerequisite for implementing such programme. The work described in the present thesis evaluated the biological potential of this larval parasitoid to fill to the gap of information on its biology and ecology. Special emphasis was put on the main factors that determine the effectiveness/ suitablility of biological control candidates, such as reproductive capacity, functional response, climatic adaptability, host foraging capacity, and non-target effects. The results revealed that two-day-old larvae were the most suitable host age, giving the highest percentage parasitism, lifetime fecundity and proportion of females. Larvae older than three days were not successfully parasitized. The percentage parasitism of two-day-old larvae was positively correlated with host density, indicating a good functional response of *A. taragamae*. Between 20 and 30 °C, the curve that described the relationship between the intrinsic rate of natural increase and the temperature for *A. taragamae* was above that of *M. vitrata,* suggesting that the parasitoid can faster build up its population than its host. The parasitoid showed its ability to use volatiles produced by cowpea flowers and host caterpillars when foraging. A host plant odour experience enhanced the capacity of the parasitoid to find uninfested flowers. The growth of non-parasitized or *A. taragamae*-parasitized larvae was slower and with reduced proportion of female wasps on some host plants compared to those reared on artificial diet. With regard to the non-target effects, the physiological host range and competitive ability of *A. taragamae* were assessed. None of the following lepidopteran species, *Eldana saccharina* Walker, *Chilo partellus* (Swinhoe), *Mussidia nigrivenella* Ragonot, *Cryptophlebia leucotreta* (Meyrick), *Sylepta derogata* Fabricius and *Corcyra cephalonica* Stainto, was successfully parasitized by *A. taragamae,* suggesting its specificity for *M. vitrata* in Benin. In no-choice competition with the egg-larval parasitoid *Phanerotoma leucobasis, A. taragamae* outcompeted the latter. All the above attributes suggest that *A. taragamae* should be a suitable biocontrol agent against *M. vitrata.* A cage release strategy involved the host plant *Sesbania cannabina*, which was artificially infested with *M. vitrata,* and inoculated with adults of *A. taragamae*. The parasitoid was released in seven selected locations in Benin but the first recovery studies did not yet yield any information on its establishment after the first generation.

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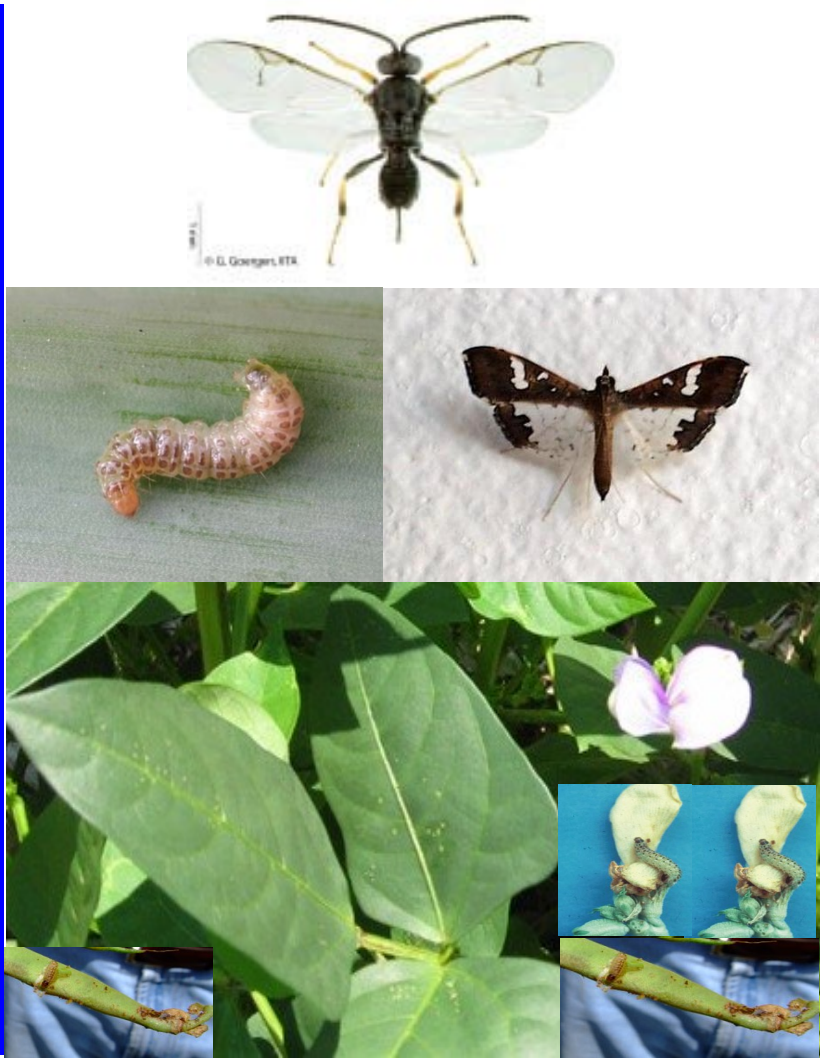
General introduction



General Introduction 01

Elie Ayitondji Dannon

Abstract

A classical biological control programme against the cowpea pod borer *Maruca vitrata* Fabricius started in 2005 at the International Institute of Tropical Agriculture (IITA), Benin station using the parasitoid wasp *Apanteles taragamae* Viereck. The pest status of *M. vitrata* has been well determined and various control methods have been developed. In this chapter, an overview of the different strategies to control the pod borer is given. The available information on the biology of *A. taragamae* is provided. The steps to implement a classical biological control programme are listed with particular attention for criteria commonly used to select or assess the efficiency of promising biological control agents. Finally, the overall and specific objectives of the present PhD thesis are presented.

1 Introduction

Cowpea, *Vigna unguiculata* (L.) Walp., is a leguminous staple crop widely cultivated in West Africa (Singh and van Emden, 1979; Zannou et al., 2004). As a major source of dietary proteins, it is expected to play a key role in human nutrition in this area of the world where few people have access to animal proteins (Phillips et al., 2003). Unfortunately, cowpea production is limited by several constraints. Of these, damage by insects remains the most important (Singh and van Emden, 1979; Egho, 2010). Several insect species have been reported attacking cowpea from the seedling stage to harvest and beyond during storage (Singh and van Emden, 1979; Jackai and Daoust, 1986; Egho, 2010). *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) has been identified as one of the most destructive insect pests of cowpea (Taylor, 1978; Sharma, 1998). Its caterpillars heavily attack both flowers and pods inducing drastic yields losses (Taylor, 1978; Sharma, 1998). A number of integrated pest control methods, reviewed in this chapter, have been developed and tested for the control of this moth. In 2005, a classical biological control against the cowpea pod borer was initiated in Benin by introducing *Apanteles taragamae* Viereck (Hymenoptera: Braconidae). This parasitoid was originally collected from Taiwan (Asia), and was imported by the International Institute of Tropical Agriculture (IITA), Benin station following the different steps required when implementing a classical biological control programme. This study focuses on the assessment of the main criteria used to select or evaluate the potential of biological control candidates prior to large scale field releases. As an ecological approach, biological control basically exploits the interactions between target pests and their natural enemies. Such interactions are mediated by cues from various sources (Vet and Dicke, 1992). Links between biological control and chemical ecology are reviewed to better understand these interactions. Furthermore, the research problem is described and the overall objective given. Specifically, this work covers some key aspects of the biology and ecology of the parasitoid wasp *A. taragamae.* Finally, the outline of the current thesis is presented.

2 Cowpea production

2.1 Importance of cowpea

Cowpea, *Vigna unguiculata* (L.) Walp., is a plant species that belongs to the Order of Leguminosales, Family Fabaceae (Papilionaceae), Tribe Phaseolae, Subtribe Phaseolinae and to the Genus *Vigna* (Singh and Rachie, 1985). Cowpea originates in Africa and is an integral part of traditional cropping systems throughout the continent, particularly in the semi-arid regions of West Africa (Ajeigbe et al., 2006). West Africa is a major centre of diversity of domesticated cowpea (Ehlers and Hall, 1997), whereas Southeastern Africa is thought to be the centre of diversity of wild *Vigna* species (Padoulosi et al., 1997). However, Rawal (1975) considers cowpea to be native to West Africa because wild and weedy forms exist in many parts of the region and can easily hybridize with cultivated forms giving viable hybrids.

Cowpea is a grain legume of worldwide importance (Jackai and Adalla, 1997). It is cultivated throughout the tropics and subtropics and is widely distributed in Africa, Asia and South America (Singh and van Emden, 1979). Cowpea is a single grain legume species. However, several varieties/cultivars have been developed with various architectures, seed types, colour and maturity length. It is mainly cultivated for its seeds, but young leaves and immature pods may also be eaten as vegetables (Uzogara and Ofuya, 1992). It provides a major source of protein in human diets, cash for farmers and fodder for livestock (Ajeigbe et al., 2006). Furthermore, the plant is especially favoured by farmers because of its ability to maintain soil fertility through nitrogen fixation and is therefore beneficial to subsequent cereal crops in rotation or association with cowpea (Singh and Rachie, 1985).

In Benin, cowpea is the most cultivated and consumed grain legume (Atachi et al., 1984; Zannou et al., 2004). It provides the cheapest source of dietary protein and is considered as “meat for poor people”. Production of dry beans in Benin averaged ca. 130,000 metric tonnes in 2007 (FAOSTAT, 2009). However, only ca 90,000 metric tonnes were really consumed providing 11.1 kg per capita and per year, which corresponds to 6.56 g of proteins per capita and per day (FAOSTAT, 2009). Given that the mean protein requirement for healthy people is 0.6 g/kg/day (Campbell et al., 2008), and considering a weight of 50 kg per person, cowpea as the major source of proteins contributes only to 22% of the need of the population in Benin. Although cowpea fits into

diverse production systems and in areas with poor soil fertility and limited rainfall, other factors mainly biotic factors limit its productivity.

2.2 Biotic constraints to cowpea production

Biotic factors are the main constraints to cowpea production in West Africa (Singh et al., 1990). In effect, cowpea is attacked during its whole cycle from seed germination to pod maturity and during seed storage by various insect pests, pathogens and rodents (Singh and Rachie, 1985). Insect pests are considered the most limiting factors to cowpea production (Singh and van Emden, 1979; Egho, 2010). Several insect species have been reported attacking cowpea. Of these, twelve species have been frequently recorded (Figure 1). During the post-flowering period, damage is made mainly by *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae), *M. vitrata* and the pod-sucking bugs with *Clavigralla tomentosicollis* Stål (Heteroptera: Coreidae) as the dominant species, whereas *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) is the most ravaging insect species during storage (Jackai and Daoust, 1986; Singh et al., 1990).

3 The flower and pod borer *M. vitrata*

3.1 Bionomics of *M. vitrata*

The origin of the moth *M. vitrata* is not yet clearly established, but is thought to be Indo-Malaysian (Tamò et al., 1997). The crambid has been reported as a pest of grain legumes throughout the tropics and subtropics (Taylor, 1967, 1978; Singh and van Emden, 1979; Singh et al., 1990; Egho, 2010). It is widely spread in cowpea production areas. In Benin, it is reported in all agro-ecosystems where it causes serious damage to cowpea.

Development of the moth from egg to adult passes through five larval stages, a prepupal and pupal stage. The length of each stage varies with rearing and/or environmental conditions (Table 1).

Eggs are deposited on buds and flowers, but oviposition on leaves, leaf axils, terminal shoots and pods has also been recorded (Taylor, 1978). The translucent eggs have a light yellow colour (Taylor, 1967), and hatch in 2-5 days (Shanower et al., 1999).

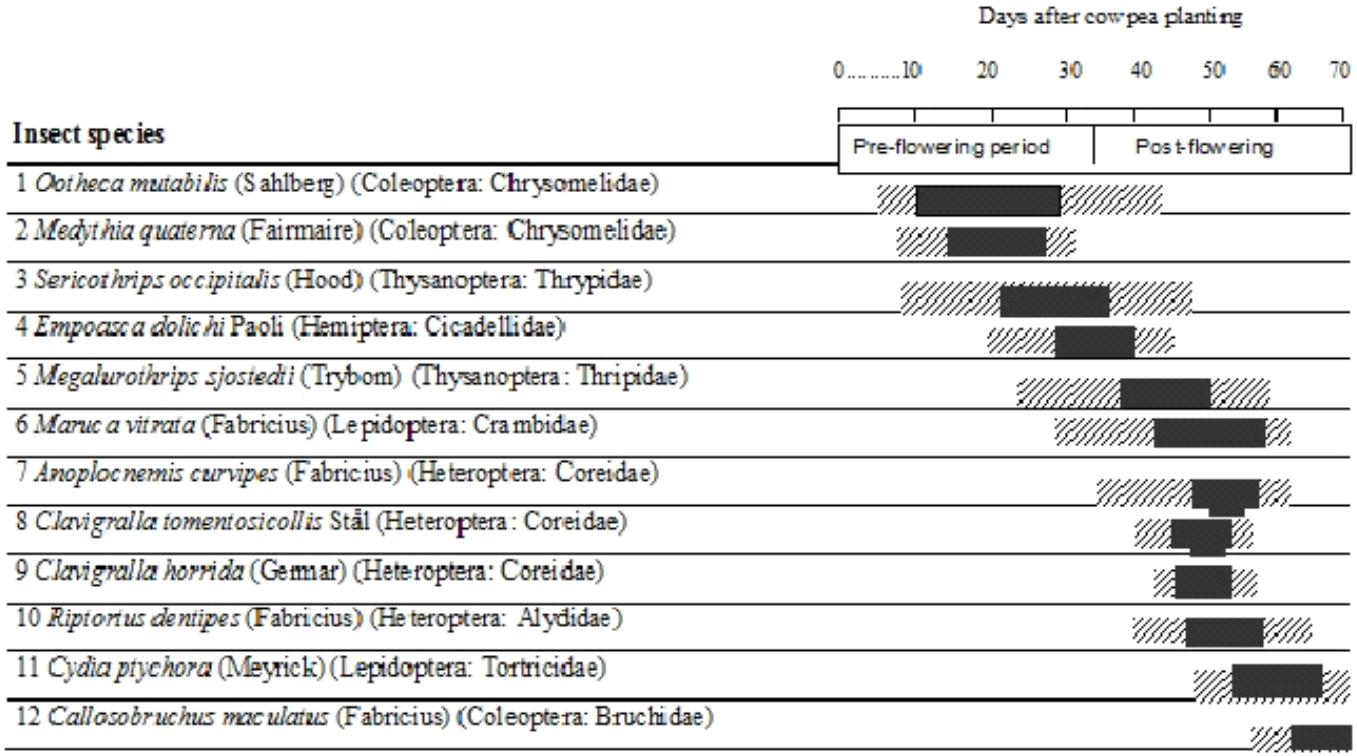


Figure 1: List of the most important insect pests of cowpea in the world and their occurrence time (Adapted from Singh et al., 1990)

First instar larvae have a clear-brown colour, and turn later into brown or green-brown at the fifth stage. Taylor (1978) reported that rearing substrates affect larval colour (Taylor, 1978). The larvae complete their development in 8-16 days (Sharma, 1998). Their size and mobility increase with age (Figure 2). The prepupal stage can be confounded with the fifth larval stage, and its duration does not exceed 2 days (Shanower et al., 1999). Pupation occurs in the soil or in plant debris and lasts 6-10

Table 1: Duration of the development stages of *Maruca vitrata*

Development stage	Duration of development stages (days)		
	laboratory study (25-29°C & 70-85% RH) (Taylor, 1967)	**laboratory study (25-28°C & 70-84% RH) (Atachi and Ahounou, 1995)**	**field study (21-30°C & 65-84% RH) (Akinfenwa, 1975)**
Egg	2-3	-	-
Larval instar: 1st	1-2	2-3	2
2nd	1-2	1-3	1-2
3rd	1-2	1-3	3
4th	2	1-3	4
5th	3-5	2-3	1-2
Pre-pupa	1-2	-	2
Pupa	6-7	6-10	6-7
Total (cycle)	17-25	13-25*	19-22*

-: indicates that data are not determined

Data followed by "*"do not include time required for egg hatching

days. Most adults emerge between 20:00 h and 23:00 h (Jackai et al., 1990). Their life span ranges from 3 to 16 days, depending on climatic factors mainly temperature and relative humidity (Table 1). Females mostly mate once or rarely many times after emergence and fecundity is affected by rearing substrates and environmental conditions (Table 2). The generation time of *M. vitrata* is typically 18-25 days (Shanower et al., 1999).

Temperature affects the development of *M. vitrata*, and optimum temperatures range between 22-28 °C for all stages. Above 34 °C, only 7.0 % of the eggs hatched and 9.5% of the pupae survived, whereas larvae and adults did not survive at such temperatures (Adati et al., 2004). An important feature of the biology of *M. vitrata* is the

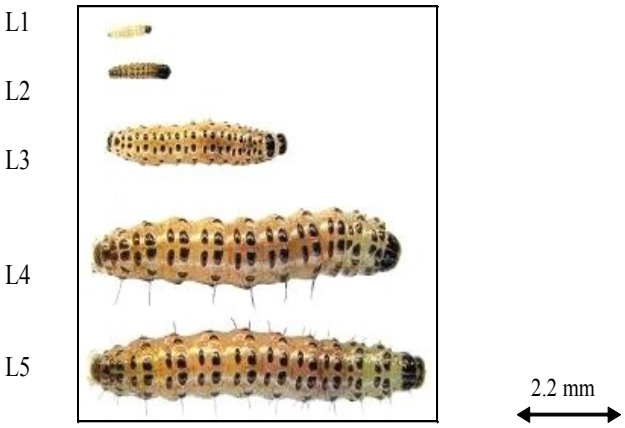


Figure 2: The different larval stages of *Maruca vitrata*
(Photos by Dr. G. Goergen, IITA-Benin)

Table 2: Fecundity of *Maruca vitrata*

Oviposition site	Number of eggs/female	Environmental conditions
Cowpea	6-189	Field study (21-30°C & 65-84% RH) (Akinfenwa, 1975)
Cowpea	8-140	Laboratory study (25-29°C & 70-85% RH) (Taylor, 1967)
Plastic cups	22-602	Laboratory study (21-27°C & 70-90% RH)(Jackai et al., 1990)

absence of diapause (Taylor, 1967, 1978). As with other tropical insects, populations of *M. vitrata* are maintained on alternative host plants during the off-season period (when cowpea is not cultivated). Many alternative host plants belonging mainly to Fabaceae (Papilionaceae) have been recorded for *M. vitrata* (Taylor, 1978; Jackai and Singh, 1983; Atachi and Djihou, 1994; Arodokoun et al., 2003). Of these, *Pterocarpus*

santalinoides L'Her. Ex DC, *Pueraria phaseoloides* (Roxb) Benth and *Centrosema pubescens* Benth play a key relay role during the long dry season, whereas *Lonchocarpus sericeus* (Poir) H.B.&K.) and *Lonchocarpus cyanescens* (Schum&Thonn.) Benth. do so during the main rainy season, and *Tephrosia platycarpa* Grill & Perr. during the short rainy season (Arodokoun et al., 2003).

Larvae of *M. vitrata* feed on flower buds, flowers and pods of cowpea. The infestation of flowers is higher than that of flower buds, terminal shoots and pods (Sharma, 1998). In West Africa, infestation of flowers reaches 80% without control measures (Afun et al., 1991) and 50% of seeds in young pods are destroyed (Dreyer and Baumgärtner, 1995). Larvae move easily from one flower to another and each larva can consume up to six flowers before completing its development. Third to fifth-instar larvae are able to bore into pods and occasionally into peduncles and stems (Taylor, 1967). *Maruca vitrata* can cause up to 80% of cowpea yield loss (Nampala et al., 2002), making it one of the key pests of cowpea which has received attention of researchers since many decades.

3.2 Control methods of *Maruca vitrata*

3.2.1 Chemical control

Control of *M. vitrata* in cowpea has mainly been achieved using synthetic insecticides such as Cypermethrin, Deltamethrin, Endosulfan, Dimethoate, Carbaryl. Both economic thresholds and calendar-based sprays have been investigated (Afun et al., 1991; Sharma, 1998). The economic threshold for *M. vitrata* on cowpea was estimated at 40% of flower infestation (Ogunwolu, 1990). Although calendar-based application of insecticides results in less cowpea infestation by *M. vitrata* compared to sprays based on economic threshold, no significant differences in grain yield were reported between the two spray methods (Afun et al., 1991). But people found it easier to apply calendar-based sprays which are so far the most used (Sharma, 1998). Thus, application of Endosulfan from 35 days after planting, two times per week in combination with one spray of Cypermethrin and of a mixture of Cypermethrin and Dimethoate provided an effective control of the pod borer (Jackai, 1995a). Atachi and Sourokou (1989) reported that a sequence of Deltamethrin-Dimethoate-Deltamethrin sprays resulted in an efficient control of *M. vitrata* and other insect pests and led to a high grain yield (1367 kg/ha).

Four high-volume sprays of Cypermethrin, i.e. at the initiation of flowering, 50% flowering, 100% flowering and pod setting, respectively, were effective against *M. vitrata* (Sharma, 1998). Liao and Lin (2000) found that the combination of Deltametrhin and Carbaryl appeared to be more effective against *M. vitrata*, when sprayed three times from 50% flowering onwards. Likewise, Opolot et al. (2006) suggested three applications of Cypermethrin (at budding, flowering and on-set pod formation) to control the pod borer and other post-flowering insect pests. Kamara et al. (2007) suggested two applications of the mixture Cypermethrin-Dimethoate to achieve a good control of all post-flowering insect pests in cowpea.

In spite of the efficacy of synthetic chemicals to control *M. vitrata* and other insect pests of cowpea, their adoption by African farmers is still low because of economic constraints (Nabirye et al., 2003). Indeed, most farmers are not able to face the high and constantly rising input costs. Farmers have to apply these insecticides at several times during the cropping period, and in every year to control the pod borer. Moreover, insecticide use has already shown its limits by leading to many side effects such as pest resurgence and secondary pest outbreaks, insecticide resistance, environmental pollution, and increased human health risks (Mackauer, 1988). Resistance of *M. vitrata* to some insecticides such as Endosulfan, Dimethoate and Cypermethrin has been reported (Ekesi, 1999), as well as secondary pest outbreaks (Atachi and Dannon, 1999; Atachi et al., 2002). Hence, more sustainable control measures seem to be more appropriate and these have been investigated.

3.2.2 Botanical pesticides

The effects of plant extracts with regard to the control of *M. vitrata* and other cowpea insect pests have also been studied. Leaf and seed extracts of *Azadirachta indica* (A. Juss.) resulted in good control of the pod borer (Jackai et al., 1992). Investigating the effect of leaf extracts of three plants (*Ocimum gratissimum* L., *Hyptis suaveolens* (L.) Poit. and *Tagetes erecta* L.), Oparaeke (2006) obtained a significant reduction in the pod borer density with yields similar to those achieved by applying synthetic insecticides. Fruit extract of *Gmelina arborea* L. was also found to reduce pod damage by *M. vitrata* (Oparaeke, 2005, 2006).

3.2.3 Cultural control

Many cultural practices have been tested with regard to cowpea infestation by *M. vitrata*. Very few practices led to the reduction of the pod borer infestation rate. Most results remained controversial (Jackai, 1995b, Tamò et al., 1997). The effects of intercropping depend on the locality, the type of crop associations and the time between sowing dates of plant communities. Cowpea intercropped with sorghum led to the reduction of the crambid populations, suggesting that sorghum provided a mechanical and visual barrier to the insect (Poswal et al., 1993). Early planting (on-set of rain) and high plant density (30 x 20 cm) have been reported to lower the pod borer density (Ekesi et al., 1996; Karungi et al., 2000a). However, these practices did not give a significant yield increase (Karungu et al., 2000b). Moreover, Asiwe et al. (2005) found that the moderately resistant variety IT86D-715 was heavily damaged by *M. vitrata* at closer spacing, although flower damage was not affected in any way by plant spacing. The implementation of these cultural practices always requires supplemental applications of synthetic pesticides in order to get a significant yield increase (Sharma, 1998; Karungi et al., 2000b).

3.2.4 Host resistance

Resistance factors in cultivated and wild *Vigna* have extensively been investigated. Breeding efforts focused on various defense mechanisms. Plant architectural characteristics, namely erect and profuse flowering cowpea cultivars, significantly reduced damage by *M. vitrata* (Oghiakhe et al., 1993). Cowpea varieties with high trichome density were found to be more resistant to pod borers (Oghiakhe et al., 1992). Similarly, Oigiangbe et al. (2002) reported that pod thickness and length of non-glandular trichomes reduced pod damage by *M. vitrata*. Investigating wild *Vigna* species, Jackai et al. (1996) found resistance in *V. unguiculata* (L) Walp. subspecies *dekindtiana* Harms., *V. oblongifolia* A. Rich and *V. vexillata* (L) A.Rich. Modalities of this resistance were both antibiosis (post-ingestion effects) and antixenosis (feeding deterrence to bore into the pods). Of these wild species, *V. vexillata* was the closest one to *V. unguiculata* (Fatokun et al., 1993). However, the strong cross-incompatibility barriers between cowpea and *V. vexillata* at both pre and post-fertilization periods did not enable the incorporation of resistant genes in cowpea lines (Fatokun, 2002). Cowpea varietal

screening for pod borer resistance has been performed since 1970 at IITA (Singh et al., 1990). To date, only moderately resistant varieties have been developed, still requiring a supplemental use of chemicals to give an efficient control of the pod borer (Fatokun, 2002). In fact, the cultivation of improved varieties such as IT93K-452-1 and IT97K-499-4 requires two sprays of Cypermethrin + Dimethoate to achieve a good control of *M. vitrata* and other post-flowering insect pests (Ajeigbe and Singh, 2006).

Adoption of cultural practices or moderately resistant cowpea varieties seems to require a complementary use of chemicals to get an effective control of *M. vitrata*. This situation has led to an overuse of chemicals with many side effects, as reported above. Therefore, it appears important to investigate other control methods that can ecologically regulate the populations of the pod borer. Of these, classical biological control remains an attractive option.

3.2.5 Biological control

Although several studies made an inventory of natural enemy species of *M. vitrata*, little attention has been given to the classical biological control of this pest (Singh et al., 1990). Monitoring of natural enemies of *M. vitrata* (Taylor, 1967; Okeyo-Owuor et al., 1991; Tamò et al., 1997; Arodokoun et al., 2006) showed that many parasitoid, predator and pathogen species attack *M. vitrata* (Table 3). The overall parasitism rate of encountered parasitoids under field conditions ranges between 10 and 15 % (Okeyo-Owuor et al., 1991; Tamò et al., 2002; Arodokoun et al., 2006). The dominant species recorded in Benin was *Phanerotoma leucobasis* Kriechbaumer (Hymenoptera: Braconidae) parasitizing on average 5.6% of *M. vitrata* larvae in cowpea fields (Tamò et al., 2002; Arodokoun et al., 2006). No effective biological control strategy has yet been developed against *M. vitrata* in Africa.

In Taiwan, *A. taragamae* has been reported parasitizing about 60% of the larvae of *M. vitrata* on *Sesbania cannabina* (Retz.) Pers. (Huang et al., 2003). Therefore, it was valued to be a promising biological agent against the legume pod borer.

Table 3: List of natural enemies of the legume pod borer *Maruca vitrata* in the world

Group	Stages parasitized	Reference
Parasitoids		
Hymenoptera		
Braconidae		
Apanteles sp.	Larva	Okeyo-Owuor et al. (1991)
Apanteles taragamae Viereck	Larva	Huang et al. (2003)
Bassus asper Chou & Sharkey	Larva	Huang et al. (2003)
Bassus javanicus Bhat & Gupta	Larva	Tamò et al. (1997)
Bassus bruesi Shenefelt	Larva	Arodokoun et al. (2006)
Bracon sp.	Larva	Okeyo-Owuor et al. (1991)
Braunsia kriegeri Enderlein	Larva	Arodokoun et al. (2006)
Braunsia sp.	Larva	Okeyo-Owuor et al. (1991)
Dolichogenidae sp.	Larva	Arodokoun et al. (2006)
Phanerotoma leucobasis Kriechbaumer	Egg-Larva	Arodokoun et al. (2006)
Phanerotoma philippinensis Ashmead	Egg-Larva	Tamò et al. (1997)
Phanerotoma sp.	Larva	Usua & Singh (1978)
Pristomerus sp.	Larva	Arodokoun et al. (2006)
Testudobracon sp.	Larva	Arodokoun et al. (2006)
Chalcididae		
Antrocephalus sp.	Pupa	Okeyo-Owuor et al. (1991)
Ichneumonidae		
Trichomma sp.	Larva-Pupa	Huang et al. (2003)
Triclistus sp.	Larva-Pupa	Huang et al. (2003)
Plectochorus sp.	Larva-Pupa	Huang et al. (2003)
Trichogrammatidae		
Trichogrammatoidea eldanae Viggiani	Egg	Tamò et al. (1997)
Eulophidae		
Tetrastichus sp.	Pupa	Usua and Singh (1978)
Tetrastichus sesamiae Risbec	Pupa	Okeyo-Owuor et al. (1991)
Diptera		
Tachinidae		
Cadurcia sp.	Larva	Arodokoun et al. (2006)
Pseudoperichaeta sp.	Larva	Usua and Singh (1978)
Nemorilla maculosa Meigen	Larva	Srinivasan et al. (2007)
Predators		
Hymenoptera		
Formicidae		
Campanotus rufoglaucus (Jerd.)	Larva	Okeyo-Owuor et al. (1991)
Campanotus sericeus Fab	Larva	Usua and Singh (1978)
Arachnida/Araneida		
Araneidae		
Selenops radiatus Latreille	Larva and adult	Usua and Singh (1978)
Pathogens		
Fungi		
Entomophthora spp.	Larva	Otieno et al. (1983)
Bacteria		
Clostridium sp.	Larva-Pupa	Okeyo-Owuor et al. (1991)
Bacillus sp.	Larva-Pupa	Okeyo-Owuor et al. (1991)
Bacillus thuringiensis Berliner	Larva	Otieno et al. (1983)
Bacillus cereus Frank. &Frank.	Larva	Otieno et al. (1983)
Protozoa		
Nosema sp.	Larva-Pupa	Okeyo-Owuor et al. (1991)
Virus		
Granulovirus	Larva	Lee et al. (2007)
Nucleopolyhedrovirus	Larva	Lee et al. (2007)

4 Carrying out a classical biological control programme

Biological control is the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be (Eilenberg et al., 2001). It is one component of an integrated pest management (IPM) strategy (Figure 3).

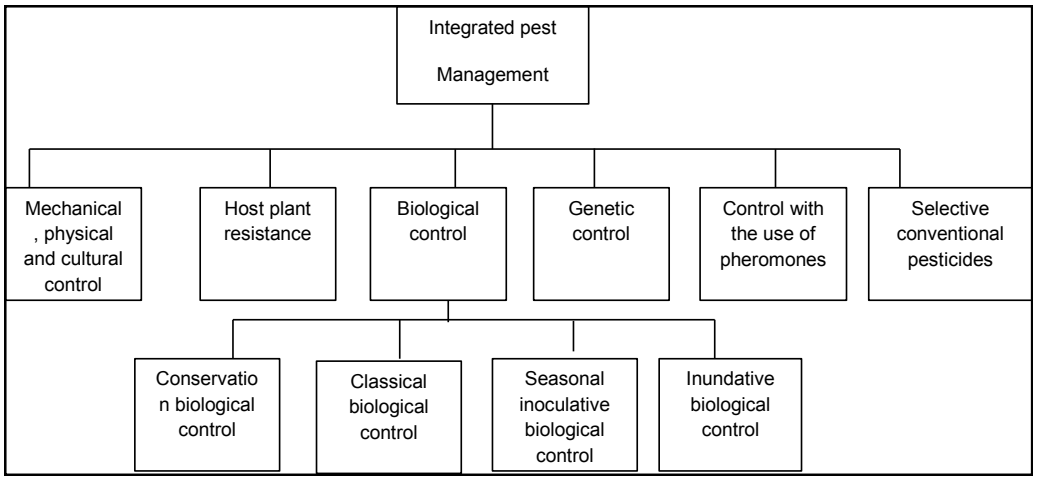


Figure 3: Components of Integrated Pest Management (Adapted from Eilenberg et al., 2001)

Four broad approaches have been distinguished in implementing biological control:

1- Conservation biological control which consists of "the modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests" (Eilenberg et al., 2001).

2- Seasonal inoculative biological control is the intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period but not permanently.

3- Classical biological control is the intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and

longterm pest control (Eilenberg et al., 2001). It differs from seasonal inoculation in that classical biological control aims at permanent establishment of the released agent (van Lenteren and Woets, 1988).

4- Inundative biological control is the use of living organisms to control pests when control is achieved exclusively by the released organisms themselves. Effects of progeny of the released organisms are not expected. Yet, this depends on the biology of the released agent. An insect species will surely multiply, whereas microorganisms such as *Bacillus thuringiensis* Berliner will decrease pest populations with no expectation of a long term control.

Some reviewers include seasonal inoculative and inundative approaches in the augmentation strategy so that they distinguish three ways to apply biological control (Yaninek and Cock, 1988; Bentley and O'Neil, 1997; van Lenteren, 2007). Augmentative biological control has therefore been defined as a periodic release (once or regular) of natural enemies to control pest for short duration (Bentley and O'Neil, 1997).

The goal of classical biological control is to find effective natural enemies, introduce them into the area of a target pest, and achieve permanent establishment so that they will provide a continuing pest control with little or no additional human intervention. In other words, it implies the importation and release of natural enemies to areas where they do not exist (anymore) in order to get a permanent establishment to control the target pest. Classical biological control has to be achieved following different steps (van Lenteren and Manzaroli, 1999; Zeddies et al., 2001; van Lenteren et al., 2003; Stiling and Cornelissen, 2005). The steps followed by most of the biological control projects are:

1. **Planning:** Biological control is developed against economically important pests. At the beginning of a biological control project/intervention, information about the pest status and identity of the target organism has to be available. Data related to the pest such as life history, damage, impact on yield, alternate host plants, and distribution, are collected. The indigenous natural enemies are also recorded and their impact and life history assessed. When none of the indigenous enemies can be used in an effective biological control strategy, the next step is made.

2. **Exploration:** Exploration to search for suitable biological candidates takes usually place in the native area of the target pest. But, sometimes, the inventory of natural enemies is extended to all areas of distribution of the pest. Literature research is often followed by studies on host range of natural enemies, parasitism rate, and the occurrence of hyperparasitism. These data help to make the first selection of promising candidates. Suitability has to be confirmed during laboratory studies. In the case that a suitable natural enemy is found, then the following steps should be made.
3. **Shipping:** After getting an import permit, a batch of the natural enemy can be prepared for shipping to the desired area.
4. **Quarantine:** The natural enemy will be contained and conditioned to minimize the risk of escape or loss of material until the authorization of release. It is also necessary to be sure that the natural enemies are not contaminated with undesirable agents. This period is of great importance. Studies are carried out on:

    - Species characterization: the strain obtained has to be clearly characterized taxonomically. The success of biological control depends greatly on the use of the appropriate strain. A too narrow genetic variability may affect the effectiveness of the biological agent (Mackauer, 1976; Hopper et al., 1993);
    - Life history: (fecundity, longevity, development time, parasitism rate, sex-ratio etc.). Do the life-history features of the parasitoid fit well with the dynamics of the target insect pest (Lane et al., 1999; Mills, 2001; van Lenteren et al., 2003; Hoelmer and Kirk, 2005)?
    - Foraging behaviour: Host range and host searching behaviour (including host plant effects) has to be investigated. Studies also focus on the oviposition and feeding behaviour, host-density responses;
    - Effects of abiotic factors: Does the climate between area of origin and area of release match for the performance of the natural enemy? The influence of temperature, relative humidity on the biology of the natural enemy are thus assessed;
    - Effect of biotic factors and assessment of environmental risks: Intra- and interspecific competition, effects on non-target organisms.

5. **Rearing:** The most suitable candidate is selected and mass reared to build up the population for release. Of importance is to establish a sufficiently large number of natural enemies to be collected (the founder population) in order to achieve enough genetic variation.
6. **Colonization:** Natural enemies are released in cages to ensure that they are adapted to environmental conditions. After that, suitable sites may be selected for experimental release. Releases may be repeated over time.
7. **Assessment of establishment:** The area of release will be monitored in order to verify whether the natural enemy has established. This can take several years. For example, it took one year after *Cotesia flavipes* Cameron was released in 1993 in the coastal area of Kenya for control of *Chilo partellus* (Swinhoe) before the parasitoid was detected (Omwega et al., 2006). After that, the parasitism rate increased slowly year after year and reached an average of 20% in 2004 i.e. 11 years after releases (Kipkoech et al., 2006).

Biological control requires the support of governments to obtain import and release permits. The Food and Agricultural Organization (FAO) has produced an International Code for the Import and Release of Exotic Biological Control Agents (FAO, 1997). By clarifying procedures and responsibilities, the Code that was approved by Member States in 1995, is particularly helpful for countries without a tradition of biocontrol.

Many parasitoid species have been introduced in several countries to implement classical biological control programmes. In Africa, classical biological control has been applied with success against some major pests (van Lenteren et al., 2006). One of the most impressive successes has been the introduction of the parasitoid wasp *Anagyrus (Epidinocarsis) lopezi* De Santis (Hymenoptera, Encyrtidae) from South America to Africa to control the cassava mealybug *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae). The parasitoid effectively attacked younger host instars, and produced more female than male offspring. After establishment, *A. lopezi* has significantly reduced damage on cassava crops and has precluded the use of insecticides targeted at the mealybug on millions of hectares (Herren and Neuenschwander, 1991).

Another example of a successful recent biological control programme in Africa,

is the introduction and establishment of *Cotesia flavipes* Cameron (Hymerotera: Braconidae) against gramineous stem borers in Africa (Overholt et al., 1997). Native to the Indo-Australian region, *C. flavipes* was imported from Pakistan to control mainly *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). The host-searching behaviour of *C. flavipes,* allows it to be more effective in attacking stems borers in small-stemmed wild grasses. The parasitoid was reported attacking medium to large (third to sixth instar) larvae of *C. partellus* (Salam et al., 2001). The impact of *C. flavipes* varied according to area and country (Kfir et al., 2002). For instance, the overall percentage of the reduction of stem borer density in the coastal region of Kenya was estimated to be 33.7 % (Kipkoech et al., 2006).

5 *Apanteles taragamae*, a promising biological agent of *M. vitrata*

Apanteles taragamae Viereck is an insect species belonging to the order of Hymenoptera, to the family of Braconidae, to the subfamily of Microgastrinae and to the genus *Apanteles* (Austin and Dangerfield, 1992). Its origin is not clear, but may be Asiatic as it has been recorded as indigenous parasitoid of *M. vitrata* in Taiwan (Huang et al., 2003) and India (Lalasangi, 1988). Some authors reported *A. taragmae* attacking other insect species in India (Ghosh and Abdurahiman, 1988; Peter and David, 1990; Mohan et al., 2000). However, the latter species, reported as being gregarious (Peter and David, 1990), seems to be different from that found in Taiwan which was solitary and specific to *M. vitrata* (Huang et al., 2003). Few studies have been done on the biology of *A. taragamae*. The wasp was reported as the dominant parasitoid species attacking *M. vitrata* on *Sesbania cannabina (*Retz) Pers. (Huang et al., 2003). Parasitism rate of *M. vitrata* larvae was as high as 63% in the field. When supplied with larvae of *M. vitrata*, *A. taragamae* exhibited a solitary behaviour (Huang et al., 2003; M. Tamò, personal communication). Based on these preliminary observations, scientists from both the World Vegetable Center (AVRDC), and the International Institute of Tropical Agriculture (IITA) identified *A. taragamae* as a promising biological control candidate against *M. vitrata.* The next step was the introduction of one hundred-twenty cocoons of *A. taragamae* into the IITA laboratories in Benin after obtaining a standard import permit from the national plant protection organization. After the successful

establishment of the parasitoid colony in the laboratories, preliminary studies were initiated to investigate some aspects of the biology of the wasp (suitable host age, development time of parasitized host fed on artificial diet, longevity of the wasp) for optimizing its rearing in the laboratory (Srinivasan et al., 2007).

6 Chemical ecology and biological control

6.1 Chemical information in plant-herbivore-carnivore interactions

Interactions are basic features observed between individuals within communities in a given environment. Living organisms mainly interact through information flows. Information flows within foods webs are important processes that affect the behaviour and dynamics of animal populations (Dicke and Grostal, 2001). Extrinsic information plays a key role in the survival of animals. Indeed, interactions between living organisms are mediated by infochemicals, which can be produced by plants, microorganisms, invertebrates and vertebrates (Dicke and Sabelis, 1988). Many terms have been proposed to describe different classes of information-conveying chemicals. The terminology proposed by Dicke and Sabelis (1988) included information-transmitting chemicals and excluded chemicals that act as toxins or nutrients (Table 4).

Infochemicals are divided into:

- Pheromones that are involved in the transmission of information between individuals of the same species
- Allelochemicals that are involved in interspecific interactions. Allelochemicals are subdivided into allomones, kairomones and synomones (Table 4).

The influence of infochemicals released from different sources (plants insect pests, their competitors and natural enemies) can be well understood when analyzing them from a tritrophic level perspective. Plants mediate the interplay between herbivores and their natural enemies. Besides preformed chemicals, plants produce secondary metabolites in response to herbivore damage. Such chemicals are termed herbivore-induced chemicals which may act directly or indirectly on herbivores. An induced direct chemical defense is e.g. the production of toxins or digestibility reducers which inhibit the growth of herbivores, while induced indirect chemical defense relates to e.g. the emission of herbivore-induced plant volatiles attracting natural enemies following herbivores damage. Volatiles are chemicals released for a short period of time, whereas

Table 4: Infochemical terminology (from Dicke and Sabelis, 1988)

Infochemical

A chemical that, in the natural context, conveys information in an interaction between two individuals, evoking in the receiver a behavioural or physiological response that is adaptive to either one of the interactants or to both.

Pheromone

An infochemical that mediates an interaction between organisms of the same species whereby the benefit is to the origin-related organism ([+, -] pheromone), to the receiver ([-, +] pheromone) or to both ([+, +] pheromone.

Allelochemical

An infochemical that mediates an interaction between two individuals that belong to different species.

Allomone

An allelochemical that is pertinent to the biology of an organism (organism 1) that, when it contacts an individual of another species (organism 2), evokes in the receiver a behavioural or physiological response that is adaptively favourable to organism 1 but not to organism 2.

Kairomone

An allelochemical that is pertinent to the biology of an organism (organism 1) that, when it contacts an individual of another species (organism 2), evokes in the receiver a behavioural or physiological response that is adaptively favourable to organism 2 but not to organism 1.

Synomone

An allelochemical that is pertinent to the biology of an organism (organism 1) that, when it contacts an individual of another species (organism 2), evokes in the receiver a behavioural or physiological response that is adaptively favourable to both organism 1 and 2.

non-volatiles chemicals can be detected for a relatively long period (Dicke and Grostal, 2001). Furthermore, some secondary plant metabolites negatively affect herbivore feeding by reducing food intake, efficiency of ingested food or by intoxicating them. Herbivores can, therefore, be deterred from feeding on plants (Dicke, 1999a). However, herbivores can develop mechanisms to detoxify plant secondary metabolites or prevent intoxication by producing enzymes such as cytochrome P450s (Feyereisen, 1999) or through physiological adaptations (for instance rapid excretion) (Wink and Schneider, 1990). The potential of herbivores to circumvent effects of secondary plant metabolites determines their host suitability and preference. Moreover, herbivores are able to recognize and avoid their enemies through infochemicals such as compounds from their enemies' exuvia, excreta (direct cues) or pheromones from alarmed, injured or dead

conspecifics (Grostal and Dicke 2000; Dicke and Grostal, 2001).

Predators and parasitoids use infochemicals to locate their host/prey habitat and to find their host/prey. They use extrinsic information to detect the presence of hosts and competitors (Vet and Dicke, 1992; Steidle and van Loon, 2003). Extrinsic information consists of visual, mechanical and chemical information. Chemical information is acquired through olfaction and taste. Direct or indirect cues are used by predators and parasitoids to recognize hosts or enemies. Direct cues may be derived from exuviae, eggs, excreta, marking pheromones or any other product of the host. Indirect cues include pheromones from alarmed, injured or dead conspecifics and infochemicals from host plants. Herbivore feeding induces volatile production by plants that attract their natural enemies (Dicke, 1999b). According to Allmann and Baldwin (2010), the rapid isomerisation of plant green leaf volatiles by herbivores through their oral secretion leads to an increase in natural enemies' recruitment by plants. Parasitoids or predators are able to discriminate among volatile blends when searching for their preferred prey or host species (Agelopoulos and Keller, 1994; Du et al., 1998), and host/prey preference can be linked to host/prey-related odour preference (Dicke, 1988). Blends of volatile compounds produced by mechanically damaged plants may differ largely from those produced by herbivore-damaged plants (Turlings et al., 1990). Moreover, different herbivore species can induce different volatile blends, indicating that plant volatiles emitted from herbivore-damaged plants can be reliable indicators of herbivore presence and identity (Vet and Dicke, 1992; Takabayashi et al., 1994). From this point of view, plants and natural enemies are considered as mutualists. However, plant damage by several herbivore species is reported to induce more complex phenotypic changes resulting in altered interactions with other community members (Dicke et al., 2009; Zhang et al., 2009). Furthermore, plants can also negatively affect performance of natural enemies by their morphology or the production of toxins (Hunter, 2003). Plant toxins that occur in tissues of immature herbivore stages may influence parasitoid larvae by stunting their growth or increasing their mortality rate (Sime, 2002). When present on the larval integument, some of these chemicals can also deter attack by predators (Montllor et al., 1991).

How plants benefit from the attraction of the herbivores' natural enemies in term of fitness is hard to quantify in complex environments (Dicke and Baldwin, 2010). But with regard to biological control implementation, reduction in pest damage by efficient

parasitoid might improve plant reproduction or fitness (Bale et al., 2008). Also, infochemicals that influence insect behaviour can be exploited by man to develop and improve environmentally benign pest management options (Dicke et al., 1990; Vite and Baader, 1990; Degenhardt et al., 2003). For instance, pheromones are used in pest monitoring and as a control measure through mating disruption, mass trapping and as a means of aggregating herbivores at delivery sites for biological control agents (Phillips, 1997; Tinzaara et al., 2007).

6.2 Chemical information and biological control

Biological control can be described as exploiting trophic interactions between living organisms in a given environment. Infochemicals have been reported to be an important component mediating these interactions (Dicke and Sabelis, 1988). Natural enemies respond to infochemicals from both herbivores and their host plants (Figure 4).

When selecting a biological control agent, it is important to combine knowledge on foraging behaviour with life history data (Force, 1974; Neuenschwander, 2001). In previous paragraphs, the influence of crops plants on the effectiveness of natural enemies has been discussed. Chemical cues from host plants play an important role in the host searching process (Dicke, 1999a). Host searching is one of the main steps in the host selection process. Besides host searching, host selection includes host evaluation and acceptance (Salt, 1935; Doutt, 1959). Host searching consists of host habitat location, host location and host finding (Vet and Dicke, 1992). In fact, host finding is the outcome (endpoint) of host searching process. It is important to notice that in each step, parasitoids use several stimuli (visual and chemical cues) of host and host -food-plant origins (Vinson, 1976; Vet and Dicke, 1992).

The performance of a parasitoid as biological control agent and its survival in a given environment are functions of how it uses information about food, mates, competitors and hyperparasitoids to make behavioural decisions (Grostal and Dicke, 2000). The success of classical biological control agents in many projects has been attributed in part to their high host searching efficiency (Dicke et al., 1990; Neuenschwander and Ajuonu 1995; Ngi-Song and Overholt, 1997; Neuenschwander, 2001; Sallam et al., 2001). And efficient host foraging is especially important at low host densities (Dicke et al., 1990).

Infochemical use by natural enemies

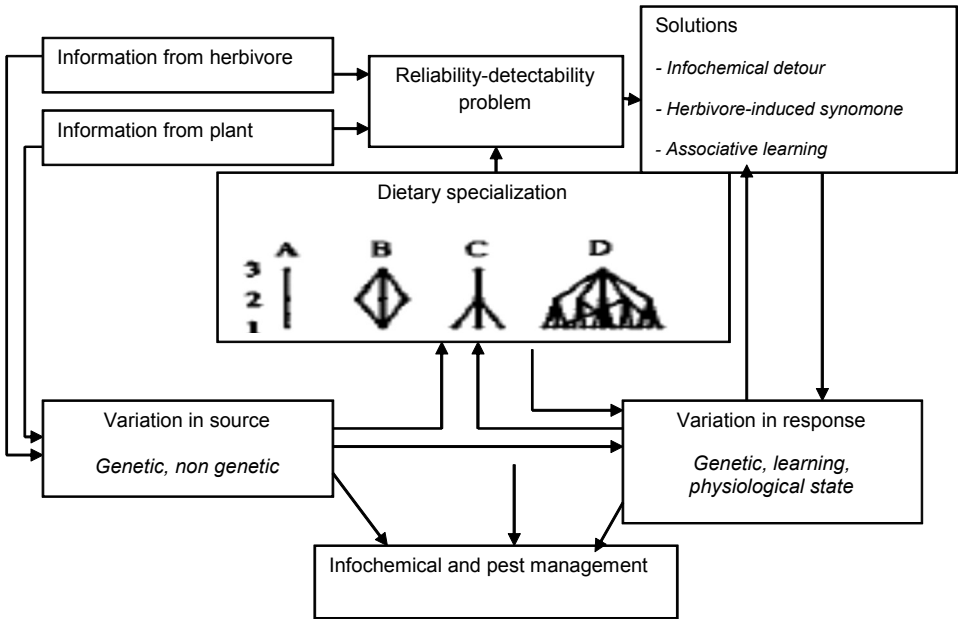


Figure 4: Use of infochemical by natural enemies in a tritrophic context (from Vet and Dicke, 1992).

1 is the first level trophic (plant), **2** is the second level (herbivore) and **3** is the third level (natural enemy)

Expected infochemical use by natural enemies of herbivores:

A: strong fixed response to kairomone, synomone and herbivore-induced synomone

B: fixed response to general kairomone component, learning of kairomone-specifics or strong response to synomone or herbivore-induced synomone;

C: strong fixed response to kairomone, learning of synomone and herbivore-induced synomone

D: no allelochemical use

Dots indicate different species.

7 Research needs

The pod borer *M. vitrata* is one of the major insect pests of cowpea that can cause up to 80% yield losses (Afun et al., 1991; Nampala et al., 2002). Control of this insect pest has been achieved mainly by the use of synthetic chemicals that led to numerous environmental consequences (pest resistance, resurgence, secondary pest outbreaks, human health hazards, environmental pollution). Alternative methods developed till now (cultural control and host resistance) still require a supplemental use of chemicals to give an effective control of the borer. Classical biological control is a sustainable approach to pest management, when host resistance levels are not high enough to control pests. One of the advantages of classical biological control is that acceptability of the method by the farmers is not an issue, contrary to the introduction of resistant varieties. Feasibility of classical biological control against the pod borer has been investigated (Tamò et al., 2003; Srinivasan et al., 2007). The fact that the larval parasitoid *A. taragamae* is able to parasitize on average 60% of the pod borer larvae in Taiwan (Huang et al., 2003) is sufficient promise to start investigating this classical biological control approach of *M. vitrata* in Africa. Therefore, the wasp has been imported from Taiwan to IITA-Benin where its potential is being evaluated. There is a lack of information on the biology of the wasp on *M. vitrata*. A thorough understanding of the biology and ecology of *A. taragamae* is a prerequisite to develop a successful classical biological control against *M. vitrata*. The present research work was designed to fill this gap of information on the biology of the wasp with particular emphasis on the interactions with environmental factors that determine the efficiency/suitability of *A. taragamae* as a biological control agent. Such factors include host stages selected for oviposition, functional response, climatic adaptability, host foraging potential, host specificity and competitive ability in the parasitoid guild (Stiling, 1993; van Lenteren and Manzaroli, 1999; Bale et al., 2008). Generally, the life history parameters of koinobiont parasitoids are mostly affected by host age/size at oviposition, temperature and host plants quality (Colinet et al., 2005; Bale et al., 2008). A good host density responsiveness is important for the stability of host-parasitoid systems after parasitoid

establishment (van Lenteren et al., 2006). Host-foraging potential plays an overriding role at low host density and shapes the extrinsic competitive behaviour of the target parasitoid in the parasitoid guild (Stiling, 1993; Neuenschwander and Ajuonu, 1995; Ngi-Song and Overholt, 1997; van Lenteren and Manzaroli, 1999; Neuenschwander, 2001; Sallam et al., 2001; Bale et al., 2008). Infochemicals from the herbivore's host plants are well-known to mediate the different steps of host foraging process by parasitoids (Vet and Dicke, 1992).). Several works showed the role of leaf volatiles in host habitat and host location by many parasitoid species (Dicke and Sabelis, 1988; Turlings et al., 1990; Vet and Dicke, 1992; Ngi-Song et al., 1996; Röse et al., 1997; Du et al., 1998; Dicke, 1999b; Fatouros et al., 2005; Dicke and Baldwin, 2010). The current work addresses a novel dimension of herbivore-induced plant volatiles (HIPV) in investigating the effects of flower volatiles primarily known for attraction of pollinators (Jervis et al., 1993; Pichershy and Gershenzon, 2002; Raguso, 2008, 2009). Another factor to be considered with regard to environmental risks of introduced parasitoids is host specificity (van Lenteren et al., 2003; Louda et al., 2003). The non-target effects of a biological agent on non-target species depends on its degree of specialization (Henneman and Memmott, 2001; Symondson et al., 2002; Louda et al., 2003). The final step in implementing a classical biological control programme is the release and establishment of the promising candidate. One factor that contributes to the successful establishment of a parasitoid is the release strategy. Parasitoids can fail to establish because of inappropriate release strategy. Designing an optimal release strategy might combine several factors such as parasitoid conditioning prior to release, release methods and rates (Grevstad, 1999; Shea and Possinghan, 2000; Bellows et al., 2006; Crowder, 2007).

8 The research objectives

The overall objective of the research presented in this thesis is to provide information on the biology and ecology of *A. taragamae* using its host *M. vitrata*. The interactions with some key environmental factors such as temperature, host age and density, host plants and non-target organisms were investigated.

The specific objectives are to:

- Study the influence of temperature, host age on the life history parameters of *A. taragamae*
- Evaluate the functional response of *A. taragamae*
- Assess the effects of volatiles from host larvae and host plants on the host foraging behaviour of *A. taragamae* with special emphasis on flower volatiles
- Investigate the effects of host plants on the life history parameters of *A. taragamae*
- Assess indirect or additional physiological traits of *A. taragamae* in relation to its suitability as biological control agent
- Explore the strategy that can optimize the release and establishment of *A. taragamae.*

9 Thesis outline

This thesis consists of six chapters.

In **chapter 1**, the pest status of *M. vitrata* is reviewed as well as several pest management strategies, and the main reasons for starting a classical biological control programme against this insect pest of cowpea. Available information on the biology of the larval parasitoid *A. taragamae* is given and the main steps in implementing classical biological control are discussed. The link between biological control and chemical ecology is stressed.

Based on the key factors that determine the efficiency/suitability of classical biological control candidates such as parasitoids, the functional response of *A. taragamae* is investigated, as well as the effects of host age and temperature on history parameters (**chapter 2**). Then, the relationship between the intrinsic rate of population increase and temperature is investigated.

The evaluation of *A. taragamae* host searching capacity is presented in **chapter 3**. Host searching efficiency is considered to be a key determinant for successful classical biological control. Therefore, the effects of volatiles from cowpea flowers and from host larvae on the foraging behaviour of the wasp are assessed using a Y-tube olfactometer.

Host plants are well-known to mediate the host foraging behaviour of parasitoid insects. But they might also influence some life history parameters of parasitoids through the production of secondary metabolites affecting herbivorous hosts. The effects of *M. vitrata*'s host plant species on the life-history parameters of *A. taragamae* are assessed in **chapter 4.** Some of the selected host plants, namely *V. unguiculata, L. sericeus,* and *P. santalinoides,* play an important role in the biology of the pod borer which is characterized by the absence of diapause in its life cycle.

Beside direct biological attributes such as climatic adaptability, host searching characteristics, functional response, some additional physiological traits are considered for assessing the performance of a classical biological control agent. These include host specificity, competitive ability and host-feeding behaviour. **Chapter 5** provides information on the physiological host range of *A. taragamae,* its competitive ability when compared to the indigenous parasitoid *P. leucobasis,* and host feeding behaviour by female wasps during oviposition.

Finally, results are discussed in a broader context with regard to factors that determine the efficiency/suitability of classical biological control agents (**chapter 6**).

Functional response and life history parameters of *Apanteles taragamae,* a larval parasitoid of *Maruca vitrata*

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Functional response and life history parameters of *Apanteles taragamae*, a larval parasitoid of *Maruca vitrata*

Elie Ayitondji Dannon, Manuele Tamò, Arnold van Huis, Marcel Dicke

Abstract

The legume pod borer *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) is a serious pest of cowpea in West Africa. The parasitoid *Apanteles taragamae* Viereck (Hymenoptera: Braconidae) that originates from Taiwan is a potential candidate for biological control of *M. vitrata*. We investigated under laboratory conditions the functional response of the parasitoid by offering each experienced female 10, 20, 30 and 40 larvae of *M. vitrata.* We studied the influence of different host larval ages on the development, longevity, sex ratio, lifetime fecundity and parasitization rate of the wasp. In a comparative study, we also investigated the life history of *A. taragamae* and *M. vitrata* at different temperatures in the range of 20 to 30 °C. The parasitoid successfully parasitized two- and three-day-old host larvae (first and second instars). Younger larvae (one-day-old) were parasitized to a lesser extent, and only males developed in them. Older larvae were not parasitized, partly because of defensive host behaviour. The success of parasitization was positively correlated with the density of two-day-old *M. vitrata* larvae. Parasitoid developmental time and longevity decreased with increasing temperature. The intrinsic rate of population increase (r_m) exhibited an optimum curve with a maximum at 24-28 °C. For the host *M. vitrata,* r_m was maximal at temperatures of 26-30 °C. The data are discussed in the context of the potential of *A. taragamae* for biological control of *M. vitrata*.

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1 Introduction

Maruca vitrata Fabricius (Lepidoptera, Crambidae) is one of the key insect pests of cowpea, causing up to 80% of yield loss (Nampala et al., 2002). It originates from the Indo-Malysian region and is a key pest of grain legumes in the tropics and subtropics (Tamò et al., 1997; Taylor, 1978). The crambid develops without diapause and relies on alternate host plants to maintain its population during the cowpea off-season (Arodokoun et al., 2003; Bottenberg et al., 1997; Taylor, 1978).

Control of *M. vitrata* in cowpea has mainly been achieved by using synthetic insecticides (Sharma, 1998; Kamara et al., 2007). Besides chemical control, cultural practices and moderately resistant varieties have been developed (Kamara et al., 2007). However, cultural practices and improved varieties still require supplemental applications of synthetic pesticides in order to obtain a substantial yield increase (Sharma, 1998). This situation often results in an overuse of chemicals with side effects such as pest resurgence and secondary pest outbreaks, insecticide resistance, environmental pollution, and increased human health risks (Ekesi, 1999). Therefore, it appears important to investigate other, environmentally benign, control methods that can regulate the populations of the legume pod borer. Of these, classical biological control remains an attractive option, in particular after *Apanteles taragamae* Viereck, a solitary endoparasitoid was recorded attacking *M. vitrata* larvae (Huang et al., 2003).

The wasp was reported parasitizing only *M. vitrata* in Taiwan. However, some reports about this wasp species indicated that it was gregarious and that it parasitized five other Pyraloidea species in India (Peter and David, 1990; Mohan and Sathiamma, 2007). For that reason, we believe that the latter wasp species is likely to be different from the one we have received from Taiwan, which is strictly solitary (Huang et al., 2003). The parasitoid was imported from Taiwan to the International Institute of Tropical Agriculture (IITA) in Benin for assessing its potential as a biocontrol agent against *M. vitrata*. In fact, prior to large scale field releases of any biocontrol agent, key aspects of its biology and ecology should be understood or evaluated (van Lenteren et al., 2003). Life history parameters provide useful information on the efficiency of biological control agents, particularly when comparing them with the ones of the target pest. Development, reproduction and survival of parasitoid insects are life history

components that depend on physiological and environmental factors (Harbison et al., 2001; Uçkan and Ergin, 2003). These factors can be either abiotic (e.g. temperature, relative humidity) or biotic (e.g. size, age, and density of the host).

The nutritional quality of an insect host is found to be correlated with its body size (West et al., 1999; Thorne et al., 2006), as large hosts contain more resources (Uçkan and Ergin, 2002). Thus, the host size selected for oviposition by a female parasitoid affects the size and sex of its progeny (Arthur and Wylie, 1959; Jones, 1982; King, 1987; Dicke, 1999a; Lacoume et al., 2006). In general, large adult parasitoids emerge from large hosts; and a larger proportion of females are produced when larger hosts are selected by the female parasitoid (Dicke, 1999a). In addition, large parasitoid females were found to have more eggs immediately available upon emergence, or to be able to generate them when needed, and have longer life expectancy than small females (Cloutier et al., 2000). However, the influence of host size on koinobiont parasitoids is complex (Harvey, 2005). Larval development of koinobiont parasitoids such as *A. taragamae* after parasitization relies on the growth rate of the host (Brodeur and Boivin, 2004; Harvey, 2005; Pennacchio and Strand, 2006). The host stage selected for oviposition by a koinobiont female is primarily based on the first host evaluation (Jones, 1982; Brodeur and Boivin, 2004). There is a host size (larval instar) threshold below which the female parasitoid rejects the host for parasitization (Jones, 1982; Brodeur and Boivin, 2004). Large hosts can exhibit strong defensive behaviour, and this can interfere with the possibility of parasitization (Brodeur et al., 1998).

Host density is reported to affect the performance of a parasitoid (Uçkan et al., 2004). The functional response of a parasitoid can be inversely or positively host-density dependent, or independent from host density (Holling, 1959). The type of functional response is another essential factor in the selection of efficient biological control agents. How a parasitoid responds to an increasing host population can determine the success of biological control.

Abiotic factors such as temperature and relative humidity influence the life history parameters and the performance of biological control agents. In classical biological control, environmental adaptability of introduced parasitoids is one of the key factors that determine their establishment and effectiveness (Kalyebi et al., 2006). There is a strong influence of temperature on developmental rate, survival, and fecundity of

parasitoids (Taylor, 1981; Roy et al., 2002; Kontodimas et al., 2004; Kalyebi et al., 2006). The relationship between temperature and developmental rate has been described as negative and linear over most of the temperature range (Campbell et al., 1974). Typically, development ceases below a lower thermal threshold; above this, the rate of development increases with temperature until an optimum is reached. Above the optimal temperature, the rate rapidly decreases to zero (Campbell et al., 1974; Brière et al., 1999).

The effect of environmental factors on the biological characteristics of *A. taragamae* has never been studied using its host *M. vitrata*. In the present study, we investigated the effect of host larval age on biological parameters of *A. taragamae*, such as development time from egg to adult, longevity, fecundity, sex ratio, and parasitization rate. We studied the functional response of the wasp, and the effect of temperature on life history parameters of *A. taragamae* and of its host *M. vitrata*. We also tested different models describing the relationship between temperature and the intrinsic rate of natural increase.

2 Materials and Methods

2.1 Mass rearing of *M. vitrata*

Pupae of *M. vitrata* were obtained from a stock culture at the field station of the International Institute of Tropical Agriculture (IITA) in Benin. They were placed in open Petri dishes that were incubated in wooden cages (44 cm x 45cm x 58 cm) with sleeves, having sides of fine screen and a glass top, and kept at 27.0 ± 0.6 °C and 60.9 ± 4.6 % relative humidity (mean ± SD). Adults emerged inside the cages and were nourished using cotton fibers moistened with 10% glucose solution. Four-day-old female moths were transferred in groups of 4 or 5 individuals in transparent small plastic cups (3 cm diameter x 3.5 cm height) and kept for 24 h to allow oviposition, which occurred on the inner surface of the cups. Ovipositing females were fed using small pieces of filter paper moistened with 10% glucose solution, which were replaced after 24 h. Cups carrying eggs were kept at the same experimental conditions till the larvae hatched. Larvae were transferred to cylindrical plastic containers (9 cm diameter x 12 cm height) provided with artificial diet prepared according to Jackai and Raulston (1988), and reared until pupation. Larvae develop through five instars. Pupae were collected and placed in

cages. All larvae used in the experiments were obtained from the mass production.

2.2 Mass rearing of *A. taragamae*

Cocoons of *A. taragamae* were obtained from the stock culture at IITA station in Benin, originally collected from the widely cultivated green manure crops *Sesbania cannabina* (Retz) Pers. infested by *M. vitrata* at the World Vegetable Center (AVRDC) in Taiwan. Emerged adults were kept in cylindrical plastic cups (4.5 cm diameter x 5 cm height). A hole (2 cm diameter) punched in the lid of the cups was covered with fine mesh. Adults of *A. taragamae* were fed with honey streaked on the fine mesh of the lid. To allow mated female wasps to parasitize hosts, they were offered, during 24 h, two-day-old larvae of *M. vitrata* in a small cylindrical cup (3 cm diameter x 3.5 cm height) containing a piece of artificial diet. The exposed larvae were reared till cocoon stage. Cocoons were collected and placed in cups (4.5 cm diameter x 5 cm height). The mass production of wasps took place in a climate chamber with a temperature of 25.3 ± 0.5 °C and a relative humidity of 78.9 ± 5.6 % (mean ± SD).

2.3 Influence of larval age of *M. vitrata* on development time, longevity, fecundity and sex ratio of *A. taragamae*

The influence of larval age of *M. vitrata* hosts on different biological parameters of *A. taragamae* was studied using three-day-old couples (male/female) of the wasp. Mating of a 24 h-old parasitoid female was allowed for 48 h by introducing a male of the same age in a small cup. This experiment was conducted in two steps. A preliminary explorative experiment was designed to assess the range of larval ages suitable to *A. taragamae* parasitization. A *M. vitrata* larva of 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 days age was exposed to one naïve (i.e. without oviposition experience) mated female for parasitization. Parasitization was monitored visually. This was done twice using different females. In parallel to this experiment, a second preliminary experiment has been conducted. Here, 10 larvae of each of the ten ages were separately placed in small cups (3 cm diameter x 3.5 cm height) containing artificial diet, and one mated female parasitoid was released in each cup for 24 h to allow parasitization. Subsequently, the larvae were reared on artificial diet till cocoon stage.

In another experiment, 100 larvae each of either 1, 2 or 3-day-old were offered to

female wasps for parasitization, 25 of each age per day during four consecutive days. Larvae of the three ages were exposed to the female wasps in different sequences to limit the effect of female physiology. Thus, a female was allowed to oviposit successively in 5 one-day-old larvae, 5 two-day-old larvae, and 5 three-day-old larvae; after that, the sequence 3, 2 and one-day-old was used for a second parasitoid individual, then 2, 1, 3, then 2, 3, 1 and so on. Each female was allowed to parasitize at most 5 larvae of each age. Parasitized larvae of each age were reared individually in small cups containing artificial diet. Larval development and survival were examined daily. The number of successfully parasitized larvae was also recorded, as well as the development time from larval to cocoon stage and from cocoon stage till adult emergence. The parasitization rate was calculated for each age from the number of parasitoid cocoons resulting from 25 parasitized larvae. Adult females were kept for mating with males of the same age. Each couple of wasps was offered twenty to thirty two-day-old *M. vitrata* larvae daily until the female died. After being exposed to the parasitoid couple, the larvae were transferred individually into small cups containing artificial diet and reared until cocoon stage. We recorded the number of cocoons of *A. taragamae* as well as the number of adult male and female parasitoids that emerged. As our Taiwan strain of *A. taragamae* is a solitary parasitoid, only one individual emerged from a cocoon. Hence, the observed life time fecundity per female was determined in terms of number of cocoons.

This study was carried out in a climate chamber under 25.3 ± 0.5 °C (mean ± SD) and 78.9 ± 5.6 % relative humidity (mean ± SD).

2.4 Effect of larval density of *M. vitrata* on the rate of parasitization by *A. taragamae*

In this experiment, 10, 20, 30, or 40 two-day-old *M. vitrata* larvae, reared in small cups (3 cm diameter x 3.5 cm height) containing artificial diet, were placed in cylindrical plastic containers (9 cm diameter x 12 cm height). One three-day-old mated and oviposition-experienced female parasitoid (by parasitizing 3 two-day-old larvae, 10 min prior to the experiment) was placed for 24 h in each container. Each density was repeated 5 times using different female parasitoids, so a total of 20 experienced mated female wasps was used. In a preliminary experiment, holes (1 cm diameter) were made

in the cover of the plastic containers, in order for the parasitoids to be able to escape from the setup. This was done in order to allow the parasitoid either to oviposit or to escape. However, since all female wasps escaped during the first runs without parasitizing larvae, all further experiments were done without the hole in the container lid. After parasitoid exposure, larvae were transferred individually into small cups containing artificial diet and reared till cocoon stage. The number of parasitized larvae in terms of number of parasitoid cocoons for each density was recorded.

This study was carried out in a climate chamber at 25.3 ± 0.5 °C, and 78.9 ± 5.6 % Relative Humidity (mean ± SD).

2.5 Influence of temperature on the life history parameters of *A. taragamae* reared on *M. vitrata*

Three-day-old mated females of *A. taragamae* were introduced into small cups (3 cm diameter x 3.5 cm height) containing about 20 two-day-old larvae of *M. vitrata* for parasitization. Parasitization was observed visually. Stung larvae were individually transferred to other small cups (3 cm diameter x 3.5 cm height) and reared at one of 5 constant temperatures, i.e. 20, 24, 26, 28 or 30°C under a relative humidity range of 70-90% in incubators. Previous studies had shown that the range of temperatures for successful development of *M. vitrata* is between 19.5 and 31.9°C (Adati et al., 2004). The constant temperatures tested in this study cover most of the ranges occurring in Benin ecosystems (Emert and Brücher, 2008). The temperature regimes are also required to estimate accurately the thermal requirements for insect development and survival using the models described below (Campbell et al., 1974). Temperature and relative humidity were measured twice a day over the whole experimental period using a thermo-hygrometer placed inside the incubator. The realized five temperatures regimes were 20.2 ± 0.6; 24.2 ± 0.4; 26.1 ± 0.6; 28.2 ± 0.8 and 29.6 ± 0.3°C, and the relative humidity for each of these temperatures 79.3 ± 11.8; 73.2 ± 10.5; 88.2 ± 7.2; 68.2 ± 10.7 and 78.2 ± 10.6 % (mean ± SD), respectively. A total of 100-150 larvae were reared on artificial diet under each temperature regime. Larval survival was checked daily until cocoon stage and the number of cocoons from which adult wasps emerged was recorded. Development time was recorded for two developmental phases (larva-cocoon and cocoon-adult) and the sex of the emerged adult was also determined. Each female

was coupled with a male of the same age in small cups. A drop of honey solution was put onto the cover of each cup to allow the adults to feed. Each couple of wasps received daily 20-30 two-day-old larvae of *M. vitrata* until the female died. A total of 30 pairs of wasps were used per temperature regime. Larvae were reared on artificial diet until cocoon formation. The number of cocoons, emerged adults and their sex ratio were recorded for each female. Realized life time fecundity of females was then recorded in terms of the number of cocoons.

In parallel, 100-150 two-day-old non-parasitized larvae of *M. vitrata* taken from the same larval population were reared at each temperature as control.

Life table parameters were calculated for *A. taragamae* and for its host *M. vitrata*. They are defined according to Birch (1948):

The net reproductive rate R_0, which is the number of female progeny per female per generation. It is given by the following formula:

$$R_o = \sum l_x \, m_x$$

Where:

x is the pivotal age of individuals in days

l_x is the age-specific survival as proportion of individuals still alive at age x

m_x is the age-specific fecundity as female offspring per female.

The intrinsic rate of increase r_m, given by the formula:

$$1 = \sum l_x \, m_x \, e^{-r_m \, x}$$

where **e** is the base of natural logarithm (ln).

The mean generation time T, calculated as follow:

$$T = \ln(R_0) / r_m \quad \text{and by approximation} \quad T = (\sum x \, l_x \, m_x) / \sum l_x \, m_x$$

The doubling time T_2 is therefore:

$$T_2 = \ln(2) / r_m$$

The finite rate of increase λ, expressed as the multiplication per female per unit

time:

$$\lambda = e^{r_m}$$

The intrinsic rate of increase r_m was calculated using the Jackknife technique (Maia et al., 2000). All these life table parameters were computed using the SAS program developed by Maia et al. (2000). This program includes multiple comparisons between groups and does not assume the same immature stage survivorship for all groups as done with the computer program developed by Hulting et al. (1990).

The effect of temperature on the intrinsic rate of increase (r_m) and the developmental rate (1/d) of *A. taragamae* and of its host *M. vitrata* was examined using mathematic models. The developmental rate is the reciprocal (1/d) of the development time (d) (Howe, 1967). Several models have been elaborated to describe the relationships between temperature and the intrinsic rate of increase or the developmental rate of insect species (Campbell et al., 1974; Brière et al., 1999; Roy et al., 2002). Of these, we selected the linear and Brière 1 models to describe the relationship between temperature and developmental rate or intrinsic rate of increase of *A. taragamae* and of its host *M. vitrata*. Equations of these models are as follows:

Linear model: $r_m = a + bT$

where:

r_m is the intrinsic rate of increase

a and b are regression coefficients

T is the temperature in °C.

Brière 1: $r_m = aT(T - T_o)(T_{\max} - T)^{1/2}$

where:

r_m is the intrinsic rate of increase

T_0 is the lower temperature threshold

T_{max} is the upper temperature threshold

a is an empirical constant.

The same models have been used to describe the relationship between the temperature and the developmental rate (d) of *A. taragamae* and of its host *M. vitrata*.

Equations of the models become:

Linear model: $1/d = a + bT$

Brière 1: $1/d = aT(T - T_o)(T_{\max} - T)^{1/2}$

where

models parameters are defined as above.

Thus, in the model equations, both r_m and 1/d were used, where d is the mean development time from egg to adult for both insect species. Brière 1 model allowed determining the lower and upper thermal thresholds for each insect species (Brière et al., 1999). We were interested in describing the relationship between temperature and the two parameters r_m and 1/d which have different ecological meanings. The intrinsic rate of natural increase r_m (measured in terms of number of female offspring/female/day) refers to population increase (Birch, 1948), while the development rate refers to individual development rate (Howe, 1967). The linear model used for describing the developmental rate enables the calculation of the thermal constant K, a kind of physiological time defined as the amount of heat units required for development; K is the inverse of the regression coefficient b of the linear model (K = 1/b).

The coefficients of the linear model were computed using the linear regression procedure, while the different parameters in the Brière 1 model were computed with the nonlinear regression procedure with the Levenberg-Marquardt iterative method (Marquardt, 1963).

3 Statistical analysis

The effect of larval host age on the duration of each parasitoid stage (larval, cocoon or pupae, adult), of the whole cycle, of the male or female cycle, and on female fecundity was tested by using the General Linear Model procedure of SAS followed by the Tukey test for the separation of means. Comparison between male and female longevity was done with a paired t-test. Likewise, the influence of temperature on the different biological parameters, development time of each stage, longevity, fecundity, immature

survivorship rate, intrinsic rate of increase, net reproduction rate, mean time generation, doubling time and the finite rate of increase have been compared by performing ANOVA using the General Linear Model procedure of SAS followed by the Tukey-test for the separation of the means. Data on sex ratio (female proportion) were analyzed by using the χ^2 test. Thus, a 2 x 2 contingency table based on the chi-square was used to test for between-larval age differences, while a 2 x 5 contingency table was used to test for between-temperatures differences in sex ratio. Comparison of development cycle and longevity between male and female were done with a paired t-test. Percentage data (p) such as proportions of parasitized larvae and proportions of immature parasitoid stages survivorship were arcsine $\sqrt{}$ (p/100) transformed prior to analysis. Proportion of immature parasitoid stages is the proportion of parasitized larvae that successfully completed the cycle and gave adults. However, untransformed data were presented in tables. Simple linear regression was applied to determine the relationship between larval density and proportion of parasitized larvae. The t-independent test was used to compare the intrinsic rate of increase of the parasitoid and of it host.

4 Results

4.1 Influence of larval host age on some biological parameters of *Apanteles taragamae*

Of the ten larval age groups (1 to 10-day-old larvae) tested only larvae of one-, two- and 3-day-old were successfully parasitized by *A. taragamae,* while older larvae escaped parasitism. Visual observation indicated that the female wasps were not able to immobilize larvae older than 3 days, which consequently escaped oviposition. The development time of immature stages (larvae and cocoon) of *A. taragamae* was not significantly influenced by the age of *M. vitrata* larvae (Table 1). However, the whole development cycle of the parasitoid was about half a day and one day shorter respectively when two-day-old and three-day-old larvae were parasitized compared to that of one-day-old parasitized larvae ($F = 10.75$; $df = 2, 44$; $P = 0.001$). The same effect was observed for males but not for females. Males that emerged from larvae which were parasitized at the age of one day, lived significantly longer than the males from larvae

Table 1: Influence of the age of *M. vitrata* larvae on the development time (mean ± SE) longevity, parasitization rate, fecundity and sex ratio of *Apanteles taragamae*.

Parasitoid life-history parameters	*Maruca vitrata* larval age at parasitization		
	One day	Two days	Three days
Development time (days):			
- egg-cocoon	8.3 ± 0.11 (30) a	7.9 ± 0.11 (67) a	7.7±0.08 (57) a
- cocoon-adult	5.0 ± 0.12 (12) a	5.2 ± 0.12 (43) a	4.9 ± 0.09 (45) a
- egg-adult (cycle)	13.6 ± 0.23 (12) a	12.9 ± 0.14 (43) b	12.5 ± 0.13 (45) c
Development cycle egg-adult (days):			
- male	13.6 ± 0.23 (12) a	12.8 ± 0.16 (25) b α	12.2 ± 0.11 (32) c α
- female	--	13.1 ± 0.25 (18) a α	13.2 ± 0.30 (13) a β
Longevity (days):			
- male	19.8 ± 3.01 (12) a	13.4 ± 1.75 (25) b α	8.5 ± 1.73 (32) b α
- female	--	11.0 ± 1.60 (15) a α	8.8 ± 1.45 (12) a α
Survival rate of larvae and cocoons (%)	7.0 ± 3.0 (4) a	43.0 ± 8.5 (4) b	46.0 ± 5.8 (4) b
Parasitization rate (%)	35.0 ± 7.9 (4) a	67.0 ± 2.5 (4) b	56.0 ± 2.8 (4) a b
Sex ratio (% of females in the population)	0.0	40.5 a. n.s.	27.3 a*
Female fecundity (number of cocoons per female)	--	55.5 ± 5.6 (14) a	42.2 ± 8.1 (11) a

Numbers in parentheses are the number of repetitions; each repetition consists of 25 host larvae

Means within each row followed by the same letters were not significantly different with Tukey test at 5% following ANOVA for development times, longevity, survival rate and parasitization rate.

For comparison between males and females, means within each column followed by Greek letters were not significantly different (paired t-test at 5%).

Sex ratios followed by the same letter (vertically) were not significantly different with χ^2 at 5% (based on 2 x 2 contingency tables) for comparison between the host larval ages of two versus three days.

* indicates that there was a difference ($P \leq 0.05$, χ^2), and n.s. no difference ($P > 0.05$, χ^2), between the sex ratio from the expected percentage of 50%, as assessed for each larval age separately.

-- indicates that the parameter is not available because only male wasps emerged from one-day-old parasitized larvae.

which were parasitized at the age of two or three days. No significant differences were observed between the longevity of male and female parasitoids when larvae of two- or three-day-old had been parasitized by their mothers.

The proportion of successfully parasitized larvae was significantly lower (about 50%) for one-day-old larvae compared to two- or three-day-old larvae (Table 1). Only male wasps emerged from larvae that had been parasitized at the age of one day, while larvae parasitized when two or three-day-old yielded both male and female wasps. No differences were observed between the fecundity of females whose mothers had parasitized two- or three-day-old larvae. The percentage of female offspring was significantly lower than the expected frequency of 50% when the parasitoids parasitized three-day-old larvae (Table 1). When comparing larval ages, there was a marginally insignificant (χ^2 = 2.9; df = 2; *P* = 0.08) difference in the percentage of females obtained from two- and three-day-old parasitized larvae.

4.2 Host density

The percentage parasitism of *M. vitrata* larvae by *A. taragamae* increased with larval density (y = 0.61x + 8.00; R^2 = 0.92; F = 25.7; df = 1, 2; *P* = 0.03) (Figure 1). Thus, the wasps exhibited a host density-dependent functional response within the range of densities tested.

4.3 Temperature

The development time of *A. taragamae* from egg to cocoon, from cocoon to adult and from egg to adult decreased significantly with an increase in temperature from 20 to 30° C (Table 2). The duration of the total life cycle from egg to adult was almost reduced by half when going from 20 to 24 °C (25 to 13 days), then it was reduced by another 3 days (10 days) at 28 and 30 °C. The same effect was observed for the development cycle of male or female wasps. Comparison between male and female development rates at the same temperature level revealed a significant difference at all temperatures except at 28 °C. The development cycle of males was significantly shorter than the cycle of females at 20, 24 and 26 °C, but longer at 20 and 30 °C. The highest lifetime fecundity was obtained at 20 °C (123 ± 12.9 cocoons per female), while the daily fecundity was lowest with 1.2 ± 0.46 cocoons per female per day at 30 °C and highest with about 7

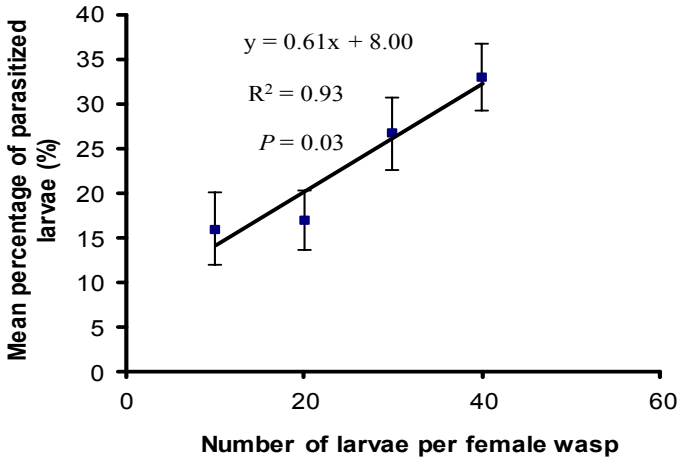

Figure 1: Relationship between the larval densities of *Maruca vitrata* and the parasitization rate

between 24 °C and 28 °C. The lowest survival rate of parasitoid larval and cocoon stages (about 70%) was obtained at 20 °C and 30 °C, and the highest at 24 °C (about 80%). The proportion of female wasps seemed not to be affected by the temperature ($\chi 2$ = 0.19; df = 4; *P* = 0.996) and was significantly lower than the expected proportion of 50% (χ^2 were 28.3; 33.3; 30.7; 29.5 and 33.3, respectively at 20, 24, 26, 28 and 30 ° C with df = 1 and *P* < 0.0001 for the five temperatures). Linear regression analysis revealed a significant negative correlation between the temperature and the following parameters: development time from egg to cocoon (y = 28.67 – 0.81x; R^2 = 0.87; F = 20.8; df = 1, 3; *P* = 0.02), development time from cocoon to adult emergence (y = 23.57 – 0.69x; R^2 = 0.76; F = 9.9; df = 1, 3; *P* = 0.05),), duration of the whole developmental cycle (from egg to adult) (y = 5.95 – 1.49x; R^2 = 0.83; F = 14.4; df = 1, 3; *P* = 0.03), the development time for males (y = 49.53 – 1.41x; R^2 = 0.83; F = 14.9; df = 1, 3; *P* = 0.03) and females (y = 54.45 – 1.56x; R^2 = 0.83; F = 14.03; df = 1, 3; *P* = 0.03), male longevity (y = 52.54 – 1.61x; R^2 = 0.87; F = 19.96; df = 1, 3; *P* = 0.02), female longevity (y = 54.80 – 1.71x; R^2 = 0.96; F = 74,4; df = 1, 3; *P* = 0.003) and the life time fecundity (y = 341.13 – 10.87x; R^2 = 0.96; F = 77.97; df = 3, 1; *P* = 0.003). There was no significant correlation between temperature and the daily fecundity (F = 1.07; df = 1, 3; *P* = 0.38) and the survival rate of parasitoid larvae and cocoons (F = 0.56; df = 1, 3; *P* = 0.51).

Table 2: Development time (mean ± SE), longevity (mean ± SE), lifetime fecundity (mean ± SE), daily fecundity (mean ± SE), immature stages survivorship (mean ± SE) and sex ratio of *Apanteles taragamae* at five different temperatures

Parameters	Temperatures regimes				
	20 ºC	24 ºC	26 ºC	28 ºC	30 ºC
Development time (days):					
- egg-cocoon	13.7 ± 0.09 (90) a	7.9 ± 0.03 (105) b	6.9 ± 0.05 (113) c	5.7 ± 0.05 (97) d	5.7 ± 0.04 (130) d
- cocoon-adult	11.3 ± 0.16 (65) a	4.8 ± 0.09 (74) b	5.2 ± 0.12 (64) b	4.4 ± 0.11 (61) b c	3.9 ± 0.11 (70) c
- egg-adult (cycle)	24.9 ± 0.24 (65) a	12.6 ± 0.10 (74) b	12.0 ± 0.12 (64) c	10.0 ± 0.10 (61) d	9.7 ± 0.08 (70) d
Development cycle (days):					
- male	23.9 ± 0.25 (34) a α	12.3 ± 0.10 (36) b α	11.9 ±0.13 (50) b α	9.9 ± 0.16 (31) c α	9.4 ± 0.09 (40) c α
- female	26.0 ± 0.32 (30) a β	13.0 ± 0.15 (37) b β	12.9 ± 0.18 (36) b β	10.03±0.12 (30) c α	10.1 ± 0.08(30) c β
Longevity (days):					
- male	18.3 ± 0.69 (30) a α	15.6 ± 2.3 (30) a α	13.8 ± 1.8 (30) a α	5.3 ± 0.76 (30) b α	3.1 ± 0.45 (30) b α
- female	21.9 ± 0.90 (30) a α	12.0 ± 0.81 (30) b α	9.2 ± 0.55 (30) b c β	7.7 ± 0.73 (30) b c β	4.1 ± 0.44 (30) d α
Lifetime fecundity (cocoons/female)	123.3 ± 12.7 (30) a	73.2 ± 5.8 (30) b	64.7 ± 6.0 (30) b	46.7 ± 4.1 (30) b	5.9 ± 1.9 (30) c
Daily fecundity (cocoons/female/day)	5.5 ± 0.37 (30) a	7.1 ± 0.80 (30) a	6.9 ± 0.51 (30) a	6.8 ± 0.61 (30) a	1.2 ± 0.46 (30) b
Survival rate of larvae and cocoons (%)	71.0 ± 2.0 (3) ac	81.7 ± 1.45 (3) b	76.0 ± 0.58 (3) a	69.3 ± 1.2 (3) c	67.3 ± 0.66 (3) c
Sex ratio (% females progeny)	34.7 a*	33.3 a*	34.3 a*	34.0 a*	33.3 a*

Numbers in parentheses are the number of repetitions
Means within each row followed by the same letters were not significantly different with Tukey test at 5% following ANOVA
Means within each column followed by Greek letters were not significantly different t-paired test at 5% for comparison between male and female
For sex ratio, female proportions followed by the same letter were not significantly different with χ^2 at 5% (based on 2 X 5 contingency table) for comparison between temperatures.
and * indicates that there was difference ($P > 0.05$, χ^2) between observed and expected (50%) percentages of the females at the same temperature.

Development time of the different stages of *M. vitrata* followed the same tendency as in the case of *A. taragamae* (Table 3). The life cycle duration was reduced from 39 to 19 days by increasing temperatures from 20 to 30°C. Male moths lived significantly longer than females at 20 and 30°C. Linear regression analysis showed a significant, negative correlation between the temperature and the following parameters: development time from egg to pupal stage ($y = 39.48 – 0.99x$; $R^2 = 0.93$; $F = 39.25$; df = 1, 3; $P = 0.008$), development time from from pupa to adult emergence ($y = 34.76 – 1.03x$; $R^2 = 0.91$; $F = 29.1$; df = 1, 3; $P = 0.012$), the whole life cycle ($y = 76.88 – 2.00x$; $R^2 = 0.92$; $F = 33.9$; df = 1, 3; $P = 0.0101$), the cycle for males ($y = 75.96 – 1.99x$; $R^2 = 0.88$; $F = 21.3$; df = 1, 3; $P = 0.019$) and females ($y = 77.36 – 2.03x$; $R^2 = 0.92$; $F = 35.65$; df = 1, 3; $P = 0.009$), male longevity ($y = 50.44 – 1.47x$; $R^2 = 0.91$; $F = 29.76$; df = 1, 3; $P = 0.012$), female longevity ($y = 39.25 – 1.07x$; $R^2 = 0.91$; $F = 30.8$; df = 1, 3; $P = 0.011$) and the immature stages survival rate ($y = 123.01 – 1.19x$; $R^2 = 0.89$; $F = 24.5$; df = 1, 3; $P = 0.016$). No significant correlation was observed between the temperature and the life time fecundity ($F = 0.17$; df = 1, 3; $P = 0.70$) and the daily fecundity ($F = 0.1$; df = 1, 3; $P = 0.94$) of *M. vitrata.* The observed lifetime fecundity reached a maximum value of 246 ± 29 eggs/female at 26°C and lower values of 122 ± 17 and 47 ± 16 eggs/female, at 20 and 30 °C respectively. Likewise, the daily fecundity followed a similar trend. The survival rate of larvae and pupae was reduced from 83% at 20°C to 63% at 30°C. Sex ratio was not affected by temperature ($\chi^2 = 0.12$; df = 4; $P = 0.998$) and no difference was obtained between proportion of females and males of *M. vitrata* (χ^2 were 0.65; 0.85; 1.6; 1.0; and 0.85, respectively at 20, 24, 26, 28 and 30 °C with df = 1 and $P > 0.05$ for the five temperatures) (Table 3).

The life table parameters of *A. taragamae* and its host *M. vitrata* are given in Table 4. The intrinsic rate of increase (r_m) and the finite rate of increase (λ) of *A. taragamae* followed a parabolic trend with a maximum between 24 and 28°C. The net reproductive rate, the mean generation time and the doubling time of the parasitoid, however, declined with increasing temperature. The intrinsic rate of increase (r_m) and the finite rate of increase (λ) of *M. vitrata* also showed a parabolic trend with a maximum between 26-30°C. Likewise, the net reproductive rate showed a parabolic trend with a maximum at 26°C while the mean generation and doubling times decreased with the temperature.

Table 3: Development time (mean ± SE), longevity (mean ± SE), lifetime fecundity (mean ± SE), daily fecundity (mean ± SE), immature stage survivorship (mean ± SE) and sex ratio of *Maruca vitrata* at five different temperatures

Parameters	Temperatures regimes				
	20ºC	24ºC	26ºC	28ºC	30ºC
Development time (days):					
- egg-pupa	20.6 ± 0.60 (75) a	14.9 ± 0.08 (70) b	12.5 ± 0.06 (100) c	11.2 ±0.07 (82) d	11.0 ± 0.12 (81) d
- pupa-adult	15.5 ± 0.06 (71) a	8.5 ± 0.06 (67) b	7.1 ± 0.07 (68) c	5.9 ± 0.13 (62) d	5.1 ± 0.07 (67) e
- egg-adult	39.0 ± 0.10 (71) a	26.4 ± 0.77 (67) b	22.8 ± 0.66 (68) c	20.2 ± 0.18 (62) d	19.2 ± 0.11 (67) e
Development cycle (days):					
- male	38.9 ± 0.14 (41) a α	26.4 ± 0.10 (34) b α	20.7 ± 0.18 (38) c α	20.2 ± 0.17 (31) c α	19.4 ± 0.17 (32) d α
- female	39.1 ± 0.21 (29) a α	26.4 ± 0.12 (31) b α	22.7 ± 0.12 (31) b β	20.1 ± 0.31 (32) d α	19.0 ± 0.08 (35) e α
Longevity (days):					
- male	22.4 ± 1.3 (30) a α	12.5 ± 0.71 (30) b α	12.9 ± 1.0 (30) b α	7.9 ± 0.81 (30) c α	7.7 ± 0.71 (30) c α
- female	6.8 ± 1.6 (30) a β	14.4 ± 1.5 (30) ab α	12.0 ± 1.1 (30) b α	10.4 ± 0.94 (30) b β	5.4 ± 0.83 (30) c β
Lifetime fecundity (eggs/female)	121.5 ± 16.6 (30) ac	140.7 ± 24.6 (30) a	246.4 ± 28.5 (30) b	145.0 ± 27.2 (30) a	46.7 ± 15.6 (30) c
Daily fecundity eggs/female/day)	8.0 ± 1.2 (30) ac	8.3 ± 1.3 (30) a	19.4 ± 1.8 (30) b	10.7 ± 1.8 (30) a	4.7 ± 1.4 (30) c
Survival rate of larvae and cocoons (%)	83.0 ± 1.5 (3) a	77.3 ± 1.8 (3) b	77.0 ± 0.0 (3) b	70.3 ± 1.3 (3) c	62.7 ± 0.67 (3) d
Sex ratio (% females progeny)	47.7 a n.s.	47.3 a n.s.	46.3 a n.s.	47.0 a n.s.	47.3 a n.s.

Numbers in parentheses are the number of repetitions
Means within each row followed by the same letters were not significantly different with Tukey test at 5% following ANOVA for comparison between temperatures.
Means within each column (same temperature) followed by Greek letters were not significantly different with t-paired test at 5% for comparison between male and female
For sex ratio, female proportions followed by the same letter were not significantly different with $\chi 2$ at 5% (based on 2 X 5 contingency table) for comparison between temperatures and n.s. indicates no difference ($p > 0.05$, $\chi 2$) between observed and expected (50%) percentages of females at the same temperature.

Table 4: Life table parameters (mean ± SE) of *Apanteles taragamae* and its host *Maruca vitrata* at five different temperatures

Parameters	Jackknife estimation				
	20ºC	24ºC	26ºC	28ºC	30ºC
-Intrinsic rate of increase (r_m) (females/female/day)					
Apanteles taragamae	0.14 ± 0.001 a	0.23 ± 0.01 b	0.23 ± 0.004 b	0.24 ± 0.01 b	0.10 ± 0.003 c
- *Maruca vitrata*	0.10 ± 0.01 a	0.14 ± 0.01 a	0.19 ± 0.002 b	0.20 ± 0.01 b	0.19 ± 0.01 b
Net reproduction rate (R_0)(females/female)					
- *Apanteles taragamae*	7.02 ± 0.46 a	23. 4± 2.40 b	24.5 ± 1.5 b	14.3 ± 0.47 c	2.6 ±0.10 c
- *Maruca vitrata*	139.5 ± 50.1 a	96.1 ± 37.8 a	168.3 ± 17.1 a	89.0 ± 13.4 a	56.3 ± 16.3 a
Mean generation time (T) (day)					
- *Apanteles taragamae*	26.2 ± 0.14 a	13.9 ± 0.04 b	14.1 ± 0.42 b	10.9 ±0.21 c	10.6 ±0.60 c
- *Maruca vitrata*	47.5 ± 2.02 b a	32.3 ± 2.07 b	27.03 ± 0.6 b c	23.2 ± 0.44 c	22.4 ± 1.6 c
Doubling time (DT)(day)					
- *Apanteles taragamae*	5.03 ±0.05 a	3.1 ± 0.087 a	3.03 ±0.06 a	2.8 ± 0.08 a	3.7 ± 1.1 a
- *Maruca vitrata*	6.7 ± 0.31 a	5.06 ± 0.35 b	3.7 ±0.03 c	3.6 ± 0.15 c	3.6 ± 0.19 c
Finite rate of increase (λ)(females/female/day)					
- *Apanteles taragamae*	1.2 ± 0.001 a	1.3 ± 0.01 b	1.3 ± 0.01 b	1.3 ± 0.01 b	1.1 ± 0.004 c
- *Maruca vitrata*	1.1 ± 0.01 a	1.2 ± 0.01 a	1.2 ± 0.002 b	1.2 ± 0.01 b	1.2 ± 0.01 b

Means are means of three cohorts of 10 females (in total 30) used to calculate life table parameters
Means within each row followed by the same letters were not significantly different with Tukey test at 5% following ANOVA for the same insect species.

The intrinsic rate of increase of *A. taragamae* was significantly larger than the intrinsic rate of increase of its host *M. vitrata* at all temperature regimes with exception to 30°C (t were 6.7; 7.8; 8.7; 4.4; and -7.8, respectively at 20, 24, 26, 28 and 30 °C with df = 2 and $P < 0.05$ for the five temperatures).

The linear model was not significant for the intrinsic rate of increase of *A. taragamae* (F = 0.02; df = 1, 3; $P = 0.9$) and was not appropriate for describing the relationship between the intrinsic rate of increase of *A. taragamae* (Table 5). The thermal constant required for the development of *A. taragamae* was lower than that of *M. vitrata*.

The curves describing the relationship between the temperature and the intrinsic rate of increase or the developmental rate of *A. taragamae* and of its host *M. vitrata* are depicted in Figure 2. The nonlinear model Brière 1, allowed estimation of the thermal thresholds for the developmental rate of *A. taragamae* and of the intrinsic rate of increase of *M. vitrata.*

5 Discussion

Larval age

The parasitoid wasp, *A. taragamae* was not able to parasitize larvae older than three days (an age corresponding to the early second *M. vitrata* larval development stage). Indeed, larvae ran faster and escaped from parasitism, suggesting a defensive behaviour (Brodeur et al., 1998). Moreover, older larvae may not be suitable for the wasp's development (Jones, 1982; Brodeur and Boivin, 2004). Parasitism success on one-day-old larvae was the lowest. This was associated with low immature survival, which may be due to limited nutritional resources available in such early instars larvae (Arthur and Wylie, 1959; Harvey et al., 2004). Hence, the parasitoid took longer to complete its development on one-day-old larvae (Table 1). The host size at oviposition was reported to affect the development duration of koinobiont parasitoids (Colinet et al., 2005). Although their hosts continue to grow after parasitism, such parasitoids were found to be selective regarding the host size or age at oviposition, which influence many of their other biological features (Jones, 1982; Brodeur and Boivin, 2004).

Table 5: Estimated parameters of four temperature-dependent models describing the influence of temperature on the intrinsic rate of increase and the developmental rate of *Apanteles taragamae* and its host *Maruca vitrata*

Model type	Parameters	*Apanteles taragamae*		*Maruca vitrata*	
		Intrinsic rate of increase	Developmental rate	Intrinsic rate of increase	Developmental rate
Linear regression	Probability	0.06 n.s.	0.006	0.04	0.0008
	a	--	-0.08 ± 0.02	-0.15 ± 0.06	-0.03 ± 0.005
	b	--	0.006 ± 0.001	0.01 ± 0.003	0.003 ± 0.0002
	K	--	157 degree-days	--	366.3 degree-days
	R^2	--	0.94	0.92	0.98
	RSS	--	1.4×10^{-4}	4.7×10^{-4}	6.6×10^{-6}
Briére 1	Probability	0.004	0.002	0.006	0.0001
	a	0.0004±0.00005	0.0001 ± 0.000	0.0002 ± 0.000	0.00003 ± 0.000
	T_0	14.9 ± 1.2	14.6 ± 1.6	13.7 ± 2.9	10.5 ± 0.91
	T_{max}	30.2 ± 0.1	35.1 ± 2.1	33.1 ± 1.7	38.1 ± 1.1
	R^2	0.97	0.98	0.91	0.99
	RSS	5.4×10^{-4}	5.3×10^{-5}	5.7×10^{-4}	6.6×10^{-7}

n.s. indicates that the model was not globally significant
-- indicates that parameters were not determined because the model was not globally significan

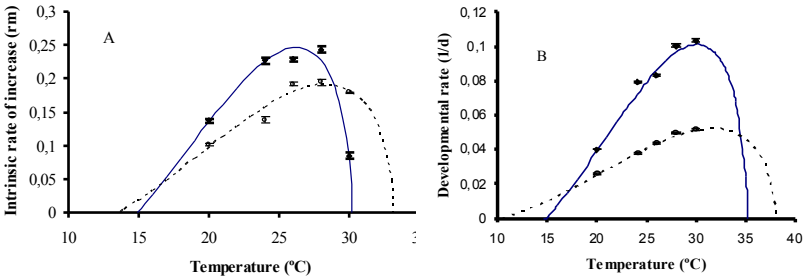

Figure 2: Relationship between the temperature and the intrinsic rate of increase (A) or the developmental rate (B) of *Apanteles taragamae* and its host *Maruca vitrata* (mean +/- SE) as described by the Brière 1 model.

_____: *A. taragamae*
- - - - : *M. vitrata*

Sex allocation in many parasitoids species was found to be influenced by host age or size at oviposition (Arthur and Wylie, 1959; Jones, 1982; King, 1987). Female wasps were reported to be selective as to the sex of the offspring they deposit in the host (Dicke, 1999a). Male offspring are often deposited in small hosts while female offspring are oviposited in larger hosts (Jones, 1982; Dicke, 1999a). In our study, *A. taragamae* also exhibited this feature of host-size dependent sex allocation. Only male offspring emerged from one-day-old parasitized larvae (Table 1). However, female parasitoids may have oviposited eggs of both sexes in one-day-old larvae, whereas larvae of this age may not provide sufficient nutritional resources for female offspring to complete their development. Indeed, female offspring were reported to require more nutritional resources to complete their development compared to male offspring (Colinet et al., 2005). Thus, female offspring might suffer higher mortality so that only males emerged from the youngest larvae (Jones, 1982; Brodeur and Boivin, 2004). In contrast, in larger hosts, i.e. two- or three-day-old larvae in our study, both male and female offspring successfully developed as found by others (Jones, 1982; Ueno, 1999; Wang et al., 2008). When supplied with only large hosts, female wasps may control their offspring sex at oviposition by depositing unfertilized eggs that give rise to sons and fertilized eggs that give rise to daughters (Henter, 2004). However, not all parasitoid species exhibit a host-size dependent sex allocation (Donaldson and Walter, 1984). For instance, host size did not influence sex allocation in the parasitoid wasp *Spalangia*

endius Walker (Hymenoptera: Pteromalidae) (Donaldson and Walter, 1984; Napoleon and King, 1999).

Comparison of males' longevity between three larval ages revealed that male wasps emerging from one-day-old parasitized larvae lived longest. This observation could be explained by differences in mating status. Mating can affect male fitness in terms of longevity (Dewbury, 1982; Onagbola et al., 2007). In fact, males and females emerging from two- or three-day-old parasitized larvae were coupled to study female fecundity, while males that emerged from one-day-old parasitized larvae remained unmated. Similar results were reported for *Anagyrus kamali* Moursi by Sagarra et al. (2002).

The proportion of female wasps that emerged from larvae parasitized at the age of 2 days was higher than that obtained in the experiments on the influence of temperature (Tables 1 and 2). This difference may be due to the mating status of female wasps involved in the two experiments. In the experiment on suitability of larval age, female wasps were allowed to mate 48 h prior to being allowed to parasitize, while in the experiment on temperature effects, female wasps at the day of emergence were allowed to mate, and parasitize immediately after. In the last case, the first eggs laid by these females may not be fertilized giving then only male progeny which would increase the proportion of males.

The fact that no differences were obtained between the two larval ages (two- and three-day-old larvae) for the rate of parasitization and the lifetime fecundity supports the existence of a host size threshold (Jones, 1982; Brodeur and Boivin, 2004) for the normal development of *A. taragamae.*

Host size may also affect the size of the emerging wasp (Dicke, 1999a; Lacoume et al., 2006). Both male and female wasps are larger when they develop in larger hosts (Lacoume et al., 2006). Many studies show a relationship between parasitoid size and its fitness in terms of female reproductive capacity (lifetime fecundity) and longevity (Visser, 1994; Ueno, 1999; Lacoume et al., 2006; Thorne et al., 2006; Wang et al., 2008). However, contrary to these findings females or males of *A. taragamae* that emerged from larvae parasitized at the age of 2 or 3 days did not significantly differ in their fecundity or longevity (Table 1).

Host density

The percentage parasitism of *M. vitrata* larvae by *A. taragamae* increased with density, which is a good indicator of the performance of a biological control agent. A natural enemy that responds positively to an increase in pest population density is considered as a good candidate (Stiling, 1987; Luna et al., 2007). If the parasitization rate increases with host density, this may contribute to host population regulation and to the system stability (Walde and Murdoch, 1988). The underlying mechanisms of accelerating functional responses include decreased host handling time, increased host searching rate and egg availability (Stiling, 1987; Walde and Murdoch, 1988). Efficiency of host searching by a parasitoid depends on its ability to use infochemicals from its host and host plants (Vet and Dicke, 1992). In this process, learning may play a determinant role. Our observations demonstrate the ability of *A. taragamae* to respond positively to an increasing population *of M. vitrata.* However, this functional response was obtained with a simplified experimental arena and may be different in a more complex environment (i.e. field conditions). Even in the laboratory, the functional response of a parasitoid is affected by many factors such as size of experimental arena, tested densities, time duration of experiments, distribution of hosts, the possibility of searching parasitoids to leave the experimental units and number of parasitoids searching together (Islam et al., 2006). Moreover, the foraging behaviour under field conditions is determined by many other factors namely host patch accessibility or distribution, presence of competitors and temperature (Fernández-Arhex and Corley, 2003; Luna et al., 2007). In the present work, all female parasitoids escaped the experimental units through holes made for this purpose a few minutes after release, without parasitizing host larvae. Because subsequent experiments did not include this possibility, we would have expected high parasitism levels at lower density since the wasps had the possibility to revisit host larvae. Consequently, we would also have expected that the percentage parasitism would decrease with host density, but this was not the case, as the percentage parasitism increased with increasing host density. Such laboratory findings are useful for assessing the performance of *A. taragamae* as a biological control agent.

Temperature

Apanteles taragamae and its host *M. vitrata* successfully developed under a range of temperatures. Linear regressions indicated significant negative correlations between the temperature and most of the life history parameters measured on both insect species. However, the relationship between the temperature and the intrinsic rate of increase (r_m) or the developmental rate is generally sigmoidal (Campbell et al., 1974; Brière et al., 1999). This thermal pattern is characteristic of poikilotherm species like insects which are not able to regulate their body temperature in response to the increase in environment temperature (Howe, 1967; Ivanović et al., 1992). And above the upper threshold, temperature affects enzyme activity or nutrient metabolism (Langridge, 1963; Sharpe and DeMichele, 1977), leading to death and rapid decline of the insect population (Ivanovic et al., 1992).

The Brière 1 model estimates quite well the lower thermal threshold for the parasitoid development but not the upper one, when compared to the values reported by Adati et al. (2004) on *M. vitrata,* which ceased to develop below 10.4-11.7°C and above 31.9°C. It also fits well to estimate the lower thermal threshold but not the optimum temperature and the upper thermal threshold of *M. vitrata*.

Comparison between the intrinsic rate of natural increase of *A. taragamae* and that of its host *M. vitrata* revealed that the parasitoid reproduced significantly faster than did its host at all the tested temperatures with the exception of 30 °C. The maximum intrinsic rate of natural increase (0.24 female/female/day) was obtained for *A. taragamae* against 0.19 for the host at 28°C. The highest r_m obtained for the moth is closer to that (0.18 female/female/day) found by Chi et al. (2005) at 27 °C. In Benin, the mean temperature over the last 30 years did not exceed 28 °C (Emert and Brücher, 2008). Minima average 22.6 °C in South Benin and 20.8 °C in the Northern part of Benin, while maxima averaging 32.5 °C in the South, reach 34.3 °C in the North during the long dry season. If we assume that the mean temperature is around 30 °C during the dry season which lasts three months, the wasp would have 8 generations against 4 generations for its host during this season. The wasp would accomplish 25 generations against 12 for its host during the remaining 9 months, assuming the mean temperature to be 28 °C. And the overall annual increase in the parasitoid population would be 75 females per female against 72 females per female for the host. These findings suggest

that *A. taragamae* is likely to exert effective biological control of *M. vitrata,* once released and established in Benin agro-ecosystems.

The present study provides basic information on the influence of larval host age, density and temperature on some biological parameters of *A. taragamae* exploiting its host *M. vitrata,* and is an essential step in the development of biological control approaches (Lane et al., 1999; van Lenteren et al., 2003) against this crambid pest.

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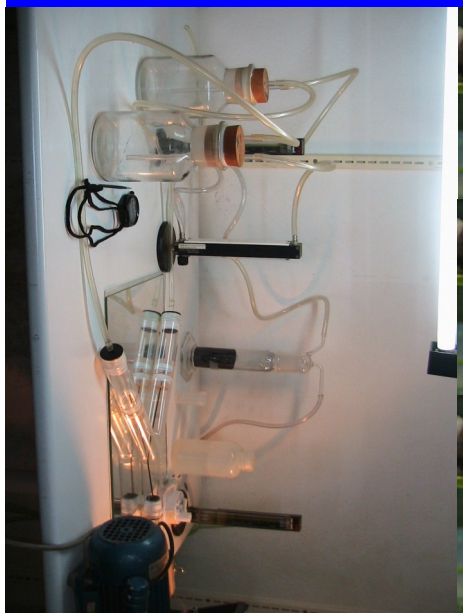
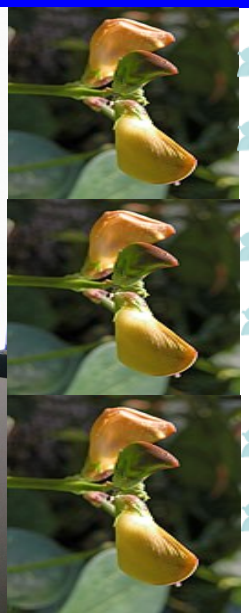
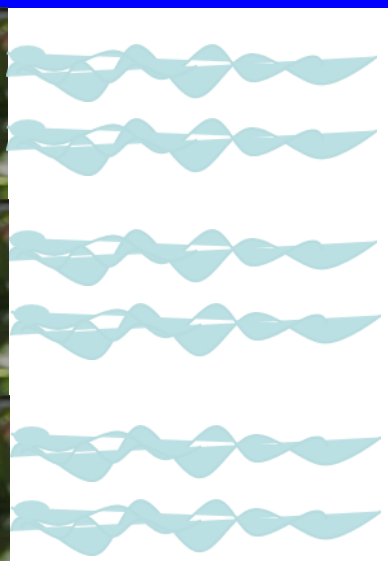
Effects of volatiles from *Maruca vitrata* larvae and caterpillar-infested flowers of their host plant *Vigna unguiculata* on the foraging behaviour of the parasitoid *Apanteles taragamae*







Effects of volatiles from *Maruca vitrata* larvae and caterpillar-infested flowers of their host plant *Vigna unguiculata* on the foraging behaviour of the parasitoid *Apanteles taragamae*

03

Elie Ayitondji Dannon, Manuele Tamò, Arnold van Huis, Marcel Dicke

Abstract

The parasitoid wasp *Apanteles taragamae* is a promising candidate introduced in Benin for the biological control of the legume pod borer *Maruca vitrata.* The effects of volatiles from cowpea and peabush flowers and *Maruca vitrata* larvae on the host selection behaviour of the parasitoid *Apanteles taragamae* were investigated under laboratory conditions using a Y-tube olfactometer. Naïve and oviposition-experienced female wasps were given a choice between several odour sources including (1) uninfested, (2) *Maruca vitrata*-infested, and (3) mechanically damaged cowpea flowers, as well as (4) stem portions of peabush plants carrying leaves and flowers, (5) healthy *M. vitrata* larvae and moribund (6) and live (7) virus-infected *M. vitrata* larvae. Responses of naïve and oviposition-experienced female wasps did not differ for all odour sources combinations. The wasps were significantly attracted to floral volatiles produced by cowpea flowers that had been infested with *M. vitrata* larvae and from which the larvae had been removed. Females of *A. taragamae* were also attracted to infested flowers after removal of both the larvae and their feces. The female wasps discriminated between volatiles from previously infested flowers and mechanically damaged flowers. Uninfested cowpea flowers attracted only oviposition experienced wasps that had a rewarding experience (that parasitized two *M. vitrata* larvae feeding on cowpea flowers) before the olfactometer test. The wasps were also attracted to uninfested leaves and flowers of peabush. Moreover, they were also attracted to healthy and live virus-infected *M. vitrata* larvae, but not when the latter were moribund. Our data show that similarly to what has been extensively been reported for foliar volatiles, also flowers of plants emit parasitoid-attracting volatiles in response to being infested with an herbivore.

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1 Introduction

The impact of insect parasitoids on host populations depends on abiotic and biotic factors. Host searching capacity is considered to be an important component of parasitoid biology, often influencing the success of inoculative biological control (Gilstrap, 1997; Wiedemann and Smith, 1997; Van Lenteren and Manzaroli, 1999; Neuenschwander, 2001; Vet, 2001; Gross et al., 2005). Effective location of hosts is especially important at low host densities. Foraging parasitoids have to deal with the so-called detectability-reliability problem (Vet and Dicke, 1992). Stimuli provided by herbivorous insects (hosts) are reliable indicators of their presence to parasitoids, but the detectability of these cues is low. On the other hand, stimuli emitted by the food plants of their herbivorous hosts are more detectable because they are emitted in larger amounts, but do not necessarily indicate the presence of the herbivores (Vet and Dicke, 1992). In order to cope with this problem, parasitoids may combine stimuli from both their hosts and host food plants (Turlings et al., 1991a, 1993; Vet and Dicke, 1992; Dicke, 1999ab; Fatouros et al., 2005; Tamò et al., 2006). Natural enemies, such as insect parasitoids, use infochemicals from host plants to locate the habitat of their hosts in order to find them (Vinson, 1976; Dicke and Sabelis, 1988; Turlings et al., 1990; Vet and Dicke, 1992; Dicke, 1999b; Mumm and Hilker, 2006; Schnee et al., 2006; Heil, 2008). Natural enemies are able to discriminate between blends of volatiles produced by mechanically damaged and herbivore-damaged plants (Eller et al., 1988; Turlings et al., 1991b; Vet and Dicke, 1992; Dicke, 1999a). In addition to herbivore-induced plant volatiles, several other factors may also affect host searching behaviour of a parasitoid, such as visual and vibrational stimuli from the host or host plant, the presence of competitors or natural enemies, previous oviposition experiences (learning) and physiological state of the parasitoid (Lawrence, 1981; Wardle, 1990; Wardle and Borden, 1990; Wäckers and Lewis, 1994; Casas et al., 1998; Dicke, 1999b; Dicke and Grostal, 2001).

The success of biological control agents in many projects has been attributed in particular to their high host searching efficiency (Neuenschwander and Ajuonu, 1995; Ngi-Song and Overholt, 1997; de Moraes et al., 1999; Neuenschwander, 2001). For instance, the superiority of *Apoanagyrus (Epidinocarsis) lopezi* De Santis over *Apoanagyrus diversicornis* Howard (which was more fecund than *A. lopezi*), was at-

tributed to its higher capacity to locate and parasitize young host instars at low densities (Neuenschwander, 2001). Similarly, *Cardiochiles nigriceps* Viereck displayed a higher host searching capacity and consequently detected and parasitized more larvae of *Heliothis virescens* (Fabricius) compared to *Microplitis croceipes* Cresson (de Moraes et al., 1999).

Apanteles taragamae Viereck, the parasitoid in use in the present study, is a solitary larval endoparasitoid of the legume pod borer *Maruca vitrata* Fabricius (Lepidoptera: Crambidae). It parasitized on average 63 % of *M. vitrata* larvae on *Sesbania cannabina* (Retz) Pers. (Huang et al., 2003). The wasp can also transmit the multi-nucleopolyhedrovirus *Mavi*MNPV to larvae of *M. vitrata* (M. Tamò, personal communication).

Maruca vitrata is one of the key insect pests of cowpea, causing up to 80% of yield loss (Nampala et al., 2002). Damage by *M. vitrata* to grain legumes is made by its larvae (Taylor, 1978). Larvae feed on flower buds, flowers and pods of cowpea (Taylor, 1978; Sharma, 1998). Infestation of flowers was found to be higher than that of flower buds and pods (Sharma, 1998). Larvae of this crambid were also reported to damage leaves of some wild leguminous plants such as the peabush, *Sesbania cannabina* (Retz) Pers. (Huang et al., 2003).

An attempt to exert biological control of this insect pest is made through the importation of *A. taragamae* from Taiwan to Benin by the International Institute of Tropical Agriculture (IITA). The potential of the wasp as biological control agent is being evaluated. In this study, we assessed the role of volatiles produced by *M. vitrata* larvae and two host plants, cowpea and peabush, in the host selection process by the parasitoid wasp *A. taragamae* using a Y-tube olfactometer. Cowpea is the main cultivated host plant of *M. vitrata* in Benin whereas peabush is the host plant on which the parasitoid was collected in Taiwan and has therefore share the same life history with the wasp.

2 Materials and Methods

2.1 Plant Materials

Seeds of the local cowpea variety Kpodji-guêguê and of peabush were sown in potted soil. The pots were placed in a greenhouse at 28 ± 1°C, and 76 ± 6 % relative humidity (means ± SD). Plants were watered every three days during the whole experimental

period. The experiments started at the onset of flowering. Flowers or stem portions were collected from *V. unguiculata* or *S. cannabina* plants to prepare the different odour sources used in the olfactometer tests.

2.2 Insect Materials

2.1.1 *Mass Rearing of M. vitrata*

Pupae of *M. vitrata* were obtained from a stock culture at the field station of the International Institute of Tropical Agriculture (IITA) in Benin. They were placed in open Petri dishes that were incubated in wooden cages (44 x 45 x 58 cm) with sleeves, having sides of fine screen and a glass top, and kept at 27.0 ± 0.6 °C and 60.9 ± 4.6 % relative humidity (mean ± SD). Adults emerged inside the cages and were fed using cotton fibres moistened with 10% glucose solution. Four-day-old female moths were transferred in groups of 4 or 5 individuals to transparent cylindrical plastic cups (3 cm diameter x 3.5 cm height) and kept for 24 h to allow for oviposition, which occurred on the inner surface of the cups. Ovipositing females were fed using small pieces of filter paper moistened with 10% glucose solution, which were replaced every 24 h. Cups carrying eggs were kept at the same experimental conditions till the larvae hatched. Larvae were transferred to large cylindrical plastic containers (9 cm diameter x 12 cm height) provided with artificial diet prepared according to Jackai and Raulston (1988), and develop through five instars until pupation. The artificial diet contains 4 l water; 59.2 g Agar-agar; 400 g cowpea grain flour; 127.2 g wheat or maize germ flour; 60 g Wesson salt; 44.4 g sorbic acid; 6.3 g Methyl p-hydroxy-benzoate; 25 g Ascorbic acid; 50 ml Acetic acid; 6 ml Formaldehyde; 11 g Aureomicin; 22 g Potassium hydroxide; 29.6ml Choline chloride and 30ml vitamin B mixture (Jackai and Raulston, 1988). Pupae were collected and placed in cages. All larvae used in the experiments were obtained from the mass production.

2.2.2 *Mass Rearing of A. taragamae*

Cocoons of *A. taragamae* were obtained from the stock culture at the IITA station in Benin that originated from parasitoids collected on *S. cannabina* infested by *M. vitrata* at the World Vegetable Center (AVRDC) in Taiwan. Emerged adults were kept in plastic cylindrical cups (4.5 cm diameter x 5 cm height). A hole (2 cm diameter) punched in the

lid of the cups was covered with fine mesh. Adults of *A. taragamae* were fed with honey streaked on the fine mesh of the lid. To allow mated female wasps to parasitize hosts, they were offered, during 24 h, two-day-old larvae of *M. vitrata* in a small cylindrical cup (3 cm diameter x 3.5 cm height) containing a piece of artificial diet. The exposed larvae were reared till cocoon stage. Cocoons were collected and placed in cylindrical cups (4.5 cm diameter x 5 cm height). The mass production of wasps took place in a climate chamber with a temperature of 25.3 ± 0.5 °C (mean ± SD) and a relative humidity of 78.9 ± 5.6 % (mean ± SD). The female wasps used for the different choice tests were obtained from this mass rearing.

2.2.3 *Maruca vitrata Multi-Nucleopolyhedrovirus (MaviMNPV)*

Maruca vitrata multi-nucleopolyhedrovirus *(Mavi*MNPV) is a baculovirus isolated from infected larvae of *M. vitrata* on peabush in Taiwan (Lee et al., 2007; Chen et al., 2008). Infected larvae were sluggish, pinkish and ceased feeding 3 to 4 days after virus exposure. When dead, the larvae were found hanging from the top of the plant with the prolegs attached to the host plant. The virus attacks all larval stages with a high susceptibility in early instars (first and second stages). *Mavi*MNPV could potentially be used as a component in an Integrated Pest Management Programme against *M. vitrata* (Lee et al., 2007). It has been introduced to the IITA-Benin laboratory from AVRDC for experimental purposes.

2.2.4 *Oviposition-experienced Female Wasps*

Emerged adult female parasitoids were kept together with males for 48 h in cylindrical plastic cups (4.5 cm diameter x 5 cm height) to allow mating. They were fed with honey. Mated females gained oviposition experience by parasitizing 2 two-day-old *M. vitrata* larvae in cylindrical plastic cups (3 cm diameter x 3.5 cm height), 30 min prior to the olfactometer tests. The host larvae had been reared on artificial diet. These oviposition-experienced parasitoid females had not received contact with the odour sources used in the present study.

2.2.5 *Odour-experienced Female Wasps*

Two-day-old mated female parasitoids were kept together with uninfested cowpea flowers for 24 h in cylindrical plastic containers (9 cm diameter x 12 cm height) where they

were fed with honey streaked on the mesh cover of containers. Thirty minutes prior to the olfactometer test, they were allowed to parasitize 2 two-day-old larvae feeding on cowpea flowers. Odour-experienced females were only used to test for their response to the volatiles from uninfested cowpea flowers against clean air.

2.3 Dynamic Olfactometer Set-up

The response of *A. taragamae* females to volatiles produced by cowpea, peabush flowers and host larvae was investigated using a glass Y-tube olfactometer similar to that used by Gnanvossou et al. (2003). The clean airflow was divided into two and each subflow passed through one of the two odour sources connected to the arms of the glass Y-tube olfactometer. The windspeed in the olfactometer was controlled at 4 l/min.

2.4 Bioassay Procedure

Naïve mated females of *A. taragamae* (without oviposition experience) and oviposition-experienced wasps were alternatingly introduced individually at the entry of the Y-shaped glass tube. Their movement was observed for maximally 10 min. A test began when the wasp started to move. Female wasps remaining motionless for more than 5 min at the release point were discarded from the analysis. The parasitoid wasps that did not reach the end of the olfactometer arm were considered as non-responding wasps. After testing 2 naïve and 2 oviposition-experienced female parasitoids, the positions of the odour sources were exchanged to correct for any unforeseen asymmetry in the experimental set-up. Odour sources were renewed after testing 8 naïve and 8 experienced female wasps. A total of 16-20 naïve and oviposition-experienced female wasps were tested daily and 60-70 naïve and oviposition-experienced females were tested in total for each choice situation. All female wasps used in this study were three-day-old.

2.5 Bioassays on the Response of Naïve and Oviposition-experienced Females Wasps to Volatiles Produced by Cowpea Flowers

The influence of volatiles produced by cowpea flowers on the host selection behaviour of both naïve and oviposition-experienced females of *A. taragamae* was investigated by testing the following odour combinations:

a. Four uninfested flowers versus clean air

b. Four caterpillar-infested flowers from which larvae were removed prior to the experiment versus clean air

c. Four caterpillar-infested flowers from which larvae and their feces were removed prior to the experiment versus clean air

d. Four mechanically damaged flowers versus clean air

e. Four uninfested versus four caterpillar-infested flowers from which larvae were removed prior to the experiment

f. Four uninfested versus four mechanically damaged flowers

g. Four caterpillar-infested flowers from which larvae and feces were removed prior to the experiment versus four uninfested flowers

h. Four caterpillar-infested flowers from which larvae were removed prior to the experiment versus four mechanically damaged flowers

Infested cowpea flowers consisted of racemes carrying 4 flowers, infested with 10 one-day-old larvae of *M. vitrata* for 24 h. Before the infestation, racemes were placed in water-filled cylindrical plastic vials (4.5 cm diameter x 11.5 cm height) sealed using parafilm to keep racemes fresh and hydrated. Larvae were removed 15 min prior to using the flowers in an olfactometer experiment. Flowers were mechanically damaged by making three scratched lines onto flowers using a clean needle, 15 min prior to the experiment.

2.6 Bioassays Assessing the Influence of Previous Contact with Cowpea and Peabush Plants on the Host Searching Behaviour of *A. taragamae*

The influence of an odour experience on the behavioural response to uninfested flower was studied by using odour experienced female wasps for the odour combination (a) (clean air versus uninfested cowpea flowers).

As the wasp strain in use has been originally collected on peabush plants, the effect of leaves and flowers of this plant on the host searching behaviour of the wasp has been evaluated using both naïve and oviposition-experienced females. The *M. vitrata* larvae cause damage to peabush by destroying mostly leaves (Huang et al., 2003). However, flowers of this leguminous shrub may also be damaged. Both naïve and oviposition-experienced female wasps were used to test the odour combination:

i. Uninfested stem portions carrying four leaves and four flowers of *S. cannabina*

versus clean air.

2.7 Bioassays on the Effect of Volatiles from Host Larvae on the Host Searching Behaviour of *A. taragamae*

The effect of volatiles produced by *M. vitrata* larvae on the host selection behaviour of *A. taragamae* was assessed using healthy, moribund and live *Mavi*MNPV-infected larvae. Virus-infection had occurred during the mass rearing of *A. taragamae*. Our objective was to assess whether the wasps, being capable of transmitting the baculovirus, avoid infected larvae. Both naïve and oviposition-experienced female wasps were used to test the following odour combinations:

j. 10 healthy larvae versus clean air

k.10 moribund *Mavi*MNPV-infected larvae versus clean air

l. 10 healthy larvae versus 10 moribund *Mavi*MNPV-infected larvae

m. 10 *Mavi*MNPV-infected larvae versus clean air

n. 10 *Mavi*MNPV-infected larvae versus 10 healthy larvae

Live *Mavi*MNPV-infected larvae were obtained by feeding larvae with virus-infected artificial diet for 2 days. For this, pieces of artificial diet were placed in a viral suspension of $2x10^4$ OB/ml (Occluded Bodies). Moribund larvae were larvae that naturally occurred in the mass rearing of *M. vitrata* and that had viral infection symptoms. The number of parasitoids that chose each odour source as first and final choice was recorded.

3 Statistical Analysis

Analysis of data on the number of parasitoids per odour source was performed using binomial tests with the null hypothesis that the distribution of the wasps over the two arms of the olfactometer was 50:50. Differences between naïve and experienced females wasps were tested with a 2 x 2 contingency table analysis based on Chi-square. Non-responding wasps were recorded but not included in the statistical analysis.

4 Results

4.1 Influence of Volatiles from Cowpea Flowers on the Host Searching Behaviour of *A. taragamae*

In all experiments, the responses of the naïve and the oviposition-experienced wasps were not significantly different (contingency table tests, $P > 0.05$). Therefore, we have combined the data for naïve and oviposition-experienced wasps.

Females of *A. taragamae* were significantly attracted to volatiles from *M. vitrata*-infested cowpea flowers from which larvae had been removed prior to the experiment, when tested against clean air or uninfested flowers (Figure 1). Similar results were obtained when the wasps were offered infested flowers from which larvae and feces had been removed prior to the experiment, against clean air or uninfested flowers. The parasitoids did not discriminate between volatiles from uninfested flowers and clean air, between volatiles from mechanically damaged flowers and clean air and between volatiles from mechanically damaged and uninfested flowers (Figure 1). Female wasps did also discriminate between volatiles from *M. vitrata*-infested flowers and mechanically damaged flowers (Figure 1).

4.2 Response to Volatiles Produced by Uninfested Peabush and Cowpea Flowers

Volatiles from stem portions carrying uninfested leaves and flowers of the peabush *S. cannabina* attracted *A. taragamae* females when tested against clean air (Figure 2). When wasps were given an odour experience, uninfested cowpea flowers were preferred over clean air (Figure 3), while without the odour experience there was no effect of volatiles from uninfested flowers on parasitoid attraction (Figure 1).

4.3 Response to Volatiles from Host Larvae

Females of *A. taragamae* displayed a preference for volatiles produced by healthy *M. vitrata* larvae when tested against clean air (Figure 4). The wasps did not show any preference for volatiles from moribund *Mavi*MNPV-infected larvae over clean air and preferred volatiles emitted by healthy larvae over volatiles from moribund *Mavi*MNPV-infected larvae. The parasitoid females did discriminate volatiles from live *Mavi*MNPV-

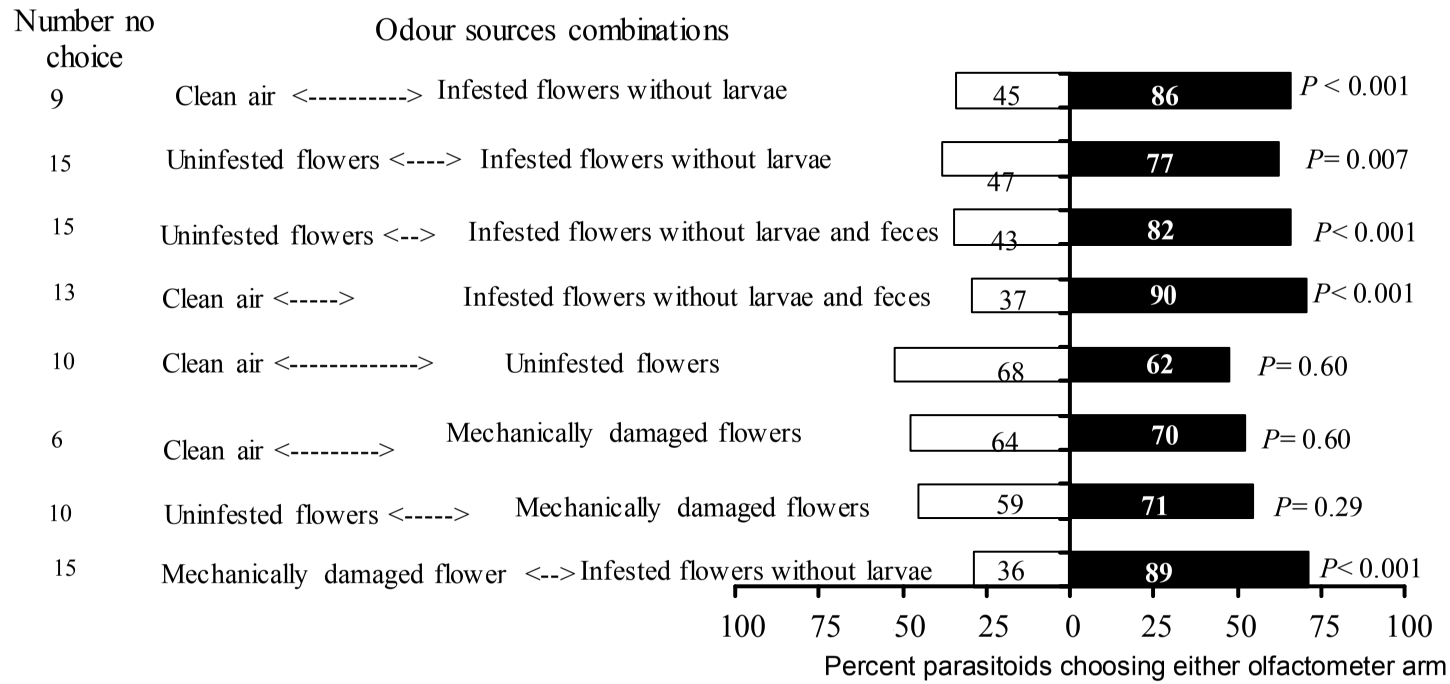

Figure 1: Behavioural responses of *Apanteles taragamae f*emales offered choices between volatiles from cowpea flowers, that were either uninfested, mechanically damaged, or infested with *Maruca vitrata*, and clean air in a Y-tube olfactometer.

Numbers in bars represent the total number of parasitoids that chose the olfactometer arm

P-values given to the right of the bars are for the two-tailed binomial test.

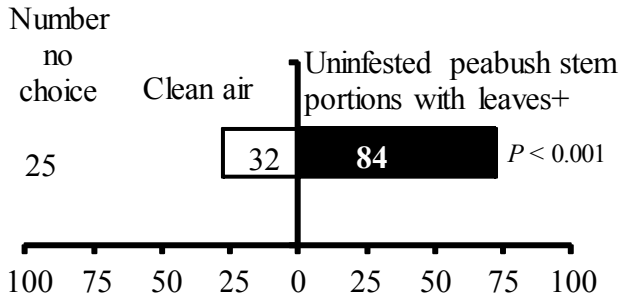

Figure 2: Behavioural response of *Apanteles taragamae* females to uninfested leaves and flowers of peabush in a Y-tube olfactometer.

Numbers in bars represent the total number of parasitoids that chose olfactometer arm

P-values given to the right of bars are for the two-tailed binomial test.

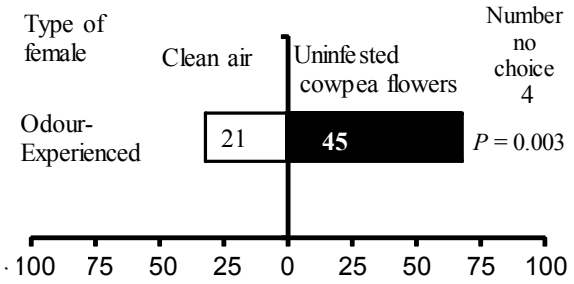

Figure 3: Behavioural response of odour-experienced females of *Apanteles taragamae* to uninfested cowpea flowers in a Y-tube olfactometer.

Numbers in bars represent the total number of parasitoids that chose olfactometer arm

P-values given to the right of bars are for the two-tailed binomial test.

*: Odour-experienced female wasps are females that that had parasitized 2 two-days-old larvae reared in the presence of host-infested cowpea flowers, 30 min prior to olfactometer test

infected larvae against clean air (Figure 4) and no preference was displayed when they were given a choice between volatiles from healthy larvae and live *Mavi*MNPV-infected larvae.

5 Discussion

Results from this study show the importance of *M. vitrata*-induced floral volatiles produced by cowpea in the host selection process of *A. taragamae*. Female wasps were attracted by volatiles emitted by *M. vitrata*-infested cowpea flowers from which the larvae had been removed. The parasitoids were not attracted to uninfested cowpea flowers, but this changed when they had received an odour experience. Indeed, long-range volatiles produced by undamaged or herbivore-damaged plants are known to attract natural enemies of herbivorous insects, increasing their efficiency in locating their hosts' habitat (Dicke and Sabelis, 1988; Turlings et al., 1990; Ngi-Song et al., 1996; Vet and Dicke, 1992; Du et al., 1998; Dicke, 1999b; Shimoda et al., 2005; Moayeri et al., 2007; Dicke and Baldwin, 2010). In most cases, studies addressed the volatiles that were produced by leaves. In the present study, however, the volatiles were emitted by the previously infested flowers. Floral volatiles are primarily known as attractants for pollinators (Jervis et al., 1993; Pichershy and Gershenzon, 2002). However, herbivorous insects that feed or oviposit on flowers have been reported to rely on cues from flowers to locate their hosts (Ekesi et al., 1998; Jönsson et al., 2005; Andrews et al., 2007) and parasitoids may also use floral volatiles to locate a food source (nectar) (Wäckers, 2004). Yet, to our knowledge only one other study has shown that herbivore-damaged flowers emit volatiles that attract a parasitoid enemy of florivorous herbivores (Jönsson and Anderson, 2008). It will be interesting to investigate how volatiles from herbivore-infested flowers affect the behaviour of pollinators. After all, herbivore-infested flowers are likely to be an inferior food source to pollinators.

The attraction of *A. taragamae* to uninfested peabush leaves and flowers may be a reflection of a genetic trait related to searching in peabush fields. Indeed, the current wasp species was imported from Taiwan, where it was collected from *M. vitrata* larvae feeding on peabush leaves. Volatiles from uninfested plants have been reported to be long-range attractants in some other parasitoid species (Elzen et al., 1983; Ding et al.,

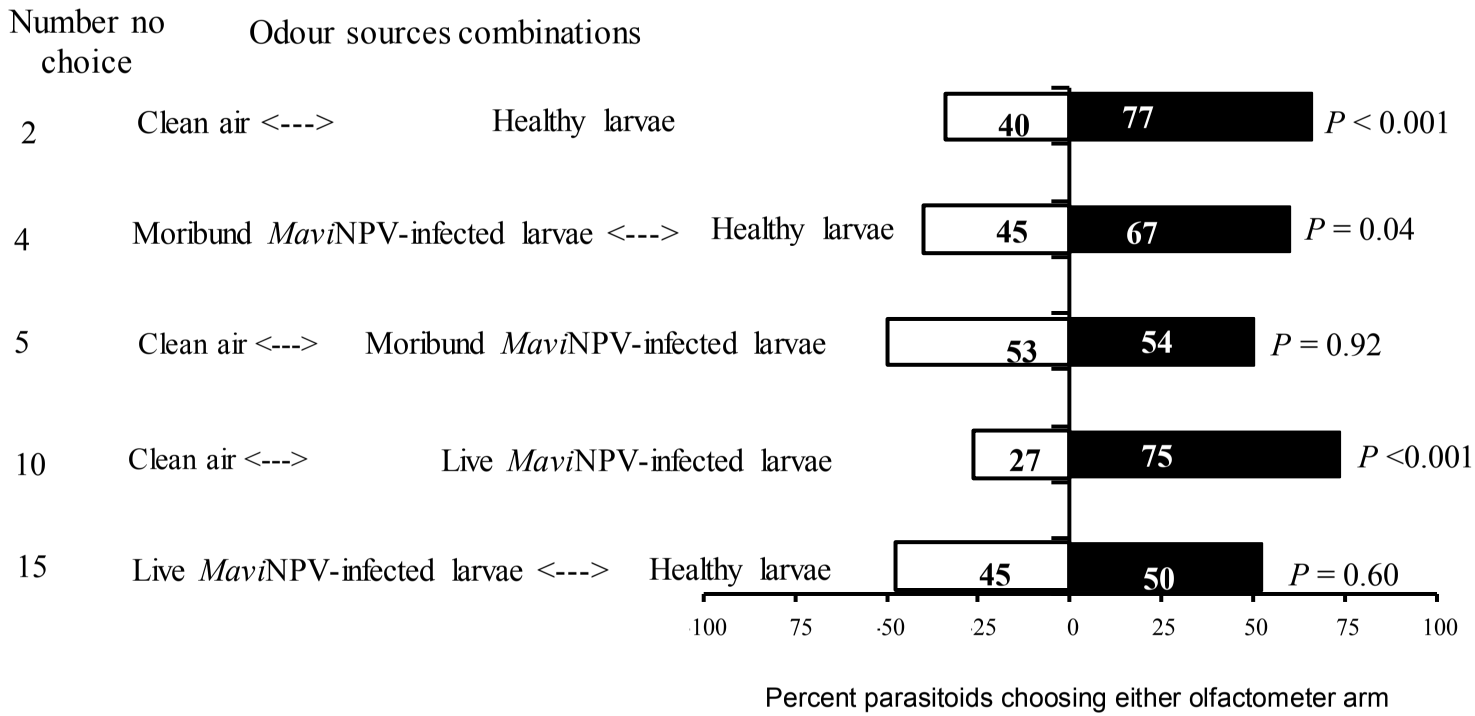

Figure 4: Behavioural responses of *Apanteles taragamae* females offered choices between volatiles from healthy *Maruca vitrata* larvae, moribund or live *Mavi*MNPV-infected larvae, and clean air in a Y-tube olfactometer.

Numbers in bars represent the total number of parasitoids that chose olfactometer arm

P-values given to the right of bars are for the two-tailed binomial test.

1989; Ngi-Song et al., 1996). Thus, *Cotesia flavipes* Cameron, originally collected in maize showed a preference for uninfested maize plants over sorghum in a dual choice olfactometer experiment (Ngi-Song et al., 1996). Likewise, the endogenous parasitoid *Cotesia sesamiae* (Cameron) preferred volatiles produced by uninfested sorghum plants over those from maize. Indeed, both sorghum and *C. sesamiae* originate from Africa and share the same environment. The braconid *Macrocentrus grandii* Goidanish, a larval parasitoid of the European corn borer, *Ostrinia nubilalis* (Hubner), was attracted to uninfested maize plants (Ding et al., 1989). However, herbivore-infested plants were found to be more attractive than uninfested plants (Turlings et al., 1991a; Du et al., 1998; Vet et al., 1998; Dicke, 1999b).

In the host habitat, short-range stimuli from the host itself are reliable indicators of its presence, but they are usually not well detectable (Vet and Dicke, 1992). Our study shows that the parasitoid wasp *A. taragamae* was significantly attracted to volatiles emitted by *M. vitrata* larvae. An oviposition experience through the parasitization of larvae fed with artificial diet did not affect the parasitoid's response to host larval volatiles. The use of kairomones by parasitoids for host location has been reported in many parasitoid species (Afsheen et al., 2008). The braconid parasitoid *M. croceipes* was attracted to odours of *H. virescens* (Fabricius) larvae (Elzen et al., 1987; Röse et al., 1997). Similarly, the bruchid larval parasitoid *Eupelmus vuilleti* (Crw) was reported to respond to volatiles from *Bruchidius atrolineatus* (Pic) larvae (Cortesero and Monge, 1994). However, usually herbivore-induced plant volatiles are more attractive to parasitoids than herbivore -produced volatiles (Turlings et al., 1991a; Steinberg et al., 1992).

Herbivore-associated organisms such as microbes may also be a source of chemical information to parasitoids during host location (Vet and Dicke, 1992). *Apanteles taragamae* was found to be a vector for the transmission of *Mavi*MNPV to larvae of *M. vitrata* and could acquire and transmit the virus over several generations (M. Tamò, personal communication). In this study, females of *A. taragamae* were attracted to *Mavi*MNPV-infected live larvae, but not to moribund larvae (Figure 4). The wasps did not discriminate between volatiles from healthy and *Mavi*MNPV-infected live larvae but they preferred the volatiles from healthy larvae over those from moribund larvae. Apparently, the viral infection only affected larvae attractiveness in a late stage of infection. The virus disease symptoms appear about 3 to 4 days after infection of the *M. vitrata* larvae (Lee

et al., 2007). Our observations are similar to those reported for the parasitoid *Biosteres longicaudatus* Ashmead, which was unable to locate immobilized or dead hosts (Lawrence, 1981). *Mavi*MNPV, like other baculoviruses, which are host-destroying viruses, is likely to negatively affect the development of *A. taragamae* and needs more attention for its management. Parasitoids and insect pathogens are often involved in scramble competition for host resources in dually infected and parasitized hosts. In such cases, some parasitoid species develop a strategy to enhance their developmental rate (Escribano et al., 2000). The temperature seemed to influence *Mavi*MNPV symptoms development in parasitized larvae. At 29° C, *A. taragamae* pupated on average 6 days after parasitization and this limited the detrimental effect of the virus infection (Dannon et al. unpublished data). Interactions between the wasp and *Mavi*MNPV need to be investigated further to evaluate the effects of the virus on the parasitoid and to identify factors that avoid or limit the detrimental effect on the wasp reproduction.

In conclusion, this study shows that floral volatiles produced by *M. vitrata*-infested cowpeas flowers attracted *A. taragamae* females. Uninfested leaves and flowers of pea-bush also attracted the parasitoid. In contrast, the wasp was attracted to uninfested cowpea flower only after an odour experience. Olfactory cues from *M. vitrata* larvae were also used by the wasp in its host selection process. Further research should assess the influence of other key host plants of *M. vitrata* such as *Pterocarpus santalinoides* L'herit ex DC.*, Lonchocarpus sericeus* (Poir) H.B.K. on the host selection behaviour of *A. taragamae.*

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Effect of *Maruca virtata* (Lepidoptera: Crambidae) host plants on the life history parameters of the parasitoid *Apanteles taragamae* (Hymenoptera: Braconidae)

Effect of *Maruca vitrata* (Lepidoptera: Crambidae) host plants on life-history parameters of the parasitoid *Apanteles taragamae* (Hymenoptera: Braconidae)

04

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Abstract

The effect of four host plant species of the herbivore *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) on development time, longevity, fecundity and sex ratio of the parasitoid *Apanteles taragamae* Viereck (Hymenoptera: Braconidae) was investigated under laboratory conditions. The larvae were parasitized when in the second instar. *Maruca vitrata* larvae were fed with flowers of four legumes, i.e. *Vigna unguiculata (*cowpea*), Sesbania rostrata, Lonchocarpus sericeus* and *Pterocarpus santalinoides,* or an artificial diet both before and after parasitization. Experiments were carried out at 25.5 ± 0.3 °C and 28.7 ± 0.6 °C. The parasitoid did not develop in hosts feeding on *L. sericeus, V. unguiculata* at 25 °C, on *P. santalinoides* at 25 or 29 °C. *Apanteles taragamae* had the shortest development time on artificial diet at both 25 and 29 ^{o}C while the longest development time was on *L. sericeus* at 29 ^{o}C. Female wasps took longer to develop compared to males at the two temperatures, regardless of the feeding substrate of their host. The longevity of the wasps at 25 °C varied among feeding substrates, but not at 29 °C. Survival rate of parasitized larvae depends on the feeding substrate. Moreover, infection of host larvae with *Maruca vitrata* multi-nucleopolyhedrovirus (*Mavi*MNPV) killed larger proportions of wasps at 25 than at 29 ° C, which was likely caused by the difference in parasitoid developmental rate. The proportion of female parasitoids was the lowest on *L. sericeus.* The daily fecundity showed a nonlinear trend regardless of the feeding substrate, indicating that *A. taragamae* is a pro-ovigenic species. The data support the slow growth-high mortality hypothesis.

1 Introduction

Maruca vitrata Fabricius (Lepidoptera: Crambidae) is one of the most ravaging insect pests of cowpea, *Vigna unguiculata* (L.) Walp. (Taylor, 1978; Sharma, 1998). Damage by *M. vitrata* to cowpea is made by its larvae feeding on flower buds, flowers and pods. The crambid develops without diapause and relies on alternate host plants to maintain its population during the cowpea off-season (Taylor, 1978; Bottenberg et al., 1997; Atachi et al., 2002; Arodokoun et al., 2003). Over 50 alternative host plant species have been recorded for *M. vitrata* (Taylor, 1978; Sharma, 1998; Arodokoun et al., 2003). Of these, *Pterocarpus santalinoides*, *Pueraria phaseoloides* and *Centrosema pubescens* play an important role during the long dry season, *Lonchocarpus sericeus* and *L. cyanescens* during the main rainy season and *Tephrosia platycarpa* during the short rainy season (Atachi et al., 2002; Arodokoun et al., 2003). Thus, these host plants constitute a source of *M. vitrata* carry-over. Therefore, an efficient method to control *M. vitrata* should target not only the main cultivated host plant (cowpea) but also the alternative one. With regard to this, biological control should be considered. Several parasitoid species have been recorded to attack *M. vitrata* larvae (Taylor, 1967; Okeyo-Owuor et al., 1991; Tamò et al., 1997; Huang et al., 2003; Arodokoun et al., 2006). Among these, *Apanteles taragamae* Viereck (Hymenoptera, Braconidae) seems to be a promising candidate for classical biological control in Africa (Srinivasan et al., 2009).

Apanteles taragamae is a solitary larval endoparasitoid of the legume pod borer *M. vitrata*. It parasitizes on average 63 % of *M. vitrata* larvae on *Sesbania cannabina* (Retz) (Huang et al., 2003). This parasitoid has been introduced from the World Vegetable Center (AVRDC) in Taiwan to the Benin research station of the International Institute of Tropical Agriculture (IITA) following standard importation procedures for evaluating its potential as a biological control candidate (FAO, 1997).

The development of koinobiont parasitoids such as *A. taragamae* depends on the host quality (Krusse and Raffa, 1999; Dicke, 1999a; Eben et al., 2000; Lill et al., 2002, Uçkan and Ergin, 2002; Harvey, 2005; Gols and Harvey, 2009). These parasitoids develop in hosts that continue to feed and grow (Brodeur and Boivin, 2004). The nutritional quality of a host plant directly affects the biology of herbivorous insects and can influence that of their natural enemies and subsequent trophic levels (Benrey et al.,

1998; Uçkan and Ergin, 2002; Bukovinszky et al*.,* 2008; Gols and Harvey, 2009). The interactions between variation in host plant quality and risk of attack by natural enemies of herbivorous insects have been formalized into the slow-growth, high-mortality hypothesis (Clancy and Price, 1987). Herbivores feeding on plants of low nutritional quality do not necessarily increase damage if their development time is prolonged (slow-growing), because they are longer vulnerable to natural enemy attack (Clancy and Price, 1987; Benrey and Denno, 1997). This hypothesis is yet to be verified for fast-growing herbivorous insects which are reportedly vulnerable to parasitism (Clancy and Price, 1987; Loader and Damman, 1991; Williams, 1999). Moreover, the quality of a host plant can also affect parasitoid development via their herbivorous insect host (Gols and Harvey, 2009). For instance, pigeonpea plants which provide suboptimal nutritional quality (when compared with cowpea and chickpea) to *Callosobruchus maculatus* (Fabricius), led to slower development and higher mortality of *Uscana lariophaga* Steffan, an egg parasitoid of *C. maculatus* (van Huis and de Rooy, 1998).

In this study, we assessed the influence of flowers of four key host plants of *M. vitrata* on the development of *A. taragamae* at different temperatures.

2 Materials and Methods

2.1 Plant materials

Flowers of *S. rostrata, V. unguiculata*, *P. santalinoides,* and *L. sericeus* were used in this study. Flowers of *S. rostrata* and *V. unguiculata* were collected in fields at the International Institute of Tropical Agriculture in Benin, while flowers of the wild host plants *P. santalinoides,* and *L. sericeus* were sampled at Agongue and Sehoue, 70 km East and 90 km North of Cotonou, respectively.

2.2 Insect species

2.2.1 *Maruca vitrata*

Pupae of *M. vitrata* obtained from a stock culture (kept for 50 generations) in the laboratory at the IITA in Benin were placed in open Petri dishes. They were incubated in wooden cages (44 x 45 x 58 cm) with sleeves, having sides of fine mesh and a glass

top, at 27.0 ± 0.6 °C and 60.9 ± 4.6 % relative humidity. Emerging adults were fed using cotton fibres moistened with 10% glucose solution. Four-day-old female moths were transferred in groups of 4 or 5 individuals to transparent cylindrical plastic cups (3 cm diameter x 3.5 cm height) and kept for 24 h to allow for oviposition, which occurred on the inner surface of the cups. Ovipositing females were fed using small pieces of filter paper moistened with 10% glucose solution, which were replaced every 24 h. Cups carrying eggs were kept at the same experimental conditions until the larvae hatched. Larvae were transferred to large cylindrical plastic containers (9 cm diameter x 12 cm height) provided with artificial diet prepared according to Jackai and Raulston (1988), and reared until pupation. Pupae were collected and placed in cages until adult emergence. Eggs used in the experiments were obtained from this mass production.

2.2.2 *Apanteles taragamae*

Cocoons of the parasitoid *A. taragamae* obtained from the stock culture at IITA in Benin were kept in plastic cylindrical cups (4.5 cm diameter x 5 cm height) till adult emergence. A hole (2 cm diameter) punched in the lid of the cups was covered with fine mesh. Adults of *A. taragamae* were fed with honey streaked on the fine mesh of the lid. To allow mated female wasps to parasitize hosts in 24 h, they were offered, two-day-old larvae of *M. vitrata* in a small cylindrical cup (3 cm diameter x 3.5 cm height) containing a piece of the artificial diet. The exposed larvae were reared until cocoon stage. Cocoons were collected and placed in cylindrical cups (4.5 cm diameter x 5 cm height). The mass production of wasps took place in a climate chamber with a temperature of 25.3 ± 0.5 °C (mean ± SD) and a relative humidity of 78.9 ± 5.6 % (mean ± SD).

2.3 Influence of *M. vitrata* host plants on development time, longevity, sex ratio and fecundity of the parasitoid *A. taragamae*

Cups (3 cm diameter x 3.5 cm height) carrying eggs of *M. vitrata* were kept at 25 °C until the larvae hatched. Three flowers of *P. santalinoides, V. unguiculata, S. rostrata, L. sericeus,* or pieces of artificial diet were put in cups containing newly hatched larvae. Flowers of *S. rostrata, L. sericeus* and *P. santalinoides* were carried by a raceme while that of *V. unguiculata* were not. Larvae were submitted to parasitization by *A. taragamae* when the larvae were two days old. Parasitized larvae were

individually transferred to cups (3 cm diameter x 3.5 cm height) and reared at 25.3 ± 0.5 (25) °C with 78.9 ± 5.6 % relative humidity and 28.7 ± 0.6 (29) °C with 68.1 ± 5.1 % relative humidity using each of the different feeding substrates until they had developed into the cocoon stage. Flowers were daily replaced with new flowers. Cocoons were kept until adult emergence. The numbers of dead larvae, cocoons and emerged adults were recorded. Emerging females were coupled with males of the same age for each feeding substrate to allow mating. Twenty to thirty two-day-old larvae were exposed to each couple of wasps daily until the females died. These larvae were fed on artificial diet. The number of cocoons that developed successfully was recorded for each feeding substrate. In total 200-250 parasitized larvae were reared using each of the feeding substrates at 25 and 29 °C. Larval mortality was recorded daily. The number of larvae that were killed by *Maruca vitrata* Multi-Nucleopolyhedrovirus (*Mavi*MNPV) was recorded at both temperatures for each feeding substrate. The virus *Mavi*MNPV was reported to infect all larval stages of *M. vitrata* (Lee et al., 2007). Infected larvae were sluggish and pinkish and died within 3 to 4 days following the first contact with the virus. The parasitoid wasp *A. taragamae* was found to acquire and transmit *Mavi*MNPV to healthy *M. vitrata* larvae (Srinivasan et al., 2009).

In parallel, 200 parasitized and non-parasitized larvae were rearing on each feeding diet for 7 days at 25 and 29 °C. The number of dead larvae was daily recorded.

The life-table parameters of the wasps for each feeding substrate were calculated at 25 and 29 °C. They are defined according to Birch (1948):

The net reproductive rate (R_0), which is the number of female progeny per female per generation. It is given by the formula:

where:

x is the pivotal age of individuals in days

$\mathbf{l_x}$ is the age-specific survival as proportion of individuals still alive at age x

$\mathbf{m_x}$ is the age-specific fecundity as female offspring per female.

The intrinsic rate of increase (r_m), given by the formula:

$$1 = \sum l_x \, m_x \, e^{-r_m \, x}$$

where **e** is the base of natural logarithm (ln).

The mean generation time (T), calculated as follows:

$$T = \ln(R_0) / r_m \quad \text{and by approximation} \quad T = (\sum x \, l_x \, m_x) / \sum l_x \, m_x$$

The doubling time T_2 is therefore:

$$T_2 = \ln(2) / r_m$$

The finite rate of increase (λ**),** expressed as the multiplication per female per unit time:

$$\lambda = e^{r_m}$$

The intrinsic rate of increase, r_m, was calculated using the Jackknife technique (Maia et al., 2000). All these life-table parameters were computed using the SAS program developed by Maia et al. (2000).

3 Statistical analysis

The effects of the different feeding substrates on the development time, longevity and fecundity of *A. taragamae*, were compared using a t-test at 25 °C and the General Linear Model (GLM) procedure of SAS followed by the Turkey-test in the case of significant differences between substrates at 29 °C. The comparison between males and females for the longevity and life cycle was done with a paired t-test. Data on sex ratio were analyzed by using the χ^2 test. Thus, a 2 x 2 or 2 x 4 contingency table based on the chi-square was used to test differences between feeding substrates at 25 °C or 29 °C. Percentage larval survival rate (p) was arcsine √ (p/100) transformed prior to the analysis of variance followed by Tukey test in the case of significant differences between feeding substrates. Comparison between survival rate of parasitized and non-parasitized larvae for each feeding substrate was done using a t-test. The t-test was also used to compare differences between feeding substrates at 25 °C and GLM

procedure of SAS followed by Tukey-test in the case of significant differences between substrates at 29 °C for the intrinsic rate of increase, the net reproductive rate, the mean generation time, the doubling time and the finite rate of increase.

4 Results

4.1 Effect of four plant species on life history parameters of *A. taragamae*

The parasitoid *A. taragamae* was unable to develop in *M. vitrata* reared on flowers of *L. sericeus* and *V. unguiculata* at 25 °C, neither on flowers of *P. santalinoides* at 25 and 29 °C (Table 1). The development time of *A. taragamae* from egg to cocoon, from cocoon to adult, and the whole cycle (from egg to adult stage) were significantly influenced by feeding substrates (Table 1).

At 25 °C

Wasps took less time to develop from egg to cocoon and from cocoon to adult when parasitized hosts were reared on artificial diet compared to flowers of *S. rostrata* (t test, t = 3.6 for egg to cocoon, t = 6.1 for cocoon to adult; $P < 0.0001$; Table 1). The life cycle was reduced by 0.8 day for male and female wasps when host larvae were fed with artificial diet compared to flowers of *S. rostrata* (t = 4.3; $P < 0.0001$; Table 1). The male wasps' cycle was 0.7 day shorter than that of females regardless of these feeding substrates. Male wasps lived more than three days longer than females when larvae were fed with flowers of *S. rostrata* (t = 2.4; $P = 0.02$; Table 1), while on artificial diet there was no significant difference. The proportion of female offspring was significantly lower than that of males when the hosts fed on flowers of *S. rostrata.* The sex ratio was not different for wasps developing in hosts feeding on *S. rostrata* compared to artificial diet ($\chi^2 = 0.2$; $P = 0.6$; Table 1).

At 29 °C

The parasitoid successfully developed in *M. vitrata* larvae feeding on three of the four tested flower species. At this temperature, the longest development time (from egg to cocoon, or cocoon to adult, or egg to adult) was observed for wasps in hosts feeding on *L. sericeus* (Table 1). The full development cycle of males was reduced by 0.8 day when compared to that of females when the host was feeding on *S. rostrata,* by 1.1 days on *V. unguiculata,* and 0.6 day on artificial diet ($P < 0.05$; Table 1). In contrast to

Table 1: Development time, longevity, and sex ratio of the parasitoid *Apanteles taragamae* when reared in hosts (*M. vitrata*) feeding on flowers of *Sesbania rostrata, Vigna unguiculata, Lonchocarpus sericeus, Pterocarpus santalinoides* or artificial diet at 25 and 29 °C

Parameter	*S. rostrata*	*V. unguiculata*	*L. sericeus*	*P. santalinoides*	Artificial diet
Development time (days):					
- egg-cocoon					
.....- at 25 °C	7.3 ± 0.05(98) a	-	-	-	7.0 ± 0.03(100) b
- at 29 °C	5.1±0.03(80) a	5.4±0.06(74) b	5.8±0.07(68) c	-	5.1 ±0.03(74) a
- cocoon-adult					
.....- at 25 °C	5.2 ± 0.07(79)a	-	-	-	4.8 ± 0.09(74) b
- at 29 °C	5.2 ± 0.06(71)a	5.3 ± 0.05(71) a	5.6±0.18(16) b	-	4.8 ± 0.09(65) c
Development cycle (days):					
- male					
.....- at 25 °C	12.2 ± 0.08(28)aα	-	-	-	11.4 ± 0.1(27)bα
- at 29 °C	10.2±0.09(24)aα	10.2±0.09(12)aα	11.4±0.1(15)b	-	9.6 ± 0.1(25) cα
- female					
.....- at 25 °C	12.9 ± 0.09(28) aβ	-	-	-	12.1 ± 0.2(27)bβ
- at 29 °C	11.0 ± 0.3(24) aβ	11.3 ± 0.3(12)aβ	-	-	10.2 ± 0.2(25)bβ
Longevity (days):					
- male					
.....- at 25 °C	11.9 ± 1.3(28) aα	-	-	-	10.9 ± 1.5(27)bα
- at 29 °C	5.5 ± 0.9(24) aα	5.0 ± 0.6(12) aα	6.3±1.1(15) a	-	5.1 ± 0.8(25) aα
- female					
.....- at 25 °C	8.8 ± 0.9(28) aβ	-	-	-	9.9 ± 0.9(27) aα
- at 29 °C	6.5 ± 0.6(24) aα	4.3 ± 0.7(12) bα	-	-	5.1 ± 0.5(25) abα
Adult emergence rate (%)					
.....- at 25 °C	79.9 ± 2.5 a	-	-	-	73.8 ± 6.6 a
- at 29 °C	88.6 ± 3.9 b	83.3 ± 4.8 b	23.5 ± 2.7 a	-	85.6 ± 3.9b
Sex ratio (% females among progeny)					
.....- at 25 °C	35.4 a*	-	-	-	39.1a
- at 29 °C	34.0 a*	16.9b*	6.3c*	-	38.4 a

Numbers in parentheses represent the number of replications

Means within each row followed by the same Latin letters were not significantly different ($P > 0.05$) with a t-test at 25 °C and ANOVA followed by Tukey test at 29 °C

- : indicates that parameters were not determined because of low number of female wasps (one female obtained from 250 parasitized larvae reared on *L. sericeus* at 29 °C*)* or unsuccessful development of parasitized larvae (*V. unguiculata* or *L. sericeus* at 25°C; *P.santalinoides* at 25 and 29 °C*)*

Comparison between male and female: means within each column followed by Greek letters are not significantly different (paired t-test at 5%).

Sex ratio: female percentages followed by the same letter were not significantly different with χ^2 at 5% (based on 2 x 2 (25 °C) or 2 x 4 (29 °C) contingency table) for comparison between feeding substrates

*: indicates that there was significant difference ($P < 0.05$, χ^2) between observed and expected (50%) percentages of the females for the feeding

male (F = 0.3; df = 2, 53; *P* = 0.8), female wasps that emerged from larvae reared on *S. rostrata* flowers lived longer when compared with females that emerged from larvae reared on cowpea flowers (F = 6.3; df = 2, 34; *P* = 0.005). The percentage of adult wasps that emerged from cocoons was the lowest on *L. sericeus* (Table 1). The wasp sex ratio was strongly affected by feeding substrates. The proportion of female wasps differed among feeding substrates of *M. vitrata* (χ^2 = 11.9; *P* = 0.007). It was lowest in hosts feeding on *L. sericeus* (6%) followed by *V. unguiculata* (17%) compared to *S. rostrata* and artificial diet (34-38%).

The mean daily fecundity was lower for parasitoids that had been reared in hosts on flowers of *S. rostrata* in comparison to artificial diet at 25 °C (0.74 against 5.85 cocoons/female/day, respectively) (t = 7.5; *P* < 0.0001; Figure 1), while no differences were observed between feeding substrates at 29 °C (values ranging from 0.16 to 2.8 cocoons/female/day; F = 0.89; df 2, 34; *P* = 0.6; Figure 1). The fecundity of parasitoids reared in hosts on artificial diet at 25 °C was much higher and peaked at 4 days after adult emergence (Figure 1). At 29 °C, the daily fecundity showed a peak at 3, 4, or 5 days after emergence for parasitoids that had developed in hosts on flowers of *S. rostrata,* artificial diet and flowers of *V. unguiculata,* respectively (Figure 1).

The survival rate of parasitized hosts was similar (t = 0.15; *P* = 0.9) on *S. rostrata* and artificial diet at 25 °C (Figure 2). Likewise, there were no significant differences for the survival rate of non-parasitized larvae at 25 °C on either of the two feeding substrates (t = 1.38; *P* = 0.2) (Figure 2).

Comparison of the survival rate of parasitized larvae reared on *S. rostrata, V. unguiculata, L. sericeus* and artificial diet did not show any significant differences at 29 ° C (F = 1.23; df = 3, 9; *P* = 0.35) (Figure 2). A similar result was obtained for non-parasitized larvae (F = 1.59; df = 3, 9; *P* = 0.26). However, at 25 °C, a significant difference was found when we compared the survival rates of parasitized and non-parasitized larvae reared on *S. rostrata* (t = 10.9; *P* < 0.0001) and artificial diet (t = 12.2; *P* < 0.0001). No differences were observed when we compared the survival rate of parasitized larvae to that of non-parasitized larvae feeding on *S. rostrata* (t = 1.92; *P* = 0.1), *V. unguiculata* (t = 1.58; *P* = 0.58), *L. sericeus* (t = 1.19; *P* = 0.28) and artificial diet (t = 1.93; *P* = 0.1) at 29 °C.

A large percentage of dead parasitized larvae showed symptoms of *Maruca vitrata* Multi-Nucleopolyhedrovirus *Mavi*MNPV disease that occurred during the rearing of *A. taragamae* (Figure 3). The percentage of larvae killed by the virus was significantly higher at 25 °C than at 29 °C for all feeding substrates (Figure 3).

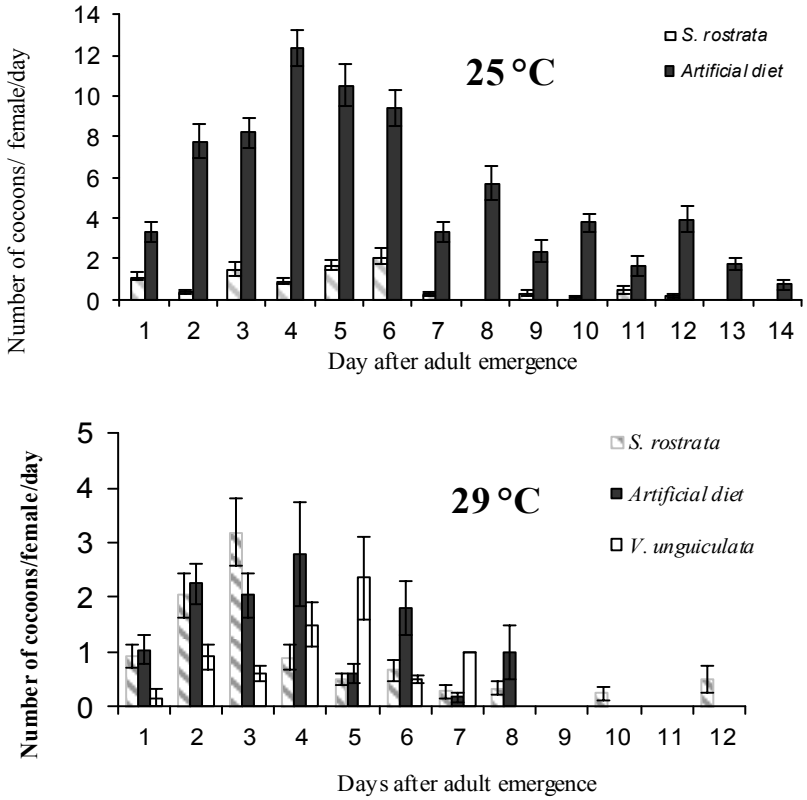

Figure 2: Daily fecundity of the parasitoid *Apanteles taragamae* after developing in its host on *Sesbania rostrata* flowers, or artificial diet at 25 °C and on *S. rostrata* flowers, *Vigna unguiculata* flowers or artificial diet at 29 °C.

Lines in bars represent standard errors of the means

Data on *L. sericeus* were not included in the Figure because of low number of replicates (only one adult female wasp was obtained from 250 parasitized larvae

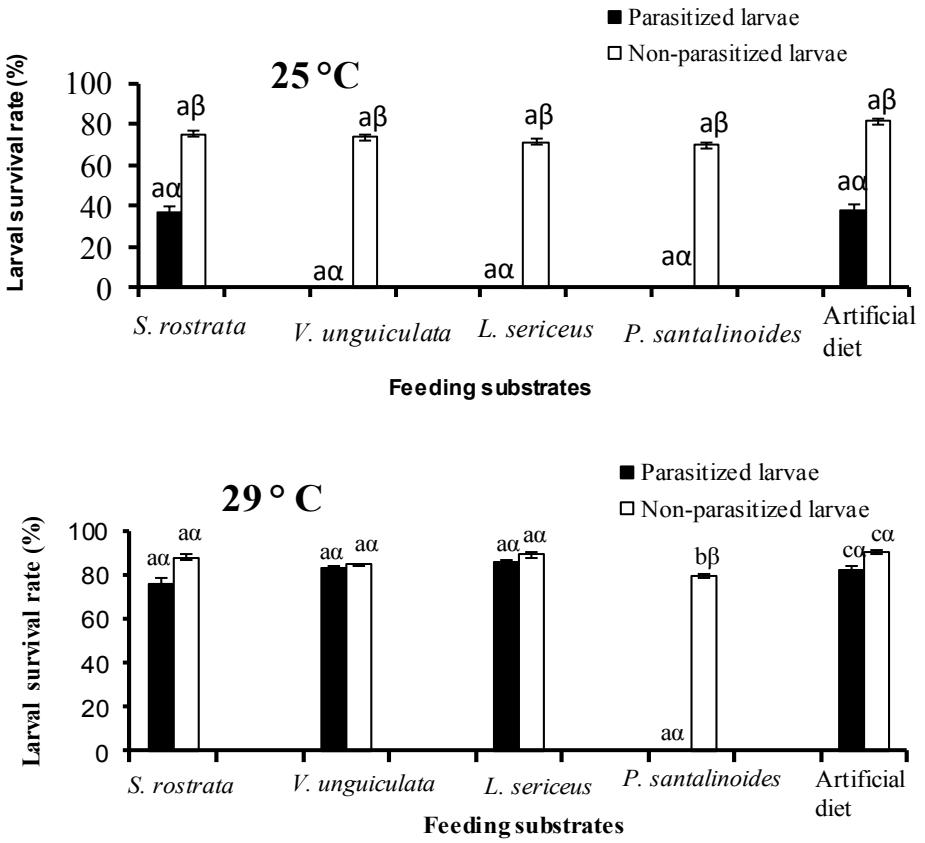

Figure 2: Survival rate of parasitized and non-parasitized *M. vitrata* larvae when reared on *Sesbania rostrata*, *Vigna unguiculata, Lonchocarpus sericeus* or *Pterocarpus santalinoides* flowers or artificial diet at 25 and 29 °C

Means were means of four replications; each replication consisted of 50 larvae

Lines in bars represent standard errors of the means

Means followed by the same letter were not significantly different with ANOVA followed by Tukey test or a t-test (α = 0.05) at each temperature. Regular letters refer to comparison (ANOVA followed by Tukey test) between feeding substrates for parasitized or non-parasitized larvae at each temperature and Greek letters to comparison (t-test) between parasitized and non-parasitized larvae for each feeding substrate at each temperature.

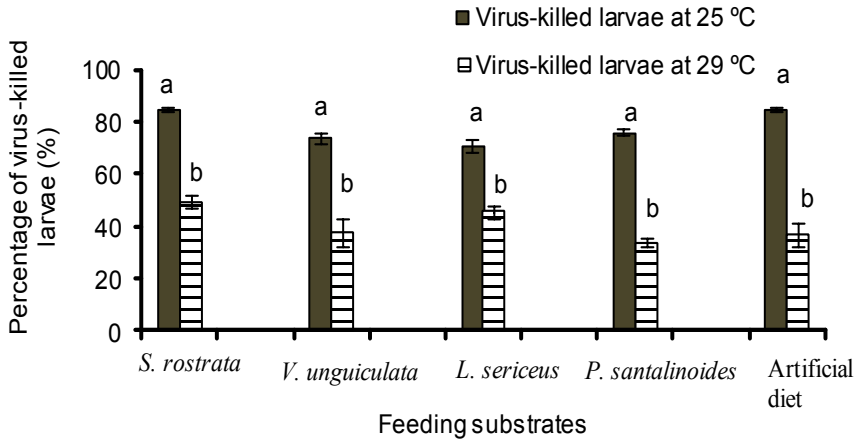

Figure 3: Percentage of dead parasitized larvae reared on flowers of *Sesbania rostrata, Vigna unguiculata, Lonchocarpus sericeus, Pterocarpus santalinoides* or artificial diet showing symptoms of *Maruca vitrata* Multi-Nucleopolyhedrovirus (*Mavi*MNPV) at 25 and 29 °C.

Means were means of four replications; each replication consisted of 50 larvae

Lines in bars represent standard errors of the means

Regular letters refer to comparison between the two temperatures 25 and 29 °C and means followed by the same letter were not significantly different with t-test within each feeding substrate

4.2 Effects of *S. rostrata, V. unguiculata,* and *L. sericeus* flowers on the life table parameters of *A. taragamae*

At 25 °C, the intrinsic rate of natural increase was 7.5 times higher for parasitoids developing in hosts feeding on artificial diet than those feeding on *S. rostrata* (Table 2). Likewise, the net reproductive rate was about 8 times higher when larvae were fed using artificial diet compared to flowers of *S. rostrata.* In contrast, no significant differences were obtained between the rearing substrates for any of the life table parameters at 29 ° C ($P > 0.05$) (Table 2).

Table 2: Life table parameters of the parasitoid *Apanteles taragamae* when reared in its host (*M. vitrata*) on flowers of *Sesbania rostrata, Vigna unguiculata, Lonchocarpus sericeus, Pterocarpus santalinoides* or artificial diet at 25 and 29 °C

Parameter	*S. rostrata*	*V. unguiculata*	*L. sericeus*	*P. santalinoides*	Artificial diet
Intrinsic rate of increase (r_m) (females/female/day)					
.....- at 25 °C	0.015 ±0.009a	-	-	-	0.145 ± 0.010a
- at 29 °C	0.054 ± 0.024a	0.087 ± 0.085a	-	-	0.112 ± 0.012a
Net reproductive rate (R0) (females/female)					
.....- at 25 °C	0.920 ± 0.187a	-	-	-	7.281 ± 1.018b
- at 29 °C	1.869 ± 0.404a	1.828 ± 1.302a	-	-	3.350 ± 0.390a
Mean generation time (T) (day)					
.....- at 25 °C	12.930 ±	-	-	-	13.515 ± 0.181b
- at 29 °C	0.150b	11.696 ±	-	-	10.370 ± 0.259a
	10.273 ±	0.646a			
	0.063a				
Doubling time (DT) (day)					
.....- at 25 °C	18.775 ±		-	-	4.830 ± 0.390 a
- at 29 °C	2.285b	4.770 ± 0.140a	-	-	6.303 ± 0.639 a
	8.935 ± 0.575a				
Finite rate of increase (λ) (females/female/day)					
.....- at 25 °C	0.994 ± 0.012a	-	-	-	1.157 ± 0.012b
- at 29 °C	1.056 ± 0.02 b	0.992 ± 0.076a	-	-	1.136 ± 0.017b

Means within each row followed by the same letters were not significantly different with a t-test (25 °C) or ANOVA followed by Tukey test (29 °C) with α = 5% for the same plant species

- : indicates that parameters were not determined because of low number of female wasps (one female obtained from 250 parasitized larvae reared on *L. sericeus* at 29 °C*)* or unsuccessful development of parasitized larvae (case of *V. unguiculata* or *L. sericeus* at 25°C and *P. santalinoides* at 25 and 29 °C*)*

5 Discussion

After parasitization, the developmental and reproductive success of koinobiont parasitoids is determined by the nutritional value of their hosts that continue to feed, grow and develop (Beckage and Riddiford, 1983). The herbivore's food plants have been reported to influence many parasitoid biological parameters such as development time, sex ratio and survivorship of immature parasitoids (Fox et al., 1990; Harvey and Vet, 1997; Bottrell and Barbosa, 1998; Gols et al*.,* 2008; Gols and Harvey, 2009). In the present study, host plants of *M. vitrata* affected the development time of the parasitoid *A. taragamae.* The longest life cycle at 29 °C was obtained when the hosts fed on flowers of *L. sericeus* followed by hosts feeding on flowers of *V. unguiculata*. This finding may be explained by the fact that *L. sericeus* is a leguminous tree with relatively small flowers in comparison to the more succulent flowers of *V. unguiculata* and *S. rostrata*. The small *L. sericeus* flowers may provide a sub-optimal food source to *M. vitrata* larvae, and as a consequence, the wasps take longer to accomplish their life cycle. Although all food substrates were leguminous plants, differences between them can also be explained by the presence of toxic secondary metabolites in the flowers (Barbosa et al., 1986). For instance, given the purplish colour of *L. sericeus* flowers, they could contain anthocyans or polyphenolic compounds which can have a detrimental effect on insect development (van Loon, 1990; Lev-Yadun and Gould, 2008). However, no information on the chemical content of these species is present in the literature.

The life cycle of female parasitoids was longer than that of males, regardless of the feeding substrates at both 25 and 29 °C. This supports the observation that female parasitoids require more nutritional resources than males to complete their development (Colinet et al*.,* 2005).

In this study, we did not succeed in rearing *A. taragamae* on flowers of *P. santalinoides* which is one of the four key host plants of *M. vitrata*. This finding may be attributed in part to interactions with the Multi-Nucleopolyhedrovirus *Mavi*MNPV that

occurred in the mass rearing of *A. taragamae.* The wasp has been reported to acquire and transmit the virus over several generations (Srinivasan et al., 2009). In our study, this virus affected the development of the wasp by killing infected parasitized larvae. As the viral disease symptoms appear about 3 to 4 days after infection of the *M. vitrata* larvae (Lee et al., 2007), and the wasp needs at least 5 and 7 days to complete its larval development at 29 °C and 25 °C, respectively (Table 1); this suggests that the wasps have better chances to escape the viral disease at 29 °C due to faster development. Furthermore, the wasp would not be able to escape the viral disease if parasitized larvae fed on sub-optimal substrates such as flowers of *P. santalinoides* that did not allow fast larval development.

The detrimental effect of *Mavi*MNPV associated with the relatively low survival rate of parasitized larvae at 25 °C supports the slow-growth, high-mortality hypothesis (Clancy and Price, 1987). Thus, a longer larval development time would increase the risk of attack by *Mavi*MNPV, leading to a higher mortality in parasitized larvae.

Host plant quality can be one of the main factors determining the sex ratio in parasitoids (Jervis et al., 2008). Host size at parasitization may affect sex allocation by female parasitoids at oviposition (Arthur and Wylie, 1959; King, 1987; van Alphen and Visser, 1990; Brodeur and Boivin, 2004; Harvey et al., 2004). Host size before parasitization is dependent upon the feeding rate and nutritional content of herbivore food plants (Harvey et al., 1994). Ovipositing parasitoid females are known to be selective for the sex of the offspring they deposit in their host (Hare and Luck, 1991; Luck et al*.,* 1992; Dicke, 1999a). Thus, male offspring are often oviposited in small hosts whereas female offspring are oviposited in the large ones (Jones, 1982; Dicke, 1999a). Our study revealed a strong effect of *M. vitrata* host plants on the proportion of *A. taragamae* females produced. In plant species where wasp development was slow (for instance *L. sericeus*), the proportion of females was reduced.

The daily fecundity showed a nonlinear pattern indicating a pro-ovigenic characteristic of *A. taragamae.* Indeed, synovigenic females may show a constant daily fecundity for a long period in their lifespan as they mature eggs over time (Jervis et al., 2001). On the other hand, pro-ovigenic insects that have a high egg load at adult emergence exhibit a nonlinear daily fecundity pattern (Ellers et al., 2000).

The high percentage of virus-killed larvae observed for parasitized larvae at 25 °C showed the influence of temperature on viral mortality. This virus-related mortality is

likely to have a differential effect on parasitoid performance in hosts feeding on flower from different plant species.

When the host was fed on artificial diet, the daily fecundity of *A. taragamae* at 25 °C was higher than at 29 °C indicating a negative effect of high temperatures on wasp performance (Dannon et al., 2010a). The optimum temperature for development of *A. taragamae* ranged from 24 to 26 °C (Dannon et al., 2010a). Indeed, insects are not able to regulate their body temperature in response to increasing temperature so that above the upper thermal threshold, enzyme activity or nutrient metabolism are affected and consequently disrupting development and survival (Langridge, 1963).

In summary, *M. vitrata* host plants affected the development time, fecundity and sex ratio of the parasitoid wasp *A. taragamae.* The wasp successfully developed on most of the tested host plants. There are many factors in plants that can affect their suitability to koinobiont parasitoids, including nutrient contents and secondary metabolites. Further research should investigate the biochemical factors in *M. vitrata* host plants that can influence the development and performance of *A. taragamae.*

Acknowledgments

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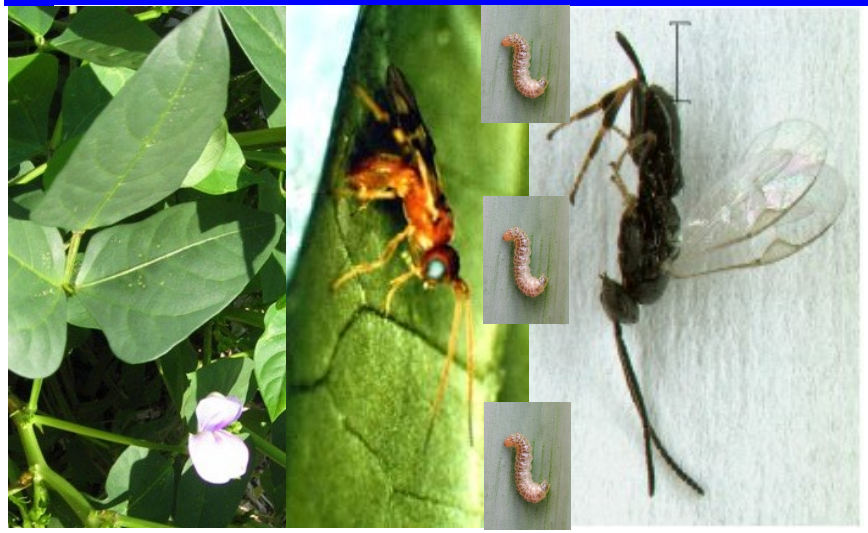
Assessing non-target effects and host-feeding of the exotic parasitoid *Apanteles taragamae,* a potential biological control agent of the cowpea pod borer *Maruca vitrata*



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Assessing non-target effects and host-feeding of the exotic parasitoid *Apanteles taragamae,* a potential biological control agent of the cowpea pod borer *Maruca vitrata*

Elie Ayitondji Dannon, Manuele Tamò, Arnold van Huis, Marcel Dicke

Abstract

Apanteles taragamae Viereck is a larval parasitoid introduced in Benin for classical biological control of the cowpea pod borer *Maruca vitrata* Fabricius. In the laboratory, we evaluated the effects of *A. taragamae* on non-target herbivore species, and on another parasitoid of *M. vitrata,* i.e. the egg-larval parasitoid *Phanerotoma leucobasis* Kriechbaumer. Furthermore, we addressed the host-feeding behaviour of *A. taragamae*. The host specificity of *A. taragamae* was assessed by offering six other lepidopteran species to the wasp. The competitive ability of *A. taragamae* was studied by providing the wasp with one- and two-days-old *M. vitrata* larvae that had hatched from eggs previously parasitized by *P. leucobasis*. Controls consisted of eggs and larvae offered only to *P. leucobasis* and *A. taragamae* respectively. None of the other six lepidoteran species was successfully parasitized by *A. taragamae.* The larval parasitoid *A. taragamae* outcompeted the egg-larval parasitoid *P. leucobasis* when offered two-day-old host larvae. Competition between the two parasitoid species did not significantly affect one-day-old host larvae that were less suitable to *A. taragamae.* Host-feeding by *A. taragamae* did not affect survival of one-day-old or two-day-old *M. vitrata* larvae. However, the percentage parasitism of two-day-old larvae was significantly reduced when exposed to female *A. taragamae* wasps that had been starved during 48 h. The data are discussed with regard to host specificity, host-feeding patterns and to factors underlying the outcome of intrinsic competition between parasitoid species.

Submitted to Biocontrol

1 Introduction

The classical biological control against the cowpea pod borer *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) has started with the introduction of the parasitoid wasp *Apanteles taragamae* Viereck (Hymenoptera: Braconidae) in Benin where its potential as biological control agent is being evaluated. In our previous studies, we have addressed the direct factors that contribute to the efficiency/suitability of the wasp. Such factors deal with the climatic adaptability of the parasitoid, its functional response, and its efficiency in searching host larvae (Dannon et al., 2010ab). However, some indirect or additional attributes might require particular attention with regard to the environmental risks of introduced species. Main areas of concern include host specificity, competitive ability and host-feeding behaviour (van Lenteren et al., 2003; Stiling and Cornelissen, 2005).

The effects of a biological agent on non-target herbivore species depend on its degree of specialization (Henneman and Memmott, 2001; Symondson et al., 2002; Louda et al., 2003). A strictly specialist parasitoid may not affect non-target herbivore species. A relative specialist parasitoid that has a narrow host range is expected to have a limited environmental risk, while a generalist is expected to affect non-target hosts (Henneman and Memmott, 2001; Symondson et al., 2002; Louda et al., 2003; van Lenteren et al., 2003). The host range of a parasitoid can be viewed physiologically or ecologically. The fundamental or physiological host range of a parasitoid is defined as the set of species that can support development of the parasitoid under laboratory conditions exclusively, while the ecological or realized host range is the current and evolving set of host species actually used for successful reproduction by the parasitoid in the field (Louda et al., 2003; Babendreier et al., 2003, 2005; Haye et al., 2005).

Competition is a process that affects the dynamics of ecological communities. It occurs between individuals of the same species (intraspecific) or different species (interspecific) that share the same resource (Birch, 1957). Interspecific or heterospecific competition is found to be an important factor that influences the outcome of biological control (Pijls et al., 1995; de Moraes et al., 1999; Ngi-Song et al., 2001; van Lenteren et al., 2003; Boivin and Brodeur, 2006). Therefore, the competitive ability should be considered when selecting a biological control agent (de Moraes et al., 1999).

Competitive interactions between parasitoid species refer to the parasitization of a host individual by more than one species and is also called multiparasitism. The outcome of the competition depends on several factors and different mechanisms have been evolved by competitors within the multi-parasitized host (Fisher, 1961; Vinson and Iwantsch, 1980; Collier and Hunter, 2001; de Moraes and Mescher, 2005; Yamamoto et al., 2007). Mechanisms involve physical attack and physiological suppression of the less competitive species (Fisher, 1961; Vinson and Iwantsch, 1980; McBrein and Mackauer, 1990; de Moraes et al., 1999).

Host-feeding is a biological characteristic that influences the performance in some parasitoid species (Jervis and Kidd, 1986). Hymenopteran parasitoid females are known to feed on host haemolymph and tissue during oviposition. Several host-feeding patterns have been reported (Benson, 1973; Hagstrum and Smittle, 1978). According to Jervis and Kidd (1986), four types of host-feeding can be distinguished: concurrent, non -concurrent, destructive and non-destructive. Concurrent host-feeding means that the female parasitoid uses the same host individual for both feeding and oviposition, while non-concurrent means that different hosts are used. In destructive host-feeding the host dies, while in non-destructive host-feeding the host survives. Parasitoid species have been reported to combine these different types (Křivan, 1997). Of all the feeding types, the non-concurrent and destructive or concurrent and non-destructive types were found to maximize parasitoid fitness (Křivan, 1997).

The current study addresses the following attributes of the biology of *A. taragamae*: host specificity, competitive capacity and host-feeding behaviour. These biological characteristics are of major consideration when selecting an effective biological control agent.

2 Materials and Methods

2.1 Plant materials

Maize: Cut parts of maize stems, collected from maize fields grown at IITA Benin station, were used to feed larvae of the African sugar-cane borer *Eldana saccharina* Walker*,* the spotted stalk borer *Chilo partellus* (Swinhoe)*,* while the ear-borer *Mussidia nigrivenella* Ragonot and the false codling moth *Cryptophlebia leucotreta* (Meyrick) were fed using young maize cobs.

Cotton: Cotton leaves collected from a cotton field at the IITA Benin station were used to feed larvae of the cotton leaf-roller *Sylepta derogata* Fabricius.

Millet: Millet grains bought from local markets in Nothern Benin were used to rear the pyralid *Corcyra cephalonica* Stainton.

2.2 Insect species

2.2.1 Maruca vitrata

Pupae of *M. vitrata* obtained from a stock culture at the laboratory of IITA Benin were placed in open Petri dishes. They were incubated in wooden cages (44 x 45 x 58 cm) with sleeves, having sides of fine mesh and a glass top, at 27.0 ± 0.6 °C and 60.9 ± 4.6 % relative humidity (mean ± SD). Emerging adults were fed using cotton fibres moistened with 10% glucose solution. Four-day-old female moths were transferred in groups of 4 or 5 individuals to transparent cylindrical plastic cups (3 cm diameter x 3.5 cm height) and kept for 24 h to allow for oviposition, which occurred on the inner surface of the cups. Ovipositing females were fed using small pieces of filter paper moistened with 10% glucose solution, which were replaced every 24 h. Cups carrying eggs were kept at the same experimental conditions until the larvae hatched. Larvae were transferred to large cylindrical plastic containers (9 cm diameter x 12 cm height) provided with artificial diet prepared according to Jackai and Raulston (1988), and reared until pupation. Pupae were collected and placed in cages up to adult emergence. *Maruca vitrata* eggs used in the different experiments were obtained from this mass production.

2.2.2 Non-target insect species

Colonies of six lepidopteran species were established from samples taken in different areas of Benin. Larvae of *E. saccharina* and *S. derogata* were initially collected in maize and cotton fields at the IITA Benin Station, respectively. Colonies of *M. nigrivenella* and *C. leucotreta* were obtained from maize cobs stored at IITA Benin. Larvae of *C. partellus* were sampled in rice fields at Ouedeme, 120 km West of Cotonou. The initial colony of *C. cephalonica* was obtained from a stock culture at IITA Benin.

2.2.3 Phanerotoma leucobasis

A colony of the egg-larval parasitoid *Phanerotoma leucobasis* Kriechbaumer (Hymenoptera: Braconidae) was established from cocoons obtained from *Lonchocarpus sericeus* flowers, collected at Sehoue, 90 km North of Cotonou. At emergence, males and females were kept together for mating. Mated females were transferred to small cups containing *M. vitrata* eggs for parasitization. Parasitized larvae were provided with artificial diet (see above) until pupation. Adult parasitoids obtained from this mass rearing were used in this study.

2.2.4 Apanteles taragamae

Cocoons of the larval parasitoid *A. taragamae,* obtained from the stock culture at IITA-Benin, were kept in plastic cylindrical cups (4.5 cm diameter x 5 cm height) till adult emergence. A hole (2 cm diameter) punched in the lid of the cups was covered with fine mesh. Adults of *A. taragamae* were fed with honey streaked on the fine mesh of the lid. To allow mated female wasps to parasitize hosts, they were offered, during 24 h, two-day-old larvae of *M. vitrata* in a small cylindrical cup (3 cm diameter x 3.5 cm height) containing a piece of artificial diet. The parasitized larvae were reared until the cocoon stage. Cocoons were collected and placed in cylindrical cups (4.5 cm diameter x 5 cm height). The mass production of wasps took place in a climate chamber with a temperature of 25.3 ± 0.5 °C (mean ± SD) and a relative humidity of 78.9 ± 5.6 % (mean ± SD).

2.3 Experiment 1: Physiological host range of *A. taragamae*

Naïve three-day-old mated females of *A. taragamae* were individually placed into plastic cups (9 cm diameter x 12 cm height), each containing one larva (one-, two- or three-day -old) of *M. vitrata*. The parasitoid was observed until it inserted its ovipositor in the larva which was then assumed to be parasitized. When the larva was parasitized, the female wasp was removed and kept for 24 h and used to parasitize a larva of one of the six lepidopteran species: *C. partellus, E. saccharina, M. nigrivenella, C. cephalonica, C. leucotreta,* and *S. derogata*. In case the larva was not stung within 10 min, the wasp was discarded and replaced by another female. Another 24 h later, the experiment was repeated with the same female wasp, offering again a larva of *M. vitrata* for

parasitization. Then, the wasp was discarded. Larvae of one-, two- and three-day-old were studied separately in different trials for each of the non-target insect species. Parasitized larvae were reared on artificial diet until pupation at 25.3 ± 0.5 °C and 78.9 ± 5.6 % relative humidity (mean ± SD). A positive control experiment was run for the three days using only larvae of *M. vitrata*.

In a second trial, naïve female parasitoids were offered a one-, two- or three-day-old larva of one of the non-target species. The following day, the same female wasp was placed in a plastic cup containing a single one-, two- or three-day-old larva of *M. vitrata*. This experiment was repeated ten times using different female wasps for each of the non-target host species.

In a third trial, *A. taragamae* was allowed during 24 h to parasitize 10 larvae of each of the non-target species, jointly placed in cups (9 cm diameter and 12 cm height). One three-day-old mated female was released in each cup. A total of 5 females were used for each non-target species. Larvae were reared until pupal stage, by using maize stem portions for the stem borers (*E. saccharina, C. partellus*), maize cobs for *M. nigrivenella* and *C. leucotreta,* millet grains for *C. cephalonica,* and cotton leaves for *S. derogata*.

2.4 Experiment 2: Interspecific competition between *A taragamae* and *P. leucobasis*

2.4.1 Development time of the parasitoids

Two naïve three-day-old mated females of *P. leucobasis* were introduced into cups (3 cm diameter x 3.5 cm height) containing on average 25 *M. vitrata* eggs. Parasitization was observed visually until all eggs were stung by female wasps. Stung eggs (assumed parasitized) were kept until hatching. Hatched larvae were individually placed in cups (3 cm diameter x 3.5 cm height) provided with a piece of artificial diet and reared until pupation. Cocoons were kept until adult emergence. Development time was recorded for each stage. Experiments were carried out in a climate chamber at 25.3 ± 0.5 °C (mean ± SD) and a relative humidity of 78.9 ± 5.6 % (mean ± SD).

The development time of the larval parasitoid *A. taragamae* was also determined using similar experimental conditions. Two-day-old host larvae were offered to three-day -old mated female parasitoids. Two female wasps were released in cups containing on

average 20 host larvae. The parasitoids were observed until they inserted their ovipositor in a larva which was then assumed to be parasitized. Larvae were individually transferred into cups (3 cm diameter x 3.5 cm height) as soon as they were parasitized and were reared on artificial diet until cocoon stage. Cocoons were collected and kept till adult emergence. The development time from larval age to cocoon, and from cocoon to adult emergence was noted. A total of 100 parasitized eggs or larvae were reared per parasitoid species.

2.4.2 Sequential no-choice tests

The outcome of the intrinsic competition between *P. leucobasis* and *A. taragamae* was assessed using one- and two-day-old larvae. Larvae hatching from eggs parasitized by *P. leucobasis* were submitted to parasitization by *A. taragamae* at the ages of one and two days. Females of *A. taragamae* were individually released in cups (3 cm diameter x 3.5 cm height) containing 20 *P-leucobasis*-parasitized larvae and kept for 24 h to allow for oviposition by *A. taragamae*. Larvae were then reared on artificial diet until pupation. The number of cocoons obtained per parasitoid species for each larval age was recorded. In parallel, we reared as control *M. vitrata* larvae that hatched from eggs parasitized by *P. leucobasis,* and those that were parasitized only by *A. taragamae*. Experiments were replicated 5 and 10 times for one-day-old and two-day-old larvae, respectively, each replicate consisting of 20 larvae or eggs.

2.5 Experiment 3: Influence of adult wasps' host-feeding on larval survival and parasitism rates

The influence of host feeding of *A. taragamae* females on larval survival was investigated using one- and two-day-old larvae. Ten larvae of each age were placed in cups (3 cm diameter x 3.5 cm height) containing artificial diet. One couple of 24 h or 48 h food-deprived wasps was released in each cup to allow larval parasitization. In parallel, honey-fed couples of wasps were released in other cups containing 10 larvae of each age. Control larvae consisted of *M. vitrata* larvae that were not submitted to parasitization by *A. taragamae.* Experiments were replicated five times for each larval age. Larval mortality was recorded daily until cocoon stage.

3 Statistical analysis

The development time of the two parasitoid species was compared using a t-test. The percentage parasitism of one- or two-day-old larvae for each parasitoid species in competition or alone, and larval survival or effect of host-feeding on percentage parasitism of *A. taragamae* were analyzed using the General Linear Model procedure of SAS followed by a Tukey test for the separation of means. A t-test was used to compare separately for each parasitoid species the percentage parasitism in or without competition per larval age. Percentage data (p) was arcsine $\sqrt{}$ (p/100) transformed prior to statistical tests.

4 Results

4.1 Physiological host range of *A. taragamae*

Although *A. taragamae* successfully parasitizes and develops in *M. vitrata* larvae, none of the other six lepidopteran species was successfully parasitized by *A. taragamae.* Visual observation of wasp behaviour revealed that females did not explore the larvae of five non-host species at all, namely *C. partellus, M. nigrivenella, S. derogata, C. leucotreta, C. cephalonica*. Only larvae of *E. saccharina* were probed during random runs but without an effective parasitization, even when female wasps were kept together with larvae for 24 h to allow for oviposition.

4.2 Development time of *P. leucobasis* and *A. taragamae*

The development time from egg to cocoon of the egg-larval parasitoid *P. leucobasis* was twice as long as that of the larval parasitoid *A. taragamae*. The cocoon stage was also longer but only by 0.6 days (Table 1). The complete cycle from egg to adult for *A. taragamae* (12.2 days) was 7.5 days shorter when compared to that of *P. leucobasis.*

Table 1: Development time of the egg-larval parasitoid *Phanerotoma leucobasis* and the larval parasitoid *Apanteles taragamae* in *Maruca vitrata* at 25 °C

Parasitoid species	Development time ± SE (days)		
	Egg-Cocoon	Cocoon-adult	Egg-adult (cycle)
A. taragamae	7.42 ± 0.06 (70)a	4.97 ± 0.06 (40)a	12.18 ± 0.06 (40)a
P. leucobasis	14.29 ± 0.05 (62)b	5.56 ± 0.08 (50)b	19.74 ± 0.08 (40)b

Numbers in parentheses are the number of replicates

Means followed by the same letter in each column are not significantly different (t-test, α=0.05).

4.3 Influence of interspecific competition with *P. leucobasis* on the percentage parasitism of one- or two-day-old *M. vitrata* larvae by *A. taragamae*

The percentage parasitism was calculated for each parasitoid species in competition with the other species or in the absence of competition considering two *M. vitrata* larval ages, i.e. one- (Figure 1) or two-day-old larvae (Figure 2).

When one-day-old *P. leucobasis*-parasitized larvae were offered to *A. taragamae* the parasitization success of *P. leucobasis* was slightly, but not significantly, reduced compared to that obtained in the absence of competition (Figure 1). Likewise, the percentage of one-day-old larvae parasitized by *A. taragamae* did not show any significant differences in competition or in the absence of competition (Figure 1).

The percentage parasitism of two-day-old *P. leucobasis*-parasitized larvae by *A. taragamae* did not differ significantly from that obtained when *A. taragamae* was offered unparasitized *M. vitrata* larvae (Figure 2). But the percentage parasitism of two-day-old larvae (Figure 2) by *A. taragamae* was at least three times higher than that of one-day-old larvae (Figure 1) when larvae were offered only to this parasitoid ($t = 3.84$; $P = 0.002$). On the other hand, the parasitization success of *P. leucobasis* was significantly lower in competition (when two-day-old *P. leucobasis*-parasitized larvae were offered to *A. taragamae*) compared to that observed when *M. vitrata* eggs were submitted only to

P. leucobasis (Figure 2). No significant differences were found between the percentage parasitism on one- (Figure 1) and two-day-old (Figure 2) larvae when *M. vitrata* eggs were offered to *P. leucobasis* only (t = 0.43; *P* = 0.67).

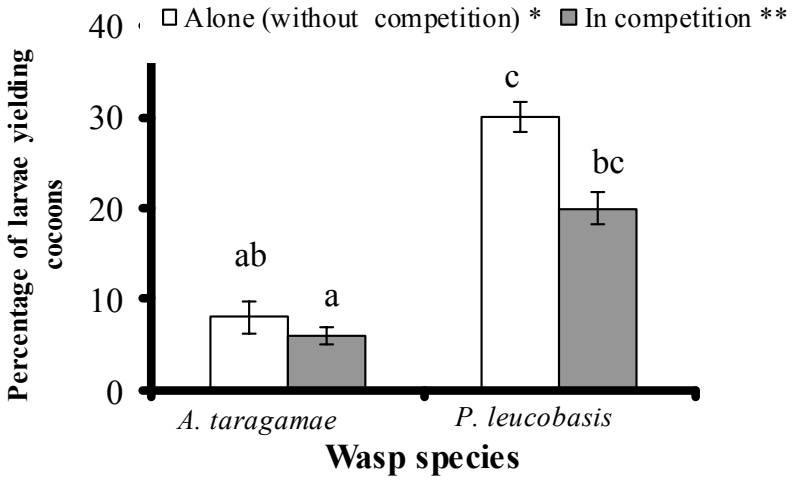

Figure 1: Parasitization success (cocoons) of *Phanerotoma leucobasis* and *Apanteles taragamae* when one-day-old *P. leucobasis*-parasitized or non-parasitized larvae were offered to *A. taragamae* either in competition or without competition.

* Alone (without competition) refers to the experiment where eggs of *M. vitrata* were parasitized by *P. leucobasis* only or where larvae of *M. vitrata* were parasitized by *A. taragamae* only

** In competition refers to the experiment where larvae hatched from *M. vitrata* eggs parasitized by *P. leucobasis* were subsequently offered to *A. taragamae*

Means are the means of 5 replications, each with 20 larvae
Lines in bars are standard errors of the means
Means followed by the same letter are not significantly different (ANOVA, followed by Tukey- test, α = 0.05)

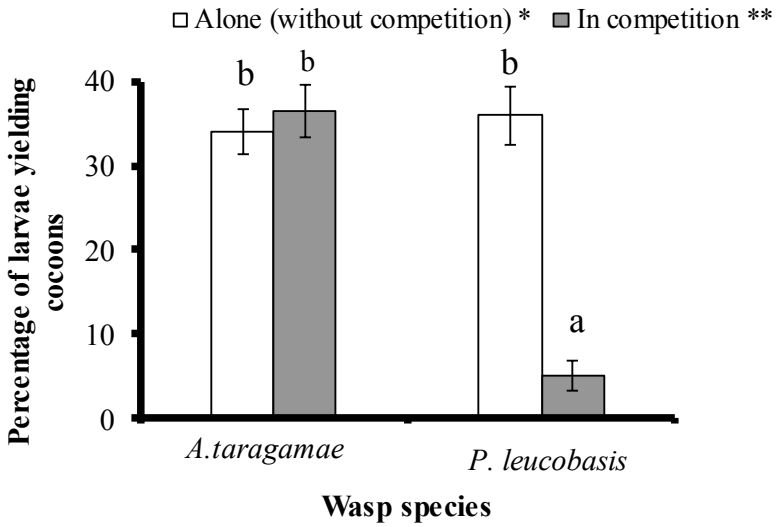

Figure 2: Parasitization success (cocoons) of *Phanerotoma leucobasis* and *Apanteles taragamae* when two-day-old *P. leucobasis*-parasitized or non-parasitized larvae of *Maruca vitrata* were offered to *A. taragamae* either in competition or without competition

* Alone (without competition) refers to the experiment where eggs of *M. vitrata* were parasitized by *P. leucobasis* only or where larvae of *M. vitrata* were parasitized by *A. taragamae* only

** In competition refers to the experiment where larvae hatched from *M. vitrata* eggs parasitized by *P. leucobasis* were subsequently offered to *A. taragamae*

Means are the means of 10 replications, each with 20 larvae
Lines in bars are standard errors of the means
Means followed by the same letter are not significantly different (ANOVA, followed by Tukey- test, $\alpha = 0.05$)

4.4 Influence of adult wasps host-feeding on larval survival and parasitism rates

Host-feeding by the parasitoid *A. taragamae* did not significantly affect the survival of *M. vitrata* larvae when one-day-old larvae were exposed to 24 h-starved parasitoid females (Figure 3). Similarly, there were no significant differences in larval survival when two-day-old larvae were exposed to 24 h or 48 h-starved females in comparison with honey-fed ones (Figure 4). Only 20% of the starved wasp couples survived beyond 48 h. No

significant differences were obtained between 24 h-starved and honey-fed female wasps for their ability to parasitize two-day-old larvae of *M. vitrata* (Figure 5). However, 48 h of starvation drastically reduced the percentage parasitism by *A. taragamae* (Figure 5).

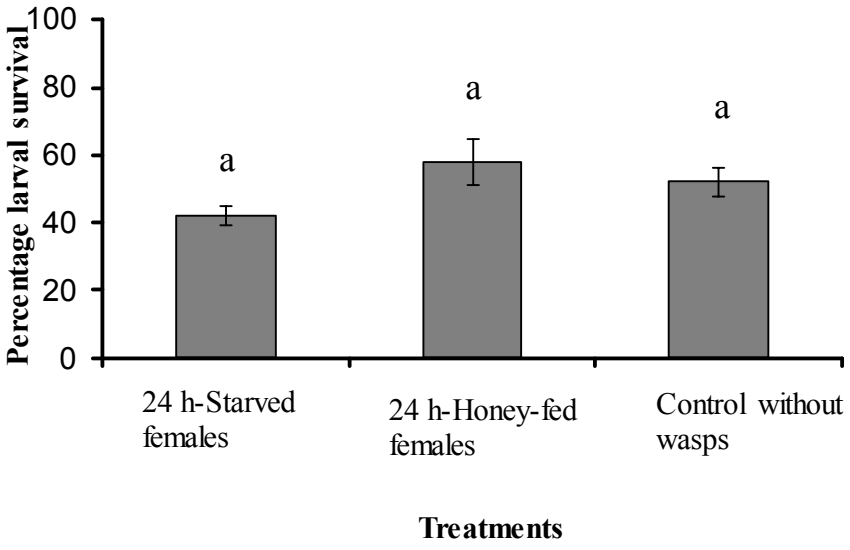

Figure 3: The survival rate of one-day-old *Maruca vitrata* larvae parasitized or not by 24 h-starved or honey-fed females of *Apanteles taragamae* .

Control without wasps consists of *M. vitrata* larvae that were not parasitized by *A. taragamae*
Means are the means of 5 replications, each with 10 larvae
Lines in bars represent standard errors of the means
Means followed by the same letter are not significantly different (ANOVA followed by Tukey test, α=0.05)

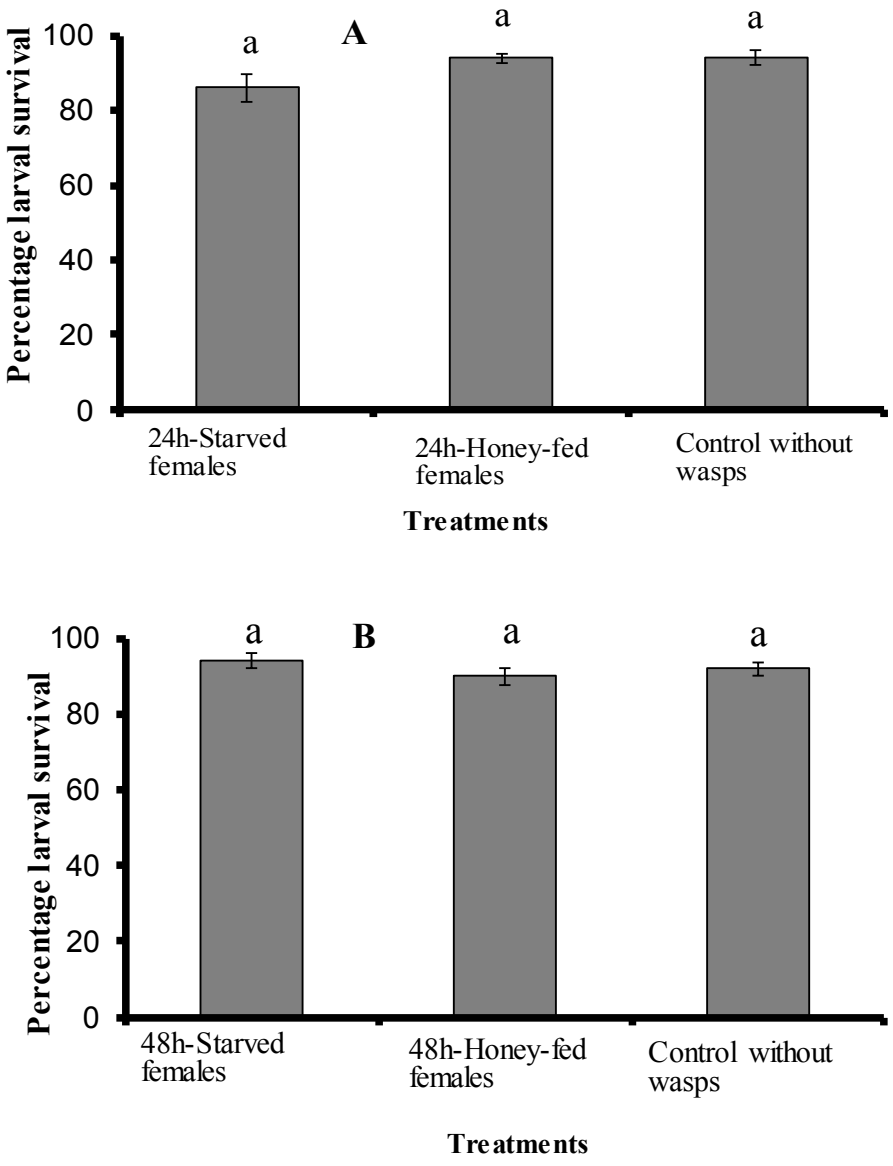

Figure 4: The survival rate of two-day-old *Maruca vitrata* larvae parasitized or not by 24 h-starved or 24 h-honey-fed (A) and 48 h-starved or 48 h-honey-fed females of *Apanteles taragamae* (B).

Control without wasps consists of *M. vitrata* larvae that were not parasitized by *A. taragamae*
Means are the means of 5 replications, each with 10 larvae
Lines in bars represent standard errors of the means
Means followed by the same letter were not significantly different (ANOVA follwed by Tukey test, α=0.05)

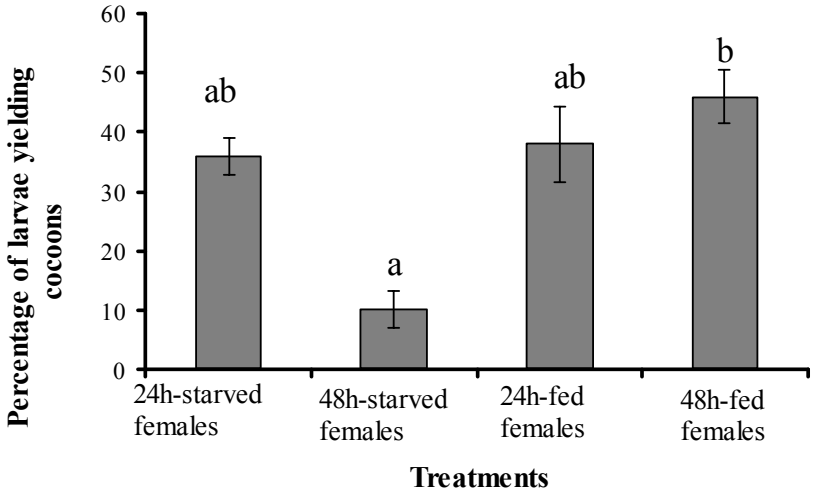

Figure 5: Percentage parasitism of two-day-old *Maruca vitrata* larvae by 24 h or 48 h-starved and honey-fed females of *Apanteles taragamae*

Means are the means of 5 replications, each with 10 larvae
Lines in bars represent standard errors of the means
Means followed by the same letter were not significantly different (ANOVA followed by Tukey test, α=0.05)

5 Discussion

Physiological host range

The parasitoid wasp *A. taragamae* displayed a strict specificity to *M. vitrata* when offered six other lepidopteran species. Most of the selected species were reported to damage either maize, cotton, or millet; and these crops often share the same agro-ecosystem with cowpea. Host suitability to parasitoid development depends on many factors including environmental influences, parasitoid ability to evade the host's internal defense system, competition with other parasitoids, presence of toxins detrimental to the parasitoid eggs or larvae, and host nutritional adequacy (Vinson and Iwantsch, 1980). Host specificity has often been reported for *Apanteles* species (Ngi-Song et al., 1999). However, some reports about *A. taragamae* indicated that it parasitized five other Pyraloidea species in India (Peter and David, 1992; Mohan and Sathiamma, 2007). Of these reported hosts, only one, i.e. *C. cephalonica,* occurs in Benin; however, it was not parasitized by the wasp in our study. For this reason, we tend to believe that *A. taragamae* reported from India is likely to be a different species from the one we have received from Taiwan. Also, the species from India is reported as being gregarious (Peter and David, 1992), while the species we have obtained from Taiwan is strictly solitary (Huang et al., 2003; Dannon et al., unpublished data).

Competition between the parasitoids A. taragamae and P. leucobasis

Community dynamics depends partly on the outcome of competition among species and may affect the management of herbivorous insect pests in tritrophic systems. The outcome of the competition between different parasitoid species sharing the same host is known to be affected by several factors (McBrein and Mackauer, 1990; de Moraes et al., 1999; Collier and Hunter, 2001; Collier et al., 2002; Pérez-Lachaud et al., 2002; de Moraes and Mescher, 2005; Yamamoto et al., 2007). Of these, development time of the parasitoids from egg to adult stage, oviposition order between parasitoid species, development stage of the host when it is parasitized, and larval morphology of the different competitors are of importance (McBrein and Mackauer, 1990; de Moraes et al.,1999; de Moraes and Mescher, 2005; Yamamoto et al., 2007). In the present study, the egg-larval parasitoid *P. leucobasis* was the first to oviposit in *M. vitrata* eggs. Its

development time from egg to cocoon was longer than that of the larval parasitoid *A. taragamae*, which may explain why the latter outcompeted *P. leucobasis.* In competition, the parasitization success of the egg-larval parasitoid *P. leucobasis* was significantly reduced compared to that obtained for the control (in the absence of competition). When offered one- or two-day-old larvae, the percentage parasitism of the larval parasitoid *A. taragamae* was not significantly affected by previous parasitization by *P. leucobasis* in comparison with the control. However, the percentage parasitism for *A. taragamae* in one-day-old larvae was significantly lower than that obtained with two-day-old larvae. This may be due to differential host suitability. These findings concur with those of Dannon et al. (2010a) who reported that one-day-old larvae were less suitable to *A. taragamae* development than two-day-old larvae. Differences observed in the development time of the two parasitoid species may affect the outcome of their competition. Because the development time of *P. leucobasis* from egg to cocoon stage is longer than that of *A. taragamae* (Table 1)*,* we hypothesize that the eggs of the latter hatch early. Therefore, larvae of *A. taragamae* might win the intrinsic competition with *P. leucobasis* by developing different mechanisms such as physical or physiological attacks. Indeed, in competition, larvae of some parasitoids are known to outcompete their competitors through direct physical or physiological attacks (Fisher, 1961; Vinson and Iwantsch ,1980; Laing and Corrigan, 1987; McBrein and Mackauer, 1990; de Moraes et al., 1999; Muturi et al., 2006). The present study did not investigate the mechanisms involved in the intrinsic competition between *A. taragamae* and *P. leucobasis*. Results from these simplified experiments cannot be extrapolated to field conditions nor used to accurately predict the overall outcome of the competition between the two parasitoid species. Previous studies showed that the percentage parasitism of *A. taragamae* increased with host density but the wasp was not able to parasitize all offered host larvae even in a small arena (Dannon et al., 2010a). Therefore, in heterogeneous and complex natural environments, a fraction of larvae may remain unparasitized. Thus, a spatio-temporal niche partitioning or dispersal-competition trade-off may lead to the coexistence of the two parasitoid species with optimum control of *M. vitrata* through complementary action. Indeed, previous studies revealed that *P. leucobasis* was found mostly on trees that are host plants of *M. vitrata* such as *Pterocarpus santalinoides* and *L. sericeus* (Tamò et al., 2002; Arodokoun et al., 2006), while *A. taragamae* was abundant on a shrub host plant *Sesbania cannabina* in Taiwan (its origin) (Huang et al.,

2003). This preference of *A. taragamae* for *M. vitrata* host plants with lower growth habit might lead to niche partitioning, thus contributing to the coexistence of *A. taragamae* with *P. leucobasis.*

Influence of parasitoid host-feeding on larval survival and parasitism rates

Host-feeding behaviour has been reported for some braconid species (Jervis and Kidd, 1986). In this study, feeding by starved *A. taragamae* on one- or two-day-old *M. vitrata* did not significantly reduce the survival of host larvae when compared with control treatments exposed to honey-fed female parasitoids. Based on this observation, we conclude that *A. taragamae* displays a non-destructive host-feeding pattern (Jervis and Kidd, 1986). The fact that no significant differences were observed between 24 h-starved and honey-fed females for the percentage parasitism of two-days-old larvae suggests a concurrent host-feeding by *A. taragamae* ovipositing females. The maximum duration of food deprivation tolerated by *A. taragamae* is 48 h, and only 20% of wasps survived after 48 h of starving, indicating that feeding on host larvae did not provide the parasitoid all required nutrients for its survival. However, no significant differences were observed between 24 h-starved and honey-fed females for their ability to parasitize *M. vitrata* larvae. This supports the notion that in pro-ovigenic parasitoids such as *A. taragamae* (Dannon et al., unpublished data), host-feeding does not improve reproductive output (Fellowes et al., 2005). Indeed, host-feeding during oviposition provides materials for somatic maintenance in pro-ovigenic parasitoids, while it supplies the synovigenic ones in materials necessary for continued egg maturation (Chan and Godfray, 1993; Fellowes et al., 2005).

This study evaluated some indirect or additional biological characteristics that influence the efficiency by the parasitoid wasp *A. taragamae,* a potential biological control agent of *M. vitrata.* However, mechanisms that underlie some attributes namely the competitive ability of the wasp remain unclear. Further research should address these mechanisms in order to better appreciate the competitive ability of *A. taragamae.*

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General Discussion

General discussion

06

Elie Ayitondji Dannon

1 Introduction

Within integrated pest management (IPM), biological control is one of the most important strategies (Eilenberg et al., 2001). Chemical control is not attractive considering its side effects. Host plant resistance and cultural control practices are not always effective enough to keep insect pests populations below economic thresholds. This proved valid for the pod borer *Maruca vitrata* Fabricius, a major insect pest of cowpea. None of cultivated cowpea lines demonstrated a satisfactory level of resistance to the pod borer (Fatokun, 2002). Only *Vigna vexillata* (L.) A. Rich, a wild *Vigna* was found to be highly resistant to *M. vitrata* (Fatokun et al., 1993). Although *V. vexillata* is close to cowpea, it was not possible to move the desirable genes into cultivated cowpea varieties because of a strong cross-incompatibility at both pre and post-fertilization levels (Fatokun, 2002). Also, a number of cultural practices did not sufficiently control *M. vitrata* (Jackai and Adalla, 1997; Karungi, 2000ab; Oso and Falade, 2010). All these measures required additional chemical control measures to achieve efficient control (Karungi et al., 2000b). This led to an overuse of chemicals with undesirable side effects such as pest resistance, resurgence, secondary pest outbreaks, environmental pollution and human health hazards. Moreover, resistance of *M. vitrata* to some key pesticides used in cowpea such as Endosulfan, Dimethoate and Cypermethrin was reported in Nigeria (Ekesi, 1999). Therefore, it became imperative to explore biological control options against this pest. An inventory of natural enemies of *M. vitrata* was carried out and the feasibility of biological control against this moth was assessed in West Africa (Taylor, 1967; Okeyo-Owuor et al., 1991; Tamò et al., 1997; Tamò et al., 2002; Tamò et al., 2003; Arodokoun et al., 2006; Srinivasan et al., 2007). However, no effective biological control strategy could be developed until a larval parasitoid of *M. vitrata,* i.e. *Apanteles taragamae* Viereck, was discovered on *Sesbania cannabina* (Retz) plants in

Taiwan (Huang et al., 2003). This strategy of introducing parasitoids or predators to control invasive pests is called classical biological control, and results have been impressive (van Lenteren, 2007). With a percentage parasitism as high as 60%, the parasitoid *A. taragamae* was considered a promising classical biological control candidate against *M. vitrata*. Hence, it was introduced in 2005 into Benin by the International Institute of Tropical Agriculture (IITA) for assessing its potential as biocontrol agent. While the pest status of *M. vitrata* was well established and extensively documented, there was a lack of information in the literature about the bioecology of *A. taragamae*. A thorough knowledge on the biology and ecology of the parasitoid is a prerequisite for its use in a classical biological control programme against *M. vitrata.* Therefore, the central focus of the present research project is to provide basic information on the biology and ecology of *A. taragamae* using *M. vitrata*. Particular emphasis is put on the biological potential of the parasitoid to achieve successful control of *M. vitrata*. In biological control programmes, successes have been attributed to key factors such as reproductive capacity, climatic adaptability, host searching efficiency, host specificity, competitive ability of parasitoids, and release strategies (Stiling, 1993; van Lenteren and Manzaroli, 1999; Bale et al., 2008). Therefore, these factors were investigated in the framework of the current PhD thesis.

The specific objectives of this thesis (**chapter 1**) are listed below, and with each objective we indicate the section of this General Discussion in which we discuss the results and the wider perspective:

- Study the influence of temperature, host age on the life history parameters of *A. taragamae* (section 2, paragraphs 1 and 3 of this chapter).
- Evaluate the functional response of *A. taragamae* (section 2, paragraph 2 of this chapter).
- Assess the effects of volatiles from host larvae and host plants on the host foraging behaviour of *A. taragamae* with special emphasis on flower volatiles section 3 of this chapter).
- Investigate the effects of host plants on the life history parameters of *A. taragamae* (section 4 of this chapter).
- Assess indirect or additional physiological traits of *A. taragamae* in relation to its suitability as biological control agent (section 5 of this chapter).

- Explore the strategy that can optimize the release and establishment of *A. taragamae* (section 6 of this chapter).

2 Reproductive potential and climatic adaptability of *A. taragamae*

Host stage selection

The selection of appropriate host stages by parasitoids for the successful development of their offspring is of great concern with regard to host acceptance or host suitability (van Lenteren and Woets, 1988; Bale et al., 2008). Host size increases with host age and affects development time, reproductive capacity, sex allocation and longevity of most parasitoid species (Jones, 1982; King, 1987; Brodeur and Boivin, 2004; Colinet et al., 2005). Even for koinobiont parasitoids such as *A. taragamae* that rely on the growth of their host after parasitism, host size at oviposition is indicative for the length of the development time, the sex of the egg deposited in host, the fecundity and adult lifespan (Jones, 1982; Brodeur and Boivin, 2004). In **chapter 2**, it is demonstrated that females of *A. taragamae* produce only male progeny and the development time was longest when one-day-old larvae were parasitized. Of the five larval development stages known for *M. vitrata, A. taragamae* successfully parasitizes only the first two, and larvae older than three days were not parasitized. The parasitoid prefers two-day-old larvae as this age gave the highest percentage parasitism, fecundity and proportion of females. Hence, the use of two-day-old larvae could optimize the mass production of *A. taragamae* in the laboratory. The parasitoid might perform better in the field when it succeeds in locating this suitable host stage. The first two host stages are predominantly found inside flowers of cowpea and are not always available for parasitism as flowers may be closed. The first two host stages can also feed on flowers or leaves of some wild host plants such as *S. cannabina* (Huang et al., 2003). When able to detect hidden hosts in open flowers, *A. taragamae* would succeed at reducing *M. vitrata* populations in cowpea fields. From the third larval stage onwards, *M. vitrata* escapes from parasitism by *A. taragamae.* These host larvae are probably able to move faster than the parasitoid. In addition, larvae from the third stage may have other defensive mechanisms such as physical attack, secretion of toxic substances, and high rate of attack by haemocytes (Gross, 1993), resulting in unsuccessful development of *A.*

taragamae. In the field, larvae that escape from parasitism by *A. taragamae* can be parasitized by other larval parasitoids such as *Braunsia* sp. (Okeyo-Owuor et al., 1991) or *Braunsia kriegeri* Enderlein (Arodokoun et al., 2006) which parasitize more advanced larval stages. Therefore, the action of *A. taragamae* could be complemented by that of *Braunsia* species to achieve a better control of *M. vitrata* in the field.

Functional response of A. taragamae

The functional response of parasitoids is an important criterion used to select candidates for biological control (van Lenteren and Bakker, 1976; van Lenteren et al., 2006). How efficient a parasitoid responds to an increasing host density, is determinant for its effectiveness in controlling the target pest. Host density responsiveness deals mainly with host regulation after the successful establishment of introduced parasitoids. Laboratory outputs are indicative for predicting the pattern of the functional responses of biological control agents under field conditions. We investigated the functional response of *A. taragamae* in a simplified arena. Results revealed that the percentage parasitism was positively correlated with host density (**chapter 2**), suggesting that *A. taragamae* can maintain its populations at low host densities. Simple linear regression assessing the relationship between the percentage parasitism and host density was reported to fit well when describing the functional response of insect parasitoids (Hassell, 1966, 1982; Trexler et al., 1988; Teder et al., 2000; Kaleybi et al., 2006; Sánchez et al., 2009). Even after applying nonlinear models, the trend of the correlation between the percentage parasitism and host density was used to confirm the pattern of the functional response (Hassell, 1966, 1982; Sánchez et al., 2009). Nonlinear models are used to assess the pattern of functional responses with consideration of factors that drive parasitoid behavioural changes or influence the parasitization rate such as host handling time, searched area, number of hosts available, number of hosts parasitized and parasitoid egg load (Hassell, 1982; Trexler et al., 1988; Jamshidnia et al., 2010).

The response of *A. taragamae* described in **chapter 1** can better be understood when looking at the factors underlying decision mechanisms for host patch exploitation by parasitoids. These mechanisms consist of three main components: response to host-associated kairomones, patch-leaving time and oviposition experience (Waage, 1979; Driessen et al., 1995). Using a model that included the parasitoid *Venturia canescens*

Gravenhorst in combination with a heterogeneous host distribution, Waage (1979) observed that the tendency of the parasitoid to stay in the host patch increased with the intensity of host-associated chemicals (kairomones), and this behaviour is called the incremental mechanism (Wajnberg et al., 2000; van Alphen et al., 2003). Over time, this response of *V. canescens* diminished as host-derived kairomones decayed at a constant rate until a specific threshold, after which the parasitoid left the patch (decremental mechanism). According to Burger et al. (2006), the initial responsiveness or patch leaving tendency in some parasitoids might be determined by volatile compounds from hosts. The decremental rule might be true for *A. taragamae,* which shows its ability to use volatiles from *M. vitrata* larvae when foraging (**chapter 3**). Waage (1979) also reported that oviposition by *V. canescens* increased its patch residence time, but Driessen et al. (1995) observed a different behaviour of the same parasitoid in patches with uniform densities. After the initial assessment of host density, ovipositing in subsequent hosts reduced the response of *V. canescens* to host kairomone which resulted in a decrease in the patch residence time. This behaviour is called the count-down mechanism which relies on the accuracy of patch density assessment by parasitoids (Driessen et al., 1995). The latter authors showed that *V. canescens* needed less time for multiple ovipositions on a patch containing four hosts than to get one oviposition on a patch with a single host. Hence, the parasitoid *V. canescens* increased its efficiency with host density. In the current study, *A. taragamae* might display a similar behaviour by increasing the percentage parasitism by 0.6% when larval density increases in one unit (see slope of the regression equation $y = 0.61 x + 8$, where x is the host density). But why in terms of number, at the density of ten larvae per female, only one or two larvae were parasitized by *A. taragamae,* remains a question that is difficult to answer with the count-down rule. On the other hand, when considering different patterns of host distribution in multi-patch environments, Vos et al. (1998) found that neither *Cotesia glomerata* (L.) nor *C. rubecula* (Marshall) followed this count-down rule and were more flexible. According to the count-down rule, the time until the first oviposition (TUFO) decreases with the increase in host density (Driessen et al., 1995). But the TUFO did not differ significantly between *C. glomerata* and *C. rubecula* on *Pieris rapae* (L.) with a patch containing different host densities. Therefore, the TUFO was not a reliable source of information on host density for *C. glomerata* or *C. rubecula,* which might adjust their patch leaving tendency to a cue like the presence or absence of host

feeding damage, and reset their patch leaving tendency after each oviposition to the initial value as it was set upon entering the patch (Vos et al., 1998). A similar behaviour can also be suggested for *A. taragamae.* In the present study, *A. taragamae* might combine both incremental and decremental patch leaving mechanisms. Switching from decreasing and increasing tendency of patch leaving may be an adaptive mechanism found in many parasitoids depending on their host distribution and ability to use host kairomones (Burger et al., 2006). Moreover, a patch-marking substance left by some female parasitoids on a patch already searched is another factor that contributes to the decremental mechanism (van Dijken et al., 1992; Höller and Hörman, 1993; Bernstein and Driessen, 1996; Hoffmeister, 2000). Similar behavioural patterns have been reported in other parasitoid species, such as some *Trichogramma* species, *Telenomus busseolae* and *Aphidius rhopalosiphi* (Wajnberg et al., 1999, 2003; Outreman et al., 2005).

The functional behaviour of *A. taragamae* needs to be confirmed in semi-field (cages) or field conditions after its successful establishment. After all, it is recognized that parasitoid behaviour observed in the laboratory cannot be directly extrapolated to behaviour under field conditions (Ives et al., 1999). In fact, the functional response of a parasitoid in the laboratory is influenced by several factors such as the size of the experimental arena, the tested densities, time and duration of experiments, distribution of hosts in the arena, possibility of foraging parasitoids to leave the experimental units, and the number of parasitoids searching together (Islam et al., 2006). On the other hand, in the field, the functional response of parasitoids is affected mostly by their host searching efficiency, host patch accessibility or distribution, presence of competitors and temperature (Fernández-Arhex and Corley 2003; Luna et al., 2007). Likewise, the availability of different host plants may affect the functional response of parasitoids by influencing their host searching efficiency. Therefore, laboratory findings should be complemented with more elaborate semi-controlled and field studies to accurately determine the functional response of parasitoids used in biological control programmes.

Influence of temperature

Climatic adaptation of classical biological control candidates is an important environmental factor on which depend their successful establishment and subsequent host regulation (Stiling, 1993; van Lenteren and Manzaroli, 1999; Bale et al., 2008). Before selecting any classical biological control agent, it is crucial to ensure the existence of climatic similarities between native and introduced areas. Such similarities are often evaluated by "climate matching" based on data from literature (van Lenteren and Manzaroli, 1999). Afterwards, acclimatization studies in quarantine are required to assess the ability of biological control candidates to adapt to a different climate.

Temperature is the climatic factor that is reported to play a major role in the survival, establishment and subsequent dispersal of parasitoids used for classical biological control (van Lenteren and Woets, 1988; van Lenteren et al., 2006; Bale et al., 2008). Insects being strictly poikilotherm, they display thermal thresholds below or above which temperature affects enzyme activity or nutrient metabolism leading to death and consequently rapid decline of their populations (Langridge, 1963; Howe, 1967; Sharpe and DeMichele, 1977; Ivanović et al., 1992). In general, the relationship between temperature and the developmental rate or intrinsic rate of natural increase of arthropods is sigmoidal (Campbell et al., 1974; Brière et al., 1999). In **chapter 2**, the thermal requirements for the development of *A. taragamae* were determined. The thermal constant (number of degree days required for development) obtained from the simple linear equation (Campbell et al., 1974) is considered a key physiological feature of insect development. The parasitoid *A. taragamae* required 153 degree days to complete its development on artificial diet. This physiological attribute of *A. taragamae* was substantially lower than that of its host *M. vitrata* (355 degree days), pointing at the ability of the parasitoid to develop faster than its host. The relationship between the temperature and the intrinsic rate of natural increase of *A. taragamae* or *M. vitrata* was described using the nonlinear model Brière 1 (Brière et al., 1999). The intrinsic rate of natural increase, a biological parameter that refers to the innate capacity of an organism to build up its population (Birch, 1948), is an aggregated indicator including the effects of developmental time, age-related mortality, longevity of ovipositing females, age-related reproductive potential (daily fecundity) and survival. Therefore, the intrinsic rate of increase is considered as the most meaningful life table parameter when evaluating

the potential of natural enemies for biological control (van Lenteren et al., 2006). The curve that describes the relationship between temperature and the intrinsic rate for *A. taragamae* was above that of its host *M. vitrata* between 20 and 30 °C, demonstrating a higher ability of the parasitoid to build up its population compared to that of its host.

3 Host foraging capacity of *A. taragamae*

Efficiency in foraging for hosts is a parasitoid feature that plays an overriding role in the success of biological control projects (Neuenschwander and Ajuonu, 1995; Ngi-Song and Overholt, 1997; de Moraes et al., 1999; Neuenschwander, 2001; van Lenteren, 2007). One famous case is the success of the parasitoid *Anagyrus (Epidinocarsis) lopezi* De Santis introduced into Africa to control the cassava mealybug *Phenacoccus manihoti* Matile Ferrero (Neuenschwander and Ajuonu, 1995; Neuenschwander, 2001). This parasitoid, with a similar reproductive capacity when compared to its congener *Anagyrus diversicornis* Howard, was found to outcompete the latter by displaying a higher capacity in locating and parasitizing young host instars of *P. manihoti* in cassava fields (Neuenschwander, 2001). Accordingly, the ability of a parasitoid to regulate host populations depends heavily not only on its reproductive capacity but also on how efficient it is in searching and finding target hosts to parasitize. Efficient foraging for hosts is especially important at low host densities (Stiling, 1993; van Lenteren and Manzaroli, 1999; Bale et al., 2008). Host foraging is a process that consists of several steps involving the combination of stimuli from different sources (Vinson, 1976; Dicke and Sabelis, 1988; Turlings et al., 1990; Vet and Dicke, 1992; Dicke, 1999ab; Grostal and Dicke, 2000; Dicke and Grostal, 2001). While cues from the herbivore's host plants are used by parasitoids to locate the host habitat, stimuli from the host itself play a role in the detection of hosts at close distance (Vet and Dicke, 1992). In **chapter 3**, it is demonstrated that volatiles produced by cowpea flowers infested with *M. vitrata* strongly attracted *A. taragamae*. Of the different odour combinations, the wasp consistently displayed its preference for volatiles produced by *M. vitrata*-infested flowers, showing the importance of these volatiles in its host foraging process.

In the host habitat, short range stimuli from the host itself are reliable indicators of its presence, but they are usually not well detectable from a distance (Vet and Dicke,

1992; Dicke, 1999b). Such cues may be directly derived from exuviae, eggs, excreta, marking pheromones or any other product of the host. In this study, *A. taragamae* was significantly attracted to volatiles from *M. vitrata* caterpillars (**chapter 3**). However, like other natural enemies, *A. taragamae* may rely on the combination of different stimuli such as visual, olfactory, acoustic, tactile or vibrational cues to find its host (Fellowes et al., 2005). The significant change in the naïve behaviour of the wasp toward uninfested cowpea flowers after an odour and rewarding experiences (**chapter 3**), suggests that, associative learning is a mechanism by which *A. taragamae* could adjust its foraging decisions as known for many other parasitoid species as well (Vet and Dicke, 1992; Vet al., 1998; Dicke, 1999b). In summary, the parasitoid showed to be able to use volatiles produced by cowpea flowers damaged by *M. vitrata* and volatiles from host caterpillars when foraging for hosts.

4 Effects of host plants quality on the life history parameters of *A. taragamae*

Host suitability is the final step in the host selection process. For koinobiont parasitoid species such as *A. taragamae,* hosts continue to feed and grow after parasitization and the final outcome depends heavily on the nutritional quality of plant materials consumed by the host. Indeed, the quality of the nutrients provided by the host plant directly affects the nutritional status of herbivorous insects and, subsequently, some biological traits of their natural enemies (Mueller, 1983; Benrey et al., 1998; Krusse and Raffa, 1999; Dicke, 1999a; Eben et al., 2000; Lill et al., 2002; Uçkan and Ergin, 2002; Harvey, 2005). Toxic secondary metabolites present in some host plants can negatively affect the development of the parasitoid via its herbivorous host (Cortesero et al., 2000). Such effects may affect even higher trophic levels (Harvey et al., 2009). In **chapter 4**, we addressed the effects of *M. vitrata* host plants on some life history parameters of *A. taragamae.* Results revealed that some host plant species slowed down the growth of non-parasitized or parasitized larvae extending their exposure to other mortality factors. The sex ratio of *A. taragamae* was also strongly affected by host plant quality. This might be due to the small size of host larvae as the result of the slower larval development observed on some host plant species before parasitization. Moreover, the

size of the host at oviposition by koinobiont parasitoids is known to influence the sex of the egg deposited in the host (Jones, 1982; Brodeur and Boivin, 2004). Although all plant species tested were leguminous, they may differ in their chemical content, making some of them less suitable to *M. vitrata* larval development. However, koinobiont parasitoids might evolve different feeding strategies to cope with the harmful effect of secondary plant metabolites ingested by hosts. These feeding strategies were reported to determine to what extent parasitoids larvae are affected by the negative effect of plant secondary compounds (Harvey and Strand, 2002). Thus, parasitoid species whose larvae are obligate tissue feeders and have to consume the host completely before pupation are likely to be exposed to toxic plant-derived compounds stored in the host's body tissues or in the gut. By contrast, parasitoids whose larvae selectively feed on host haemolymph and fat body, and pupate externally from host larvae, might avoid exposure to toxic substances ingested by the latter (Harvey and Strand, 2002). Moreover, secretion and detoxification of toxic compounds by the host may dilute the effect of these compounds on the development of parasitized hosts. Parasitoids might also adapt to toxic plant secondary metabolites or select the host plants that optimize their life history parameters (Hunter, 2003). Also, toxic plant secondary compounds may differently influence the performance and survival of specialist and generalist endoparasitoids larvae. For instance, glucosinolates stored in the body tissue of *Plutella xylostella* (L.) were reported to affect more strongly the performance and survival of *Diadegma fenestrale* (Holmgren) (a generalist parasitoid) compared to *Diadegma semiclausum* Hellen (a specialist parasitoid) (Gols et al., 2008). The parasitoid wasp *A. taragamae* pupates externally from *M. vitrata* larvae but whether it feeds on haemolymph or consumes host tissues, remains unclear and needs more attention in further research. Another explanation for the significant variation in the effect of the host plants of *M. vitrata* on some life history parameters of *A. taragamae* such as survival of parasitized larvae and sex ratio may be found in the infection of parasitized larvae by *Maruca vitrata* Multi-Nucleopolyhedrovirus (*Mavi*MNPV). Infection by *Mavi*MNPV significantly affected the survival rate of parasitized larvae and subsequently increased the detrimental effect of toxic plant-derived compounds. Indeed, *A. taragamae* is reported as a vector of the virus *Mavi*MNPV, being capable of acquiring and spreading the virus through its ovipositor (Srinivasan et al., 2009; M. Tamò, personal

communication). Infected *M. vitrata* larvae start to show viral disease symptoms 3-4 days after the first contact with the virus (Lee et al., 2007). However, higher temperatures seemed to decrease the detrimental effect of the virus infection through the reduction of larval development time. For instance, the development time of parasitized larvae was 2 days shorter at 29 °C than at 25 °C, and this has contributed to the reduction of the impact of *Mavi*MNPV infection on parasitoid development (**chapter 4**). The slower growth of non-parasitized or *A. taragamae*-parasitized *M. vitrata* larvae observed on some host plants exposed the insect to the harmful effect of *Mavi*MNPV, thus supporting the slow growth high mortality hypothesis (Clancy and Price, 1987). According to this hypothesis, herbivores developing slower on some feeding substrates are more exposed to factors that cause their mortality.

5 Potential effects of *A. taragamae* on non-target organisms

Risk assessment of exotic natural enemies is of growing concern with regard to the permanent nature of classical biological control agents (Louda et al., 2003; van Lenteren et al., 2003, 2006). The “best” biological control agent is expected to have no or very limited effects on non-target organisms (van Lenteren et al., 2003, 2006). Evaluation of non-target effects of an exotic parasitoid involves studies on its host specificity and competitive behaviour to native beneficial insects. In **chapter 5**, we assessed the physiological host range of *A. taragamae* and its competitive ability in no-choice test involving the most dominant parasitoid species occurring in Benin, *Phanerotoma leucobasis* Kriechbaumer (Hymenoptera: Braconidae) (Arodokoun et al., 2006).

The parasitoid wasp *A. taragamae* did not successfully parasitize any of the following six other lepidopteran species: *Eldana saccharina* Walker, *Chilo partellus* (Swinhoe), *Mussidia nigrivenella* Ragonot *Cryptophlebia leucotreta* (Meyrick) *Sylepta derogata* Fabricius, *Corcyra cephalonica* Stainton. This parasitoid is likely to be specific to *M. vitrata* in Benin. This first laboratory host-range assessment has included herbivorous species that were of economic importance and in the future it might be extended to other lepidopteran species occurring in Benin ecosystems. Furthermore, the physiological host range needs to be complemented with the ecological one but this

is only possible after the parasitoid's establishment.

In no-choice experiments, *A. taragamae* won the competition with the indigenous egg-larval parasitoid *P. leucobasis.* This finding, observed in a simplified arena, cannot be used to accurately predict the outcome of the competition between the two parasitoid species under field conditions. Indeed, several factors are reported to affect interspecific competitive interactions among parasitoid species sharing the same host. Such factors include host specificity, host searching efficiency, reproductive capacity, ability to synchronize with the right host stage and functional response of the given parasitoid species (Lewis et al., 1990; de Moraes et al., 1999; Boivin and Brodeur, 2006). Our results showed that the percentage parasitism of *A. taragmae* did not increase when offering *P. leucobasis*-parasitized larvae compared to non-parasitized larvae of *M. vitrata.* Therefore, in a simplified arena *A. taragamae* is likely to equally parasitize non-parasitized and *P. leucobasis*-parasitized *M. vitrata* larvae. From this point of view, given that the percentage parasitism of *M. vitrata* eggs by *P. leucobasis* did not exceed 6% in cowpea fields (Arodokoun et al., 2006), and the percentage parasitism of *A. taragamae* was on average 60% (**chapter 2**), and assuming that the chance for *A. taragamae* to choose between non-parasitized and *P. leucobasis*-parasitized larvae is 50%, the choice of larvae in a population containing both non-parasitized and *P. leucobasis*-parasitized larvae by *A. taragamae* follows a binomial distribution. Thus, the probability (P) for *A. taragamae* to choose 6 *P. leucobasis*-parasitized larvae when parasitizing 60 larvae is given by:

$$P(x=6) = C_{60}^{6}(0.5)^{6}(0.5)^{(60-6)} = 4.10^{-11}$$

where C is the combination of possible choice scenario.

Thus, the probability of *A. taragamae* to parasitize all larvae that hatch from *M. vitrata* eggs that were previously parasitized by *P. leucobasis,* remains very low. The competitive behaviour of *A. taragamae,* observed in the current study, could therefore be viewed as an additional attribute for its efficiency in the parasitoids guild. Actually, a lack in competitive ability of introduced parasitoids is one of the factors that lead to failures of classical biological control programmes because of parasitism or predation by native

natural enemies (Stiling, 1993). Moreover, following the establishment of *A. taragamae* in Benin agro-ecosystems, there might be limited threat that *P. leucobasis* would not survive as it also parasitizes *Euzopherodes vapidella* Mann (Lepidoptera: Pyralidae), a major insect pest of stored yam in Ivory Coast (Sauphanor and Ratnadass, 1985; Girardin and Nindjin, 1996). Sampling of stored yam is on-going at the International Institute of Tropical Agriculture (IITA) to confirm the presence of *E. vapidella* in Benin (M. Tamò, personal communication).

6 Strategy developed for the release of *A. taragamae*

The final goal of any classical biological control programme is a successful establishment of a natural enemy and the subsequent control of the target pest. Initial settlement of parasitoids in a new environment depends on several factors related to the parasitoid (reproductive potential, host searching efficiency, competitive ability), adaptability to environmental factors (host quality and availability, temperature, relative humidity) and to the release rate, method and timing of release (Grevstad, 1999; Shea and Possinghan, 2000; Bellows et al., 2006; Crowder, 2007). The results of the research experiments described in previous chapters (**chapter 2, 3, 4, and 5**) demonstrate that *A. taragamae* is potentially suitable as classical biological control agent of the cowpea pod borer *M. vitrata.* Therefore, a release strategy has been developed considering some factors that are reported to optimize the successful dispersion and establishment of parasitoids used as biological control agents. Such factors are related to the release timing, parasitoid conditioning immediately before release, release rate and host synchronization. We chose the option to release parasitoid pupae in selected sites that were abundant in wild host plants of *M. vitrata.* This release strategy included the host plant *S. cannabina,* an artificial infestation of plants with *M. vitrata,* the covering of plots with nets supported by an iron framework, the release of adults of *A. taragamae* in cages and removing of nets after cocoon formation (Figure 1). This cage release strategy was thought to optimize the lifespan of adults that would emerge from cocoons and to avoid thermal shock that often affects the release of adults. In a preliminary study, we compared the performance of two host plants *S. cannabina* and *S. rostrata* (a local host plant of *M. vitrata*). The two host plants were sown in plots of 2 x 2 m in field at

the station of IITA-Benin. These plots were artificially infested each with 2000 eggs of *M. vitrata* and covered with nets when plants were 45 days old. Then, two days after the infestation, 100 couples of *A. taragamae* were released in each cage. Ten days later, plants were collected and cocoons counted per plant species. Results from this study revealed a good performance of *A. taragamae* on *S. cannabina* with on average 227.3 ± 7.8 cocoons/4 m^2 compared to the local host plant *S. rostrata* with 40 ± 15.4 cocoons/4 m^2. This difference between the two host plant species might be explained by the fact that larvae of *M. vitrata* prefer feeding on glabrous leaves of *S. cannabina* (Huang et al., 2003), while on *S. rostrata* the leaves are slightly pubescent so they feed mainly on flowers, which are much less abundant than leaves (M Tamò, personal communication). Therefore, the release strategy included *S. cannabina* and consisted of three main steps. First, *S. cannabina* is planted on 2 x 2 m plots in seven selected sites in Benin. Then, 45 days after planting, plants were infested with 2000 eggs of *M. vitrata* and covered with a net. One hundred couples of *A. taragamae* were released in each cage at the selected sites when larvae were two-day-old. The wasps were fed with honey droplets put on plant leaves. Finally, cages were opened ten days later, i.e. when the parasitoid cocoons were formed and adult wasps that emerged were expected to disperse throughout the different locations (Figure 1). Release of *A. taragamae* was carried out during the flowering period of some host plants such as *Vigna unguiculata, Cajanus cajan, Lonchocarpus sericeus, L. cyanescens, Sesbania* spp*., Centrosema pubescens* and *Tephrosia platycarpa.* Recovery studies through sampling of host plant flowers started one month after the release of *A. taragamae.* However, they did not yield information on the presence of the parasitoid after its first generation. Colonization of the different agro-ecosystems and establishment might take time. For instance, it took one year to find *Cotesia flavipes* Cameron back after it was released in 1993 in the coastal area of Kenya to control the crambid *Chilo partellus* (Swinhoe) (Omwega et al., 2006).

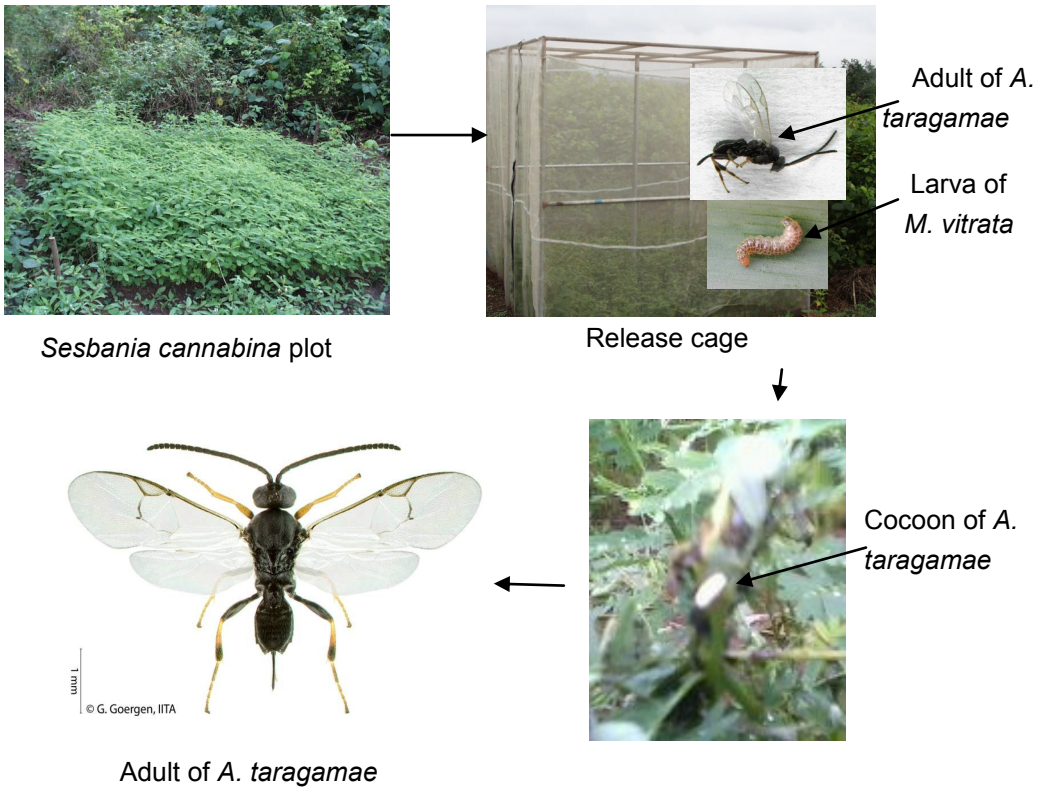

Figure 1: Strategy developed for *Apanteles taragamae* release in Benin

7 Concluding remarks and future perspectives

This thesis addresses some basic aspects of the biology and ecology of the parasitoid *A. taragamae* with regard to the attributes that determine its effectiveness as classical biological control agent. It adds a novel dimension to the better understanding of the foraging behaviour of parasitoids in demonstrating the production of herbivore-induced volatiles by cowpea flowers.

Several biological characteristics of the wasp need to be investigated under field conditions. Therefore, the first major challenge for future research is to ensure the establishment of *A. taragamae* through repeated releases using the release strategy elaborated above. Intensive recovery studies should then be undertaken on a large range of *M. vitrata* host plants. After a successful establishment of *A. taragamae*, field studies will be necessary to validate its functional response described in this thesis in order to better understand the interactions of *M. vitrata* and *A. taragamae* in systems including cowpea and/or other host plants. Prior to this, the functional response might be investigated in large arenas or field cages in semi-field conditions including several female parasitoids searching together. Another aspect to be targeted during future investigations is the determination of the ecological host range of *A. taragamae* in its new environment; this with regard to the assessment of environmental risks of the parasitoid. Also some laboratory, semi-field or field experiments are required to elucidate the mechanisms underlying the intrinsic interspecific competition between *A. taragamae* and the egg-larval parasitoid *P. leucobasis*. This will contribute to a good evaluation of the competitive interactions between the two braconid species.

We found that the parasitoid *A. taragamae* uses volatiles from cowpea flowers when foraging. The composition of the volatile blend from uninfested or *M. vitrata*-infested cowpea flowers is not known and could be determined to study the role of individual volatile components in the attraction of *A. taragamae* to cowpea flowers. Furthermore, the influence of flower volatiles of other key host plants of *M. vitrata* on the host selection behaviour of *A. taragamae* should be investigated such as *Pterocarpus santalinoides* L'herit ex DC*., L. sericeus*, *L. cyanescens* , and *Pueraria phaseoloides* (Roxb) Benth. Some of the alternative host plants might be crucial for the survival of parasitoid populations during the cowpea off-season, particularly because the pod borer does not undergo diapause during the dry season and is known to maintain its

population throughout the year using alternative hosts. Another area of interest might be the changes induced in the volatile composition of flowers damaged by herbivores. Flower volatiles are primarily known to attract pollinators (Jervis et al., 1993; Pichershy and Gershenzon, 2002; Raguso, 2008, 2009). Changes in volatile blends following herbivore damage were reported to affect the attraction of pollinators by flower volatiles (Effmert et al., 2008; Kessler and Halitschke, 2009) particularly for cross-pollinating plant species. Quantifying or understanding the interactions between induced plants defences, pollinators' attraction and reproduction success should be targeted by future research. The investigation of host plant quality in relation to parasitoid performance (**chapter 4**) suggests that secondary metabolites in some *M. vitrata* host plants might be the main factors reducing the survival rate and growth of caterpillars before and after parasitization by *A. taragamae.* However, no information is available on the chemical content of these plants. Future investigations may aim at investigating the flower chemical composition of some key host plants of *M. vitrata* such as *P. santalinoides, L. sericeus, L. cyanescens* and *P. phaseoloides.* Finally, the impact of *A. taragamae* on *M. vitrata* populations should be evaluated at regular (yearly) intervals after an establishment of the parasitoid.

Overall, by assessing the biological potential of *A. taragamae* complemented with an elaborate release strategy, this work provides thorough knowledge on the parasitoid's ecology and constitutes a major step in implementing the first classical biological control programme against the cowpea pod borer *M. vitrata* in Africa. Although time was the major practical constraint of the research project (three years of field works only), it gives interesting insights in several open research areas related to the parasitoid *A. taragamae*. Success of the present programme in Benin would bring hope to farmers and consumers in other cowpea production areas of West Africa where it could be extended.

Another issue to resolve in order to optimize the implementation of biological control programmes in Africa is to be able to attract increased support by national decision makers, i.e. governments. Most classical biological control projects in Africa have been developed and carried out by international research organizations with external financial support, while national programmes delivered import and release permits. National governments should get more involved in the development and

deployment of biological control programmes as a sustainable pest management strategy and this concerns not only classical biological control, but also inoculation strategies and in particular conservation biological control. They should bring it at the forefront of their agricultural development priorities through technical and financial support; in fact, it is speculated that efficient modern pest management strategies will strongly depend on biological control (van Lenteren, 2007). For this to happen, agricultural research in Africa needs to be redirected by giving particular emphasis to biological control. At the same time, public awareness campaigns on the benefits of biological control should be carried out in order to reach all actors on the value chain, from the producers to the consumers, including researchers and policy makers.

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Summary

Apanteles taragamae Viereck is a larval parasitoid introduced into Benin by the International Institute of Tropical Agriculture (IITA) to start a classical biological control project against the cowpea pod borer *Maruca vitrata* Fabricius. The work described in the current thesis was initiated to evaluate the biological potential of *A. taragamae*. This work focuses on the main criteria used when selecting candidates for biological control, such as reproductive capacity, functional response, climatic adaptability, host foraging capacity, and non-target effects.

The pest status of *M. vitrata* was well studied and different management methods such as chemical control, cultural practices and moderately resistant varieties have been developed (**chapter 1**). Chemical control is so far the most used practice against *M. vitrata*, while the other methods always require additional applications of chemicals. This has led to an overuse of chemicals with side effects such as pesticide resistance, secondary pest outbreaks, environmental pollution, and human health hazards. Therefore, it is imperative to explore other environmentally sound methods, especially (classical) biological control. The first effective classical biological control programme against *M. vitrata* started in 2005 with the introduction of *A. taragamae* from Taiwan into Benin. The different steps to implement a classical biological control programme have been conducted (**chapter 1**) and a rearing colony of the parasitoid was established at IITA, Benin station. The present PhD research project aimed at providing thorough knowledge on the biology and ecology of *A. taragamae,* a prerequisite for its use in any biological control programme.

Experiments were performed to assess the suitable host stages, the functional response and the influence of temperature on the life history parameters of *A. taragamae* (**chapter 2**). Of the five larval stages known for *M. vitrata, A. taragamae* successfully parasitized only the first two stages, and the highest percentage parasitism, lifetime fecundity, and female proportions were obtained when two-day-old larvae were parasitized. The percentage parasitism of two-day-old larvae was positively correlated with host density, suggesting a good functional response of the parasitoid. When reared at five different temperatures, representing averages that occur in Benin, *A. taragamae* performed better than its host *M. vitrata.* The thermal constant of *A. taragamae* (153 degree days) was lower than that of *M. vitrata* (355 degree days), pointing at the ability of the parasitoid to develop faster than its host. Between 20 and 30 °C, the curve that

describes the relationship between the temperature and the intrinsic rate of natural increase of *A. taragamae* was above that of its host *M. vitrata*. This demonstrates a higher ability of the parasitoid to build up its population compared to its host. So the reproductive capacity of the parasitoid is higher than that of its host. However, other biological attributes such as the foraging capacity should be taken into account when evaluating biological control candidates.

The host foraging behaviour of *A. taragamae* was investigated in a Y-tube olfactometer (**chapter 3**). When foraging, the parasitoid used volatiles produced by cowpea flowers in response to damage by *M. vitrata* caterpillars. It discriminated between volatiles from caterpillar-damaged flowers and those produced by uninfested or mechanically damaged flowers. Learning host plant odours influenced the host-foraging decisions of *A. taragamae.*

The endpoint of host selection is host acceptance or suitability. For koinobiont parasitoids such as *A. taragamae,* growth of hosts after parasitization relies on the nutritional quality of plant materials consumed. The suitability of four key host plants of *M. vitrata* to the development of larvae parasitized by *A. taragamae* was assessed (**chapter 4**). When reared on flowers of *Vigna unguiculata, Sesbania rostrata, Lonchocarpus sericeus,* or *Pterocarpus santalionoides,* the growth of parasitized larvae was slower than when they were reared on artificial diet. The survival rate of larvae varied between host plant species. The proportion of female progeny also varied between feeding substrates and was lowest on *L. sericeus*. Only the daily fecundity did not show any differences between host plants. The parabolic trend of the daily fecundity observed on most of the feeding substrates pointed at a pro-ovigenic oviposition pattern of *A. taragamae.* The infection by *Maruca vitrata* Multi-NucleoPolyhedroVirus (*Mavi*MNPV) influenced in a negative way the development of *A. taragamae* on most host plants. However, temperature reduced this detrimental effect of *Mavi*MNPV through the reduction of larval development time. Thus, at 29 °C the development time of parasitized larvae was on average 2 days shorter than at 25 °C and subsequently the mortality rate of *Mavi*MNPV-infected larvae was reduced.

The non-target effects of *A. taragamae* were evaluated with regard to environmental risks. Experiments were performed to determine the host specificity and competitive ability of *A. taragamae* (**chapter 5**). The parasitoid did not successfully

parasitize any of the following lepidopteran species: *Eldana saccharina* Walker*, Chilo partellus* (Swinhoe)*, Mussidia nigrivenella* Ragonot, *Cryptophlebia leucotreta* (Meyrick), *Sylepta derogata* Fabricius*,* and *Corcyra cephalonica* Stainton. *Apanteles taragamae* appears to be specific to *M. vitrata* in Benin. In a no-choice test, *A. taragamae* outcompeted the egg-larval parasitoid *Phanerotoma leucobasis.* Another additional attribute of *A. taragamae* investigated during this work is the host-feeding behaviour of ovipositing females. There were no significant differences between the percentage parasitism obtained when females of *A. taragamae* were 24 h-starved and when they were fed with honey. Also the survival rate of larvae parasitized by 24 h-starved females did not differ significantly from that obtained when they were parasitized by honey-fed females. These observations suggest a non-concurrent and non-destructive feeding pattern of ovipositing females of *A. taragamae.*

Overall, the above biological features displayed by *A. taragamae* indicate that it is a potentially suitable classical biological control agent of *M. vitrata.* The parasitoid was released in seven selected locations in Benin using an elaborated deployment strategy (**chapter 6**). This strategy was based on the dispersal of *A. taragamae* adults from pupae field cages. These pupae were obtained by artificially infesting *Sesbania cannabina* plants with *M. vitrata,* and inoculated with adults of *A. taragamae* in cages. Sampling during the first release season did not yet reveal the establishment of *A. taragamae* after the first generation of released wasps. Therefore, a major future challenge should be to optimize the establishment of the parasitoid through repeated releases followed by intensive recovery studies.

By evaluating the biological potential of *A. taragamae,* the work described in the present thesis contributes to studying the feasibility of the first classical biological programme against the cowpea pod borer *M. vitrata.* The success of this programme would make biological control a viable alternative for a more sustainable cowpea production in Africa.

Samenvatting

Apanteles taragamae Viereck is een sluipwesp die in Benin is geïntroduceerd door het International Institute of Tropical Agriculture (IITA) voor de biologische bestrijding van de ogenboon peulenbooder *Maruca vitrata* Fabricius. Het onderzoek dat is beschreven in dit proefschrift had als doel het evalueren van de potentie van deze sluipwesp als natuurlijke vijand van *M. vitrata*. Hierbij zijn met name de belangrijkste selectiecriteria voor een natuurlijke vijand bestudeerd, zoals reproductieve capaciteit, functionele respons, vermogen tot aanpassing aan klimaatsomstandigheden, efficiëntie van gastheerzoeken, en neveneffecten op niet-doel-organismen.

In eerste instatie werd de status van *M. vitrata* als plaaginsect onderzocht en verschillende eerder ontwikkelde bestrijdingsmethoden zoals chemische bestrijding, kweekmaatregelen en het gebruik van resistente gewasvariëteiten, op een rij gezet (**hoofdstuk 1**). Chemische bestrijding is tot dusver de meeste gebruikte vorm van bestrijding van *M. vitrata*, terwijl er bij de andere methoden altijd sprake is van extra inzet van chemische middelen. Dit heeft geleid tot overmatig gebruik van chemische stoffen met neveneffecten zoals resistentie tegen pesticiden, wederom uitbreken van een plaag, milieuvervuiling en humane gezondheidsproblemen. Het is daarom noodzakelijk om andere milieuvriendelijke vormen van bestrijding, met name de (klassieke) biologische bestrijding, te onerzoeken. Het eerste effectieve klassieke biologische bestrijdingsproject tegen *M. vitrata* is in 2005 begonnen met het introduceren van *A. taragamae* uit Taiwan in Benin. De eerste stappen voor een klassiek biologisch bestrijdingsproject werden genomen (**hoofdstuk 1**) en een kweek van de sluipwesp werd opgezet op het onderzoeksstation van het IITA in Benin. Dit promotieonderzoek had als doel het vergaren van belangrijke kennis van de biologie en ecologie van *A. taragamae,* als eerste vereiste voor het gebruik van deze sluipwesp in de biologische bestrijding van *M. vitrata*.

Verschillende experimenten werden uitgevoerd om de geschikte gastheerstadia en de functionele respons van *A. taragamae* te bepalen, en de invloed van temperatuur op de ontwikkeling van de sluipwesp (**hoofdstuk 2**). Twee van de vijf larvale stadia van *M. vitrata* werden succesvol geparasiteerd door *A. taragamae.* Het hoogste percentage parasitering, het hoogste percentage vrouwelijke nakomelingen en het hoogste aantal nakomelingen werd verkregen als twee-dagen-oude rupsen werden geparasiteerd. Er was een positieve correlatie tussen het percentage parasitering van twee-dagen-oude rupsen en de gastheerdichtheid. Dit laatste duidt op een goede functionele respons. Bij

vijf verschillende temperaturen, allemaal gemiddelde waarden voor Benin, presteerde *A. taragamae* beter dan het plaaginsect *M. vitrata.* De thermische constante van *A. taragamae* (153 graaddagen) was lager dan die van *M. vitrata* (355 graaddagen). Dit wijst op een snellere ontwikkeling van de sluipwesp dan die van de gastheer. Tussen de 20 en 30 °C lag de curve die de relatie beschrijft tussen de temperatuur en de intrinsieke populatiegroensnelheid van *A. taragamae*, boven de curve van *M. vitrata*. Dit duidt op het vermogen van de sluipwesp om sneller in aantal toe te nemen dan de gastheer. Al met al is de reproductieve capaciteit van de natuurlijke vijand dus groter dan die van het plaaginsect. Andere biologische kenmerken zoals het vermogen om gastheren te vinden en te parasiteren moet echter ook in aanmerking genomen worden bij het evalueren van een natuurlijke vijand.

Het gastheerzoekgedrag van *A. taragamae* werd onderzocht in een Y-buis olfactometer (**hoofdstuk 3**). Tijdens het zoeken gebruikte de sluipwesp vluchtige stoffen die door bloemen van ogenenboon worden geproduceerd na schade veroorzaakt door vraat van *M. vitrata* rupsen. De wesp maakte onderscheid tussen de geur van door rupsen beschadige bloemen, onbeschadigde en mechanisch beschadigde bloemen. Het vermogen tot leren van plantengeuren bevorderde het vinden van gastheren door *A. taragamae.*

Het accepteren van een gastheer door een sluipwesp wordt uiteindelijk bepaald door de geschiktheid van de gastheer. Voor sluipwespen waarvan geparasiteerde gastheren nog een tijdje blijven leven, zoals in het geval van *A. taragamae*, hangt de groei van de gastheer na parasitering af van de kwaliteit van het plantenmateriaal dat de gastheer gebruikt als voedselbron. De geschiktheid van de vier belangrijkste voedselplanten van *M. vitrata* voor de ontwikkeling van door *A. taragamae* geparasiteerde rupsen werd bepaald (**hoofdstuk 4)**. Als de rupsen werden gekweekt op bloemen van *Vigna unguiculata, Sesbania rostrata, Lonchocarpus sericeus* en *Pterocarpus santalionoides,* was de groei van geparasiteerde rupsen trager dan die van rupsen gekweekt op kunstmatig dieet. Het overlevingspercentage van rupsen verschilde tussen de plantensoorten. Het percentage vrouwelijke nakomelingen variëerde ook tussen voedingssubstraten en was het laagst op *L. sericeus*. Alleen het dagelijkse aantal nakomelingen verschilde niet tussen de plantensoorten. Het verloop van het dagelijkse aantal nakomelingen over de tijd op de meeste voedingssubstraten duidde op een pro-

ovigeen ovipositiepatroon van *A. taragamae.* Een infectie van de rupsen met het *Maruca vitrata* Multi-NucleoPolyhedroVirus (*Mavi*MNPV) had een negatief effect op de ontwikkeling van *A. taragamae* op de meeste plantensoorten. Temperatuur verminderde echter dit negatieve effect van *Mavi*MNPV door de ontwikkelingsduur van de rupsen te verkorten. Bij 29 °C was de ontwikkelingsduur van geparasiteerde rupsen gemiddeld twee dagen korter dan bij 25 °C. Deze kortere intwikkelingsduur resulteerde vervolgens in het verlagen van het sterftepercentage van rupsen die waren geinfecteerd met het *Mavi*MNPV.

De neveneffecten van *A. taragamae*, met name op andere organismen, werden bestudeerd. Experimenten werden uitgevoerd om de gastheerspecificiteit en concurrentiekracht van *A. taragamae* te bepalen (**hoofdstuk 5**). De sluipwesp kon geen van de volgende lepidoptera soorten succesvol parasiteren: *Eldana saccharina* Walker, *Chilo partellus* (Swinhoe), *Mussidia nigrivenella* Ragonot, *Cryptophlebia leucotreta* (Meyrick), *Sylepta derogata* Fabricius en *Corcyra cephalonica* Stainton. *Apanteles taragamae* lijkt in Benin alleen *M. vitrata* te kunnen parasiteren. In een experiment waarin de wespen geen keuze werd aangeboden, concurreerde *A. taragamae* de ei-larve sluipwesp *Phanerotoma leucobasis* weg. Vervolgens werd onderzocht of de parasiterende *A. taragamae* vrouwtjes zelf gastheervoedingsgedrag vertoonden. Er was geen significant verschil in het percentage parasitering tussen *A. taragamae* vrouwtjes die 24 uur niet gevoed waren en vrouwtjes die wel waren gevoed met honing. Ook het percentage overleving van rupsen die waren geparasiteerd door deze twee groepen sluipwespenvrouwtjes verschilde niet. Deze observaties suggereren dat het gastheervoeden door *A. taragamae* niet destructief is.

Al met al wezen de onderzochte biologische kenmerken van *A. taragamae* er op dat deze sluipwesp in potentie een geschikte natuurlijke vijand voor de biologische bestrijding van *M. vitrata* is. De sluipwep werd daarom buiten het laboratorium losgelaten op zeven geselecteerde locaties in Benin waarbij een zogenaamde “deployment” strategie werd gebruikt (**hoofdstuk 6**). Deze strategie was gebaseerd op de verspreiding van volwassen *A. taragamae* wespen uit poppen in kooitjes. Deze poppen waren verkregen door *Sesbania cannabina* planten kunstmatig met *M. vitrata* rupsen te infecteren, en de rupsen vervolgens door volwassen *A. taragamae* wespen te laten parasiteren. Uitgebreide bemonstering in het eerste veldseizoen wees echter niet op een

succesvolle vestiging van *A. taragamae* na de eerste generatie van losgelaten wespen. De belangrijkste uitdaging in de toekomst blijft dus het optimaliseren van de vestiging van de sluipwesp door herhaaldelijk loslaten in het veld, gevolgd door een uitgebreide bemonstering.

Door het evalueren van de potentie van *A. taragamae* als natuurlijke vijand in de biologische bestrijding, draagt dit proefschrift bij aan het onderzoek naar de haalbaarheid van het eerste klassieke biologische bestrijdingsproject van de ogenboon peulenboorder *M. vitrata.* Het succes van dit project kan er voor zorgen dat biologische bestrijding een levensvatbaar alternatief voor een duurzamere productie van ogenboon in Afrika wordt.

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Curriculum vitae

Elie Ayitondji Dannon was born in Houeke (Laïnta), a small village of Cove (South Benin) in 1970. He attended Laïnta-Cogbe Primary School (1976 to 1980) and joined the Secondary School of Cove (1981 to 1985) and the Protestant College of Cotonou (1985 to 1988) for ordinary and advanced secondary education, respectively. He graduated at Faculté des Sciences Agronomiques (FSA) of Université d'Abomey-Calavi (UAC) in 1995 as Agricultural Engineer majoring in plant protection "with distinction". After a brief training at the International Institute of Tropical Agriculture (IITA), Benin station in 1995, he worked as research assistant at FSA/UAC from 1996 to 2001. In between he was involved in research training in 1997 at IITA, Benin station. In 2001, he was awarded a "Deustcher Akademischer Austausch Dienst" (DAAD) scholarship to pursue his postgraduate studies in Agricultural Sciences at Georg-August University of Goettingen (Germany). After his Master of Sciences (MSc) in 2003, he returned to (FSA)/ (UAC) as research assistant and worked in a collaborative project with the Agricultural and Food Technology Programme of INRAB (Benin National Institute of Agricultural Research). In 2007, he has got the opportunity to start his PhD thesis at Wageningen University with a grant from NUFFIC/NFP. The PhD research project focused on the biological potential of the parasitoid wasp *Apanteles taragamae* to control the cowpea pod borer *Maruca vitrata.* This project was successfully carried out at the Laboratory of Entomology/Wageningen University in collaboration with IITA, Benin and ended with some exciting findings reported in the present thesis.

Publications

1 Peer reviewed publications

Dannon EA, Tamò M, van Huis A, Dicke M (2010) Functional response and life history parameters of *Apanteles taragamae,* a larval parasitoid of *Maruca vitrata.* BioControl 55: 363-378.

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Dannon EA, Tamò M, van Huis A, Dicke M. Assessing non-target effects and host feeding of the exotic parasitoid *Apanteles taragamae,* a potential biological control agent of the cowpea pod borer *Maruca vitrata.* Submitted to BioControl.

Atachi P, **Dannon EA**, Rurema DG (2006) Seed damaging field pests in an intercropping of pigeonpea (*Cajanus cajan* L. Millsp) in pest management of cowpea (*Vigna unguiculata* L. Walp.) in Southern Benin: Dynamics of seed attack. Annales des Sciences Agronomiques du Benin 8:197-218.

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probabilities in differn patterns of cowpea-pigeonpea intercropping in South Benin. Bulletin de la Société Zologique de la France 124: 239-260.

2 Other scientific publications

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Education statement

PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)

Towards the biological control of *Maruca Vitrata* a key pest of cowpea using *Apanteles taragamae*: main steps and required studies prior to field release (2007)

Writing of project proposal (4.5 ECTS)

Biology and ecology of *Apanteles taragamae* Viereck (Hymenoptera: Braconidae), a larval parasitoid of *Maruca vitrata* Fabricius (Lepodoptera: Crambidae), a key pest of cowpea, *Vigna unguiculata* (L.) Walp (2007)

Post-graduate courses (3.6 ECTS)

Advanced statistics; PE&RC (2007)

Basic statistics; PE&RC (2007)

Parasitoid taxonomy and ecology; PE&RC (2010)

Laboratory training and working visits (7 ECTS)

Training on rearing of *Maruca vitrata*, *Apanteles taragamae* preparation of artificial diet; International Institute Tropical Agriculture (IITA) Benin station (2007)

Deficiency, refresh, brush-up courses (6 ECTS)

Insect-Plant Interactions; ENTO, Wageningen (2007)

Competence strengthening / skills courses (2.1 ECTS)

Information Literacy including introduction; WGS (2011)

Writing of scientific projects; FSA/UAC/Benin (2008)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.5 ECTS)

PE&RC Weekend (2011)

The Netherlands Annual Ecological Meeting; Congresscentrum, De Werelt, Lunteren, the Netherlands (2011)

Discussion groups / local seminars / other scientific meetings (10.3 ECTS)

Biocontrol and campus seminars at IITA, Benin (2007-2010)

Insect-Plant Interactions Discussions; Laboratory of Entomology, Wageningen University (2007, 2010-2011)

PhD Discussion; Laboratory of Entomology (2007, 2010-2011)

Annual Meeting of the Netherlands Entomological Society; De Reehorst, Ede, the Netherlands (2010)

International symposia, workshops and conferences (4.1 ECTS)

5th Insect-Plant Interaction Workshop; Wageningen, the Netherlands (2010)

14th International Symposium on Insect-Plant Interactions; Wageningen, the Netherlands (2011)

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