

**Inter- and intraspecific variation of nutritional and environmental adaptation
of egg-parasitoids of the genus *Trichogramma*
(Hymenoptera, Trichogrammatidae)**

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Affectionately dedicated to:

My Mother & the soul of my Father

My wife

AND

My children

(Mohamad, Saja & Yamen)

Declaration

I declare that the thesis hereby submitted for the Ph. D. degree, is my own original research work and has not been previously submitted by me at another University for any degree. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due references to the literature, and acknowledgment of collaborative research and discussions. The work was done under the guidance of Professor Dr. Dr. C. P. W. Zebitz, at the Phytomedicine Institute of Agricultural Faculty of Hohenheim University.

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List of abbreviations

List of abbreviations

ANOVA	analysis of variance
Bt	<i>Bacillus thuringiensis</i>
°C	temperature in degree Celsius
ca.	<i>circa</i>
CpGV	<i>Cydia pomonella</i> granulovirus
cv.	cultivar
d	day
DDT	Dichlordiphenyltrichlorethan
e.g.	for example
et al.	<i>et alii</i>
Fig.	figure
h	hour
l	liter
L:D	photoperiod indication
IGRs	insect growth regulators
m	minute
mg	milligram = 10^{-3} g
µg	microgram = 10^{-6}
R ₀	net reproductive rate
r_m	intrinsic rate of natural increase
r.h.	relative humidity
s	second
s.d.	standard deviation
S.E.	standard error

List of abbreviations

SIT	sterile insect technique
sp.	species (singular)
spp.	species (plural)
T_c	mean cohort generation time
λ	finite rate of increase

CHAPTER I

GENERAL INTRODUCTION

General introduction

1. General introduction

1.1. Life history and distribution of the Codling moth

The Codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) is the most serious pest of apple and pear worldwide. When apple orchards are not protected, up to 95 percent fruit damage can occur only due to infestation by the Codling moth. The Codling moth occurs on all continents where apple and pear are grown, with a distribution from Europe, Asia, North and South Africa to Australia (Fig. 1). Also, *C. pomonella* is found as key pest in other fruit crops such as peach, plum, quince and walnut (Barnes, 1991; Hoyt et al., 1983; Quarles, 2000; van Frankenhuyzen and Stigter, 2002).

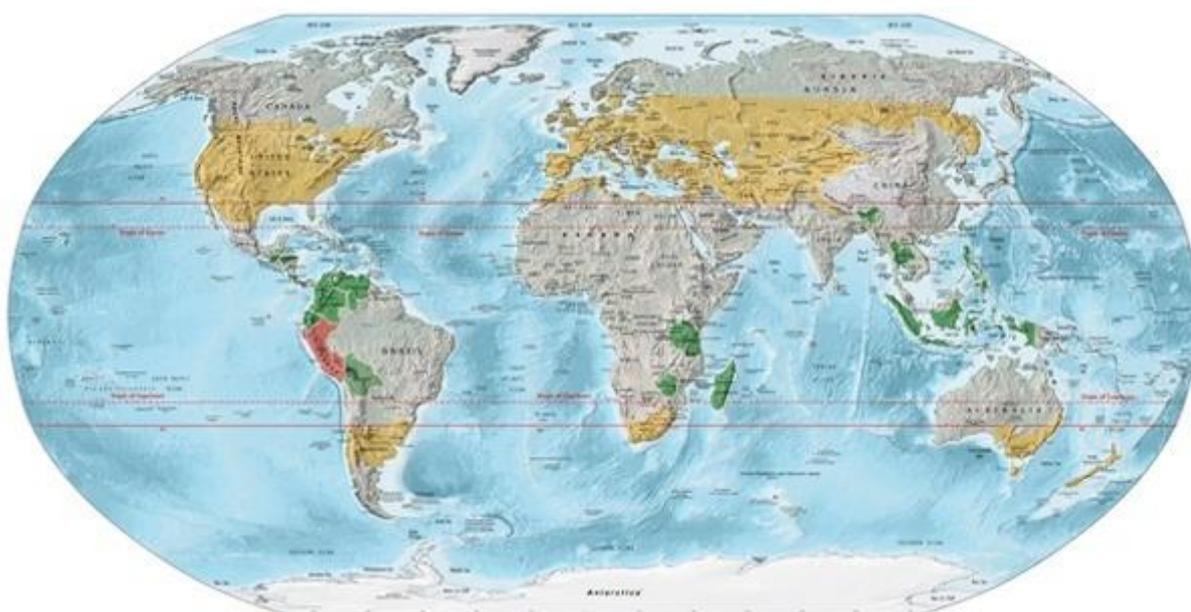


Figure 1. Geographical distribution of the Codling moth, *Cydia pomonella*. (Map Color Key: Yellow = distribution of Codling moth and its host plants, green = distribution of host plants, but presence of Codling moth not confirmed, red = distribution of host plants, Codling moth reported but confirmation is pending.

(http://entomology.tfrec.wsu.edu/Cullage_Site/CM_Map.html)

The Codling moth has one to four generations per year depending on the different geographic conditions (Bleton, 1938; El-Gamil et al., 1977; Shel'deshova, 1967), where temperature is considered as the determining factor for the number of generations per year (Croft and Hoyt, 1983). In southwestern Germany for example, the Codling moth hibernates

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as mature larvae in a silken cocoon under loose bark in protected areas at the base of the tree, but also in fruit bins, orchard trash piles, brush, wooden or bamboo posts, in soil cracks, and later in harvesting crates (Barnett et al., 1991). Larvae develop to pupal stage when spring temperatures exceed 10 °C. Pupation occurs inside silken cocoons of brownish colour of ca. 13 mm length. Due to sexual dimorphism of the pupae, sex determination in the pupal stage is possible. First flight of adults in the vegetation period usually coincides with end of petal fall from apple blossoms (BBCH 67 - 96). The peak of adult emergence occurs 4 to 12 days later, depending on weather conditions (Anonymous, 2000). These moths begin deposition of single eggs or egg clusters on the leaves. Each female deposits ca. 50 to 60 eggs (Almatni, 2003). The egg is tiny, 1.2 mm in diameter, whitish to transparent, and flattened disc-shaped. First instar larvae hatch after 8 - 14 days after oviposition (Anonymous, 2000). The newly hatched larvae immediately begin crawling to search for a fruit to feed upon (Barnett et al., 1991). After a first nibbling on the apple fruit peel, larvae bore into the fruit and feed as a fruit miner until maturity for 2 to 4 weeks. Mature larvae leave the fruit and pupate in protected places. A part of these pupae hibernate until the next year and the other, not quantified yet, emerge as adults after 8 to 20 days after pupation, around early June. Females of this second generation oviposit directly on apple fruits and the hatched larvae enter the fruit with continuous feeding inside the apple fruits. This direct damage is considered the most important from economical aspects. Mature larvae of this generation then hibernate in remote, protected places, as mentioned above. A third generation may show up in Middle Europe in very warm years in September, however not entering larval maturity to hibernate.

1.2. Codling moth control

1.2.1. Cultural control

Cultural control and hygiene relies on sanitation and removal of potential hibernation sites of the Codling moth to reduce pest population density and infestation pressure. Although it seems to be simple, any cultural control has not been quantified and efficacy has not been proved, requiring more research in a holistic pest control approach. Removing hibernation sites or hibernating Codling moth may be effective and be integrated with other methods, since the 96 percent reduction in number of overwintering Codling moth larvae has been observed in the Wenas valley in 1970 (Butt et al., 1973).

1.2.2. Chemical control

Since the 1960s, chemical control has been based on the use of broad-spectrum insecticides, such as organophosphates, carbamates, and to a limited extent, synthetic pyrethroids, replacing soon the even more dangerous DDT, which has been used until the early 1970s (Brader, 1977; Croft and Hoyt, 1983; Fisher, 1960; Madsen et al., 1970; Oatman and Libby, 1965; Varela et al., 1993). The number of worldwide references on the Codling moth exceeds 3,600 articles, among which papers on chemical control dominate. The sometimes highly toxic insecticides provided effective control of Codling moth and other pests but they have had the disadvantage of unwanted side effects on natural enemies, such as predators and parasitoids. Furthermore, the residual effect of the insecticides on the environment, including low crop yield, destruction of soil micro-fauna and flora, and undesirable residue accumulation in food crops (Edwards, 1986), in addition to pollution of air, soil, groundwater and surface waters, and pesticide resistance lead to a complete turn in Codling moth control towards environmentally safe methods (Cossentine and Vincent, 2002). More recently, the chemical industry has developed effective insect growth regulators (IGRs), affecting chitin synthesis as well as hormonal regulation of insect development which combine high toxicity to Codling moth with safety to many important beneficial species (Mulder and Gijswijt, 1973), and these compounds have shown high efficacy in controlling this pest (Hiemstra et al., 1999).

1.2.3. Mating disruption

The application of the mating disruption technique by using sex pheromones has been investigated as a selective control for Codling moth since the early 1970s (Cardé and Minks, 1995), but it was not until the early 1990s that reliable commercial products were available to growers in the world. The most important application technique for lepidopteran species is to permeate the atmosphere with synthetic pheromone, and to thereby prevent olfactory communication and mate finding. Successful, area-wide applications of the mating disruption technique in Europe concern three lepidopteran insects from horticultural crops, Codling moth *C. pomonella*, and the grape berry moths *Eupoecilia ambiguella* (Hb.) and *Lobesia botrana* (Schiff.) (Ridgway et al., 1990; Witzgall and Arn, 1990; Witzgall et al., 1997, 2008).

Despite that this technique has many positive aspects, such as ease of application and being less expensive than other methods in areas with low Codling moth density, also it has

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negative aspects as less effective in orchards with dense Codling moth populations, less effective in small orchards and in areas with untreated adjacent orchards, and it is not effective in orchards with a steep inclination and have uneven canopies. This technique also requires excellent mitigation procedures for optimal application (Barnes et al., 1992; Knight, 2006; Moffitt and Westigard, 1984).

1.2.4. Biological control

The management of Codling moth based on control by both native and introduced natural enemies has proven elusive, significant reductions in population densities (Blommers, 1994; Glen, 1982). General predators such as birds, predaceous insects, and spiders have been reported as suppressive agents of Codling moth; these include woodpeckers, carabid beetles, and mirid bugs (Dondale et al., 1979; Huber, 1980, 1986). More than 100 parasitoid species have been recorded from Codling moth (Lloyd, 1958). *Trichomma enecator* (Rossi) and *Pristomerus vulnerator* (Panzer) (Hymenoptera: Ichneumonidae) and *Elodia tragica* (Meigen) and *E. morio* (Fall.) (Diptera: Tachinidae) all are koinobiont parasitoids of early-mid stage larvae, killing and emerging from the cocooned prepupae (Mills, 2005). In addition to these larval parasitoids, also egg parasitoids contribute to pest control. Besides *Ascogaster quadridentata* (Wesmael) (Hymenoptera: Braconidae) (Brown and Kainoh, 1992), nine (Lloyd, 1958) or more (as assumed by Pinto et al. 2002) *Trichogramma* species are considered the most effective antagonists.

Although Codling moth is susceptible to several diseases, commercially available bacteria (*Bacillus thuringiensis* (Berliner)) (abbreviated as Bt), fungi (*Spicaria farinosa* (Holm ex S. F. Gray), *Lecanicillium (Verticillium) lecanii* (Zimm.) Zare and W. Gams and *Fusarium oxysporum* (Schlecht.)), *C. pomonella* granulovirus (CpGV) (Baculoviridae), and nematodes (Mermithidae and Steinernematidae) can cause significant reductions in Codling moth densities (Andermatt et al., 1988; Dutky, 1959; Poinar, 1991; Tanada, 1964).

1.2.4.1. Sterile insect technique

Since the 1950s, it is known that insect pests can be controlled through a ‘birth control’ method based on genetic manipulation and known as autocidal pest control or the SIT (Knipling, 1955). It involves the colonization and mass-rearing of the target pest species, sterilization by exposure of the pupae to X-ray or gamma (γ) –irradiation, or exposure to high

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or low temperature (Bloem et al., 1999). The sustained release into the target area in sufficient numbers to achieve appropriate sterile to wild insect ratios, inundating the wild, fertile males with sterile ones. After release, the sterile males will locate and mate with wild females and transfer the sperm with the dominant lethal mutations. The sperm can fertilize the eggs, but because of the dominant lethal mutations, embryogenesis is arrested resulting in no offspring. SIT technique was widely used to control this pest in many countries like Canada (Proverbs and Newton, 1966), USA (Butt et al., 1970), Western Europe (Wildbolz and Mani, 1975), Australia and New Zealand (Suckling, 2003). Except in very rare cases, SIT and inherited sterility (IS) approaches to control Codling moth was not sufficiently successful yet and needs improvement (Vreysen et al., 2010).

1.2.4.2. Bacteria, viruses and nematodes

Bacteria *Bacillus thuringiensis* (Bt) is the most widely used entomopathogenic bacterium to control lepidopterous pests (Lacey et al., 2001). Although Codling moth larvae are susceptible to crystalline proteins (Cry), which are toxic to many insect taxa (Andermatt et al., 1988; Rang et al., 2000), its significance as a control agent of Codling moth is very limited by the improbability of ingesting a lethal dose of Bt toxin until entering the fruit by neonate larvae, because the larvae take up only very few Bt-contaminated surface material and mainly consume the fruit flesh which is not contaminated with Bt (Almatni and Jamal, 2003).

Fungi One of the important species of fungi attacking the Codling moth is *Beauveria bassiana* ((Bals.-Criv.) Vuill.), particularly during hibernation (Cross et al., 1999; Falcon and Huber, 1991; Garcia-Gutierrez et al., 2004). Ferron (1978) and Falcon and Huber (1991) reported that *B. bassiana* is most successful when combined with chemical pesticides.

Viruses One of the most efficient and highly selective pathogens of Codling moth is the *Cydia pomonella* granulovirus (*CpGV*). Its specificity to the Codling moth and very few closely related species, and its safety to non-target organisms are well documented (Falcon et al., 1968; Gröner, 1986, 1990; Lacey et al., 2005) and contribute to the conservation of other natural enemies in the orchard agroecosystem. However, since *C. pomonella* could develop resistance against *CpGV*, which had been broken by a new *CpGV* isolate, additional control methods are required to for a future resistance management (Asser et al., 2007; Fritsch et al., 2005; Kienzle et al., 2008; Zingg, 2008).

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Nematodes The most vulnerable developmental stage of the Codling moth to entomopathogenous nematodes is the cocooned overwintering larva. The significant reduction of hibernating larvae could provide complete or substantial protection of fruits as the initial population (1st generation) in the following growing is reduced by 60 %, but needs further optimisation (Dutky, 1959; Dutky and Hough, 1955; Kienzle et al., 2008, 2010; Weiser, 1955).

1.2.4.3. Predators

Birds are the most apparent predators of the Codling moth, such as woodpeckers (Picidae) or crows (Corvidae) will attack large larvae, either walking to or already in their cocooning sites (Glen and Milsom, 1978; Lawrence et al., 2005). Spiders and mites, and also some beetles, as from the family Carabidae, which dominate the epigeal zone around the tree base where Codling moth cocoons are likely to be found, may play a minor role in Codling moth control (Basedow and Dickler, 1981; Glen and Milsom, 1978; Riddick and Mills, 1994) and can, in addition to other insects from different orders such as Neuroptera, Thysanura, and Heteroptera, attack the Codling moth, but do not contribute to a substantial reduction of population density (Jaynes and Marucci, 1947; Glen, 1982).

1.2.4.4. Parasitoids

A diverse complex and several types of parasitoids (*e.g.* egg parasitoids, larval parasitoids, pupal parasitoids and larval-pupal parasitoids) have been recorded attacking all life stages of the Codling moth with the exception the adult stage (Athanasov et al., 1997; Rosenberg, 1934; Glen, 1982). Among the larval parasitoids *Trichomma enecator* (Rossi), *Microdus rufipes* (Nees), *Ascogaster quadridentata* (Wesmael), *Microdus conspicuous* (Wesmael), *Cryptus sexmaculatus* (Gravenhorst), *Elodia tragica* (Meigen), *Mastrus ridibundus* (Gravenhorst), and *Ephialtes caudatus* (Ratzeburg) are estimated to be the most important species (Bezemer and Mills, 2001, 2003; Darcy and John, 1990; Simmonds, 1944; Turnbull and Chant, 1961). Moreover, also the larval-pupal parasitoid *Pristomerus vulnerator* (Panz) has been reported to suppress effectively Codling moth populations (Athanasov et al., 1997).

One of the more thoroughly studied natural enemies are the egg parasitoid species of the genus *Trichogramma*, which have been employed in biological control programs of fruit pests

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the pests. Since these species can be mass-reared and used in augmentative inundative control, they are considered the most important direct biocontrol agents.

1.3. *Trichogramma* spp.

1.3.1. Importance of *Trichogramma* as biological control agent

Among the order of Hymenoptera the family Trichogrammatidae represent the most important egg parasitoids. *Trichogramma* species are reported to attack eggs of more than 200 lepidopterous host species, including the pest groups of borers, webworms, loopers, leafworms, fruitworms, cutworms, bollworms and armyworms (Knutson, 1998). Therefore it was used more than any other entomophagous species in inundative and inoculative biological control programs in more than 30 countries in field and fruit crops (*e.g.* corn, cotton, sugarcane, fruit trees, vegetables), and forests (Hase, 1925; King et al., 1985; Li, 1994; Smith, 1996; Stinner, 1977; Sweetman, 1958; van Lenteren, 2000). Hassan (1997) and van Lenteren (2000) reported that over 16 million ha receive parasitoid inundative releases. But it is very difficult to estimate precisely the correct size of the area in which egg parasitoids are released every year. There is a continuous increase in the number of countries in which biofactories are being established for the mass production and release of egg parasitoids, as in Europe, the Americas, and Asia (van Lenteren and Bueno, 2003). However, the statistics and reports on biocontrol and mass-rearing activities are not always available or reliable in many countries as in (former) socialist countries *e.g.* Russia, China, or Cuba. Therefore, estimates vary from author to author, but Smith (1996) estimated the use of *Trichogramma* spp. for biocontrol of insect pests on 32 million ha.

1.3.2. Biology of *Trichogramma*

Trichogramma species are among the smallest parasitic wasps (0.2–1.5 mm, and 8 µg in weight), but despite their minute size have been the subjects of more studies than any other parasitoid genus (Silvia et al., 1999).

1.3.2.1. Host acquisition

Successful acquisition and parasitisation of a host egg can be distinguished into four different steps: host habitat location, host location, host acceptance and host suitability testing, (Flanders, 1937; Salt, 1935). Godfray (1994), Vet and Dicke (1992) and Vinson (1998)

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reported that the females of the parasitoids encounter and explore a great variety of chemical and physical stimuli during host location process. *Trichogramma* females search for their hosts following various environmental stimuli. Host habitat location is guided by general stimuli like colour and host plant chemicals (Nordlund, 1994; Romeis et al., 1998, 2005; Vinson, 2010). However, this step is affected also by the host-density in the field, the kind and the structure of the pests's foodplants, and the distance between the plants, as well as by abiotic environmental factors (Laing, 1937).

The exact host egg location is stimulated (i) *directly* by using volatile kairomones originating from the host eggs, or (ii) *indirectly* by host sex-pheromones, and /or host scales and other adult traces, and /or synomones induced from the host plants after host egg deposition, or by host (adult or larvae) feeding activity (Colazza et al., 2010). Following the host finding process, host suitability is the next step (Pak et al., 1990). This process is affected by mechanical, chemical and physiological factors, which allow distinction between host-egg age, chorion resistance during drilling with the ovipositor and results in egg preferences (Quednau, 1955). After finding a host egg, a *Trichogramma* female examines the host surface, walking back and forth and turns around and around on it and drumming with her antennae for 10–40 seconds, where the duration of examination depends on the curvature of the egg surface (Hajek, 2004; Salt, 1935). The female can also detect marker pheromones deposited when other *Trichogramma* females have been walking on the egg and ovipositing to avoid superparasitization (Hajek, 2004). If an egg had been accepted up to this behavioural step, the female begins to drill with her ovipositor and, with some experience, she can detect whether parasitoid eggs are already parasitized or not (Vinson, 1976). If a female does not find unparasitized host eggs within a certain time span, *Trichogramma* females either will give up searching or they will oviposit into an already parasitized host egg (Pak et al., 1986).

These behavioural steps can be used to distinguish the performance of *Trichogramma* species or strains to describe inter- and intraspecific variability. For example, Pak (1988) found that the acceptance of *Pieris brassicae* (L.) eggs varied among *Trichogramma* strains, while *Mamestra brassicae* (L.) eggs were promptly accepted by almost all the strains tested. Also, *T. aurosum* strains differed in both their preference for a host species and drilling time to penetrate the host eggs chorion (Samara et al., 2011).

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The number of *Trichogramma* eggs laid per host egg is determined by the size of the host egg, ranging from 1 to 4 *Trichogramma* eggs per host egg. A single *Trichogramma* female lay one to ten eggs per day, and total egg production per female may range from 10 to 190 eggs during adult life, depending on the respective species. Within a *Trichogramma* species / strain of a species, the total number of eggs produced depend on the body size of the respective female, which itself depends on biomass consumed during preimaginal development as well as from female nutrition as adult (Ruberson and Kring, 1993). Preimaginal development of *Trichogramma* species comprises three larval instars which develop within 3-4 days into the subsequent pupal stage. After 4-5 days as a pupa, the adults emerge from the pupae and escape from the host eggs by chewing a circular hole in the egg-shell (Strand, 1986). As a rough estimate, *Trichogramma* complete their lifecycle from egg to adult in 10 days at 25 ± 1 °C.

1.3.2.2. *Trichogramma* feeding

The larval stages of *Trichogramma* obtain sufficient nutrients from host egg yolk until they pupate stage and ultimately emerging as adults (Flanders, 1937; Volkoff et al., 1995).

Adult nutrition however, is necessary for post-emergence survival and affects adult longevity and parasitic performance. In the field, nectar and pollen are the most important food sources for predacious or parasitic insects, including also *Trichogramma* (Andow and Risch, 1987; Knutson, 1998; Wellinga and Wysoki, 1989). These nutrients enhance longevity (Shearer and Athanassov, 2004), fecundity (Shearer and Athanassov, 2004; Somchoudhury and Dutt, 1988), fertility and flight propensity of these wasps (Forsse et al., 1992). Any alternative or supplementary food provided from naturally-occurring sources in the field such as aphid honeydew, flower nectar and pollen offered in the field can improve the efficacy of augmentative biocontrol programs. In case of lacking natural supplementary food, artificial food sources can be provided as supplements either in the release container before, or applied as a foliage spray in conjunction with releases. Furthermore, the appropriate supplementary food can improve mass-rearing of *Trichogramma*.

1.3.3. Life table parameters

Life-table analysis is an appropriate tool to study the dynamics of animal populations, especially arthropods, providing very important demographic parameters (Maia et al., 2000). These parameters enable development of biological control programmes (Pratissoli and Parra, 2000), and may also be useful in constructing population models (Carey, 1993), and understanding interactions with insect pests and natural enemies (Omer et al., 1996). The cohort life-table gives the most comprehensive description of the survivorship, development and reproduction of a population that are fundamental factors in both theoretical and applied population ecology (Taghizadeh et al., 2008).

The parameters estimated from the life tables are: *the intrinsic rate of natural increase* (rm), used to measure the growth rate of a population per female (Pak and Oatman, 1982), *the net reproductive rate* (R_0), *the mean cohort generation time* (T_c) and *the finite rate of increase* (λ) (Maia et al., 2000; Nagarkatti and Nagaraja, 1978; Southwood, 1978).

1.3.4. Dispersal ability of *Trichogramma*

Trichogramma species are very minute in size; therefore they are believed to be very sensitive to climatic conditions (Keller et al., 1985). Qualitative observations have related *Trichogramma* flight and efficacy with good climatic conditions, namely warm and sunny weather, as opposed to cloudy, rainy and windy weather (van Steenburgh, 1934; Yu et al., 1984). The activity of *Trichogramma* spp. was greatly reduced when temperatures were below 17 °C (Smith, 1994). Also the relative humidities affected on the activity of the *Trichogramma* wasps, whereas the rate of emergence and longevity of *T. ostriniae* were reduced when the relative humidities were < 45% and > 95% (Zhang et al., 1983), which is likely to result in reduced parasitism of eggs in the field. In addition to the great effects of wind speed and direction on the dispersal of *Trichogramma* in the field (Allen and Gonzalez, 1974), Hendricks (1967) observed that the dispersal wasps of *T. semifumatum* (Perkins) shifted from regular to downwind skewed when wind speed exceeded 3 m/s but remained below 5.5 m/s (10.8-19.8 km/h).

Moreover there are several additional factors affecting the dispersal of *Trichogramma* wasps, among them; plant architecture (Smith, 1996), plant volatiles (Laing, 1937 and McCall et al., 1993), plants and leaf surface area (Ables et al., 1980). For example, the parasitisation

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rate of the European corn borer in the field decreased when the leaf areas of corn plants increased (Andow and Prokrym, 1991; Ables et al., 1980; Burbutis and Koepke, 1981). The density and location of the host also influence *Trichogramma* releases (Ables et al., 1980). Also the parasitoids of *Trichogramma* generally show either functional responses to host density with better parasitism seen in hosts that lay eggs in clusters rather than singly (van Lenteren et al., 1982).

1.3.5. Overwintering of *Trichogramma*

Diapause is a period of developmental arrest combined with adaptive physiological changes, with development resuming not necessarily on return of suitable conditions (Blum, 1985; Chapman, 1998). The diapause in *Trichogramma* species has already attracted high attention because these minute egg parasitoids are not only widely used for regular augmentative biological control but also represent an important component of natural biocoenoses (Boivin, 1994; Smith, 1996; Sorokina, 1987; Telenga, 1956). It was repeatedly shown that the low temperature is the most important environmental factor, acting on the larvae and inducing the pre-pupal diapause (Boivin, 1994; Garcia et al., 2002).

Later, it was reported that the diapause may depend on the photoperiodic conditions of the parental generation development, although this maternal influence could be revealed only under near-threshold temperatures (Boivin, 1994; Reznik et al., 2002; Reznik and Kats, 2004; Zaslavski and Umarova, 1990). There are also many factors affecting the wasps to induce the diapause as food quality and population density may play a role in diapause regulation.

1.4. Study objectives

Because of the efficacy of *Trichogramma* and their easy rearing on many factitious hosts, such as *Ephestia kuehniella* (Keller), *Sitotroga cerealella* (Oliver), *Plodia interpunctella* (Hubner), *Galleria mellonella* (Linnaeus), and *Corcyra cephalonica* (Stainton) (Ebrahimi, 2004; Smith, 1996), they are used worldwide against many serious pests in the world (Li, 1994; Smith, 1996). For example, in Germany many species of *Trichogramma* as: *T. embryophagum* (Htg.), *T. dendrolimi* (Masumura), *T. cacoeciae* (Marchal) and *T. principium* (Sugonyaev and Sorokina) are used to control the Codling moth (*C. pomonella*) (Hassan, 1988; Hassan, 2005; Schütte and Franz, 1961). However, most of the previous studies based on trial and error experiments (Hassan et al., 1994), therefore, the principal goals of this study

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were: first to choose the effective species of *Trichogramma* among many species were collected from different places, and second to improve and increase the efficacy of these wasps for use in the biological control program.

To study the impact of food on the biology (longevity, fecundity and fertility) of different species of *Trichogramma*, honey was selected as a main food source for many species of *Trichogramma* (chapter 2). Moreover, in chapter 2, different varieties of pollen also were chosen as additional and/or alternative food to the parasitoids to find out which kind of food is the best (honey alone, honey with pollen or pollen alone).

The response of different strains of *Trichogramma* to infochemicals during host location on different surfaces and effect the host plant surfaces on the behaviour of different strains of *Trichogramma* also studied (chapter 3).

In addition, to study the effect of low temperatures on the quality and biology of different species of *Trichogramma*, the diapause under outdoor conditions of these species was also investigated (chapter 4). In chapter 5 general discussion and conclusion were obtained from the data of all experiment mentioned in the thesis.

References

- Ables, J.R., McCommas, D.W. jr., Jones, S.L., Morrison, R.K., 1980. Effect of cotton plant size, host egg location, and location of parasite release on parasitism by *Trichogramma pretiosum*. *Southwestern Entomologist.*, 5, 261-265.
- Allen, J.C., Gonzalez, D., 1974. Spatial attack patterns of *Trichogramma pretiosum* around release sites compared with a random diffusion model. *Environmental Entomology* 3, 647-652.
- Almatni, W., Jamal, M., 2003. Primary field comparison for the efficacy of various bio- and growth-regulator insecticides against Codling moth, *Cydia pomonella* (L.), in apple orchards under southern Syrian conditions. *Arab Journal of Plant Protection* 21, 109-115.
- Almatni, W.S., 2003. Survey and study of natural enemies of Codling moth *Cydia pomonella* (L.) in As-Sweida and evaluation of some bio-agent measures. PhD-thesis, University of Damascus. 295 pp.

General introduction

- Andermatt, M., Mani, E., Wildbolz, T., Lüthy, P., 1988. Susceptibility of *Cydia pomonella* to *Bacillus thuringiensis* under laboratory and field conditions. *Entomologia Experimentalis et Applicata* 49, 291-295.
- Andow, D.A., Risch, S.J., 1987. Parasitism in diversified agroecosystems: Phenology of *Trichogramma minutum* (Hym.: Trichogrammatidae). *Entomophaga* 32, 255-260.
- Andow, D.A., Prokrym, D.R., 1991. Release density, efficiency and disappearance of *Trichogramma nubilale* for control of European corn borer. *Entomophaga* 36, 105-113.
- Anonymous, 2000. Codling moth on fruit trees. Ohio State University Extension Fact Sheet HYG-2203-92 (<http://ohioline.osu.edu/hyg-fact/2000/2203.html>). (10.07.2011)
- Asser-Kaiser, S., Fritsch, E., Undorf-Spahn, K., Kienzle, J., Eberle, K.E., Gund, N.A., Reineke, A., Zebitz, C.P.W., Heckel, D.G., Huber, J., Jehle, J.A. 2007. Rapid emergence of baculovirus resistance in Codling moth due to dominant, sex-linked inheritance. *Science* 317, 1916-1918.
- Athanassov, A., Charmillot, P.J., Jeanneret, P., Renard, D., 1997. The larval and pupal parasitoids of Codling moth *Cydia pomonella* L. *Revue Suisse de viticulture, Arboriculture, Horticulture* 29, 99-1067.
- Barnes, M.M., Millar, J.G., Kirsch, P.A., Hawks, D.C., 1992. Codling moth (Lep.: Tortricidae) control by dissemination of synthetic female sex pheromone. *Journal of Economic Entomology* 85, 1274-1277.
- Barnes, M.M., 1991. Codling moth occurrence, host race formation and damage. In: van der Geest, L.P.S., Evenhuis, H.H. (Eds.), *World Crop Pests*. pp. 313-327.
- Barnett, W.W., Bentley, W.J., Bethell, W.J., Pickel, C., Weddle, P.W., Zalom, F.G., 1991. Integrated pest management for apples and pears. University of California, Publication 3340, 68-89.
- Basedow, T., Dickler, E. 1981. Untersuchungen über die Laufkäfer in einer Obstanlage anhand von Boden- und Lichtfallenfangen (Col.: Carabidae). *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie* 3, 36-39.
- Bezemer, T.M., Mills, N.J., 2001. Host density responses of *Mastrus ridibundus*, a parasitoid of the Codling moth, *Cydia pomonella*. *Biological Control* 22, 169-175.

General introduction

- Bezemer, T.M., Mills, N.J., 2003. Clutch size decisions of a gregarious parasitoid under laboratory and field conditions. *Animal Behaviour* 66, 1119-1128.
- Bleton, C. 1938. Observations sur quelques lepidoptères parasites des peupliers dans la region de Fes (Maroc). *Revue de pathologie végétale et d'entomologie agricole de France* 25, 218-225.
- Bloem, S., Bloem, K.A., Carpenter, J.E., Calkins, C.O., 1999. Inherited sterility in Codling moth (Lepidoptera: Tortricidae): Effect of substerilizing doses of radiation on field competitiveness. *Environmental Entomology* 28, 669-674.
- Blommers, L.H.M., 1994. Integrated pest management in European apple orchards. *Annual Review of Entomology* 39, 213-241.
- Blum, M.S., 1985. *Fundamentals of insect physiology*. Wiley, New York, pp. 616.
- Boivin, G., 1994. Overwintering strategies of egg parasitoids, In: Wajnberg, E., Hassan, S.A. (Eds.), *Biological control with egg parasitoids*, pp. 219-244.
- Brader, L., 1977. Resistance in mites and insects affecting orchard crops. In: Watson, D.L., Brown, A.W.A. (Eds.), *Pesticide management and insecticide resistance*. Academic Press, New York, pp. 235-377.
- Brown, J.J., Kainoh, Y., 1992. Host castration by *Ascogaster* spp. (Hymenoptera: Braconidae). *Annals of the Entomological Society of America* 85, 67-71.
- Burbutis, P.P., Koepke, C.H., 1981. European corn borer control in peppers by *Trichogramma nubilale*. *Journal of Economic Entomology* 74, 246-247.
- Butt, B.A., Hathaway, D.O., White, L.D., Howell, J.F., 1970. Field releases of Codling moths sterilized by tepa and gamma irradiation. *Journal of Economic Entomology* 63, 912-915.
- Butt, B.A., White, L.D., Moffitt, H.R., Hathaway, D.O., Schoenleber, L.G., 1973. Integration of sanitation, insecticides, and sterile moth releases for suppression of populations of Codling moths in the Wenas valley of Washington. *Environmental Entomology* 2, 208-212.
- Cardé, R.T., Minks, A.K., 1995. Control of moth pests by mating disruption, successes and constraints. *Annual Review of Entomology* 94, 211-216.

General introduction

- Carey, J.R., 1993. Applied demography for biologists, with special emphasis on insects. Oxford University Press, U.K, pp. 211.
- Chapman, R.F., 1998. The Insects: Structure and function, 4th ed. Cambridge University Press, UK, pp. 770.
- Colazza, S., Peri, E., Salerno, G., Conti, E., 2010. Host searching by egg parasitoids: Exploitation of host chemical cues. In: Cònsoli, F.L., Parra, J.R.P., Zucchi, R.A. (Eds.), Egg parasitoids in agroecosystems with emphasis on *Trichogramma*. Springer, Dordrecht, pp. 97-147.
- Cossentine, J.E., Vincent, C., 2002. *Cydia pomonella* (L.), Codling moth (Lep.: Tortricidae). In: Mason, P.G., Huber, J.T. (Eds.), Biological programmes in Canada. CAB International, 90-94.
- Croft, B.A., Hoyt, S.C., 1983. Integrated management of insect pests pome and stone fruits. Wiley Interscience. New York, NY, pp. 454.
- Cross, J.V., Solomon, M.G., Chandler, D., Jarrett, P., Richardson, P.N., Winstanley, D., Bathon, H., Huber, J., Keller, B., Langenbruch, G.A., Zimmermann, G., 1999. Biocontrol of pests of apples and pears in northern and central Europe: 1. Microbial agents and nematodes. *Biocontrol Science and Technology* 9, 125-149.
- Darcy, A.R., John, J.B., 1990. Embryonic castration of the Codling moth, *Cydia pomonella* by an endoparasitoid, *Ascogaster quadridentata*. *Journal of Insect Physiology*, 36, 111-118.
- Dondale, C.D., Parent, B., Pitre, D., 1979. A 6-year study of spiders (Araneae) in a Quebec apple orchard. *The Canadian Entomologist* 111, 377-380.
- Dutky, S.R., 1959. Insect microbiology. *Advances in Applied Microbiology* 1, 175-200.
- Dutky, S.R., Hough, W.S., 1955. Note on parasitic nematode from Codling moth larvae, *Carpocapsa pomonella*. *Proceedings of the Entomological Society of Washington* 57, 244.
- Ebrahimi, E., 2004. Investigation and identification of species of *Trichogramma* Westwood. In: Azema, M., Mirabzadeh, A. (Eds.), Issues on different aspects of applying natural enemies for biological control of insect pests, Nashre Markaz, Tehran, Iran, pp. 1-71.

General introduction

- Edwards, C.A., 1986. Agrochemicals as environmental pollutants. In: Van Hofsten, B., Eckstrom, G. (Eds.), Control of pesticide applications and residues in food. A guide and directory. Swedish Science Press, Uppsala, pp. 1-19.
- EL-Gamil, F.M., Gaaboub, I.A.; EL-Sawaf, S.K., 1977. Population study of Codling moth *Cydia pomonella carpocapsa laspeyresia* olethreutidae lepidoptera infesting pear orchards in the eastern outskirts of Alexandria, Egypt. Journal of Agricultural Science 89, 655-658.
- Falcon, L.A., Huber, J., 1991. Biological control of the Codling moth, In: van der Geest, L.P.S., Evenhuis, H.H. (Eds.), Tortricid Pests, Their biology, natural enemies and control. Elsevier Science Publishers, Amsterdam, The Netherlands, pp. 355-369.
- Falcon, L.A., Kane, W.R., Bethel, R.S., 1968. Preliminary evaluation of a granulosis virus for control of the Codling moth. Journal of Economic Entomology 61, 1208-1213.
- Ferron, P., 1978. Biological control of entomogenous pests by entomogenous fungi. Annual Review of Entomology 23, 409-442.
- Fisher, R.W., 1960. Note on resistance to DDT in the Codling moth *Carpocapsa (Cydia) pomonella* (L.) in Ontario. Canadian Journal of Plant Science 40, 580-582.
- Flanders, S.E., 1937. Notes on life history and anatomy of *Trichogramma*. Annals of Entomological Society of America 30, 304-308.
- Forsse, E., Smith, S.M., Bouchier, R.S., 1992. Flight initiation in the egg parasitoid *Trichogramma minutum*: Effects of ambient temperature, mates, food and host eggs. Entomologia Experimentalis Applicata 62, 47-154.
- Fritsch, E., K. Undorf-Spahn, Kienzle, J., Zebitz, C.P.W., Huber, J., 2005. Apfelwickler-Granulovirus: Erste Hinweise auf Unterschiede in der Empfindlichkeit lokaler Apfelwickler-Populationen. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 57, 29-34.
- Garcia, P.V., Wajnberg, E., Pizzol, J., Olivejra, M.L.M., 2002. Diapause in the egg parasitoid *Trichogramma cordubensis*: Role of temperature. Journal of Insect Physiology 48, 349-355.
- Garcia-Gutierrez, C., Gonzales Maldonado, M.B., Medrano-Roldan, H., Chairez-Hernandez, H., 2004. Evaluación de la cepa BbP1 de *Beauveria bassiana*, Mycotrol, Metain y

General introduction

- azinfos-metilico contra *Cydia pomonella* L. (Lepidoptera: Tortricidae) en laboratorio y campo. *Folia Entomology Mexicana* 43, 1-7.
- Glen, D.M., 1982. Effects of natural enemies on a population of Codling moth, *Cydia pomonella*. *Annals of Applied Biology* 101, 199-201.
- Glen, D.M., Milsom, N.F., 1978. Survival of mature Codling larvae in relation to predation by birds and beetles. In: Dickler, E. (Eds.), *The use of integrated control and the insect sterile technique for control of the Codling moth*. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 180, 97-99.
- Godfray, H.C.J., 1994. *Parasitoids: behavioral and evolutionary ecology*. Princeton University Press, Princeton, New Jersey, pp. 488.
- Gröner, A., 1986. Specificity and safety of baculoviruses. In: Granados, R.R., Federici, B.A. (Eds.), *The Biology of Baculoviruses, Vol. I, Biological properties and molecular biology*. CRC Press, Boca Raton, FL, pp. 177-202.
- Gröner, A., 1990. Safety to non target invertebrates of baculoviruses, In: Laird, M., Lacey, L.A., Davidson, E.W. (Eds.), *Safety of microbial insecticides*. CRC Press, Boca Raton, FL, pp. 135-147.
- Hajek, E.A., 2004. *Natural Enemies: An introduction to biological control*. Cambridge University Press. Cambridge, pp. 396.
- Hase, A., 1925. Beitrage zur Lebensgeschichte der Schlupfwespe *Trichogramma evanescens* Westwood. *Biolog. Reichsanst. Land- Forstwirtschaft Berlin-Dahlem* 14, 171-224.
- Hassan, M., 2005. Factors influencing the parasitism of Codling moth eggs by *Trichogramma cacoeciae* March. and *T. principium* Sug. et Sor. (Hym.: Trichogrammatidae). *Journal of Pest Science* 78, 31-39.
- Hassan, S.A., 1988. Selection of suitable *Trichogramma* strains to control the Codling moth *Cydia pomonella* and the two summer fruit tortrix moths *Adoxophyes orana*, *Pandemis heparana* (Lep.: Tortricidae). *Entomophaga* 33, 19-27.
- Hassan, S.A., 1997. Seleção de espécies de *Trichogramma* para o uso em programas de controle biológico. In: Parra, J.R.P., Zucchi, R.A (Eds.), *Trichogramma e o Controle Biológico Aplicado*. FEALQ, Piracicaba, Brasil, pp. 183-206.

General introduction

- Hassan, S.A., Wührer, B.G., Rost, W.M., 1994. Die Anwendung von Eiparasiten der Gattung *Trichogramma* zur Bekämpfung des Pflaumenwicklers *Cydia funebrana* (Treitschke). Mitt Biol Bundesanst Berlin-Dahlem, 301-379.
- Hendricks, D.E., 1967. Effect of wind on dispersal of *Trichogramma semifumatum*. Journal of Economic Entomology 60, 1367-1373.
- Hiemstra, M., Toonen, A., De Kok, A., 1999. Determination of benzoylphenylurea insecticides in pome fruit and fruiting vegetables by liquid chromatography with diode array detection and residue data obtained in the Dutch national monitoring program. Journal of AOAC International 82, 1198-1205.
- Hoyt, S.C., Leeper, J.R., Brown, G.C., Croft, B.A., 1983. Basic biology and management components for insect IPM. In: Croft, B.A. Hoyt, S.C. (Eds.), Integrated management of insect pests of pome and stone fruits. Wiley, New York, pp. 93-151.
- Huber, J., 1980. Field persistence of the Codling moth granulosus virus. Bull. IOBC/WPRS, 3: 58-59.
- Huber, J., 1986. Use of baculoviruses in pest management programs. In: Granados, R.R., Federici, B.A. (Eds.), The Biology of baculoviruses. Vol. II. Practical Application for Insect Control. CRC Press, Boca Raton, FL, pp. 181-202.
- Jaynes, H.A., Marucci, P.E., 1947. Effect of artificial control practices on the parasites and predators of Codling moth. Journal of Economic Entomology 40, 9-25.
- Keller, M.A., Lewis, W.J., Stinner, R.E., 1985. Biological and practical significance of movement by *Trichogramma* species: a review The Southwestern Entomologist 8, 138-155.
- King, E.G., Bull, D.L., Bouse, L.F., Phillips, J.R., 1985. Biological control of bollworm and tobacco budworm in cotton by augmentative releases of *Trichogramma*. The Southwestern Entomologist 8, 1-10.
- Kienzle, J., Zimmer, J., Volk, F., Zebitz, C.P.W., 2008. Experiences with entomopathogenic nematodes for the control of overwintering Codling moth larvae in Germany. In: FÖKO (ed.) eco-fruit – 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing, 18.-20. February 2008, Weinsberg/Germany, 277-283.

General introduction

- Kienzle, J., Heinisch, D., Kiefer, J., Trautmann, M., Volk, F., Zimmer, J., Zebitz, C.P.W., 2010. Three years experience with entomopathogenic nematodes for the control of overwintering Codling moth larvae in different regions of Germany. In: FÖKO (ed.) eco-fruit – 14th International Conference on Organic Fruit-Growing, 22-24. February 2010, Stuttgart-Hohenheim/Germany, 163-168.
- Knight, A.L., 2006. Assessing the mating status of female Codling moth (Lepidoptera: Tortricidae) in orchards treated with sex pheromone using traps baited with ethyl (E, Z)-2, 4- decadienoate. *Environmental Entomology* 35, 894-900.
- Knipling, E.F., 1955. Possibilities of insect control or eradication through the use of sexually sterile males. *Journal of Economic Entomology* 45, 459-462.
- Knutson, A., 1998. The *Trichogramma* Manual. Agricultural communications, Texas agriculture extension service. A and M University System College Station, TX, USA, pp. 37.
- Lacey, L.A., Frutos, R., Kaya, H.K., Vail, P., 2001. Insect pathogens as biological control agents: do they have a future? *Biological Control* 21, 230-248.
- Lacey, L.A., Arthurs, S.P., Headrick, H., 2005. Comparative activity of the Codling moth granulovirus against *Grapholita molesta* and *Cydia pomonella* (Lepidoptera: Tortricidae). *Journal of the Entomological Society of British Columbia* 102, 79-80.
- Laing, J., 1937. Host finding by insect parasites. I, Observations on the finding of hosts by *Alysia manducator*, *Mormoniella vitripennis* and *Trichogramma evanescens*. *Journal of Animal Ecology* 6, 298-317.
- Lawrence, A., Lacey, L.A., Unruh, T.R., 2005. Biological control of Codling moth (*Cydia pomonella*) (Lepidoptera: Tortricidae) and its role in integrated pest management, with emphasis on entomopathogens. *VEDALIA* 12, 33-60.
- Li, L.Y., 1994. Worldwide use of *Trichogramma* for biological control on different crops: a survey. In: Wajnberg, E., Hassan, S.A. (Eds.), *Biological control with egg parasitoids*. CAB International, Wallingford, CT, pp. 37-53
- Lloyd, D.C., 1958. Memorandum on natural enemies of the Codling moth, *Carpocapsa pomonella* (L.). Commonwealth Institute of Biological Control, Trinidad, pp. 40.

General introduction

- Madsen, H.F., Morgan, C.V.G., 1970. Pome fruit pests and their control. *Annual Review of Entomology* 15, 295-320.
- Maia, A.H.N., Luiz, A.J.B., Campanhola, C., 2000. Statistical inference on associated fertility life table parameters using jackknife technique: Computational aspects. *Journal of Economic Entomology* 93, 511-518.
- McCall, P.J., Turlings, T.C.J., Lewis, W.J., Tumlinson, J.H., 1993. Role of plant volatiles in host location by the specialist parasitoid *Microplitis croceipes* Cresson (Braconidae: Hymenoptera). *Journal of Insect Behaviour* 6, 625-639
- Mills, N.J., 2005. Selecting effective parasitoids for biological control introductions: Codling moth as a case study. *Biological Control* 34, 274-282.
- Moffitt, H.R., Westgard, P.H., 1984. Suppression of the Codling moth (Lep.: Tortricidae) population on pears in southern Oregon through mating disruption with sex pheromone. *Journal of Economic Entomology* 77, 1513-1519.
- Mulder, R., Gijswijt, M.J., 1973. The laboratory evaluation of two promising new insecticides which interfere with cuticle deposition. *Pesticide Science* 4, 737-745.
- Nagarkatti, S.U.D.H.A., Nagaraja, H., 1978. Experimental comparison of laboratory-reared vs. wild-type *Trichogramma confusum* Vigg. (Hym.: Trichogrammatidae). I, Fertility, fecundity and longevity. *Entomophaga* 23, 129-136.
- Nordlund, D.A., 1994. Habitat location by *Trichogramma*. In: Wajnberg E, Hassan, S.A. (Eds.), *Biological control with egg parasitoids*. CAB International, Wallingford, CT, 155-163.
- Northwest Horticultural Council, 2011. World distribution of Codling moth. Washington State University. (http://entomology.tfrec.wsu.edu/Cullage_Site/CM_Map.html) (22.03.2011)
- Oatman, E.R., Libby, J.L., 1965. Progress on insecticidal control of apple insects. *Journal of Economic Entomology* 58, 766-770.
- Omer, A.D., Johnson, M.W., Tabashnik, B.E., 1996. Demography of the leafminer parasitoid *Ganaspidium utilis* Beardsley (Hym.: Eucoilidae) at different temperatures. *Biological Control* 6, 29-34.

General introduction

- Pak, G.A., Oatman, E.R., 1982. Biology of *Trichogramma brevicapillum*. *Entomologia Experimentalis et Applicata* 32, 61-67.
- Pak, G.A., Van Dalen, A., Kaashoek, N.K., Dijkman, H., 1990. Host egg chorion structure influencing host suitability for the egg parasitoid *Trichogramma* Westwood. *Journal of Insect Physiology* 36, 869-875.
- Pak, G.A., 1988. Selection of *Trichogramma* for inundative biological control. Ph.D. Dissertation, Wageningen Agricultural University, The Netherlands, pp. 224.
- Pak, G.A., Buis, H.C.E.M., Heck, I.C.C., Hermans, M.L.G., 1986. Behavioral variations among strains of *Trichogramma* spp.: host-age selection. *Entomologia Experimentalis et Applicata* 40, 247-258.
- Pinto, J.D., Koopmanschap, A.B., Platner, G.R., Stouthamer, R., 2002. The North American *Trichogramma* (Hym.: Trichogrammatidae) parasitizing certain Tortricidae (Lepidoptera) on apple and pear, with ITS2 DNA: characterizations and description of a new species. *Biological Control* 23, 134-142.
- Poinar, G.O. Jr., 1991. Nematode parasites. In: van der Geest, L.P.S., Publishers, H.H., Amsterdam, The Netherlands, pp. 273-281.
- Prattisoli, D., Parra, J.R.P., 2000. Fertility and life table of *Trichogramma pretiosum* (Hym.: Trichogrammatidae) in eggs of *Tuta absoluta* and *Phthorimaea operculella* (Lep.: Gelechiidae) at different temperatures. *Journal of Applied Entomology* 124, 330-342.
- Proverbs, M.D., Newton, J.R., 1966. Orchard assessment of the sterile male technique for control of the Codling moth, *C. pomonella* (L.). *Canadian Entomologist* 98, 90-95.
- Quarles, W., 2000. Mating disruption success in Codling moth IPM. *IPM Practitioner* 22, 1-12.
- Quednau, W., 1955. Über einige neue *Trichogramma* -Wirte und ihre Stellung im Wirt-Parasit-Verhältnis. Ein Beitrag zur Analyse des Parasitismus bei Schlupfwespen. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, Berlin (N.F.)* 7, 145-148
- Rang, C., Lacey, L.A., Frutos, R., 2000. The crystal proteins from *Bacillus thuringiensis* subsp. *thompsoni* display a synergistic activity against the Codling moth, *Cydia pomonella*. *Current Microbiology* 40, 200-204.
- Reznik, S.Y., Kats, T.S., Umarova, T.Y., Voinovich, N.D., 2002. Maternal age and endogenous variation in maternal influence on photoperiodic response in the progeny

General introduction

- diapause in *Trichogramma embryophagum* (Hym.: Trichogrammatidae). European Journal of Entomology 99, 175-181.
- Reznik, S.Y.a., Kats, T.S., 2004. Exogenous and endogenous factors inducing diapause in *Trichogramma principium* Sug. et Sor. (Hym.: Trichogrammatidae). Entomological Review 84, 963-970.
- Riddick , E.W., Mills, N.J., 1994. Potential of adult carabids (Coleoptera: Carabidae) as predators of fifth-instar Codling moth (Lep.: Tortricidae) in apple orchards in California. Environmental Entomology 23, 1338-1345.
- Ridgway, R.L., Silverstein, R.M., Inscoc, M.N., 1990. Behaviour-modifying chemicals for insect management: Applications of pheromones and other attractants. Marcel Dekker, New York, pp. 281-314.
- Romeis, J., Babendreier, D., Wäckers, F.L., Shanower, T.G., 2005. Habitat and plant specificity of *Trichogramma* egg parasitoids underlying mechanisms and implications. Basic and Applied Ecology 6, 215-236.
- Romeis, J., Shanower, T.G., Zebitz, C.P.W., 1998. Response of *Trichogramma* egg parasitoids to colored sticky traps. BioControl 43, 17-27.
- Rosenberg, H.T., 1934. The biology and distribution in France of the larval parasites of *Cydia pomonella*. Bulletin of Entomological Research 25, 201-256.
- Ruberson, J.R., Kring, T.J., 1993. Parasitism of developing eggs by *Trichogramma pretiosum* (Hym.: Trichogrammatidae): host age preference and suitability. Biological Control 3, 39-46.
- Salt, G., 1935. Experimental studies in insect parasitism III- Host selection. Proceedings of the Royal Society of London. Series B, Biological Sciences, 117, 413-435.
- Samara, R., Monje, J.C., Qubbaj, T., Zebitz, C.P.W., 2011. Studies on host preference and oviposition behaviour of *Trichogramma aurosum* Sugonjaev and Sorokina strains in choice and non-choice tests. Arab Journal of Plant Protection 29, 259-266.
- Schütte, F., Franz, J.M., 1961. Untersuchungen zur Apfelwicklerbekämpfung (*Carpocapsa pomonella* (L.)) mit Hilfe von *Trichogramma embryophagum* Hartig. Entomophaga 6, 237-247.

General introduction

- Shearer, P.W., Athanassov, A., 2004. Impact of peach extra floral nectar on key biological characteristics of *Trichogramma minutum* (Hym.: Trichogrammatidae). *Journal of Economic Entomology* 97, 789-792.
- Shel'deshova, G.G., 1967. Ecological factors determining distribution of Codling moth *Laspeyresia pomonella* (L.) (Lep.: Tortricidae) in northern and southern Hemispheres. *Entomological Review* 46, 349-361.
- Silvia, I.M.M.S., Honda, J., Van Kan, F.J.P.M., Hub, J., Neto, L., Pintureau, B., Stouthamer, R., 1999. Molecular differentiation of five *Trichogramma* species occurring in Portugal. *Biological Control* 16,177-184
- Simmonds, F.J., 1944. Observations on the parasites of *Cydia pomonella* L. in southern France. *Scientific Agriculture* 25, 1-30.
- Smith, S.M., 1994. Methods and timing of releases of *Trichogramma* to control lepidopterous pests. In: Wajnberg, E., Hassan, S.A. (Eds.), *Biological control with egg parasitoids*. CAB International, Oxon, UK, pp. 113-144.
- Smith, S.M., 1996. Biological control with *Trichogramma*: advances successes and potential of their use. *Annual Review of Entomology* 41, 375-406.
- Somchoudhury, A.K., Dutt, N., 1988. Evaluation of some flowers as a nutritional source of *Trichogramma* spp. *Indian Journal of Entomology* 50, 371-373.
- Sorokina, A.P., 1987. Biological and morphological confirmation of a new species, *Trichogramma telengai* sp. n. (Hym.: Trichogrammatidae). *Entomologicheskoe Obozrenie* 66, 32-46.
- Southwood, T.R.E., 1978. *Ecological methods with particular reference to the study of insect populations*, 2nd ed. Chapman and Hall, London. pp. 524 .
- Suckling, D.M. 2003. Applying the sterile insect technique for biosecurity: benefits and constraints. *New Zealand Plant Protection* 56, 21-26.
- Stinner, R.E., 1977. Efficacy of inundative releases. *Annual Review of Entomology* 22, 515-531.
- Strand, M.R., 1986. The physiological interactions of parasitoids with their host and their influence on reproductive strategies. In: Waage, J., Greathead, D. (Eds.), *Insect Parasitoids*. Academic Press, London, UK, pp. 97-136.

General introduction

- Sweetman, H.L., 1958. The principles of biological control. Wm. C. Brown Co., Dubuque, Iowa, pp. 560.
- Taghizadeh, R., Fathipour, Y., Kamali, K., 2008. Influence of temperature on life table parameters of *Stethorus gilvifrons* (Mulsant) (Coleoptera, Coccinellidae) fed on *Tetranychus urticae* Koch. Journal of Applied Entomology 132, 638-645.
- Tanada, Y., 1964. A granulosis virus of the Codling moth, *Carpocapsae pomonella* (L.) (Olethreutidae: Lepidoptera). Journal of Insect Pathology 6, 378-380.
- Telenga, N.A. 1956. Investigations on *Trichogramma evanescens* Westw. and *T. pallida* Meyer and their use for the control of injurious insects in the USSR. Entomologischeskoe Obozrenie 35, 599-610.
- Turnbull, A.L., Chant, D.A., 1961. The practice and theory of biological control of insects in Canada. Canadian Journal of Zoology 39, 697-753.
- van Frankenhuyzen, A., Stigter, H., 2002. Schädliche und nützliche Insekten und Milben an Kern- und Steinobst in mitteleuropa. Ulmer Verlag, Stuttgart, pp. 288.
- van Lenteren, J.C., 2000. Success in biological control of arthropods by augmentation of natural enemies. In: Gurr, G.M., Wratten, S.D. (Eds.), Biological Control: Measures of Success. Kluwer Academic Publishers, Dordrecht, pp. 77-103.
- van Lenteren, J.C., Bueno, V.H.P., 2003. Augmentative biological control of arthropods in Latin America. BioControl 48, 123-139.
- van Lenteren, J.C., Glas, R.C.G., Smith, P.H., 1982. Evaluation of control capabilities of *Trichogramma* and results of laboratory and field research on *Trichogramma* in the Netherlands. Les trichogrammes, Ier Symposium International, Antibes, 74, 257-269.
- van Steenburgh, W.E., 1934, *Trichogramma minutum* Riley as a parasite of the oriental fruit moth (*Laspeyresia molesta* Busck) in Ontario. Canadian Journal of Research 10, 287-314.
- Varela, L.G., Welter, S.C., Jones, V.P., Brunner, J.F., Riedl, H., 1993. Monitoring and characterization of insecticide resistance in Codling moth (Lepidoptera: Tortricidae) in four western states. Journal of Economic Entomology 86, 1-10.
- Vet, L.E.M., Dicke, M., 1992. The ecology of infochemical use by natural enemies of herbivores in a tritrophic context. Annual Review of Entomology 37, 141-172.

General introduction

- Vinson, S.B., 1976. Host selection by insect parasitoids. *Annual Review of Entomology* 21, 109-134.
- Vinson, S.B., 1998. The general host selection behaviour of parasitoid Hymenoptera and a comparison of initial strategies utilized by larviphagous and oophagous species. *Biological Control* 11, 79-96.
- Vinson, S.B., 2010. Nutritional ecology of insect egg parasitoids. In: Cõnsoli, F.L., Parra, J.R.P., Zucchi, R.A. (Eds.), *Egg parasitoids in agroecosystems with emphasis on Trichogramma*. Springer, Dordrecht, pp. 25-56.
- Volkoff, A., Daumal, J., Barry, P., Fancois, M., Harlitzky, N., Ross, M.M., 1995. Development of *Trichogramma cacoeciae* Marchal (Hym.: Trichogrammatidae): Time table and evidence for a single larva instar. *International Journal of Insect Morphology and Embryology* 24, 459-466.
- Vreysen, M.J.B., Carpenter, J.E., Marec, F., 2010. Improvement of the sterile insect technique for Codling moth *Cydia pomonella* (L.) (Lep.: Tortricidae) to facilitate expansion of field application. *Journal of Applied Entomology* 134, 165-181.
- Weiser, J., 1955. *Neoaplectana carpocapsae* n. sp. (Anguillulata, Steinernematidae) novy cizopasník housenek obalece jablecneho, *Carpocapsae pomonella* L. *Vestník Československé Společnosti Zoologické* 19, 44-52.
- Wellinga, S., Wysoki, M., 1989. Preliminary investigation of food source preferences of the parasitoid *Trichogramma platneri* Nagarkatti (Hym.: Trichogrammatidae). *Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz* 62, 133-135.
- Wildbolz, T., Mani, E., 1975. Zum Stand der Sterilpartnermethode beim Apfelwickler. *Zeitschrift für Angewandte Entomologie* 77, 359-362.
- Witzgall, P., Stelinski, L., Gut, L., Thomson, D., 2008. Codling moth management and chemical ecology. *Annual Review of Entomology* 53, 503-522.
- Witzgall, P., Arn, H., 1990. Direct measurement of the flight behavior of male moths to calling females and synthetic sex pheromones. *Zeitschrift für Naturforschung* 45, 1067-1069.

General introduction

- Witzgall, P., Unelius, C.R., Rama, F., Chambon, J.P., Bengtsson, M., 1997. Mating disruption of pea moth, *Cydia nigricana*, and Codling moth *C. pomonella*, using blends of sex pheromone and attraction antagonists. IOBC/wprs Bulletin 20, 207-215.
- Yu, D.S.K., Laing, J.E., Hagley, E.A.C., 1984. Dispersal of *Trichogramma* spp. (Hym.: Trichogrammatidae) in an apple orchard after inundative release. Environmental Entomology 13, 371–374.
- Zaslavski, V.A., Umarova, T.Ya., 1990. Environmental and endogenous control of diapause in *Trichogramma* species. Entomophaga 35, 23-29.
- Zhang, J., Wang, J., Liu, G., Yian, Y., 1983. The effect of relative humidity and temperature on *Trichogramma ostriniae*. Natural Enemies of Insects 5, 129-134.
- Zingg, D. 2008. Madex Plus and Madex I12 overcome virus resistance of Codling moth. In: FÖKO (Eds.) eco-fruit – 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing, 18.-20. Feb. 2008, Weinsberg/Germany, 256-260.

CHAPTER II

**Intra- and interspecific performance of *Trichogramma* species
(Hymenoptera: Trichogrammatidae) alimented with honey, pollen
or honey-pollen mixtures.**

Intra- and interspecific performance of *Trichogramma* species (Hymenoptera: Trichogrammatidae) alimented with honey, pollen or honey-pollen mixtures.

Abstract: The effect of different diets of honey and pollen on life-table parameters of four strains of *Trichogramma cacoeciae*, and one strain of *T. evanescens* and *T. oleae* respectively (Hymenoptera: Trichogrammatidae) was evaluated in a series of experiments under laboratory conditions. The intraspecific variability of adult performance was assessed with four strains of *T. cacoeciae* only fed on water or honey diets. Honey diet improved significantly adult mean longevity, mean fecundity, mean fertility, and net reproduction rate (R_0). Significant intraspecific variability was shown only in mean fecundity and mean fertility, but not in longevity. The best performing strain of *T. cacoeciae* (HOH 1) of these experiments has been taken to assess the nutritional value of pollen collected from birch, maize and sunflower. Pollen as honey additive had a significant positive effect on female longevity as compared to honey alone, but longevity did not differ significantly between the honey-pollen diets. Pollen added to water had no ameliorative effect on longevity except sunflower pollen, which had a significant detrimental effect. Mean total fecundity and fertility of females was not improved when fed honey and birch or maize pollen. Sunflower pollen added to honey, however, had a detrimental effect on these parameters. When these three pollens were offered with water, all parameters were not or negatively affected, revealing sunflower pollen having the worst nutritional value. Tests on the suitability of bee-pollen to ameliorate *Trichogramma* mass-rearing were done with *T. cacoeciae*, *T. evanescens* and *T. oleae*. Except fertility, longevity and fecundity differed significantly between the species. Basic diet (water or honey) and pollen added showed interactive effects on the performance of *Trichogramma* species. Interactions between species and added pollen only revealed significant differences in fecundity and fertility, whereas longevity was only slightly affected. Species - basic diet - pollen interactions could only be shown for fertility. *T. oleae* had a shorter adult lifespan and the lowest number of parasitized eggs compared with the other two species, but it had the highest rate of emergence when fed on honey mixed with bee pollen. The results indicate that (i) *Trichogramma* adults are feeding upon pollen, and (ii) nutritional quality of pollen differs between plant species, and (iii) honeybee-pollen may be used to improve mass-rearing of *Trichogramma* species.

Key words: *Trichogramma* species, life-table parameters, insect-plant interactions, mass rearing.

2.1. Introduction

Trichogramma spp. egg parasitoids are the most important agents for augmentative biological control of lepidopterous pests worldwide. Since the last review (Smith 1996), release of mass-produced *Trichogramma* to control pest Lepidoptera in various important cash and staple crops boosted in the last few years, such as olive (Agamy, 2010; Hegazi et al., 2007, 2012), cotton (Bastos et al., 2010; Davies et al. 2011a,b), fruit orchards (Mathews et al. 2011; Pluciennik and Olszak, 2010; Rodrigues et al., 2011; Samara et al., 2011; Zimmermann, 2004a,b), grapes (El Wakeil et al., 2008; Hommay et al., 2002; Nagarkatti et al., 2003; Zimmermann, 2004a,b), maize and sugarcane (Albert et al., 2008; Gardner et al., 2007; Hoffmann et al., 2006; Oztemiz, 2009; Vejar-Cota et al., 2005; Zhang et al., 2010; Zimmermann, 2004a,b), vegetables (Ballal and Singh, 2003; Faria et al., 2008; Hwang et al., 2010; Klug and Meyhöfer, 2009; Miura et al., 2001; Romeis et al., 1999; Russell and Bessin, 2009; Sithanatham et al., 2001; Zimmermann, 2005), or ornamentals (Zimmermann, 2004a, b).

Beside uptake of the host's body fluid (host-feeding), adult parasitoids of different families are known to feed upon floral and extrafloral nectar, and honeydew in the field (Andow and Risch, 1987; Jervis et al., 1992; Quicke, 1997; Wellinga and Wysoki, 1989) which can maximize parasitoid performance and increase female sex ratios (Tylianakis et al., 2004; Berndt and Wratten, 2005; Irvin et al., 2006; Saavedra et al., 1997; Shearer and Atanassov, 2004). For *Trichogramma* species, the alternative or additional food sources are poorly investigated, yet.

Pollen as a food source for *Trichogramma* sp. is reported contradictory. Wellinga and Wysoki (1989) found no evidence of pollen feeding by *T. platneri* Nagarkatti and Somchoudhury and Dutt (1988) found no significant effect of maize or sorghum pollen on longevity and fecundity of *T. perkinsi* Girault and *T. australicum* Girault, indicating that females of these species are either not able to take up or digest this pollen or that even after digestion this pollen had no effect at all. On the other hand, Zhang et al. (2004) found that *T. brassicae* Bezdenko feeds on maize pollen with a positive effect on female longevity and fecundity. In the latter case, Romeis et al. (2005) assumed that these females were feeding on (pseudo) germinated pollen, releasing pollen nutrients. However, even then, they feed on pollen and *Trichogramma* females may opportunistically exploit pollen as food source when available which may occur particularly during pollination of anemophilous plants. However, nectar, honeydew, or pollen may differ in food quality depending from plant species (Forrest and

Knights, 1972; Irvin et al., 2006, 2007; Irvin and Hoddle, 2007; Lundgren, 2009; Romeis et al., 2005).

To obtain the best performance of *Trichogramma* sp. as biocontrol agent, it is inevitable to select the most appropriate species or strain and to mass-rear *Trichogramma* under optimal conditions. These are not only abiotic conditions or the species of the factitious host, but also the food provided during mass rearing, or found and fed upon opportunistically in the field after release (Hassan, 1993, 1994; Jervis and Kidd, 1986; Jervis et al., 1992). Adult *Trichogramma* are known to feed upon nectar, pollen or the host's body fluid (host-feeding) to satisfy the demand for energy, proteins or micronutrients (Zhang et al., 2004).

Nectar, honeydew, and host fluid are possible natural food sources in the field (Jervis et al., 1992; Quicke, 1997). These natural food sources were substituted in the laboratory with diluted honey and sucrose. Several authors showed that feeding *Trichogramma* adults greatly increased longevity and fecundity in the laboratory. Food sources tested included honey (Lund, 1938; Stinner et al., 1974; Pu, 1979; Yu et al., 1984; Hohmann et al., 1988), sugar (Narayanan and Mookherjee, 1956), honey and protein diets (Ashley and Gonzales, 1974), honey, sugar, and yeast (Leatemala et al., 1995). Leius (1963) showed that pollen mixed with sugar and water extended the life span and increased the fecundity of *Scambus buolianae* Hartig (Hymenoptera: Ichneumonidae).

Like most adult parasitoids, also *Trichogramma* species feed on carbohydrate sources and pollen, using sugars as an energy source and pollen as protein source (Jervis and Kidd, 1986; Heimpel and Jervis, 2005). Such feeding may be critical to enhancing biological pest control or mass rearing of parasitoids by a better adult performance (Andow and Risch, 1987; Wellinga and Wysoki, 1989; Leatemala et al., 1995; McDougall and Mills, 1997; Olson and Andow, 1998; Knutson, 1998; Gurr and Nicol, 2000; Wäckers et al., 2008; Jonsson et al., 2008). A better food supply of *Trichogramma* lead to increased longevity of *T. platneri* (Hohmann et al., 1989), a higher flight tendency of *T. minutum* Riley (Forsse et al., 1992), and a higher parasitisation rate by *T. perkinsi* and *T. australicum* (Somchoudhury and Dutt, 1988). Although protein, free amino acids, and lipids are also taken up by host-feeding on the hemolymph and tissues, these sources may be rare under field conditions. Thus, pollen may serve as substitute to be used primarily to support egg production, while other non-host foods are probably used primarily for maintenance (Jervis and Kidd, 1986; Jervis et al., 1992, 1996; Quicke, 1997).

However, additional food sources are crucial in *Trichogramma* mass rearing to produce the necessary quantities of species with defined qualities for field release. Therefore, Lund (1938) suggested that feeding adult *Trichogramma* prior to their release can increase their effectiveness. Adult longevity, fecundity, fertility, and searching capacity are the most important criteria to determine the quality of *Trichogramma* species (Greenberg, 1991; Cerutti and Bigler, 1991; Bigler, 1994; Dutton et al., 1995; van Lenteren, 2000).

The aim of the present study was to describe the effects of different diets on the longevity, development, capacity of parasitism and other life-table parameters of different species and strains of *Trichogramma*.

2.2. Material and methods

2.2.1. Source of parasitoids and rearing procedures

Trichogramma cacoeciae Marchal and *T. oleae* Voegelé wasps collected in 2006 from different countries by exposing bait cards with factitious host eggs (*Sitotroga cerealella* Oliver (Lep., Gelechiidae)) for 48 h in the field and subsequent incubation under laboratory conditions (25 ± 1 °C, 40-60% R.H and 16:8 L: D) (Table 1). *Trichogramma evanescens* Westwood wasps were obtained from the Institute of Biological Control of the Julius-Kühn-Institute (JKI), Darmstadt, Germany (Table 1). The females used in all experiments were not older than 24h old, unfed, naive, and had experienced no previous contact with a host egg except for emergence. Fresh eggs of the Angoumois grain moth *S. cerealella* were used as factitious hosts for all strains in all experiments.

2.2.2. Pollen sources

The birch pollen used in this study was collected in large quantities from blooming birch trees (*Betula pendula* Roth) collected from the botanical garden of the University of Hohenheim. Sunflower pollen was collected using a hand vacuum cleaner by aspirating the pollen directly from the flowers. Then the pollen was dried for 24 h under room temperatures. Maize pollen was collected directly from maize plants by cutting the tassel into plastic bags, sieved on a clean paper and dried for 36-48 h, in order to keep the moisture of the pollen below 6%. Bee pollen was purchased from a supermarket and stored in the refrigerator under -4 °C. All other pollen was stored in a small glass with a lid at -20 °C until further use in the experiment. Under these

conditions, the pollen can be stored up to two years (Bogdanov et al., 1999; Li et al., 2008; Perveen and Ali, 2008).

2.3. Experimental set-up

Thirty females (16-24h old) of each strain were individually separated and released in a small plastic tube (5×3.5 cm) covered with black nets (0.7 mesh). *Sitotroga cerealella* eggs glued with non-toxic glue (Arabic Gum) on circular paper cards, each of them containing 100 ± 15 eggs and a little amount of test diet. These cards with eggs and test diet were offered daily to every individual female until the female died. The parasitized eggs from each tube were transferred to another clean tube and kept in the laboratory for parasitoid development. After 4-5 days the number of parasitized eggs were recorded when they turned black, by counting them under a binocular microscope. The number of emerging females in each tube was assessed after transferring them into 10% alcohol in a new clean glass tube.

In the first experiment, the effect of honey as food and the intraspecific variability in performance was assessed. 30 females each of four strains of *T. cacoeciae* were released separately into individual clean tubes closed with cotton clothing. Host eggs were offered every day and only a small drop of water was given onto the cotton for drinking, until the female died.

In the second experiment, eight diets were designed: water and honey respectively without any additive, and water and honey, each mixed either with maize, sunflower, or birch pollen (20 mg pollen / ml water or honey). All food sources were prepared daily and offered to *T. cacoeciae* HOH 1.

In the third experiment, four diet combinations were designed as in the second experiment, using bee pollen (20 mg pollen / ml water or honey) alone offered to *T. cacoeciae* HOH 1, *T. oleae* see 2, and *T. evanescens* 195 DE 06. Bee pollen was used to assess the suitability of a commercial pollen source to be independent from pollen collected from plants.

2.4. Data analysis

Statistical processing of data was done using JMP[®] 9.0.0 software (2010, SAS Institute Inc., Cary, NC 27513, USA). All experimental data were normally distributed. Outliers were eliminated after Jackknife-procedure if necessary. To compare data of different feeding regimes, one-way ANOVA followed by all pairwise multiple comparison procedure Tukey-Kramer HSD-test was applied.

Intra- and interspecific performance of *Trichogramma* species

Table 1 *Trichogramma* species and strains used, their original hosts, geographical location, and year of the collection.

Species	Strain	Original host	Collected in: Culture	Country of origin	Latitude	Longitude	Collector	Year of collection
<i>T. cacoeciae</i>	HOH 1	<i>S. cerealella</i> ¹	Apple	Hohenheim Germany	48° 11' N	9° 13' E	H. Alkarrat	2006
<i>T. cacoeciae</i>	HOH 2	<i>S. cerealella</i> ¹	Apple	Hohenheim Germany	48° 11' N	48° 11' N	H. Alkarrat	2006
<i>T. cacoeciae</i>	KIO2 TN	<i>Ectomyelois ceratoniae</i>	Pomegranate	Tunisia	36° 50' N	10° 11' E	I. Ksentini	2004
<i>T. cacoeciae</i>	SY 98 01	<i>C. pomonella</i>	Apple	As Sweida Syria	32° 45' N	36° 45' E	W. Almatni	1998
<i>T. oleae</i>	See 2	<i>S. cerealella</i> ¹	Weeds	Hohenheim Germany	48° 11' N	48° 11' N	C. P. W. Zebitz	2006
<i>T. evanescens</i>	195 DE 06	<i>S. cerealella</i> ¹	Lab rearing	Germany			JKI ²	2006

¹ = bait eggs

² = Julius-Kühn-Institute, Institute for Biological Control, Darmstadt, Germany

2.5. Results

2.5.1. Effect of honey as food and intraspecific variability

No significant differences were found in longevity between strains within honey-fed females, ranging between 10.60 ± 3.36 days for *T. cacoeciae* KIO 2 and 12.96 ± 4.47 days for *T. cacoeciae* HOH 1 or between strains within water-fed females (ranging between 5.79 ± 2.70 days for *T. cacoeciae* HOH 1, and 7.50 ± 3.02 days for *T. cacoeciae* HOH 2). However, mean longevity differed significantly between honey-fed and water-fed females (one-way ANOVA, $F = 18.8666$, $p < 0.0001$; Tukey-Kramer HSD-test) (Table 2).

The fecundity of all honey-fed strains was significantly higher than from water-fed strains (Table 2). The mean number of blackened eggs of honey-fed *T. cacoeciae* HOH 1 (86.21 ± 13.80 parasitized eggs/ female) was higher compared with the other three strains. Also the fecundity of *T. cacoeciae* KIO 2 TN (76.67 ± 10.29) was higher compared with *T. cacoeciae* HOH 2 (57.71 ± 9.10) and *T. cacoeciae* SY 98 01 (57.93 ± 14.67). No significant differences in fecundity were found between the water-fed strains ranging between 23.69 ± 9.67 parasitized eggs for *T. cacoeciae* HOH 1 and 29.41 ± 6.92 parasitized eggs for *T. cacoeciae* SY 98 01.

Also, the fertility (mean % emerged adults) of all honey-fed females of *T. cacoeciae* was higher than in females supplied with water only, but not differing significantly between strains when supplied with honey (Table 2). However, the fertility of water-fed strains of *T. cacoeciae* differed significantly, with strain HOH 2 and KIO 2 TN performing best (62.83 ± 19.00 and 63.21 ± 17.33 % emerged adults, respectively).

As a consequence, the net reproductive rate (R_0) of honey-fed strains was superior to water-fed strains. *Trichogramma cacoeciae* HOH1 showing the highest value (66.23), followed by *T. cacoeciae* KIO 2 TN (56.03), *T. cacoeciae* HOH 2 (46.40) and *T. cacoeciae* SY 98 01 (45.20), while the net reproductive rate of water-fed strains ranged between 12.97 for *T. cacoeciae* HOH 2 and 18.43 for *T. cacoeciae* KIO 2 TN (Table 2).

Intra- and interspecific performance of *Trichogramma* species

Table 2 Longevity, fecundity, fertility and net reproduction rate (R_0) (mean \pm sd respectively) of honey-fed and water-fed strains of *T. cacoeciae* (one-way ANOVA; for longevity: $F = 18.8666$, $P < 0.0001$, d.f. = 7, 221; for fecundity: $F = 164.0204$, $P < 0.0001$; d.f. = 7, 221; for fertility: $F = 25.3976$, $P < 0.0001$; d.f. = 7, 221, followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level).

Strains	Diet	Mean longevity * (day)	Ratio **	Mean fecundity * (eggs parasitized / female)	Ratio **	Mean fertility * (% emerged adults)	Ratio **	R_0 ($\sum l_x m_x$)	Ratio **	no. of repeats
<i>T. cacoeciae</i> HOH 1	Honey	12.96 \pm 4.47 ^a	2.24	86.21 \pm 13.80 ^a	3.64	79.49 \pm 8.42 ^a	1.45	66.23	5.11	28
	Water	5.79 \pm 2.70 ^b		23.69 \pm 9.67 ^d		55.00 \pm 12.17 ^{de}		12.97		29
<i>T. cacoeciae</i> HOH 2	Honey	10.71 \pm 4.09 ^a	1.43	57.71 \pm 9.10 ^c	2.13	82.97 \pm 7.70 ^a	1.22	46.40	2.52	28
	Water	7.50 \pm 3.02 ^b		27.10 \pm 9.94 ^d		67.83 \pm 19.00 ^{bc}		18.43		30
<i>T. cacoeciae</i> KIO 2 TN	Honey	10.93 \pm 3.15 ^a	1.85	76.67 \pm 10.29 ^b	3.14	76.53 \pm 7.28 ^{ab}	1.21	56.03	3.73	27
	Water	5.90 \pm 2.40 ^b		24.43 \pm 6.79 ^d		63.21 \pm 17.33 ^{cd}		15.03		30
<i>T. cacoeciae</i> SY 98 01	Honey	10.60 \pm 3.36 ^a	1.51	57.93 \pm 14.67 ^c	1.97	81.68 \pm 8.98 ^a	1.55	45.20	2.92	28
	Water	7.03 \pm 1.72 ^b		29.41 \pm 6.92 ^d		52.64 \pm 14.16 ^e		15.50		29

* Within a column, means with the same letter are not significantly different.

** Ratio honey-fed / water-fed

Intra- and interspecific performance of *Trichogramma* species

Except in strain HOH 2 (Fig 1b), the mean number of parasitized eggs per female and day differed significantly between honey-fed and water-fed strains at least in the first eight days of female life. t-test was only performed when the number of replicates per treatment in pairs to compare was not lower than $n = 7$ (Fig. 1).

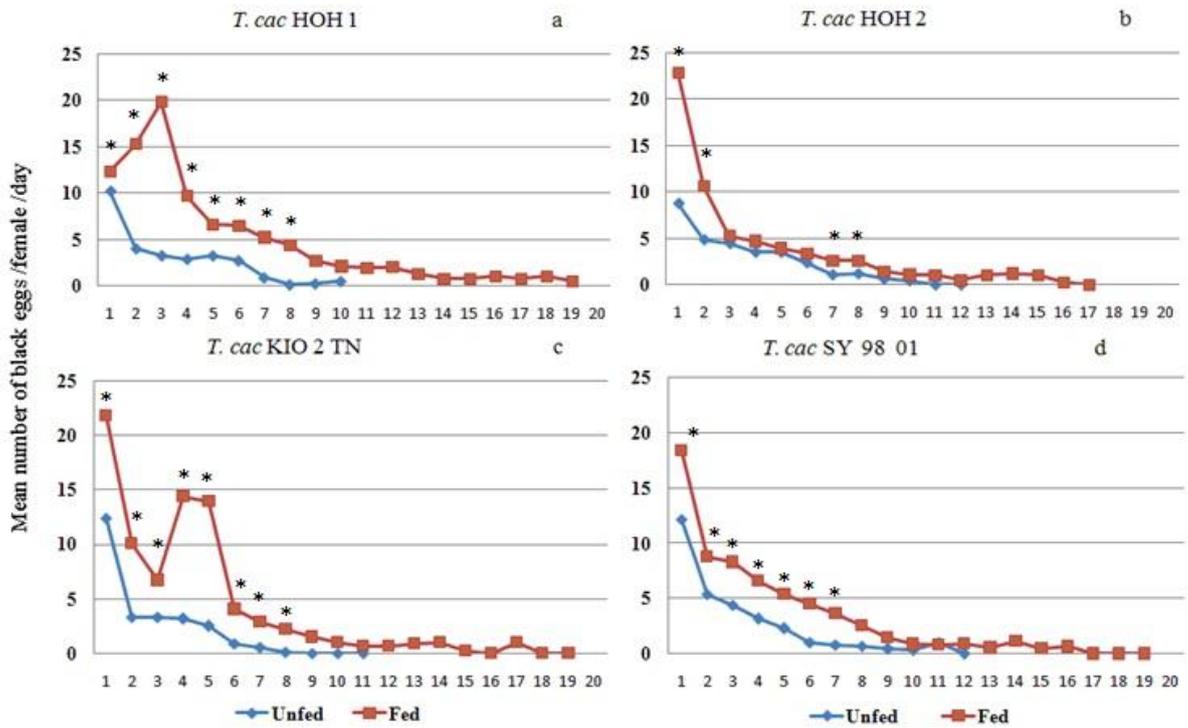


Figure 1 Daily mean parasitization per female (as assessed as number of blackened eggs) of *S. cerealella* eggs by four strains of *T. cacoeciae* (asterisks indicate statistical significance between food regimes within the strains after t-test at $P < 0.01$).

Median survival time as calculated by the WEIBULL-function (JMP procedure Survival/Reliability analysis) ranged from 6 - 8 days in water-fed strains and from 11 - 13 days in honey-fed strains (Figure 2).

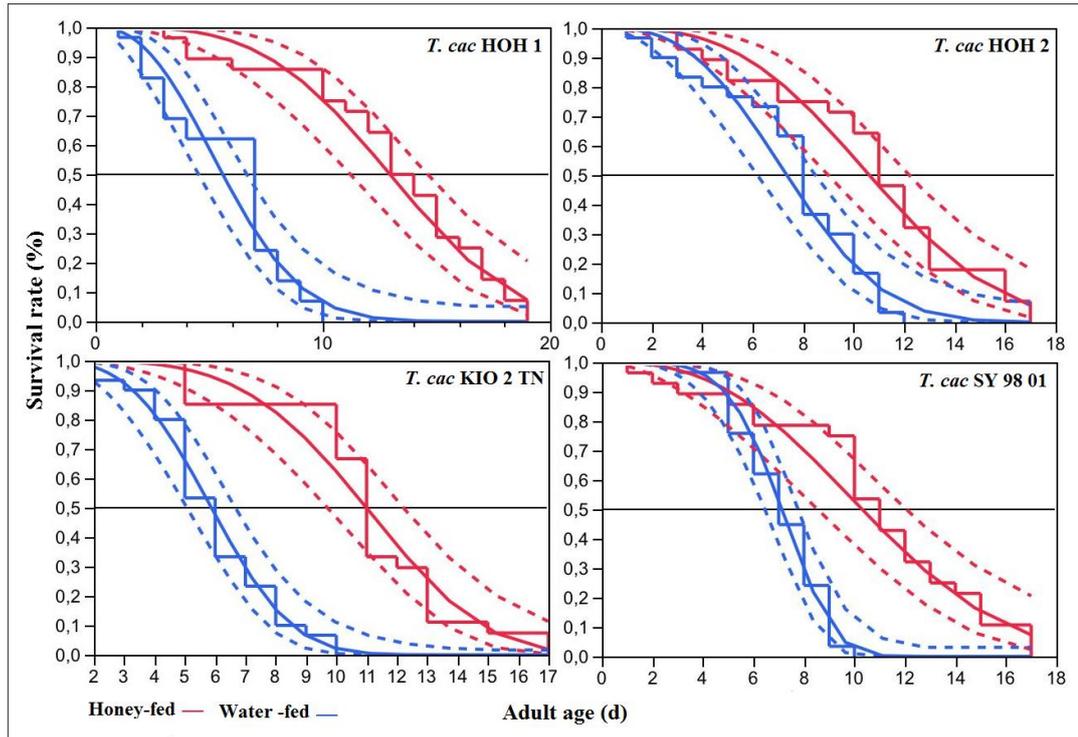


Figure 2 Survival plot of honey-fed and water-fed strains of *T. cacoeciae*. (the broken line indicates 95 % confidence limits of the WEIBULL-plot)

2.5.2. Nutritional value of different pollens

Mean longevity and survival rates of *T. cacoeciae* HOH 1 fed on honey-based diets were significantly better than those of the insects fed on water-based diets (GLM; basic diet: L-R- X^2 : 172.5791, $P < 0.0001$; d.f. = 1). Pollen as honey additive had a significant positive effect on female longevity as compared to honey alone, but longevity did not differ significantly between the honey-pollen diets (one-way ANOVA, $F = 4.3958$, $P = 0.0057$; d.f. = 3,116; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level). Pollen added to water had no ameliorative effect on longevity except sunflower pollen, which had a significant detrimental effect (one-way ANOVA, $F = 8.5201$, $P < 0.0001$; d.f. = 3,116; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level) (Table 3; Figure 3).

Intra- and interspecific performance of *Trichogramma* species

Table 3 Longevity, fecundity, fertility, and net reproductive rate R_0 (mean \pm sd respectively) of *T. cacoeciae* strain HOH 1 fed with different diets of pollen mixed with honey or with water.

Diets	Mean longevity * (days)	Estimated 50 % survival ** (days)	Mean total fecundity * (eggs parasitized / female)	Mean total fertility * (no. of emerged adults)	Emergence rate (%)	R_0 ($\Sigma l_x m_x$)
Honey	12.33 \pm 4.96 ^b n = 30	12.12 10.47 / 14.04	86.21 \pm 13.80 ^a n = 28	68.43 \pm 12.33 ^a n = 28	79.38	68.52
Honey + Birch	16.87 \pm 6.12 ^a n = 30	16.83 14.71 / 19.26	91.90 \pm 30.30 ^a n = 30	58.61 \pm 19.91 ^{ab} n = 28	63.78	62.93
Honey + Maize	17.13 \pm 7.18 ^a n = 30	16.75 14.33 / 19.58	80.57 \pm 32.93 ^{ab} n = 30	54.03 \pm 21.91 ^b n = 30	67.06	54.03
Honey + Sunflower	14.00 \pm 5.75 ^{ab} n = 30	13.81 11.86 / 16.08	64.59 \pm 18.64 ^b n = 29	36.93 \pm 12.43 ^c n = 29	57.18	38.03
Water	6.37 \pm 3.00 ^a n = 30	6.13 5.12 / 7.35	23.79 \pm 9.66 ^a n = 29	12.55 \pm 5.44 ^a n = 29	52.75	13.03
Water + Birch	5.53 \pm 2.06 ^a n = 30	5.48 4.78 / 6.29	16.21 \pm 5.86 ^b n = 29	12.58 \pm 4.65 ^a n = 29	77.61	13.13
Water + Maize	6.47 \pm 2.97 ^a n = 30	6.24 5.23 / 7.45	11.30 \pm 6.75 ^c n = 30	6.86 \pm 4.05 ^b n = 29	60.71	7.17
Water + Sunflower	3.6 \pm 1.67 ^b n = 30	3.47 2.90 / 4.16	8.45 \pm 4.89 ^c n = 29	4.66 \pm 3.53 ^b n = 29	55.15	5.03

* Within a column, means with the same letter within a basic diet (honey or water) are not significantly different.

** With lower and upper 95 % confidential limits as calculated from the WEIBULL-function (JMP procedure Survival/Reliability analysis)

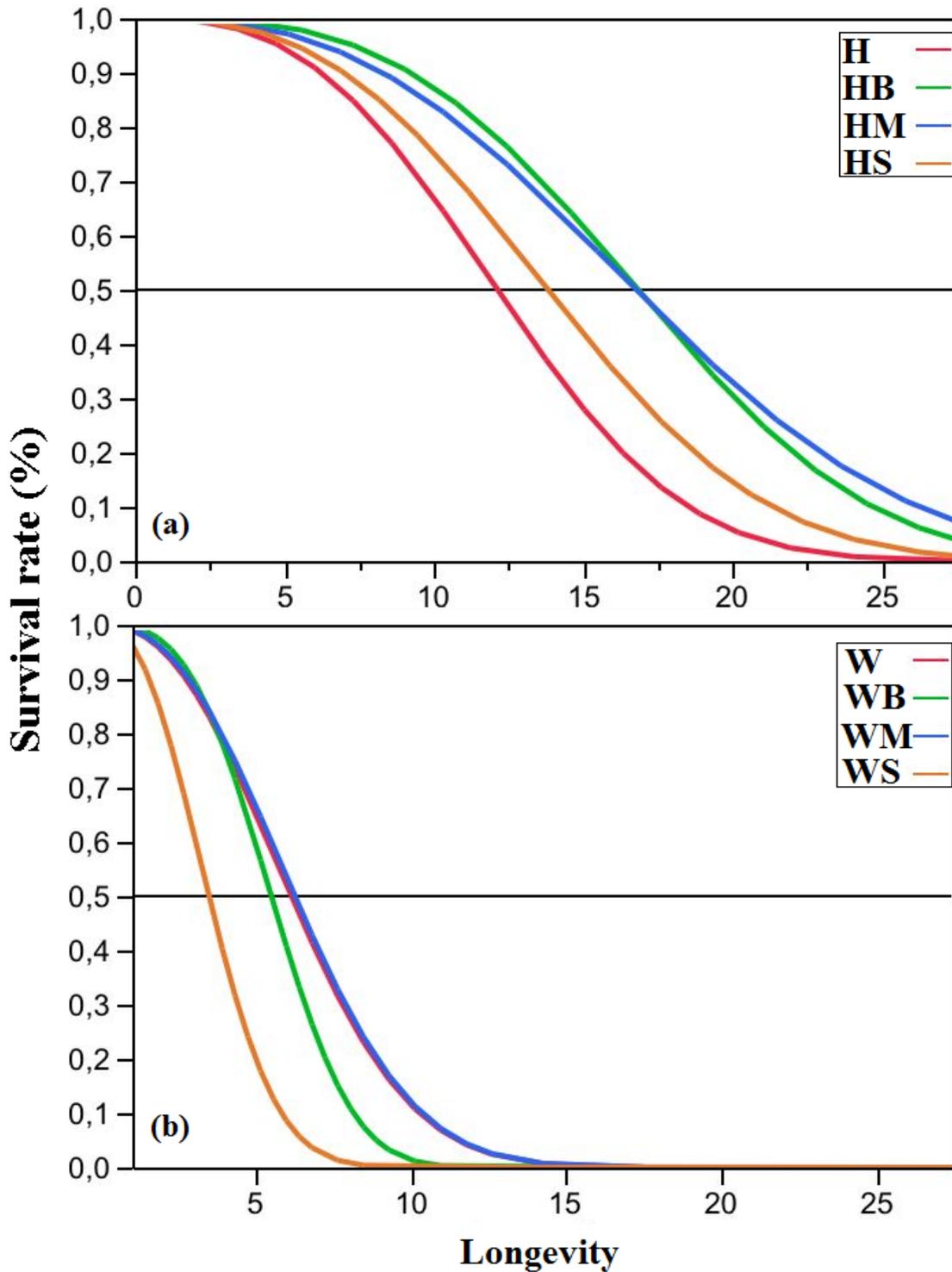


Figure 3 Survival plot of honey-fed (a) and water-fed (b) *T. cacaoeciae* strain HOH 1.

Mean total fecundity and total fertility was significantly affected by the basic diet and pollen added where both the dietary components also interacted (Table 4). Females fed with

honey-based diets had a significantly higher fecundity than those fed on water-based diets. However, except sunflower pollen which had a significantly negative effect, birch and maize pollen did not improve fecundity when mixed with honey as basic diet (one-way ANOVA, $F = 6.2699$, $P = 0.0006$; d.f. = 3,113; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level). However, each pollen added to water exerted a significantly adverse effect on fecundity (one-way ANOVA, $F = 26.6706$, $P < 0.0001$; d.f. = 3,113; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level) (Table 3).

Table 4 Analysis of variance (ANOVA, Standard Least Squares) of total fecundity and total fertility of *T. cacoeciae* as affected by honey or water as basic diets and pollen added.

Total fecundity			
Source	d.f.	F-value	P
Basic diet	1, 226	730.5275	< 0.0001
Added pollen	3, 226	12.3357	< 0.0001
Basic diet * added pollen	3, 226	3.0133	0.0310
Total fertility			
Source	d.f.	F-value	P
Basic diet	1, 223	749.4698	< 0.0001
Added pollen	3, 223	25.8128	< 0.0001
Basic diet * added pollen	3, 223	8.6589	< 0.0001

Fertility of deposited eggs of females fed with honey-based diets was significantly higher than of eggs produced by females supplied with a water-based diet (Table 3 and 5). Sunflower pollen added to honey (one-way ANOVA, $F = 16.6301$, $P < 0.0001$; d.f. = 3,111; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level), and maize and sunflower pollen added to water (one-way ANOVA, $F = 23.5715$, $P < 0.0001$; d.f. = 3,112; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level) had an adverse effect on fertility, whereas the other pollen did not influence fertility (Table 3).

The highest net reproductive rate (R_0) was found for females fed with honey only followed by honey mixed with birch, maize, and sunflower pollen. The same ranking could be found also in water-based diets (Table 3).

As in practical biocontrol, fecundity and fertility of the first three days in adult lifespan is taken to estimate the control efficacy of *Trichogramma* sp. under laboratory conditions. As in total fecundity and total fertility, fecundity and fertility after three days of adult life was affected by the basic diet and the pollen added. Both, basic diet and pollen showed significant interactions in their effect on the production of progeny (Table 5).

Table 5 Analysis of variance (ANOVA, Standard Least Squares) of fecundity and fertility after three days of adult lifespan of *T. cacoeciae* as affected by honey or water as basic diets and pollen added.

Fecundity			
Source	d.f.	F-value	P
Basic diet	1, 218	921.0929	< 0.0001
Added pollen	3, 218	16.5229	< 0.0001
Basic diet * added pollen	3, 218	2.8917	0.0363

Fertility			
Source	d.f.	F-value	P
Basic diet	1, 218	816.3205	< 0.0001
Added pollen	3, 218	26.7830	< 0.0001
Basic diet * added pollen	3, 218	2.9790	0.0324

Birch pollen added to honey resulted in a significantly higher fecundity after 3 days compared to honey alone, whereas maize and sunflower pollen had no effect (one-way ANOVA, $F = 9.3550$, $P < 0.0001$; d.f. = 3,108; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level). Birch pollen added to water only slightly increased fecundity, however, maize and sunflower pollen exerted a negative effect (one-way ANOVA, $F = 10.7553$, $P < 0.0001$; d.f. = 3,110; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level).

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Pollen of birch and maize added to honey did not improve fertility. Sunflower pollen reduced slightly fertility as compared with honey and honey with maize pollen slight but the reduction was significant if compared with honey with birch pollen (one-way ANOVA, $F = 3.2152$, $P = 0.0258$; d.f. = 3,108; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level). Fertility increased significantly when birch pollen was added to water. Maize pollen only marginally improved fertility compared to water alone and sunflower pollen had a negative effect on fertility, however not being significant to water but significant to water added with birch or maize pollen (one-way ANOVA, $F = 10.4454$, $P < 0.0001$; d.f. = 3,108; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level) (Table 6).

Table 6 Fecundity, fertility (mean \pm sd respectively) of *T. cacoeciae* after three days fed on different basic diets mixed with pollen.

Diets	Fecundity (eggs parasitized / female)	Fertility (% emerged adults)
Honey	37.35 \pm 5.10 ^b	76.05 \pm 8.67 ^{ab}
Honey + Birch	46.21 \pm 12.01 ^a	76.17 \pm 15.85 ^a
Honey + Maize	37.97 \pm 10.67 ^b	74.55 \pm 12.00 ^{ab}
Honey + Sunflower	33.93 \pm 6.35 ^b	66.32 \pm 17.89 ^b
Water	10.96 \pm 3.55 ^a	59.31 \pm 12.03 ^{bc}
Water + Birch	13.03 \pm 5.92 ^a	77.55 \pm 13.89 ^a
Water + Maize	7.17 \pm 4.51 ^b	67.61 \pm 32.97 ^{ab}
Water + Sunflower	7.18 \pm 4.69 ^b	45.40 \pm 22.47 ^c

* Within a column, means with the same letter within a basic diet (honey or water) are not significantly different.

2.5.3. Suitability of commercial bee-pollen

For *Trichogramma* mass-rearing it may be advantageous to substitute pure plant species specific pollen by commercially available bee-pollen to overcome plant species specific differences in

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nutritional quality and to provide a better availability of pollen. Performance of the three *Trichogramma* species was significantly affected when fed with honey or with honey ameliorated with bee pollen. Except fertility, longevity and fecundity differed significantly between the species. As expected, basic diet and pollen added showed interactive effects on the performance of *Trichogramma* species. Interactions between species and added pollen only revealed significant differences in fecundity and fertility whereas longevity was only slightly affected. Species-basic diet-pollen interactions could only be shown for fertility (Table 7 and 8).

Table 7 Analysis of variance (ANOVA, Standard Least Squares method) of total longevity, total fecundity and total fertility of three *Trichogramma* species as affected by honey or water as basic diets and bee pollen added.

Total longevity			
Source	d.f.	F-value	P
Basic diet	1, 325	313.2352	< 0.0001
Added pollen	1, 325	67.7756	< 0.0001
Species	2, 325	19.6187	< 0.0001
Basic diet * added pollen	1, 325	26.7406	< 0.0001
Species * basic diet	2, 325	6.9089	0.0012
Species * added pollen	2, 325	2.4124	0.0912
Species * basic diet * added pollen	2, 325	1.6342	0.1967
Total fecundity			
Source	d.f.	F-value	P
Basic diet	1, 325	1668.962	< 0.0001
Added pollen	1, 325	101.1738	< 0.0001
Species	2, 325	40.1207	< 0.0001
Basic diet * added pollen	1, 325	103.9245	< 0.0001
Species * basic diet	2, 325	15.2415	< 0.0001
Species * added pollen	2, 325	4.6789	0.0099
Species * basic diet * added pollen	2, 325	0.5084	0.6019
Total fertility			
Source	d.f.	F-value	P
Basic diet	1, 325	70.3760	< 0.0001
Added pollen	1, 325	33.3339	< 0.0001
Species	2, 325	1.3003	0.2739
Basic diet * added pollen	1, 325	31.1219	< 0.0001
Species * basic diet	2, 325	0.4684	0.6264
Species * added pollen	2, 325	11.5965	< 0.0001
Species * basic diet * added pollen	2, 325	7.2317	0.0008

Trichogramma oleae had a shorter adult lifespan compared with the other two species studied (Table 8). Bee pollen improved female performance only when added to honey (Table 8, Figure 4). As expected, net reproductive rate R_0 also reflects the positive effects of honey or pollen as diet constituents (Table 8).

Table 8 Longevity, fecundity, fertility and net reproductive rate (R_0) (mean \pm s.d. respectively) of three *Trichogramma* species fed with water alone (oneway ANOVA; for longevity: $F = 6.2365$, $P = 0.0030$, d.f. = 2,82; for fecundity: $F = 37.5359$, $P < 0.0001$; d.f. = 2,82; for fertility: $F = 2.2552$, $P = 0.1113$; d.f. = 2,82, followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level); water plus bee pollen (oneway ANOVA; for longevity: $F = 9.4864$, $P = 0.0002$, d.f. = 2,79; for fecundity: $F = 5.9559$, $P = 0.0039$; d.f. = 2,79; for fertility: $F = 16.1298$, $P < 0.0001$; d.f. = 2,79); honey alone (oneway ANOVA; for longevity: $F = 4.0737$, $P = 0.0205$, d.f. = 2,83; for fecundity: $F = 19.0574$, $P < 0.0001$; d.f. = 2,83; for fertility: $F = 1.1145$, $P = 0.3329$; d.f. = 2,83, followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level) or honey plus bee pollen (oneway ANOVA; for longevity: $F = 11.2108$, $P < 0.0001$, d.f. = 2,81; for fecundity: $F = 11.0068$, $P < 0.0001$; d.f. = 2,81; for fertility: $F = 4.0922$, $P = 0.0203$; d.f. = 2,81; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level).

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Species	Mean longevity* (days)	Mean fecundity * (eggs parasitized / female)	Mean fertility * (% emerged adults)	$R_0(\sum l_x m_x)$
Water				
<i>T. evanescens</i>	4.69 ± 1.39 ^b n = 29	11.66 ± 3.83 ^b n = 29	62.92 ± 24.86 ^a n = 29	7.17
<i>T. oleae</i>	4.15 ± 0.86 ^b n = 27	11.26 ± 2.43 ^b n = 27	64.10 ± 17.36 ^a n = 27	6.90
<i>T. cacoeciae</i>	5.86 ± 2.75 ^a n = 29	23.79 ± 9.66 ^a n = 29	54.30 ± 12.75 ^a n = 29	6.66
Water + Bee pollen				
<i>T. evanescens</i>	7.71 ± 2.01 ^a n = 24	16.83 ± 3.61 ^a n = 24	69.07 ± 10.87 ^b n = 24	11.47
<i>T. oleae</i>	5.50 ± 1.94 ^b n = 30	13.60 ± 3.86 ^b n = 30	73.57 ± 11.79 ^b n = 30	9.77
<i>T. cacoeciae</i>	5.54 ± 2.25 ^b n = 28	15.57 ± 2.90 ^{ab} n = 28	86.49 ± 12.11 ^a n = 28	13.20
Honey				
<i>T. evanescens</i>	11.90 ± 6.51 ^a n = 29	64.59 ± 13.80 ^b n = 29	82.16 ± 12.56 ^a n = 29	55.20
<i>T. oleae</i>	8.17 ± 5.19 ^b n = 30	54.47 ± 22.39 ^b n = 30	78.19 ± 9.19 ^a n = 30	42.07
<i>T. cacoeciae</i>	11.19 ± 3.86 ^{ab} n = 27	86.30 ± 14.06 ^a n = 27	79.79 ± 8.43 ^a n = 27	43.14
Honey + Bee pollen				
<i>T. evanescens</i>	18.86 ± 6.42 ^a n = 29	106.97 ± 25.60 ^a n = 29	77.03 ± 6.00 ^b n = 29	81.20
<i>T. oleae</i>	12.50 ± 4.01 ^b n = 28	87.11 ± 19.65 ^b n = 28	82.30 ± 8.16 ^a n = 28	69.97
<i>T. cacoeciae</i>	17.59 ± 5.26 ^a n = 27	116.07 ± 24.71 ^a n = 27	81.62 ± 8.39 ^{ab} n = 27	88.50

* Within a subcolumn, means with the same letter are not significantly different.

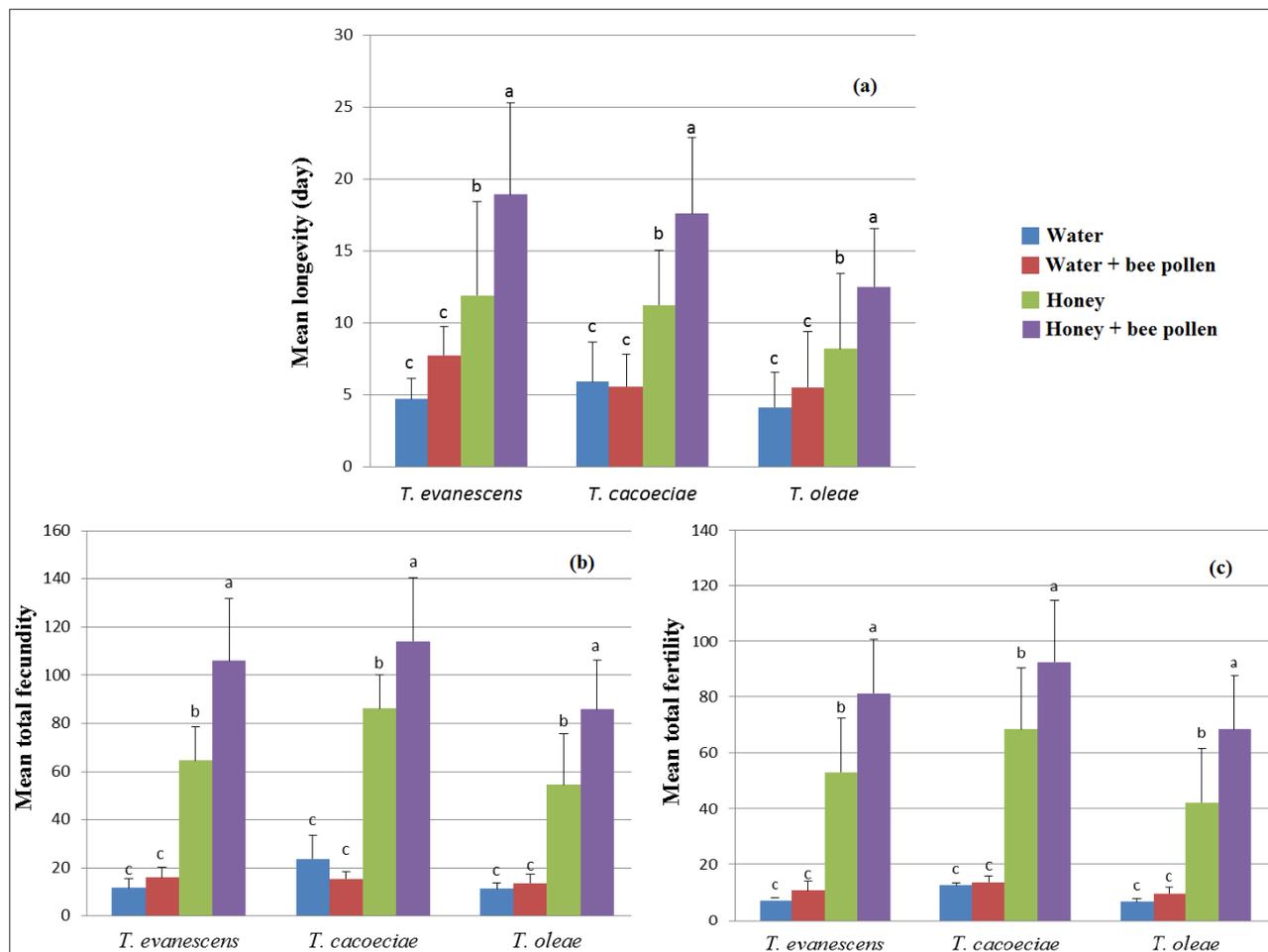


Figure 4 Longevity (a), total fecundity (b), and total fertility (c) (mean ± sd) of three *Trichogramma* species fed on water or honey alone or mixed with bee pollen.

2.6. Discussion

Pollen, as a plant tissue, is capable of protein synthesis, and its protein content may range from 2.5 - 61% (Roulston and Cane, 2000). Moreover, pollen is rich in free amino acids, sterols, lipids, and carbohydrates (Solberg and Remedios, 1980; Dobson, 1988; Nepi and Franchi, 2000; Pacini et al., 2006). With this broad spectrum of nutritive components, pollen may serve as sole diet or complement dietary deficiencies, of parasitoids. However, pollen also may contain secondary plant compounds, such as alkaloids, phenolics, and terpenes, or mannose sugars, which are known to be toxic to honeybees (Stanley and Linskens, 1974; Roulston and Cane, 2000; Wäckers, 2005). Adult *Trichogramma* are known to feed upon nectar, pollen and the

host's body fluid to satisfy the demand for energy, proteins or micronutrients (Zhang et al., 2004).

Because of the high variability in chemical pollen constituents its nutritional quality may vary. If consumed as complementary food to ameliorate poor diets or energy rich diets, pollen containing diets should affect adult performance of *Trichogramma* and differences in nutritional quality of pollen should be uncovered. Furthermore, intraspecific differences in adult life-table parameters of a *Trichogramma* species have been reported (Smith and Hubbes, 1986; Ram et al., 1995; Samara et al., 2008). It may be assumed, that conversion of food also differs with genotype within a species.

Food consumption by adults of parasitoids is considered to be one of the most critical factors affecting survival, searching efficiency, reproduction, and, thus, the success of biological control (Jervis et al., 1996; Lundgren, 2009). Unlike for larval and pupal parasitoids, the majority of recent studies on *Trichogramma* sp. aimed towards carbohydrate sources. With few exceptions, pollen as additional or complementary food source for egg parasitoids has not been assessed (Wellinga and Wysoki, 1989; Somchoudhury and Dutt, 1988; Rohi et al., 2002; Zhang et al., 2004; Geng et al., 2006). Our experiments have shown that the carbohydrate source honey improved all adult life-table parameters of four *T. cacoeciae* strains compared to water. A similar positive effect has been reported earlier for *T. brassicae* (Gurr and Nicol, 2000; Lundgren and Heimpel, 2003), *T. chilonis* Ishii (Leatemia et al., 1995), *T. evanescens* (Narayanan and Mookherjee, 1955), *T. maxacalii* Voegelé and Pointel (de Oliveira et al., 2000), *T. minutum* (Marston and Ertle, 1969; Yu et al., 1984; Smith et al., 1986; Leatemia et al., 1995; Lundgren and Heimpel, 2003), *T. ostriniae* Pang and Chen (Hoffmann et al., 1995; Fuchsberg et al., 2007), *T. platneri* (Hohmann et al., 1988; Mansfield and Mills, 2002), *T. pretiosum* Riley (Ashley and Gonzalez, 1974; Lundgren and Heimpel, 2003). Particularly the fecundity, the most important parameter in biocontrol, was increased by honey-feeding 3.3 to 6-fold in *T. minutum* (Yu et al., 1984; Leatemia et al., 1995), and 1.3 to 2.3-fold in *T. evanescens* (Lund, 1938; Ashley and Gonzales, 1974).

In contrast to these positive results, the presence of honey reduced the percentage of ovipositing females of *T. principium* Sugonjaev and Sorokina and the mean number of eggs laid during 2 days (Reznik et al., 1997).

Our experiments with *T. cacoeciae* revealed significant differences in life-table parameters between the four strains tested. *Trichogramma cacoeciae* strains HOH 2 and SY 98 01

performed significantly better than the strains HOH 1 and KIO 2 TN as indicated by longevity and fecundity when offered water only. Fertility, however, was erratic between the four strains. Offering honey improved adult longevity, fecundity and fertility significantly, which has also been found in part for *T. platneri* (McDougall and Mills, 1997). The significant differences in adult longevity and fertility disappeared, only mean fecundity still differed significantly. However, the relative positive effect of honey reveals best the different capability of the four strains to utilize this energy source. Considering the relativized data (Ratio honey-fed / water-fed), strain HOH 1 experienced a considerable stronger improvement of longevity, fecundity, and net reproduction rate than any other strain tested, although the performance was very poor when water was offered only. This level of enhanced performance corresponds to results obtained with *T. platneri* and *T. minutum* and species-specific differences in performance may be minimized by food of higher quality (Marston and Ertle, 1969; Yu et al., 1984; Smith et al., 1986; Hohmann et al., 1988; Leatemia et al., 1995; Garcia et al., 2001; Zhang et al., 2004; Kalyebi et al., 2005; Samara et al., 2008). We assume that these differences observed might be due to the different effects of ecological pressure from local climatic conditions and, consequently, to genetic variability of these strains. Intraspecific genetic variability has been reported in *T. brassicae* (*T. maidis* Voegelé and Pintureau) for various traits such as locomotion (Pompanon et al., 1997), the area searched by a female (Bruins et al., 1994; Wajnberg and Colazza, 1998), and progeny allocation (Wajnberg, et al., 1989). Metabolisation of food may be assumed also differing with genotypes as shown by our results.

The question whether pollen can ameliorate poor (water) or energy-rich (honey) diets has been examined poorly and the results and interpretations were contradictory. Our results with a *T. cacoeciae* strain (HOH 1) performing poorly with water-supply only, but experiencing the best improvement when honey was offered should reveal best the dietary effects of pollen collected different plant species. All assessed parameters were, either as a trend or significantly, affected by pollen-containing diets. The effects observed in females leave no doubt that pollen may serve as food source and is taken up by *T. cacoeciae*, and it may be possible, that more *Trichogramma* species may exploit pollen as additional food source as it has been reported for *T. cacoeciae* in our study or *T. bourarachae* Pintureau and Babault, *T. brassicae* and *T. chilonis* (Rohi et al., 2002; Zhang et al., 2004; Geng et al., 2006). However, the different pollen offered revealed diverging results. When pollen was added to water, only maize pollen had no effect on female longevity but an adverse effect on reproduction. Birch and sunflower pollen, at least as a trend, deteriorated longevity and fecundity. When added to honey, *per contra*, some pollen could

significantly improve longevity and reproduction. Comparing the different pollen, irrespectively the basic diet, sunflower had no positive or even a detrimental effect on longevity, fecundity and fertility. In contrast, birch and maize pollen improved female longevity and did affect fecundity adversely. Obviously pollen suitability as food depends on what the pollen is mixed with. Regular or transgenic cotton pollen with honey prolonged longevity of *T. chilonis* over honey alone (Geng et al., 2005, 2006). Wang et al. (2007) reported that *T. ostrinae* fed on honey only or honey with maize pollen lived longer and produced more offspring than those fed on water and water with maize pollen. Mixing maize pollen with water improves longevity of *T. brassicae* over water alone, but when added to honey it attains no measurable benefits (Zhang et al., 2004). *Lycopersicon* sp. pollen was not conceded a significant positive effect on *T. bourarachae* although the authors reported a slightly longer lifespan compared to unfed adults (Rohi et al., 2002). Our experiments revealed that the effects of pollen mixed with water or honey vs. water or honey alone also depend on the *Trichogramma* species tested, as shown for *T. cacoeciae*, *T. evanescens*, and *T. oleae* when bee pollen was offered. Performance of *T. evanescens* and *T. oleae* was improved by 30 - 64 % for longevity and 20 - 70 % for fecundity when bee pollen was added to both, water or honey, whereas *T. cacoeciae* was negatively affected by bee pollen added to water but benefit when added to honey.

Due to a complex composition of small molecular nutrients, high levels of free amino acids, protein, lipids, and polysaccharides, pollens are considered important valuable alternate or additional food sources for predators and parasitoids improving life-table parameters (Barbier, 1970; Stanley and Linskens, 1974).

The highly variable performance of *Trichogramma* species might be dependent on either an interspecific variability in pollen utilization or in food quality of different pollens. Our results obtained with *T. cacoeciae*, *T. evanescens*, and *T. oleae* show that utilization of bee pollen, a mixture of pollen from different plant species (species composition not analyzed) differs with *Trichogramma* species. On the other hand, our results obtained with *T. cacoeciae* fed with pollen of different plant species indicate that also pollen differs in nutritional quality. However, comparisons of the nutritional value of pollens from different plant species and their effect on life-table parameters are rare. Only Ouyang et al. (1992) demonstrated that development, survivorship, and reproduction of *Euseius tularensis* Congdon (Acari, Phytoseiidae) varied markedly with the pollen source. This might due to the different contents of amino acids in pollen originating from different plant species as also reported for butterfly longevity and

fecundity after ingestion of amino acids and carbohydrates in pollen of various plant species (O'Brien et al., 2003; Mevi-Schütz and Erhardt, 2005; Beck, 2007).

In our results, the differences in nutritional values of pollens for *T. cacoeciae* are notable and a reliable explanation requires a detailed analysis of pollen constituents and a subsequent correlation with life-table parameters which remains to be subjected to further studies.

Reports of *Trichogramma* species visiting open flowers in the field are lacking and have been described from laboratory experiments only, resulting in a better parasitoid performance (Begum et al., 2004, 2006; Witting-Bissinger et al., 2008). From these results it may be deduced that active searching for open inflorescences and pollen-feeding besides nectar-uptake could be possible. However, it can be taken as highly probable that they will opportunistically feed on pollen of anemochorous plants wherever these pollen may be deposited, as it may be observed in maize fields or in fields in the vicinity to anemochorous trees like birch.

2.7. Conclusion

Our results demonstrate that honey has positive effects on longevity, fecundity, and fertility of *Trichogramma* species. Pollen may improve *Trichogramma* performance as well, however, differences in honey and pollen utilization as food source occur between *Trichogramma* species and pollen sources. Carbohydrates and pollen, when taken up either opportunistically or after directed search may provide a better control efficacy after augmentative release of *Trichogramma*. Furthermore, honey-pollen mixtures may improve mass-rearing. Further studies on pollen constituents and their effect on egg parasitoids will provide a better insight into *Trichogramma* nutritional physiology.

2.8. Acknowledgements

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References

- Agamy, E., 2010. Field evaluation of the egg parasitoid, *Trichogramma evanescens* West. against the olive moth *Prays oleae* (Bern.) in Egypt. *Journal of Pest Science* 83, 53-58.
- Albert, R., Maier, M., Dannemann, K., 2008. Maiszünslerbekämpfung Bekämpfung und neue Entwicklungen beim *Trichogramma brassicae* Einsatz. *Gesunde Pflanzen* 60, 41-54.
- Andow, D.A., Risch, S.J., 1987. Parasitism in diversified agroecosystems: Phenology of *Trichogramma minutum* (Hym.: Trichogrammatidae). *Entomophaga* 32, 255-260.
- Ashley, T.R., Gonzalez, D., 1974. Effect of various food substances on longevity and fecundity of *Trichogramma*. *Environmental Entomology* 3, 169-561.
- Ballal, C.R., Singh, S.P., 2003. The effectiveness of *Trichogramma chilonis*, *Trichogramma pretiosum* and *Trichogramma brasiliense* (Hym.: Trichogrammatidae) as parasitoids of *Helicoverpa armigera* (Lep.: Noctuidae) on sunflower (*Helianthus annuus*) and redgram (*Cajanus cajan*). *Biocontrol Science and Technology* 13, 231-240.
- Barbier, M., 1970. Chemistry and biochemistry of pollens. *Progress in Phytochemistry* 2, 1-34.
- Bastos, C.S., Torres, J.B., Suinaga, F.A., 2010. Parasitism of cotton leafworm *Alabama argillacea* eggs by *Trichogramma pretiosum* in commercial cotton fields. *Journal of Applied Entomology* 134, 572-580.
- Beck, J., 2007. The importance of amino acids in the adult diet of male tropical rainforest butterflies. *Oecologia* 151, 741-747.
- Begum, M., Gurr, G.M., Wratten, S.D., Nicol, N.I., 2004. Flower colour affects tri-trophic biocontrol interactions. *Biological Control* 30, 584-590.
- Begum, M., Gurr, G.M., Wratten, S.D., Hedberg, P.R., Nicol, H.I., 2006. Using selective food plants to maximize biological control of vineyard pests. *Journal of Applied Ecology* 43, 547-554.
- Berndt, L.A., Wratten, S.D., 2005. Effects of alyssum flowers on the longevity, fecundity, and sex ratio of the leafroller parasitoid *Dolichogenidea tasmanica*. *Biological Control* 32, 65-69.

- Bigler, F., 1994. Quality control in *Trichogramma* production. In: Wajnberg, E., Hassan, S.A., (Eds.), Biological control with egg parasitoids. CAB International, Wallingford, UK, pp. 93-111.
- Bogdanov, S., Bieri, K., Iff, D., Seiler, K., Schenk, E., Stöckli, H., Zürcher, K., 1999. Pollen: Umschreibung und Richtlinien zur Beuteilung - Auszüge aus einem Entwurf für das Schweizerische Lebensmittelbuch.
(www.culturaapicola.com.ar/apuntes/polen/polen_aleman.pdf; last access: 15th March 2012)
- Bruins, E.B.A., Wajnberg, W., Pak, E.G.A., 1994. Genetic variability in the reactive distance in *Trichogramma brassicae* after automatic tracking of the walking path. *Entomologia Experimentalis et Applicata* 72, 297-303.
- Cerutti, F., Bigler, F., 1991. Methods for the quality evaluation of *Trichogramma evanescens* Westw. used against the European corn borer. In: Bigler, F., (Eds.), Proceedings of the fifth workshop of the IOBC working group, quality control of mass reared arthropods. Swiss Federal Research Station, Wageningen NL, pp. 119-126.
- Davies, A.P., Carr, C.M., Scholz, B.C.G., Zalucki, M.P., 2011b. Using *Trichogramma* Westwood (Hym.: Trichogrammatidae) for insect pest biological control in cotton crops: an Australian perspective. *Australian Journal of Entomology* 50, 424-440.
- Davies, A.P., Pufke, U.S., Zalucki, M.P., 2011a. Spatio-temporal variation in *Helicoverpa* egg parasitism by *Trichogramma* in a tropical Bt-transgenic cotton landscape. *Agricultural and Forest Entomology*. 13, 247-258.
- Dobson, H.E.M., 1988. Survey of pollen and pollenkitt lipids - chemical cues to flower visitors? *American Journal of Botany* 75, 170-182.
- Dutton, A., Bigler, F., 1995. Flight activity assessment of the egg parasitoid *Trichogramma brassicae* (Hym, Trichogrammatidae) in laboratory and field conditions. *Entomophaga* 40, 223-233.
- El-Wakeil, N.E., Farghaly, H.Th., Ragab, Z.A., 2008. Efficacy of inundative releases of *Trichogramma evanescens* in controlling *Lobesia botrana* in vineyards in Egypt. *Journal of Pest Science* 81, 49-55.

- Faria, C.A., Torres, J.B., Fernandes, A.M.V., Farias, A.M.I., 2008. Parasitism of *Tuta absoluta* in tomato plants by *Trichogramma pretiosum* Riley in response to host density and plant structures. *Ciência Rural* 38, 1504-1509.
- Flanders, S.E., 1937. Notes on life history and anatomy of *Trichogramma*. *Annals of the Entomological Society of America* 30, 304-308.
- Forrest, J.M.S., Knights, B.A., 1972. Presence of phytosterols in the food of the aphid, *Myzus persicae*. *Journal of Insect Physiology* 18, 723-728.
- Forsse, E., Smith S.M., Bouchier R.S., 1992. Flight initiation in the egg parasitoid *Trichogramma minutum*: Effects of ambient temperature, mates, food and host eggs. *Entomologia Experimentalis et Applicata* 62, 147-154.
- Fuchsberg, J.R., Yong, T.H., Losey, J.E., Carter, M.E., Hoffmann, M.P., 2007. Evaluation of corn leaf aphid (*Rhopalosiphum maidis*; Homoptera: Aphididae) honeydew as a food source for the egg parasitoid *Trichogramma ostrinae* (Hym.: Trichogrammatidae). *Biological Control* 40, 230-236.
- Garcia, P.V., Wajnberg, E., Oliveira, M.L.M., Tavares, J., 2001. Is the parasitisation capacity of *Trichogramma cordubensis* influenced by the age of the female?. *Entomologia Experimentalis et Applicata* 98, 219-224.
- Gardner, S. M., Dissevelt, M., van Lenteren, J.C., 2007. Behavioural adaptations in host finding by *Trichogramma evanescens* (Westw.): the influence of oviposition experience on response to host contact kairomones. *Bulletin of Insectology* 60, 23-30.
- Geng, J-H., Shen, Z-R., Li Z-X., Zheng, L., 2005. Effect of pollen of conventional cotton and transgenic Cry1Ac+CpTI cotton on reproduction and survival of the parasitoid wasp *Trichogramma confusum* Viggiani (Hym.: Trichogrammatidae). *Acta Ecologica Sinica* 25, 1575-1582.
- Geng, J.H., Shen, Z.R., Song, K., Zheng, L., 2006. Effect of pollen of regular cotton and transgenic Bt+CpTI cotton on the survival and reproduction of the parasitoid wasp *Trichogramma chilonis* (Hym.: Trichogrammatidae) in the laboratory. *Environmental Entomology* 35, 1661-1668.

- Greenberg, S.M., 1991. Evaluation techniques for *Trichogramma* quality. In: Bigler, F. (Eds.), Proceedings of fifth workshop of the IOBC working group, quality control of mass reared arthropods. Swiss Federal Research Station, Wageningen, NL, pp. 138-145.
- Gurr, G. M., Nicol, H.I., 2000. Effect of food on longevity of adults of *Trichogramma carverae* (Oatman and Pinto) and *Trichogramma brassicae* (Bezdenko) (Hym.: Trichogrammatidae). Australian Journal of Entomology 39, 185-187.
- Hassan, S.A., 1993. The mass rearing and utilizing of *Trichogramma* to control lepidopterous pests achievement and outlook. Pesticide Science 37, 387-391.
- Hassan, S.A., 1994. Strategies to select *Trichogramma* species for use in biological control. In: Wajnberg, E, Hassan, S.A. (Eds.), Biological control with egg parasitoids. Wallingford, UK: CAB International, pp. 55-71.
- Hegazi, E., Herz, A., Hassan, S.A., Khafagi, W.E., Agamy, E., Zaitun, A., El-Aziz, G.A., Showeil, S., El-Said, S., Khamis, N., 2007. Field efficiency of indigenous egg parasitoids (Hym.: Trichogrammatidae) to control the olive moth (*Prays oleae*, Lep.: Yponomeutidae) and the jasmine moth (*Palpita unionalis*, Lep.: Pyralidae) in an olive plantation in Egypt. Biological Control 43, 171-187.
- Hegazi, E., Khafagi, W.E., Herz, A., Konstantopoulou, M., Hassan, S.A., Agamy, E., Atwa, A., Shweil, S., 2012. Dispersal and field progeny production of *Trichogramma* species released in an olive orchard in Egypt. BioControl 57, 481-492 (doi: 10.1007/s10526-011-9420-4).
- Heimpel, G.E., Jervis, M.A., 2005. Does floral nectar improve biological control by parasitoids? In: Wäckers, F.L., van Rijn, P.C.J., Bruin, J. (Eds.), Plant-provided food for carnivorous insects: A protective mutualism and its applications. Cambridge University Press, Cambridge, UK, pp. 267-304.
- Hoffmann, M.P., Walker, D.L., Shelton, A.M., 1995. Biology of *Trichogramma ostriniae* (Hym.: Trichogrammatidae) reared on *Ostrinia nubilalis* (Lep.: Pyralidae) and a survey for additional hosts. Entomophaga 40, 387-402.
- Hoffmann, M.P., Pitcher, S.A., Cheever, S.A., Gardner, J., Losey, J.E., Kuhar, T.P., Laub, C.A., Youngman, R.R., 2006. Efficacy of inoculative releases of *Trichogramma ostriniae* (Hym.: Trichogrammatidae) against European corn borer *Ostrinia nubilalis* (Lep.: Crambidae) in field corn. Biological Control 36, 345-349.

- Hohmann, C.L., Luck, R.F., Oatman, E.R., Platner, G.R., 1989. Effects of different biological factors on longevity and fecundity of *Trichogramma platneri* Nagarkatti (Hym.: Trichogrammatidae). *Anais da Sociedade Entomologica do Brasil* 18, 61-70.
- Hohmann, C.L., Luck, R.F., Oatman, E.R., 1988. A comparison of longevity and fecundity of adult *Trichogramma platneri* (Hym.: Trichogrammatidae) reared from eggs of the cabbage looper and the angoumois grain moth with and without access to honey. *Journal of Economic Entomology* 81, 1307-1312.
- Hommay, G., Gertz, C., Kienlen, J.C., Pizzol, J., Chavigny, P., 2002. Comparison between the control efficacy of *Trichogramma evanescens* Westw. (Hym.: Trichogrammatidae) and two *Trichogramma cacoeciae* Marchal strains against grapevine moth (*Lobesia botrana* (Den. and Schiff.)), depending on their release density. *Biocontrol Science and Technology* 12, 569-581.
- Hwang, I.C., Park, C., Kang, D.K., Jin, N.Y., Jung, S.Y., Seo, M.J., Kim, J.E., Youn, Y.N., Yu, Y.M., 2010. Combined application of *Trichogramma ostrinae* and *Bacillus thuringiensis* for eco-friendly control of *Plutella xylostella*. *Journal of Applied Biological Chemistry* 53, 316-322.
- Irvin, N.A., Hoddle, M.S., Castle, S.J., 2007. The effect of resource provisioning and sugar composition of foods on longevity of three *Gonatocerus* spp., egg parasitoids of *Homalodisca vitripennis*. *Biological Control* 40, 69-79.
- Irvin, N.A., Hoddle, M.S., 2007. Evaluation of floral resources for enhancement of fitness of *Gonatocerus ashmeadi*, an egg parasitoid of the glassy-winged sharpshooter, *Homalodisca vitripennis*. *Biological Control* 40, 80-88.
- Irvin, N.A., Scarratt, S.L., Wratten, S.D., Frampton, C.M., Chapman, R.B., Tylianakis, J.M. 2006. The effects of floral understoreys on parasitism of leafrollers (Lep.: Tortricidae) on apples in New Zealand. *Agricultural and Forest Entomology* 8, 25-34.
- Jervis, M.A., Kidd N.A.C., 1986. Host-feeding strategies in hymenopteran parasitoids. *Biological Reviews* 61, 395-434.
- Jervis, M.A., Kidd N.A.C. Heimpel G.E., 1996. Parasitoid adult feeding behaviour and biocontrol a review. *Biocontrol News and Information* 17, 11N-26N.

- Jervis, M.A., Kidd, N.A.C. Walton, M., 1992. A review of methods for determining dietary range in adult parasitoids. *Entomophaga* 37, 565-574.
- Jonsson, M., Wratten, D.S., Landis, D.A., Gurr, M.G., 2008. Recent advances in conservation biological control of arthropods by arthropods. *Biological Control* 45, 172-175.
- Kalyebi, A., Overholt, W.A., Sithanatham, S., Mueke, J.M., Hassan, S.A. Schulthess, F., 2005. Parasitism, longevity and progeny production of six indigenous Kenyan trichogrammatid egg parasitoids (Hym.: Trichogrammatidae) at different temperature and relative humidity regimes. *Biocontrol Science and Technology* 15, 255-270.
- Klug, T., Meyhöfer, R., 2009. Performance of two *Trichogramma brassicae* strains under greenhouse and field conditions for biocontrol of the silver Y moth in spinach cultures. *Journal of Pest Science*. 82, 73-79.
- Knutson, A., 1998. The *Trichogramma* Manual. Agricultural communications, Texas agriculture extension service. A and M University System College Station, TX, USA, pp. 37.
- Leatemia, J.A., Laing, J.E., Corrigan, J. E., 1995. Effects of adult nutrition on longevity, fecundity, and offspring sex ratio of *Trichogramma minutum* (Hym.: Trichogrammatidae). *Canadian Entomologist* 127, 245-254.
- Leius, K., 1963. Effects of pollens on fecundity and longevity of adult *Scambus buoliana* (Htg.) (Hym.: Ichneumonidae). *Canadian Entomologist* 95, 202-207.
- Li, J., Chen, J., Zhang, Z., Pan, Y., 2008. Proteome analysis of tea pollen (*Camellia sinensis*) under different storage conditions. *Journal of Agricultural and Food Chemistry* 56, 7535-7544.
- Loper, G.M., Cohen, A.C., 1987. Amino acid content of dandelion pollen, a honey bee (Hym.: Apidae) nutritional evaluation. *Journal of Economic Entomology* 80, 14-17.
- Lund, H.O., 1938. Studies on longevity and productivity in *Trichogramma evanescens*. *International Journal of Agricultural Research* 56, 421-439.
- Lundgren, J.G., 2009. Relationships of natural enemies and non-prey foods. Springer International, Dordrecht, The Netherlands. pp. 453.
- Lundgren, J.G., Heimpel, G.E., 2003. Quality assessment of three species of commercially produced *Trichogramma* and the first report of thelytoky in commercially produced *Trichogramma*. *Biological Control* 26, 68-73.

- Mansfield, S., Mills, N.J., 2002. Host egg characteristics, physiological host range, and parasitism following inundative releases of *Trichogramma platneri* (Hym.: Trichogrammatidae) in walnut orchards. *Environmental Entomology* 31, 723-731.
- Marston, N., Ertle, L.R., 1969. Host age and parasitism by *Trichogramma minutum* (Hym.: Trichogrammatidae). *Annals of the Entomological Society of America* 62, 1476-1481.
- Mathews, C.R., Bottrell, D.G., Brown, M. W., 2011. Interactions between extrafloral nectaries, ants (Hymenoptera: Formicidae), and other natural enemies affect biological control of *Grapholita molesta* (Lep.: Tortricidae) on peach (Rosales: Rosaceae). *Environmental Entomology* 40, 42-51.
- Mc Dougall, S.J., Mills, N.J., 1997. The influence of hosts, temperature and food sources on the longevity of *Trichogramma platneri*. *Entomologia Experimentalis et Applicata* 83, 195-203.
- Mevi-Schütz, J., Erhardt, A., 2005. Amino acids in nectar enhance butterfly fecundity: a long-awaited link. *The American Naturalist* 165, 411-419.
- Miura, K., Tanaka, H., Shibao, M., Ohta, I., 2001. Effect of release of egg parasitoids, *Trichogramma* spp., with a net-type row cover on the diamondback moth, *Plutella xylostella* (L.). *Japanese Journal of Applied Entomology* 45, 151-155.
- Nagarkatti, S., Tobin, P.C., Sauders, M.C., Muza, A. J., 2003. Release of native *Trichogramma minutum* to control grape berry moth. *Canadian Entomologist* 135, 589-598.
- Narayanan, E.S., Mookherjee, P.B., 1955. Effect of nutrition on the longevity and rate of reproduction in *Trichogramma evanescens minutum* (Chalcidoidea: Hymenoptera). *Indian Journal of Entomology*. 17, 376-382.
- Nepi, M., Franchi, G. G., 2000. Cytochemistry of mature angiosperm pollen. *Plant Systematic and Evolution* 222, 45-62.
- O'Brien, D.M., Boggs, C.L., Fogel, M.L., 2003. Pollen feeding in the butterfly *Heliconius charitonia*: isotopic evidence for essential amino acid transfer from pollen to eggs. *Proceedings of the Royal Society of London B* 270, 2631-2636.
- Olson, D.M., Andow, D.A., 1998. Larval crowding and adult nutrition effects on longevity and fecundity of female *Trichogramma nubilale* Ertle and Davis (Hym.: Trichogrammatidae). *Environmental Entomology* 27, 508-514.

- Ouyang, Y., Grafton-Cardwell, Elizabeth, E., Bugg, R.L., 1992. Effects of various pollens on development, survivorship, and reproduction of *Euseius tularensis* (Acari: Phytoseiidae). *Environmental Entomology* 21, 1371-1376.
- Oztemiz, S., 2009. Natural parasitism and release efficiency of *Trichogramma evanescens* Westwood in *Ostrinia nubilalis* Hübner attacking maize in Turkey. *Journal of Entomological Science* 44, 132-140.
- Pacini, E., Guarnieri, M., Nepi, M., 2006. Pollen carbohydrates and water content during development, presentation, and dispersal: a short review. *Protoplasma* 228, 73-77.
- Perveen, A., Ali, K.SH., 2008. Maintenance of pollen germination capacity of *Malus pumila* L., (Rosaceae). *Pakistan Journal of Botany* 40, 963-966.
- Pluciennik, P., Olszak, R.W., 2010. The role of parasitoids in limiting the harmfulness of leafrollers in apple orchards. *Journal of Plant Protection Research* 50, 1-8.
- Pompanon, F., Scheipper, B., Mourer, Y., De Fouillet, P., Bouletreau, M., 1997. Evidence for a substrate-borne sex pheromone in the parasitoid wasp *Trichogramma brassicae*. *Journal of Chemical Ecology* 23, 1349-1360.
- Quicke, D.L.J., 1997. *Parasitic Wasps*. Chapman and Hall, London, 470 pp.
- Ram, P., Tshernyshey, W.B., Afonina, V.M., Greenberg, Sh. M., 1995. Studies on the strains of *Trichogramma evanescens* Westwood (Hym.: Trichogrammatidae) collected from different hosts in northern Maldoval. *Journal of Applied Entomology* 119, 79-82.
- Reznik, S.Ya., Umarova, T.Ya., Voinovich, N.D., 1997. The influence of previous host age on current host acceptance in *Trichogramma*. *Entomologia Experimentalis et Applicata* 82, 153-157.
- Rodrigues, M.L., Garcia, M.S., Nava, D.E., Botton, M., Parra, J.R.P., Guerrero, M., 2011. Selection of *Trichogramma pretiosum* lineages for control of *Grapholita molesta* in peach. *Florida Entomologist* 94, 398-403.
- Rohi, L., Bourarach, K., Chemseddine, M., Pintureau, B., 2002. Effet de la nourriture sur la longévité de deux Hyménoptères parasitoïdes oophages. *Bulletin Mensuel de la Societe Linneenne de Lyon* 71, 118-122 (abstract only).

- Romeis, J., Babendreier, D., Wäckers, F.L., Shanower, T.G., 2005. Habitat and plant specificity of *Trichogramma* egg parasitoids - underlying mechanisms and implications. *Basic and Applied Ecology* 6, 215-236.
- Romeis, J., Shanower, T.G., Zebitz, C.P.W., 1999. *Trichogramma* egg parasitism of *Helicoverpa armigera* on pigeonpea and sorghum in southern India. *Entomologia Experimentalis et Applicata* 90, 69-81.
- Roulston, T.H., Cane, J.H., 2000. Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution* 222, 187-209.
- Russell, K., Bessin, R., 2009. Integration of *Trichogramma ostriniae* releases and habitat modification for suppression of European corn borer (*Ostrinia nubilalis* Hübner) in bell peppers. *Renewable Agriculture and Food Systems* 24, 19-24.
- Saavedra, J.L.D., Torres, J.B., Ruiz, M.G., 1997. Dispersal and parasitism of *Heliothis virescens* eggs by *Trichogramma pretiosum* (Riley) in cotton. *Journal of Pest Management* 43, 169-171.
- Samara, R., Monje, C.J., Zebitz, C.P.W., 2008. Comparison of different European strains of *Trichogramma aurosum* (Hym.: Trichogrammatidae) using fertility life tables. *Biocontrol Science and Technology* 18, 75-86.
- Samara, R., Monje, J.C., Zebitz, C.P.W., Qubbaj, T., 2011. Comparative biology and life tables of *Trichogramma aurosum* on *Cydia pomonella* at constant temperatures. *Phytoparasitica* 39, 109-119.
- Schöller, M., Hassan, S.A., 2001. Comparative biology and life tables of *Trichogramma evanescens* and *T. cacoeciae* with *Ephestia elutella* as host at four constant temperatures. *Entomologia Experimentalis et Applicata* 98, 35-40.
- Shearer, P.W., Atanassov, A., 2004. Impact of peach extrafloral nectar on key biological characteristics of *Trichogramma minutum* (Hym.: Trichogrammatidae). *Journal of Economic Entomology* 97, 789-792.
- Sithanantham, S., Abera, T.H., Baumgärtner, J., Hassan, S.A., Löhr, B., Monje, J.C., Overholt, W.A., Paul, A.V.N., Fang Hao, Wan, Zebitz, C.P.W., 2001. Egg parasitoids for augmentative biological control of lepidopteran vegetable pests in Africa: research status and needs. *Insect Science and its Application* 21, 189-205.

- Smith, S.M., Hubbes. M., Carrow, J.R., 1986. Factors affecting inundative releases of *Trichogramma minutum* against the spruce budworm. *Journal of Applied Entomology* 101, 29-39.
- Smith, S.M., 1996. Biological control with *Trichogramma*: advances success and potential of their use. *Annual Review of Entomology* 41, 375-406.
- Solberg Y., Remedios G., 1980. Chemical composition of pure and bee-collected pollen. *Meldinger fra Norges Landbrukshogskole* 59, 1-13.
- Somchoudhury, A.K., Dutt N., 1988. Evaluation of some flowers as a nutritional source of *Trichogramma* spp. *Indian Journal of Entomology* 50, 371-373.
- Stanley, R.G., Linskens, H.F., 1974. *Pollen: biology, biochemistry, management*. Berlin, Springer-Verlag 117-257.
- Stinner, R.E., Ridway, R.L., Morrison, R.K., 1974. Longevity, fecundity, and searching ability of *Trichogramma pretiosum* reared by three methods. *Environmental Entomology* 3, 558-560.
- Tylianakis, J.M., Didham, R.K., Wratten, S.D., 2004. Improved fitness of aphid parasitoids receiving resource subsidies. *Ecology* 85, 658-666.
- van Lenteren, J.C., 2000. Success in biological control of arthropods by augmentation of natural enemies. In: Gurr, G., Warten, S. (Eds.), *Biological control: measures of success*. Kluwer Academic Publishers, Hingham, USA, pp. 77-103.
- Vejar-Cota, G., Echeverria, N.E., Rodriguez-Del-Bosque, L.A., 2005. Parasitism and development of *Conura acuta* (Hym.: Chalcididae) on sugarcane stalk borers (Lep.: Crambidae) in Mexico. *Environmental Entomology* 34, 1122-1128.
- Wäckers, F.L., van Rijn, P.C.J., Bruin, J., 2005. *Plant-provided food for carnivorous insects: A protective mutualism and its applications*. Cambridge University Press, UK, pp. 368.
- Wäckers, F.L., van Rijn, P.C.J., Heimpel, G.E., 2008. Honeydew as a food source: making the best of a bad meal? *Biological Control* 45, 176-184.
- Wajnberg E., Pizzol, J., Babault, M., 1989. Genetic variation in progeny allocation in *Trichogramma maidis*. *Entomologia Experimentalis et Applicata* 53, 177-187.
- Wajnberg, E., Colazza, S., 1998. Genetic variability in the area searched by a parasitic wasp. Analysis from automatic video tracking of the walking path. *Journal of Insect Physiology* 44, 437-444.

- Wang, Z.Y., Wu, Y., He, K.L., Bai, S.X., 2007. Effects of transgenic Bt maize pollen on longevity and fecundity of *Trichogramma ostrinae* in laboratory conditions. *Bulletin of Insectology* 60, 49-55.
- Wellington, S., Wysoki, M., 1989. Preliminary investigation of food source preferences of the parasitoid *Trichogramma platneri* Nagarkatti (Hym.: Trichogrammatidae). *Anzeiger für Schädlingskunde Pflanzenschutz Umweltschutz* 62, 133-135.
- Witting-Bissinger, B.E., Orr, D.B., Linker, H.M., 2008. Effects of floral resources on fitness of the parasitoids *Trichogramma exiguum* (Hym.: Trichogrammatidae) and *Cotesia congregata* (Hym.: Braconidae). *Biological Control* 47, 180-186.
- Yu, D.S.K., Hangle, E.A.C., Laing, J.E., 1984. Biology of *Trichogramma minutum* collected from apples in southern Ontario. *Environmental Entomology* 13, 1324-1329.
- Zhang, F., Babendreier, D., Wang, Z.Y., Il, K.S., Zheng, L., Pyon, Y.C., Bai, S.X., Song, K., Ri, J.O., Grossrieder, M., Kuhlmann, U., 2010. Mass releases of *Trichogramma ostrinae* increase maize production in DPR Korea. *Journal of Applied Entomology*. 134, 481-490.
- Zhang, G.R., Zimmermann, O., Hassan, S.A., 2004. Pollen as a source of food for egg parasitoids of the genus *Trichogramma* (Hym.: Trichogrammatidae). *Biocontrol Science and Technology* 14, 201-209.
- Zimmermann, O., 2004a. Der Einsatz von *Trichogramma*-Schlupfwespen in Deutschland. Zum aktuellen Stand der Forschung und Nutzung von Eiparasitoiden gegen Schadlepidopteren im biologischen Pflanzen- und Vorratsschutz. *Gesunde Pflanzen* 56, 157-166.
- Zimmermann, O., 2004b. Die Anwendung von Nützlingen im biologischen Pflanzenschutz in Deutschland - Aktuelle Anmerkungen. *Gesunde Pflanzen* 56, 151-156.
- Zimmermann, O., 2005. Der Baumwollkapselwurm ein neuer Schädling in Gemüsekulturen und seine biologische Bekämpfung mit *Trichogramma* Schlupfwespen. *Gemüse* 3, 21-24.

CHAPTER III

Parasitization of Codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) eggs by three strains of *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) as mediated by apple plant properties.

Parasitization of Codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae) eggs by three strains of *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) as mediated by apple plant properties.

Abstract: The properties of plant surfaces are important factors affecting the success of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) egg parasitoids released for augmentative biological control. This study evaluated the effect of leaf's upper surface of three apple cultivars ("Golden Delicious", "Boskoop" and "Topaz") on the foraging success of three strains of *T. cacoeciae* (HOH1, KIO 2 TN and SY 98 01) for host eggs of the Codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae). 20 naive females were released into arenas with different surfaces (non exposed to Codling moth females (*blank*), exposed to the females without eggs (*adult kairomones*) and exposed to ovipositing Codling moth females (*eggs + adult kairomones*)). Foraging time differed significantly between the strains tested, showing strain HOH 1 performing best. Host handling time and time spent post parasitization did not differ between the strains. The apple cultivar did not affect any of these parameters. Leaving time was significantly affected by Codling moth stimuli abundant on the leaves, where giving up time was shortest on blank leaves and significantly longer on leaves with adult stimuli only but no host eggs. Time spent on leaves was the longest when host adult stimuli and host eggs were abundant on the leaves. Leaving time was further affected by apple cultivar and *T. cacoeciae* strain tested. Compared with the other cv. tested, time spent on leaves of cv. "Golden Delicious" was longest for any treatment. *T. cacoeciae* strain HOH 1 always spent the shortest on any cv. and treatment.

In another experiment, parasitization of eggs on apples placed at different heights (0, 25, and 50 cm above cage ground) was assessed. The parasitization rate of *Cydia* eggs was slightly affected by apple varieties; however the differences were not consistent. Parasitization rates decreased with height of apples above ground, with the highest rate on apples placed on ground, near the releasing point of *Trichogramma* females. Significant differences in parasitization rates between strains could be detected, but they were not consistent also.

Keywords: genetic variation, apple cultivars, searching behaviour, giving-up time.

3.1. Introduction

Egg parasitoids of the genus *Trichogramma* are among the most important natural enemies used in augmentative biocontrol of lepidopterous pests in forests, orchards, and row crops (Gross et al., 1981; Hamada, 1992; Hassan, 1988; 1993; Hirai et al., 1996; Keller et al., 1985; Li, 1994; Lopez et al., 1982; Oatman and Platner, 1971; Parra et al., 1987; Segers et al., 1984; Smith, 1996; Sun et al., 1990; van Lenteren, 2000). As they kill the pests before hatching, larvae have no chance to inflict plant damage. In the last decades, several reviews have been published on the relationships among plants, hosts and parasitoids in general, which reflect a strong interest in these antagonists, both as models for behavioural ecologists and as important organisms for classical (inoculative) and inundative biological control programs (Bale et al., 2008; Hawkins et al., 1999; Li, 1994; Makee, 2005; Vet, 1999; Romeis et al., 1997). Parasitoid foraging is governed by plant surface properties. Andow and Prokrym (1990) and Gingras et al. (2002) define plant structure by three components: (i) plant size (height) or surface area, (ii) heterogeneity (abundance and diversity of plant parts), and (iii) connectivity (absolute number of connections between plant components). However, these components are morphological characters at a macro- and micro-scale and do not consider chemical plant characters, which can also impair movement, searching time and foraging success of parasitoids (Andow and Prokrym, 1990; Lukianchuk and Smith, 1997; Lovinger et al., 2000; Suverkropp et al., 2010; Wang and Keller, 2001). Among parasitoids, trichogrammatids are possibly the best studied antagonists on the species level as well as in multi-trophic level interactions. The wasps of *Trichogramma* use egg stimuli which originated from the host eggs to find their hosts in two ways: direct host-related cues, or indirect host-related cues (Colazza et al., 2010) and the parasitization rates achieved by *Trichogramma* may vary greatly among different habitats, plant species, and plant structures or, region of plant on which the host eggs are located (Romeis et al., 2005).

Trichogrammatids search the plant surface by walking to locate their host. Success in host egg location and parasitization is (i) inversely correlated with foliage area as reported for various crops (see review by Romeis et al., 2005), (ii) positively or negatively affected by physical properties, such as abundance and density of trichomes (Headrick et al., 1996; Mc Gregor et al., 2002; Obrycki, 1986; Romeis et al., 2005; Sütterlin and van Lenteren, 1995), and chemical properties, such as surface volatiles, non-volatile surface chemicals, or sticky trichome exudates (Altieri et al., 1982; Laing, 1937; Romeis et al., 1998; Thorpe, 1985; Turlings et al., 1990; Lewis et al., 1972; Renou et al., 1992; Nordlund, 1984; 1985; Romeis et al., 1997).

In addition to plant factors, searching behaviour of *Trichogramma* is mediated by host chemical cues comprising lepidopteran egg and scale volatiles or contact chemicals, host pheromones, host frass or female moth accessory gland secretions (Noldus, 1988; Noldus et al., 1990; Lewis and Martin, 1990; Lewis et al., 1982).

The main objective of this study was to demonstrate the effect of leaf or fruit surfaces of three apple varieties on searching behaviour of three strains of *Trichogramma cacoeciae* Marchal and to assess any intraspecific variation in searching performance.

3.2. Material and methods

3.2.1. Insect sources

Trichogramma cacoeciae strains used in this study were collected in the field from different countries and subsequently reared under laboratory conditions (25 ± 1 °C, 40-60% R.H and 16:8 L: D) on eggs of the Angoumois grain moth, *Sitotroga cerealella* Oliver (Lep.: Gelechiidae) for several generations in clean plastic tubes (PS Multipurpose Container, 15 ml, 38.80 * 23.00 mm, Cat.-No.: 203170, Greiner bio-one) (Table 1). Honey was offered as supplementary food to the adults.

The Codling moth, *Cydia pomonella* L. (Lep.: Tortricidae), was obtained as a starter population from the Institute of Biological Control, Julius-Kühn-Institute, at Darmstadt and reared in the laboratory on an artificial diet according to Bathon et al. (1991) at 25 °C, 40-60% R.H and 18:6 L: D photoperiod. To obtain eggs for the experiments, the respective test substrates (fruits and shoots with leaves) were exposed in a cage (frame 30×30× 50 cm, covered with black mosquito gauze) for one night to ovipositing female Codling moths.

3.2.2. Plant material

Apple fruits and leaves were collected from three apple varieties ("Golden Delicious", "Boskoop" and "Topaz") from pesticide-free orchards at the research station of the University of Hohenheim. Fruits collected were of approximately the same age and size. Leaves were collected by cutting twigs with *ca.* 25 – 30 leaves. These shoots were kept turgescient during transport and until use of leaves by putting them into beakers with tap water.

3.3. Experimental set-up:

3.3.1. Effects of host location on searching behaviour

This experiment was conducted to compare searching behaviour of three strains of *T. cacoeciae* on different apple leaves surfaces, with presence and absent of host. For each test, nine branches of each apple cultivar were taken. The branches were divided into three groups: the first one exposed to 15 females of *C. pomonella* for 24 h, the second group was exposed to Codling moth females for 2-3 h with no oviposition but leaving any chemical stimuli or wing or abdominal scales, and the third was not exposed to any insect to keep them free of any stimuli. From each group 20 leaves were taken and individually placed in the center of a petri dish (10 cm diam.) on a moistened filter paper (Fig 1a). One *Trichogramma* female was placed on the center of the test leaves of each group using a fine brush (size 000) and subsequently observed for 10 min. Searching of *Trichogramma* females was distinguished as: time until location of the host egg, time until parasitizing, and time until leaving the leaf. The experiment was repeated 20 times for leaves of all apple varieties and all strains of *T. cacoeciae*.

3.3.2. Effect of apple fruit surfaces of different varieties on searching capacity

It could be observed in the field (unpublished data) and in cage experiments that female *Trichogramma* searching behaviour is either negatively phototactic or positively geotactic, and host eggs on fruits in a position below the releasing point of *Trichogramma* females, were found and parasitized easily. Thus, this experiment was designed to scrutinize the short-range bottom-up searching capacity of 3 *Trichogramma* strains.

Two apple fruits of each cultivar containing 24 h old Codling moth eggs were exposed at three heights (0, 25 and 50 cm) resp. in the centre of a glass cage (50×50×65 cm) (Fig. 1). Then, 20 *Trichogramma* females were released on the bottom of the cage and left to parasitize the host eggs for 24 hours.

Subsequently, the fruits were taken out of the cage and fruit surfaces containing *Cydia* eggs were cut out and placed in a sterile petri dish and embedded with the cut sides into paraffin to keep the fruit pieces fresh and turgescient (Fig. 2b). The petri dishes were covered with the lid,

closed tightly with Parafilm[®], and incubated in a climatic chamber at 25 ± 1 °C, 40 – 60 % R.H and 16:8 L:D photoperiod. After 4-5 days, parasitized eggs turned black and the number of parasitized eggs was recorded. Each test series was repeated four times for each apple cultivar and each strain of *Trichogramma*, resulting in 24 apples tested for each height per *Trichogramma* strain.

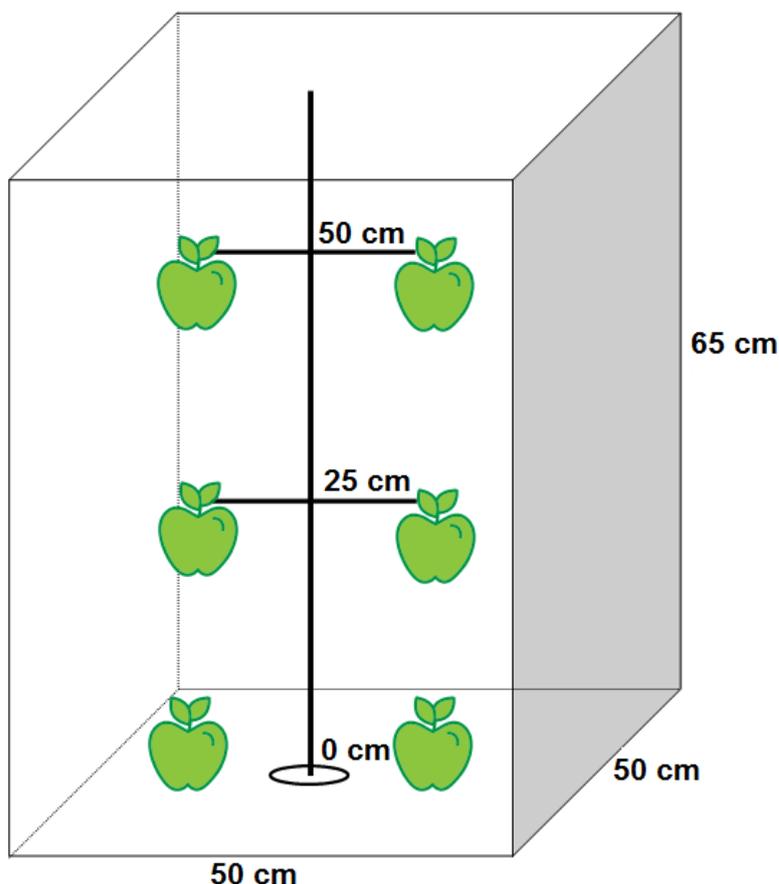


Figure 1 Glass cage for *Trichogramma cacoeciae* (50×50×65 cm) with apple fruits exposed in three different heights.

3.4. Data analysis:

All data were submitted to statistical analysis using JMP[®] 9.0.0 software (2010, SAS Institute Inc., Cary, NC 27513, USA). All experimental data were normally distributed. The respective statistical tests are given in the legends of tables and figures.

Table 1 List of the collected *T. cacoeciae* strains, their, hosts, culture, latitude, location, latitude, longitude, and time of the collection.

Species	Strain	Host	Collected in	Country of origin	Latitude	Longitude	Collector	Year of collection
<i>T. cacoeciae</i>	Hohenheim 1	<i>Sitotroga cerealella</i>	Apple	Germany	48° 11' N	9° 13' E	H. Alkarrat	2006
<i>T. cacoeciae</i>	KIO2 TN	<i>Ectomyelois ceratoniae</i>	Pomegranate	Tunisia	36° 50' N	10° 11' E	I. Ksentini	2004
<i>T. cacoeciae</i>	CAC SY 98 01	<i>Cydia pomonella</i>	Apple	Syria	32° 45' N	36° 45' E	W. Almatni	1998

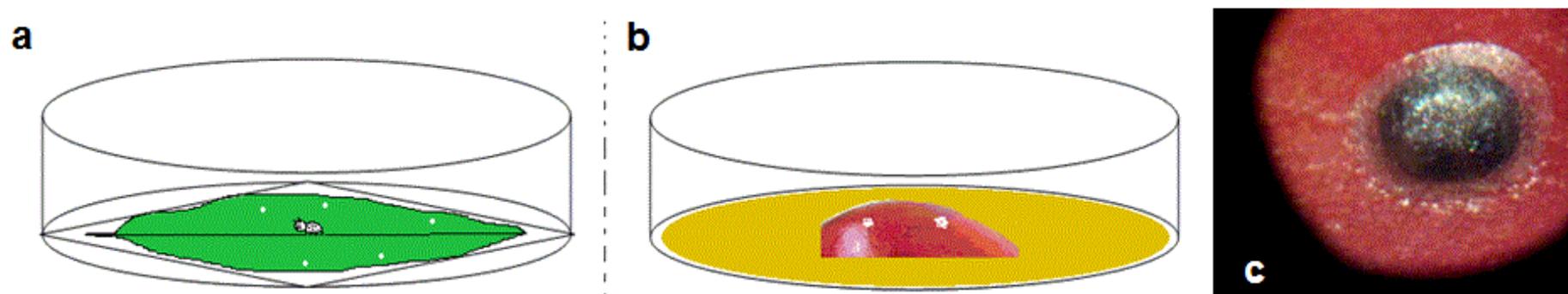


Figure 2 a: Petri dish with filter paper and infested apple leaf with *C. pomonella* eggs and *Trichogramma* insect; b: Petri dish with a cut apple fruit embedded into paraffin, containing Codling moth eggs; c: cut apple fruit piece with parasitized Codling moth egg.

3.5. Results:

3.5.1. Effect of apple leave surface on searching behaviour

Time spent foraging differed significantly between HOH 1 and both the other strains only on leaves of cv. “Boskoop” and “Topaz” but not on cv. “Golden Delicious”. Handling time and time spent on the leaves post parasitization until leaving the leaf did neither differ between strains nor between the apple cultivars (Table 2 and 5).

Searching time on leaves was significantly affected by the presence of eggs and *C. pomonella* stimuli. Females spent the shortest time on leaves without any Codling moth stimulus followed by leaves with adult stimuli (scales and possible secretions) and leaves with host eggs and adult stimuli (Table 3 and 5).

Significant differences in time spent foraging until leaving were found between strains on leaves with eggs and adult stimuli, whereas strain of HOH 1 spent the shortest time compared to the other strains. Moreover, our results showed significant differences between strains on leaves of all apple cultivars with adult stimuli. Female of KIO 2 TN stayed the longest time on all cultivars compared with the other two strains studied, while the female of HOH 1 spent the shortest time on leaves of cv. “Topaz”. No significant differences in time until leaving were found between strains on blank leaves of cv. “Boskoop” and “Golden Delicious” while the mean time spent by strain of HOH 1 on cv. “Topaz” was significantly shorter than that spent by KIO 2 TN and SY 98 01 (Table 4 and 5).

Table 2 Searching behaviour (foraging, host handling and post parasitization time) of three strains of *T. cacoeciae* on the upper leaf surface of three apple varieties with the presence of eggs of *C. pomonella* (one-way ANOVA; for “Boskoop”: foraging time: F = 5.7961, P < 0.0051, d.f. = 2, 57; host handling: F = 1.3160, P < 0.2767, d.f. = 2, 54; post parasitization: F = 0.3691, P < 0.6930, d.f. = 2, 57; for “Golden Delicious”:foraging time: F = 2.6006, P < 0.0832, d.f. = 2, 56; host handling: F = 2.3108, P < 0.1087, d.f. = 2, 55; post parasitization: F = 0.4447, P < 0.6432, d.f. = 2, 56; for “Topaz”: foraging time: F = 11.4206, P < 0.0001, d.f. = 2, 55; host handling: F = 1.0542, P < 0.3554, d.f. = 2, 55; post parasitization: F = 0.4007, P < 0.6717, d.f. = 2, 56; followed by Tukey-Kramer HSD-test, P≤ 0.05 level).

Mean foraging time (sec)			
	“Golden Delicious”*	“Boskoop”*	“Topaz”*
<i>T. cacoeciae</i> HOH 1	296.947 ± 59.948 ^a n = 19	303.900 ± 79.042 ^b n = 20	268.722 ± 45.640 ^b n = 18
<i>T. cacoeciae</i> KIO 2 TN	340.000 ± 98.193 ^a n = 20	377.300 ± 71.312 ^a n = 20	340.050 ± 75.473 ^a n = 20
<i>T. cacoeciae</i> SY 98 01	346.600 ± 53.702 ^a n = 20	363.900 ± 66.955 ^a n = 20	365.850 ± 66.669 ^a n = 20
Mean host handling time (sec)			
	“Golden Delicious”*	“Boskoop”*	“Topaz”*
<i>T. cacoeciae</i> HOH 1	68.427 ± 35.450 ^a n = 19	79.211 ± 33.095 ^a n = 19	74.263 ± 33.808 ^a n = 19
<i>T. cacoeciae</i> KIO 2 TN	91.211 ± 47.341 ^a n = 19	77.947 ± 36.522 ^a n = 19	62.263 ± 34.461 ^a n = 19
<i>T. cacoeciae</i> SY 98 01	73.900 ± 33.811 ^a n = 20	64.473 ± 21.464 ^a n = 19	61.800 ± 20.537 ^a n = 20
Mean post parasitization time until leaving the leaf (sec)			
	“Golden Delicious”*	“Boskoop”*	“Topaz”*
<i>T. cacoeciae</i> HOH 1	38.90 ± 22.678 ^a n = 20	41.200 ± 19.449 ^a n = 20	40.450 ± 18.398 ^a n = 20
<i>T. cacoeciae</i> KIO 2 TN	42.90 ± 20.440 ^a n = 20	45.550 ± 22.940 ^a n = 20	42.895 ± 22.588 ^a n = 19
<i>T. cacoeciae</i> SY 98 01	36.420 ± 21.900 ^a n = 19	40.350 ± 18.980 ^a n = 20	46.200 ± 20.070 ^a n = 20

* Means within each column followed by the same letter are not significantly different.

Table 3 Leaving time of three strains of *Trichogramma* affected by presence and absence of host egg or adult stimuli on leaves of three apple cultivars (one-way ANOVA; *T. cacoeciae* HOH 1: for “Boskoop”: F = 216.2194, P < 0.0001, d.f. = 2, 56; for “Golden Delicious”: F = 166.4495, P < 0.0001, d.f. = 2, 56; for “Topaz”: F = 252.8857, P < 0.0001, d.f. = 2, 55; *T. cacoeciae* KIO 2 TN: for “Boskoop”: F = 486.3897, P < 0.0001, d.f. = 2, 55; for “Golden Delicious”: F = 184.3277, P < 0.0001, d.f. = 2, 57; for “Topaz”: F = 169.9014, P < 0.0001, d.f. = 2, 54; *T. cacoeciae* SY 98 01: for “Boskoop”: F = 349.5249, P < 0.0001, d.f. = 2, 56; for “Golden Delicious”: F = 300.2368, P < 0.0001, d.f. = 2, 55; for “Topaz”: F = 200.0228, P < 0.0001, d.f. = 2, 56; followed by Tukey-Kramer HSD-test, P ≤ 0.001 level).

<i>Trichogramma cacoeciae</i> HOH 1			
	“Golden Delicious” *	“Boskoop”*	“Topaz”*
Eggs + adult kairomones	413.25 ± 69.410 ^a n = 20	429.85 ± 70.204 ^a n = 20	388.45 ± 63.078 ^a n = 20
Adult kairomones	255.11 ± 46.090 ^b n = 19	210.00 ± 61.038 ^b n = 20	163.21 ± 45.359 ^b n = 19
Blank	101.75 ± 41.967 ^c n = 20	55.79 ± 28.425 ^c n = 19 ^c	53.95 ± 25.072 ^c n = 19
<i>Trichogramma cacoeciae</i> KIO 2 TN			
	“Golden Delicious”*	“Boskoop”*	“Topaz”*
Eggs + adult kairomones	483.45 ± 83.509 ^a n = 20	514.84 ± 57.961 ^a n = 19	464.26 ± 65.438 ^a n = 19
Adult kairomones	325.60 ± 59.263 ^b n = 20	243.00 ± 41.984 ^b n = 20	299.63 ± 61.162 ^b n = 19
Blank	98.65 ± 41.150 ^c n = 20	58.53 ± 32.740 ^c n = 19	118.32 ± 44.974 ^c n = 19
<i>Trichogramma cacoeciae</i> SY 98 01			
	“Golden Delicious”*	“Boskoop”*	“Topaz”*
Eggs + adult kairomones	468.90 ± 53.394 ^a n = 19	473.80 ± 53.677 ^a n = 20	473.85 ± 63.268 ^a n = 20
Adult kairomones	277.05 ± 33.117 ^b n = 19	173.75 ± 64.299 ^b n = 20	218.65 ± 69.246 ^b n = 20
Blank	123.80 ± 43.216 ^c n = 20	59.74 ± 24.456 ^c n = 19	107.68 ± 38.467 ^c n = 19

* Means within each column followed by the same letter are not significantly different.

Table 4 Leaving time of *Trichogramma* strains affected by three apple cultivars in presence and absence of host egg on the leaves (one-way ANOVA; Eggs + adult kairomones: for “Boskoop”: F = 9.4474, P < 0.0003, d.f. = 2, 56; for “Golden Delicious”: F = 5.5641, P < 0.0063, d.f. = 2, 56; for “Topaz”: F = 10.6570, P < 0.0001, d.f. = 2, 56; Adult kairomones: for “Boskoop”: F = 7.4809, P < 0.0013, d.f. = 2, 57; for “Golden Delicious”: F = 11.2459, P < 0.0001, d.f. = 2, 55; for “Topaz”: F = 25.1740, P < 0.0001, d.f. = 2, 55; Blank: for “Boskoop”: F = 0.0941, P < 0.9104, d.f. = 2, 54; for “Golden Delicious”: F = 2.1201, P < 0.1294, d.f. = 2, 57; for “Topaz”: F = 16.4291, P < 0.0001, d.f. = 2, 54; followed by Tukey-Kramer HSD-test, P ≤ 0.05 level).

Eggs + adult kairomones			
	“Golden Delicious” [*]	“Boskoop” [*]	“Topaz” [*]
<i>T. cacoeciae</i> HOH 1	413.250 ± 69.410 ^b n = 20	429.850 ± 70.204 ^b n = 20	388.450 ± 63.078 ^b n = 20
<i>T. cacoeciae</i> KIO 2 TN	483.450 ± 83.509 ^a n = 20	514.842 ± 57.961 ^a n = 19	464.263 ± 65.438 ^a n = 19
<i>T. cacoeciae</i> SY 98 01	468.895 ± 53.394 ^a n = 19	473.800 ± 53.677 ^{ab} n = 20	473.850 ± 63.268 ^a n = 20
Adult kairomones			
	“Golden Delicious” [*]	“Boskoop” [*]	“Topaz” [*]
<i>T. cacoeciae</i> HOH 1	255.105 ± 46.090 ^b n = 19	210.000 ± 61.038 ^{ab} n = 20	163.211 ± 45.359 ^c n = 19
<i>T. cacoeciae</i> KIO 2 TN	325.600 ± 59.263 ^a n = 20	243.000 ± 41.984 ^a n = 20	299.632 ± 61.162 ^a n = 19
<i>T. cacoeciae</i> SY 98 01	277.053 ± 33.117 ^b n = 19	173.750 ± 64.299 ^b n = 20	218.650 ± 69.246 ^b n = 20
Blank			
	“Golden Delicious” [*]	“Boskoop” [*]	“Topaz” [*]
<i>T. cacoeciae</i> HOH 1	101.750 ± 41.967 ^a n = 20	55.790 ± 28.425 ^a n = 19	53.947 ± 25.072 ^b n = 19
<i>T. cacoeciae</i> KIO 2 TN	98.650 ± 41.150 ^a n = 20	58.526 ± 32.740 ^a n = 19	118.316 ± 44.974 ^a n = 19
<i>T. cacoeciae</i> SY 98 01	123.800 ± 43.216 ^a n = 20	59.737 ± 24.456 ^a n = 19	107.684 ± 38.467 ^a n = 19

* Means within each column followed by the same letter are not significantly different.

Table 5 Analysis of variance (ANOVA, GLM: General Linear Model, normal distribution, link: identity, full module test) of foraging time (number of observations = 177, Chi-square = 34.8159, d.f. = 4, p = 0.0001); host handling time (number of observations = 173, Chi-square = 5.6756, d.f. = 4, p = 0.2247), post handling time (number of observations = 178, Chi-square = 2.1054, d.f. = 4, p = 0.7164) and leaving time (number of observations = 527, Chi-square= 1085.200, d.f. = 6, p = 0.0001) of three strains of *T. cacoeciae*, affected by upper leaf surface of three apple varieties with *C. pomonella* eggs and adult kairomones, with adult kairomones only, and without any stimulus (blank).

Foraging time			
Source	d.f.	Chi-square	P
Strains	2	31.846027	0.0001
Apple cultivar	2	3.9533804	0.1385
Host handling time			
Source	d.f.	Chi-square	P
Strains	2	2.7466908	0.2533
Apple cultivar	2	2.9591924	0.2277
Post parasitization			
Source	d.f.	Chi-square	P
Strains	2	1.0278809	0.5981
Apple cultivar	2	1.1058869	0.5753
Leaving time			
Source	d.f.	Chi-square	P
Strains	2	82.380405	0.0001
Apple cultivar	2	36.102663	0.0001
Stimulus	2	1070.4013	0.0001

When apples with eggs were placed on the bottom of the cages, significant differences in the parasitization rate were found between the strains compared. *Trichogramma cacoeciae* KIO 2 parasitized fewer eggs on the cultivars "Golden Delicious" (66.34 %) and "Boskoop" (81.50 %) compared with the other two strains studied, while there were no significant differences between the strains on cv. "Topaz". At 25 cm height and at 50 cm height the differences between strains were not significant, only the parasitization rate of strain SY 98 01 on cv. "Topaz" was lower than the strain HOH 1 at 25 cm height. At 50 cm height, parasitization rate was only lower on cv. "Golden Delicious" (Table 7). The position of apple fruits above ground affected the parasitization rate significantly more than the both factors "strain" or "cultivar" (Table 6).

Table 6 Analysis of variance (ANOVA, GLM: General Linear Model, normal distribution, link: identity, full module test) of parasitization rate (%) of three strains of *T. cacoeciae* on *C. pomonella* eggs as host, affected by three heights (0, 25 and 50 cm) and three apple varieties: (Number of observations = 216, Chi-square = 248.6890, d.f. = 6, $p < 0.0001$).

Source	d.f.	Chi-square	P
Strains	2	16.3644	0.0003
Cultivar	2	15.08254	0.0005
Height	2	238.1191	< 0.0001

Table 7 Parasitization rate of three strains of *Trichogramma cacoeciae* on apple fruits of different cultivars in different heights (number of repeats for each individual test set = 8; one-way ANOVA; for “Boskoop”: height 0 cm: F = 5.0403, P < 0.0163, d.f. = 2, 21; height 25 cm: F = 0.5027, P < 0.6120, d.f. = 2, 21; height 50 cm: F = 6.9881, P < 0.0047, d.f. = 2, 21; for ”Golden Delicious”: height 0 cm: F = 16.8709, P < 0.0001, d.f. = 2, 21; height 25 cm: F = 0.4400, P < 0.6499, d.f. = 2, 21; height 50 cm: F = 0.9643, P < 0.3975, d.f. = 2, 21; for “Topaz”: height 0 cm: F = 3.6066, P < 0.0450, d.f. = 2, 21; height 25 cm: F = 5.1610, P < 0.0150, d.f. = 2, 21; height 50 cm: F = 2.4125, P < 0.1140, d.f. = 2, 21; followed by Tukey-Kramer HSD-test, P ≤ 0.05 level).

Parasitization rate (%) at 0 cm			
	“Golden Delicious” [*]	“Boskoop” [*]	“Topaz” [*]
<i>T. cacoeciae</i> HOH 1	88.38 ± 5.59 ^a	92.26 ± 5.11 ^a	75.53 ± 13.59 ^a
<i>T. cacoeciae</i> KIO 2 TN	66.35 ± 13.18 ^b	81.50 ± 6.39 ^b	85.82 ± 6.40 ^a
<i>T. cacoeciae</i> SY 98 01	86.65 ± 2.94 ^a	85.43 ± 8.61 ^{ab}	86.20 ± 4.25 ^a
Parasitization rate (%) at 25 cm height			
	“Golden Delicious” [*]	“Boskoop” [*]	“Topaz” [*]
<i>T. cacoeciae</i> HOH 1	63.33 ± 15.33 ^a	70.28 ± 14.20 ^a	67.38 ± 12.67 ^a
<i>T. cacoeciae</i> KIO 2 TN	70.34 ± 17.98 ^a	63.96 ± 16.70 ^a	43.27 ± 20.48 ^b
<i>T. cacoeciae</i> SY 98 01	66.56 ± 10.58 ^a	63.89 ± 12.77 ^a	56.52 ± 9.90 ^{ab}
Parasitization rate (%) at 50 cm height			
	“Golden Delicious” [*]	“Boskoop” [*]	“Topaz” [*]
<i>T. cacoeciae</i> HOH 1	42.97 ± 14.06 ^a	56.85 ± 19.34 ^a	36.86 ± 13.48 ^a
<i>T. cacoeciae</i> KIO 2 TN	34.97 ± 9.98 ^a	41.15 ± 16.30 ^{ab}	27.18 ± 12.30 ^a
<i>T. cacoeciae</i> SY 98 01	33.69 ± 18.25 ^a	27.26 ± 10.62 ^b	24.27 ± 9.97 ^a

* Means within each column followed by the same letter are not significantly different.

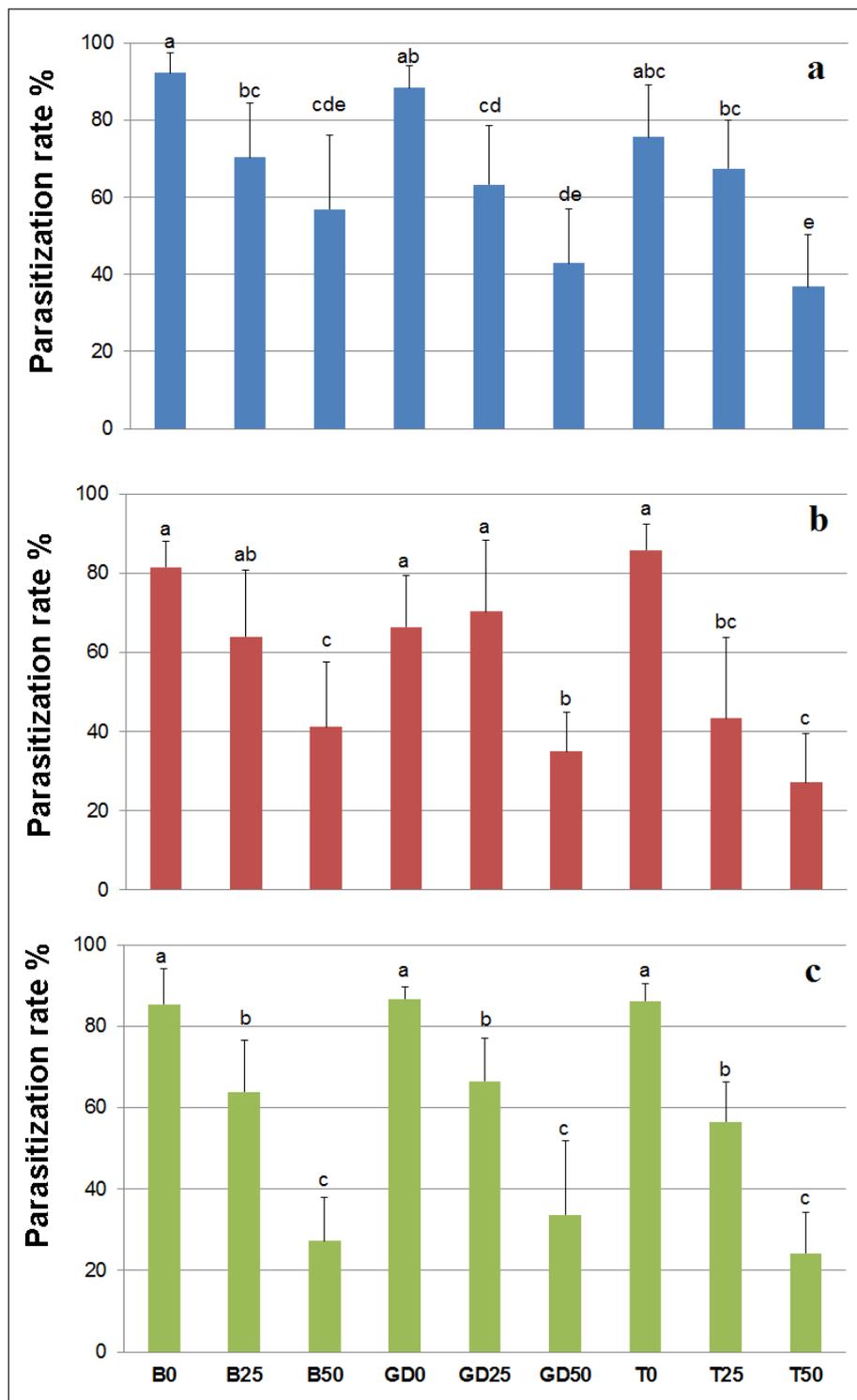


Figure 3 Parasitization rate of Codling moth eggs on fruits of three apple varieties (B: “Boskoop”, GD: “Golden Delicious”, and T: “Topaz”) exposed in three heights (0: 0 cm, 25: 25 cm and 50: 50 cm) by three strains of *T. cacoeiae* (a: HOH 1, b: KIO 2 TN and c: SY 98 01). Columns with different letters indicate significant differences between treatments at $p = 0.05$ by one way ANOVA followed by Tukey-Kramer HSD-test.

3.6. Discussion

Searching behaviour of *T. cacoeciae* (foraging, handling the host, and leaving the host plant) differed significantly between the strains but was not affected by the leaf surfaces of the apple cultivars tested. As leaf surface complexity was reported to affect *Trichogramma nubilale* Ertle and Davis searching activity and parasitization rate (Andow and Prokrym, 1990), the differences of apple leaf surfaces between apple cultivars (Al Bitar et al., 2012) did not affect foraging time until host-egg finding, handling time or post-parasitization time until leaving the leaf by *T. cacoeciae*. Only leaving time (= giving-up time) on leaves with no stimulus (blank) or adult stimuli without host eggs was significantly affected by the factor apple cultivar. A possible explanation of these differences may be seen in the number of trichomes and the trichome-covered area on the upper leaf side of apple leaves, where “Golden Delicious”-leaves have a relative trichome-covered area of only 3.4 % compared to the almost doubled area on leaves of the cultivars “Boskoop” and “Topaz” with 7.1 and 6.3 % respectively (Al Bitar et al., 2012). This relation is also found in the giving-up time on blank leaves of cv. “Golden Delicious” twice the time on both the other cultivars. On leaves with adult stimuli only, this relation was less pronounced, but visible. Thus, it may be concluded that with presence of host eggs, the possibly disturbing effects of trichomes on *Trichogramma* walking (Carrillo et al., 2008; Kauffman and Kennedy, 1989; Kashyap et al., 1991; Keller, 1987; Rabb and Bradley, 1968; Romeis et al., 1998; Suverkropp et al., 2010; Treacy et al., 1985; 1986) may be neglected by the parasitoid females and only may play a role when any positive stimulus is absent.

Females of all *T. cacoeciae* strains tested were able to detect stimuli left by Codling moth females during walking on an apple leaf without ovipositing as giving up time for host-egg searching was significantly lower on blank leaves than on leaves with adult stimuli but no host eggs. This implies that (i) Codling moth females leave traces like scales or volatile chemicals while visiting an apple leaf, possibly during search for an appropriate oviposition site, and (ii) *T. cacoeciae* females are able to perceive these olfactorial or possibly also mechanical stimuli. *Trichogramma* species are known to exploit sex pheromones of potential host species as kairomones result in increased wasp residence times, walking times, and path lengths (Boo and Yang, 2000, Kaiser et al., 1989, Lewis et al., 1982, McGregor and Henderson, 1998, Milonas et al., 2009a, 2009b, Noldus, 1988, Noldus and van Lenteren, 1985, Noldus et al., 1990, 1991, Reddy et al., 2002, Schöller and Prozell, 2002). However, they also are able to detect other stimuli than sex-pheromones because production of sex pheromones by lepidopterans is

terminated after mating (Ahn et al., 2002, Ando et al., 1996, Coffelt and Vick, 1987, Foster, 1993, Giebultowicz et al., 1991, Hanin et al., 2011, Raina, 1989, Ramaswamy et al., 1996). This assumption is supported by the attraction of *T. bournieri* Pintureau and Babault females by odours of non-calling females of stemborers, although to a lesser extent than by calling females (Bruce et al., 2009). Scales left by ovipositing moths and hexane surface extracts stimulated host-seeking response of and increased parasitism by *T. evanescens* Westwood (Lewis et al., 1972). Presence of stimuli released by mated Codling moth females during walking on a surface are of higher reliability and may, at least, indicate to *Trichogramma* females a higher probability to find a host egg and not to waste time and energy for fruitless host egg searching.

Our results show significant differences between the strains in searching behaviour, where strain *T. cacoeciae* HOH 1 performs best among the strains studied, because they needed the shortest time to find the host. Significant genetic variation in host egg location has also been reported for *T. brassicae* Bezdenko (Bruins et al., 1994). Considering the significantly differing parasitization rates of the three strains used in this study, genetic variation in performance is also given in *T. cacoeciae*.

Moreover, host egg location on fruits as shown by the parasitization rate differed between, *Trichogramma* strains, apple varieties and height of apple fruits in the cage. Generally, the parasitization rate decreased with distance of apples above ground, where the *Trichogramma*-females have been released. This may be due to onset of searching in the vicinity of the releasing point, and then moving to higher strata to continue searching for further host eggs. This result corresponds to reports by Yu et al (1984) for *T. minutum* Riley, and Ables et al. (1980) for *T. pretiosum* Riley where the vertical distribution of these parasitoids within apple trees was skewed toward the lower part of the tree canopy. It may be concluded that distribution of *Trichogrammatids* in fruit orchards should be more even than achieved by hanging *Trichogramma*-cards to reduce the distance between the places of adult parasitoid emergence to the next possible Codling moth oviposition site and to compensate gaps in parasitoid dispersion. An even distribution of *Trichogramma* in orchards can be accomplished by spray-application of parasitized factitious host eggs.

Although parasitization rates differed significantly between apple cultivars or *Trichogramma* strain tested, the differences between apple cultivars were not consistent and, thus, should not be overestimated. The differences in the parasitization rate between the *Trichogramma* strains were not very pronounced and should be taken as a trend, where strain

KIO 2 TN performed slightly less than both the other strains. It may be assumed that searching behaviour of *Trichogramma* females can be affected by apple fruit surface because the apple cultivars are characterized by sometimes tremendous differences in fruit cuticle microstructure (Curry, 2005).

The complete searching behaviour as affected by various plant surfaces has to be scrutinized in detail to explain differences in parasitization performance of trichogrammatids. Light should particularly be shed upon the genetic variation in searching and parasitization behaviour of *Trichogramma* species to choose the most efficient strain for biocontrol of lepidopterous pests in a given crop.

References

- Ables, J.R., Mc Commas, D.W., Jones, S.L., Morrison, R.K., 1980. Effects of cotton plant size, host egg location, and location of parasite release on parasitism by *Trichogramma pretiosum*. *The Southwestern Entomologist* 5, 261-264.
- Ahn, S.J., Choi, M.Y., Boo, K.S., 2002. Mating effect on sex pheromone production of the oriental tobacco budworm, *Helicoverpa assulta*. *Journal of Asia-Pacific Entomology* 5, 43-48.
- Al Bitar, L., Gorb, S.N., Zebitz, C.P.W., Voigt, D., 2012. Egg adhesion of the Codling moth *Cydia pomonella* L. (Lep.: Tortricidae) to various substrates: I. Leaf surfaces of different apple cultivars. *Arthropod-Plant-Interactions* 6, 471-488.
- Altieri, M.A., Annamalai, S., Katiyar, K.P., Flath, R.A., 1982. Effects of plant extracts on the rates of parasitization of *Anagasta kuehniella* (Lep.: Pyralidae) eggs by *Trichogramma pretiosum* (Hym.: Trichogrammatidae) under greenhouse conditions. *Entomophaga* 27, 431-438.
- Ando, T., Kasuga, K., Yajima, Y., Kataoka, H., Suzuki, A., 1996. Termination of sex pheromone production in mated females of the silkworm moth. *Archives of Insect Biochemistry and Physiology* 31, 207-218.
- Andow, D.A., Prokrym, D.R., 1990. Plant structural complexity and host-finding by a parasitoid. *Oecologia* 82, 162-165.

- Bale, J.S., van Lenteren, J.C., Bigler, F., 2008. Biological control and sustainable food production. *Philosophical Transactions of the Royal Society* 363, 761-776.
- Bathon, H., Singh, P., Clare, G.K., 1991. Rearing methods. In: van der Geest, L.P.S., Evenhuis, H.H. (Eds.), *Tortricid pests: Their biology, natural enemies and control*. Vol. 5 of *World Crop Pests*, W. Helle, ed. Elsevier, Amsterdam, The Netherlands, pp. 283-293.
- Boo, K.S., Yang, J.P., 2000. Kairomones used by *Trichogramma chilonis* to find *Helicoverpa assulta* eggs. *Journal of Chemical Ecology* 26, 359-375.
- Bruce, A.Y., Schulthess, F., Mueke, J., Calatayud, P.A., 2009. Olfactory attraction of egg parasitoids to virgin females of noctuid stemborers. *Biological Control* 54, 763-772.
- Bruins, E.B.A., Wajnberg, E., Pak, G.A., 1994. Genetic variability in the reactive distance in *Trichogramma brassicae* after automatic tracking of the walking path. *Entomologia Experimentalis et Applicata* 72, 297-303.
- Carrillo, D., Pena J.E., Capinera, J., 2008. Effects of host plants on successful parasitism by *Haeckelianas peralta* (Hym.: Trichogrammatidae) on *Diaprepes abbreviatus* (Coleoptera: Curculionidae) eggs. *Environmental Entomology* 37, 1565-1572.
- Coffelt, J.A., Vick, K.W., 1987. Sex pheromones of *Ephestia cautella* (Walker) (Lep.: Pyralidae): Influence of mating on pheromone titer and release rate. *Journal of Stored Products Research* 23, 119-123.
- Colazza, S., Peri, E., Salerno, G., Conti, E., 2010. Host searching by egg parasitoids: Exploitation of host chemical cues. In: Consoli et al. (Eds.), *Egg Parasitoids in agroecosystems with emphasis on Trichogramma*. Springer, New York, NY, USA, pp. 97-147.
- Curry, E.A., 2005. Ultrastructure of epicuticular wax aggregates during fruit development in apple (*Malus domestica* (Borkh.)). *Journal of Horticultural Science and Biotechnology* 80, 668-676.
- Foster, S.P., 1993. Neural inactivation of sex pheromone production in mated light brown apple moths, *Epiphyas postvittana* (Walker). *Journal of Insect Physiology* 39, 267-273.
- Giebultowicz, J.M., Raina, A.K., Uebel, E.C., Ridgway, R.L., 1991. Two-step regulation of sex-

- pheromone decline in mated gypsy moth females. Archives of Insect Biochemistry and Physiology 16, 95-105.
- Gingras, D., Boivin, G., 2002. Effect of plant structure, host density and foraging duration on host finding by *Trichogramma evanescens*. Environmental Entomology 31, 1153-1157.
- Gross, H.R., Lewis, W.J., Donald, A., Norlund, D.A., 1981. *Trichogramma pretiosum*: effect of rerelease parasitization experience on retention in release areas and efficiency. Environmental Entomology 10, 554-556.
- Hamada, R., 1992. Egg parasitoids of common cutworm, *Spodoptera litura* (Fabricius) (Lep.: Noctuidae). Japanese Journal of Applied Entomology and Zoology 36, 258-259.
- Hanin, O., Azrielli, A., Applebaum, S.W., Rafaeli, A. 2011. Functional impact of silencing the *Helicoverpa armigera* sex-peptide receptor on female reproductive behaviour. Insect Molecular Biology 21, 161-167.
- Hassan, S.A., 1988. *Trichogramma* News. v. 4. Federal research center for agriculture and forestry Braunschweig, Germany, pp. 32.
- Hawkins, B.A., Mills, N.J., Jervis, M.A., Price, P.W., 1999. Is the biological control of insects a natural phenomenon? Oikos 86, 493-506.
- Headrick, D.H., Bellows, T.S., Perring, T.M., 1996. Behaviours of female *Eretmocerus* sp. Nr. *Californicus* (Hym.: Aphelinidae) attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae) on cotton, *Gossypium hirsutum* (Malvaceae) and melon, *Cucumis melo* (Curcubitaceae). Biological Control 6, 64-75.
- Hiari, K., Miyakawa, Y., Takeuchi, H., 1996. Parasitization efficacy and movement distance by *Trichogramma dendrolimi* Matsumura on green peppers in vinylhouse. The Kanto-Tosan Plant Protection Society 43, 217-219.
- Kaiser, L., Pham-Delegue, M.H., Bakchine, E., Masson, C., 1989. Olfactory responses of *Trichogramma maidis* (Pint. et Voeg.): Effects of chemical cues and behavioural plasticity. Journal of Insect Behaviour 2, 701-712.

- Kashyap, R.K., Kennedy, G.G., Farrar, R.R.Jr., 1991. Behavioural response of *Trichogramma pretiosum* Riley and *Telenomus sphingis* (Ashmead) to trichome/ methyl ketone mediated resistance in tomato. *Journal of Chemical Ecology* 17, 543-556.
- Kauffman, W.C., Kennedy, G.G., 1989. Relationship between trichome density in tomato and parasitism of *Heliothis* spp. (Lep.: Noctuidae) eggs by *Trichogramma* spp. (Hym.: Trichogrammatidae). *Environmental Entomology* 18, 698-704.
- Keller, M.A., 1987. Influence of leaf surfaces on movements by the hymenopterous parasitoid *Trichogramma exiguum*. *Entomologia Experimentalis et Applicata* 43, 55-59.
- Keller, M.A., Lewis, W.J., Stinner, E., 1985. Biological and practical significance of movement by *Trichogramma* species: a review. *The Southwestern Entomologist* 8, 138-155.
- Laing, J., 1937. Host-finding by insect parasites. I. Observations on the finding of hosts by *Alysiamanducator*, *Mormoniella vitripennis* and *Trichogramma evanescens*. *Journal of Animal Ecology* 6, 298-317.
- Lewis, W.J., Jones, R.L., Sparks, A.N., 1972. A host-seeking stimulant for the egg parasite *Trichogramma evanescens*: its source and a demonstration of its laboratory and field activity. *Entomological Society of America* 65, 1087-1089.
- Lewis, W.J., Martin, J., 1990. Semiochemicals for use with parasitoids: status and future. *Journal of Chemical Ecology* 16, 3067-3089.
- Lewis, W.J., Nordlund, D.A., Gueldner, R.C., Teal, P.E.A., Tumlinson, J.H., 1982. Kairomones and their use for management of entomophagous insects. XIII. Kairomonal activity for *Trichogramma* spp. of abdominal tips, excretion and a synthetic sex pheromone blend of *Heliothis zea* (Boddie) moths. *Journal of Chemical Ecology* 8, 1323-1331.
- Li, L.Y., 1994. Worldwide use of *Trichogramma* for biological control on different crops: a survey, In: Wahnberg, E., Hassan, S.A. (Eds.), *Biological Control with Egg Parasitoids*. CAB International, UK; pp. 37-53.
- Lopez, J.D., Jones, S.L., House, V.S., 1982. Species of *Trichogramma* parasitizing eggs of *Heliothis* spp. and some associated lepidopterous pests in Central Texas. *The Southwestern Entomologist* 7, 87-93.

- Lovinger, A., Liewehr, D., Lamp, W.O., 2000. Glandular trichomes on alfalfa impede searching behaviour of the potato leafhopper parasitoid. *Biological Control* 18, 187-192.
- Lukianchuk, J.L., Smith, S.M., 1997. Influence of plant structural complexity on the foraging success of *Trichogramma minutum*: a comparison of search on artificial and foliage models. *Entomologia Experimentalis et Applicata* 84, 221-228.
- Makee, H., 2005. Factors influencing the parasitism of Codling moth eggs by *Trichogramma cacoeciae* March. And *T. principium* Sug. et Sor. (Hym.: Trichogrammatidae). *Journal of Pest Science* 78, 31-39.
- McGregor, R.R., Henderson, D.E., 1998. The influence of oviposition experience on response to host pheromone in *Trichogramma sibericum* (Hym.: Trichogrammatidae). *Journal of Insect Behaviour* 11, 621-632.
- McGregor, R.R., Prasad, R.P., Henderson, D.E., 2002. Searching behaviour of *Trichogramma* wasps (Hym.: Trichogrammatidae) on tomato and peppers. *Journal Entomological Society British Columbia* 99, 93-98.
- Milonas, P.G., Mazomenos, B.E., Konstantopoulou, M.A., 2009a. Kairomonal effect of sex pheromone components of two lepidopteran olive pests on *Trichogramma* wasps. *Insect Science* 16, 131-136.
- Milonas, P.G., Martinou, A.F., Kontodimas, D.C., Karamaouna, F., Konstantopoulou, M.A., 2009b. Attraction of different *Trichogramma* species to *Prays oleae* sex pheromone. *Annals of the Entomological Society of America* 102, 1145-1150.
- Noldus, L.P., 1988. Response of the egg parasitoid *Trichogramma pretiosum* to the sex pheromone of its host *Heliothis zea*. *Entomologia Experimentalis et Applicata* 48, 293-300.
- Noldus, L.P., Lewis, W.J., Tumlinson, J.H., 1990. Beneficial arthropod behaviour mediated by airborne semiochemicals. IX. Differential response of *Trichogramma pretiosum*, an egg parasitoid of *Heliothis zea*, to various olfactory cues. *Journal of Chemical Ecology* 16, 3531-3544.
- Noldus, L.P., van Lenteren, J.C., 1985. Kairomones for the egg parasite *Trichogramma evanescens* Westw., I. Effect of volatile substances released by two of its hosts, *Pieris brassicae* L. and *Mamestra brassicae*, L. *Journal of Chemical Ecology* 11, 781-791.

- Noldus, L.P., van Lenteren, J.C., Lewis, W.J., 1991. How *Trichogramma* parasitoids use moth sex pheromones as kairomones: orientation behaviour in a wind tunnel. *Physiological Entomology* 16, 313-327.
- Nordlund, D.A., Chalfant, R.B. and Lewis, W.J., 1984. Response of *Trichogramma pretiosum* females to extracts of two plants attacked by *Heliothis zea*. *Agriculture Ecosystems and Environment* 12, 127-133.
- Nordlund, D.A., Chalfant, R.B. and Lewis, W.J., 1985. Response of *Trichogramma pretiosum* females to volatile synomones from tomato plants. *Journal of Entomological Science* 20, 372-376.
- Oatman, E.R., Platner, G.R., 1971 .Biological control of the tomato fruit worm, cabbage looper, and horn worm on processing tomatoes in southern California, using mass releases of *Trichogramma pretiosum*. *Journal of Economic Entomology* 64, 501-506.
- Obrycki, J.J., 1986. The influence of foliar pubescence on entomophagous species. In: Boethel, D.J., Eikenbary, R.D. (Eds.), *Interactions of plant resistance and parasitoids and predators of insects*. John Wiley and Sons, New York, pp. 61-83.
- Parra, J.R.P., Zucchi, R.A., Silveira, N.S., 1987. Biological control of pests through egg parasitoids of the genera *Trichogramma* and *Trichogrammatoidea*. *Memórias do Instituto Oswaldo Cruz, Rio Janeiro* 82, 153-160.
- Rabb, R.L., Bradley, J.R., 1968. The influence of host plants on parasitism of the eggs of the tobacco budworm. *Journal of Economic Entomology* 61, 1249-1252.
- Raina, A.K., 1989. Male-induced termination of sex pheromone production and receptivity in mated females of *Heliothis zea*. *Journal of Insect Physiology* 35, 821-826.
- Ramaswamy, S.B., Qiu, Y., Park, Y.I., 1996. Neuronal control of post-coital pheromone production in the moth *Heliothis virescens*. *Journal of Experimental Zoology* 274, 255-263.
- Reddy, G.V.P., Holopainen, J.K., Guerrero, A. 2002. Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. *Journal of Chemical Ecology* 28, 131-143.

- Renou, M., Nagnan, P., Berthier, A., Durier, C., 1992. Identification of compounds from the eggs of *Ostrinia nubilalis* and *Mamestra brassicae* having kairomone activity on *Trichogramma brassicae*. *Entomologia Experimentalis et Applicata* 63, 291-303.
- Romeis, J., Shanower, T.G., Zebitz, C.P.W., 1997. Volatile plant infochemicals mediate plant preference of *Trichogramma chilonis*. *Journal of Chemical Ecology* 23, 2455-2465.
- Romeis, J., Shanower, T.G., Zebitz, C.P.W., 1998. Physical and chemical plant characters inhibiting the searching behaviour of *Trichogramma chilonis*. *Entomologia Experimentalis et Applicata* 87, 275-284
- Romeis, J., Babendreier, D., Wäckers, F.L., Shanower, G., 2005. Habitat and plant specificity of *Trichogramma* egg parasitoids underlying mechanisms and implications. *Basic and Applied Ecology* 6, 215-236.
- Schöller, M., Prozell, S., 2002. Response of *Trichogramma evanescens* to the main sex pheromone component of *Ephestia* spp. and *Plodiain terpunctella*, (Z, E) - 9, 12-tetradecenyl acetate (ZETA). *Journal of Stored Products Research* 38, 177-184.
- Segers, J.C., Benedict, J.H. Treacey, M.F, 1984 .Natural parasitism of *Heliothis* spp. eggs on cotton and corn in the lower Gulf Coast of Texas. *The Southwestern Entomologist* 9, 245-248.
- Smith, S.M., 1996. Biological control with *Trichogramma*. *Advances, success, and potential of their use. Annual Review of Entomology* 41, 375-406.
- Sun, X.; Tan, Y., Chen, J., 1990. Study on the utilization of *Trichogramma dendrolimi* to control the Masson pine moth *Dendrolimus punctatus* at Longshan forest farm, China. *Forest Research* 4, 407-410.
- Sütterlin, S., van Lenteren, J.C., 1997. Influence of hairiness of *Gerbera jamesonii* leaves on the searching efficiency of the parasitoid *Encarsia formosa*. *Biological Control* 9, 157-165.
- Suverkropp, B.P., Bigler, F., van Lenteren, J.C., 2010. Movement and host finding of *Trichogramma brassicae* on maize plants. *Bulletin of Insectology* 63, 115-127.
- Thorpe, K.W., 1985. Effects of height and habitat type on egg parasitism by *Trichogramma minutum* and *T. pretiosum*. *Agricultural Ecosystems and Environment* 12, 117-126.

- Treacy, M.F., Benedict, J.H., Segers, J.C., Morrison, R.K., Lopez, J.D., 1986. Role of cotton trichome density in bollworm (Lep.: Noctuidae) egg parasitism. *Environmental Entomology* 15, 365-368.
- Treacy, M.F., Zummo, G.R., Benedict, J.H., 1985. Interactions of host-plant resistance in cotton with predators and parasites. *Agriculture Ecosystems and Environment* 13, 151-157.
- Turlings, T.C.J., Loughrin, J.H., McCall, P.J., Roese, U.S.R., Lewis, W.J., Tumlinson, J.H., 1995. How caterpillar damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America* 92, 4169-4174.
- Turlings, T.C.J., Scheepmaker, J.W.A., Vet, L.E.M., Tumlinson, J.H., Lewis, W.J., 1990. How contact foraging experiences affect preferences for host related odors in the larval parasitoid *Cotesia marginiventris* (Cresson) (Hym.: Braconidae). *Journal of Chemical Ecology* 16, 1577-1589.
- van Lenteren, J.C., 2000. Success in biological control of arthropods by augmentation of natural enemies. In: Gurr, G., Wratten, S. (Eds.), *Biological control: measures of success*. Kluwer Academic Publishers, Hingham, USA, pp. 77-103.
- van Lenteren, J.C., Li Zhoa, H., Kamerman, J.W., Rumei, X., 1995. The parasite-host relationship between *Encarsia formosa* (Hym.: Aphelinidae) and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). XXVI. Leaf hairs reduce the capacity of *Encarsia formosa* to control greenhouse whitefly on cucumber. *Journal Applied of Entomology* 119, 553-559.
- Vet, L.E.M., 1999. From chemical to population ecology: Infochemical use in an evolutionary context. *Journal of Chemical Ecology* 25, 31-49
- Wang, X.G., Keller, M.A., 2002. A comparison of searching efficiency of two larval parasitoids of *Plutella xylostella*. *Ecological Entomology* 27, 105-114.

CHAPTER IV

**Effect of hibernation on different strains of *Trichogramma*
(Hymenoptera: Trichogrammatidae) and influence of low
temperature on adult survival.**

Effect of hibernation on different strains of *Trichogramma* (Hymenoptera: Trichogrammatidae) and influence of low temperature on adult survival.

Abstract One German and one Tunisian strain of *Trichogramma oleae* Voegelé and two German, one Tunisian, and one Syrian strain of *T. cacoeciae* Marchal (Hym., Trichogrammatidae) were hibernated from October 10th 2007, up to parasitoid emergence in 2008 under field conditions in Stuttgart-Hohenheim, Germany. 60 Days after exposure to outdoor conditions and after complete hibernation life table parameters of survivors was assessed. Our results demonstrate that all strains studied were able to overwinter successfully on eggs of the factitious host *Sitotroga cerealella* Olivier (Lep., Gelechiidae). Mean development time of trichogrammatids until adult emergence after full hibernation ranged between 183 and 189 days. Emergence rate ranged between 53.59 and 60.98 %. Net reproduction rate (R_0) of *T. oleae* strains decreased with duration of field exposure, whereas in *T. cacoeciae* strains R_0 was lowest after 60 days field exposure. Longevity of all adults emerged from parasitized eggs was affected by field exposure compared to the control. Total fecundity, total fertility and percent fertility after 60 days and/or after hibernation showed neither significant differences between strains nor within the strains studied. Total fertility of *T. cacoeciae* strains decreased significantly after midterm and/or after full hibernation compared with the control, while no significant differences were detected within *T. oleae* strains. Time until adult emergence, mortality, and percentage of deformed adults increased with storage duration at 4 °C. *Trichogramma oleae* (German strain) showed best adaptation to cold storage for 50 days.

Keywords: *Trichogramma cacoeciae*, *T. oleae*, Syria, Tunisia, Germany, diapause, life-table, performance, parasitization

4.1. Introduction

Trichogramma spp. are the most widely used biocontrol agents against lepidopterous pests in biological control programmes (Hassan, 1993; Hunter, 1994; King, 1993; Olkowski and Zhang, 1989, Smith, 1996). Both, native and allochthonous species have been mass-reared and released (Li, 1994; van Lenteren, 2000) in different crops. Their success in pest control depends on their quality as represented by emergence rate, longevity, fecundity, fertility and searching capacity (Bigler, 1994; Bueno et al., 2012; Cerutti and Bigler, 1991; Dutton and Bigler, 1995; Dutton et al., 1996; Greenberg, 1991; van Lenteren, 1991). In addition to the short life cycle of *Trichogramma* species and the fact that they can be mass-reared on factitious hosts allow these wasps to be produced quickly and affordably, compared to other parasitoids (Bueno et al., 2009; Parra and Zucchi, 2004; Smith, 1996) and make *Trichogramma* species highly suitable for inundative releases.

For inundative biological control programs and to improve storage conditions and the potential for mass releases, diapause of *Trichogramma* has received high attention (Pizzol and Voegelé, 1988; Voegelé et al., 1988; Zaslavskii and Umarova, 1990; for reviews see Boivin, 1994; Smith, 1996). Although indigenous and also allochthonous *Trichogramma* species are released for control of target pests for a limited time within the vegetation period, often in the same area year by year, settling potential and environmental safety concerning their relatively high polyphagy, have not been considered in due diligence (Babendreier et al., 2003a, b; Kuske et al., 2004; Nafus, 1993; Yong and Hoffmann, 2006). Concerns may be particularly raised whether allochthonous parasitoids can overwinter in the area of release. *Trichogramma* hibernation may occur by quiescence or diapause (Garcia et al., 2002; Rundle and Hoffmann, 2003), induced as a direct response to unfavourable environmental conditions, with resuming development immediately after favourable conditions return (Blum, 1985; Chapman, 1998; Lee and Denlinger, 1991; Saunders, 1982). Some species of the genus *Trichogramma* enter diapause in the prepupal stage governed by the photoperiod during their preimaginal development (Norris, 1994; Reznik et al., 2002; Reznik and Kats, 2004; Saunders, 2002; Tauber et al., 1983, 1984, 1986; Zaslavskii and Umarova, 1990). However, the main factor inducing diapause is assumed to be temperature (Anunciada, 1983; Boivin, 1994; Bonnemaïson, 1972; Babendreier, 2003c; Laing and Corrigan, 1995; Ma and Chen, 2006; Maslennikova, 1959; Norris, 1994; Parker and Pinnel, 1971; Pintureau and Daumal, 1995; Pizzol, 1978; Pizzol et al., 2010; Voegelé et al., 1986; Sorokina and Maslennikova, 1988; Zaslavskii and Umarova, 1990). Hibernation ability

and diapause is also an issue in maintaining *Trichogramma* species beyond mass-rearing and to improve storage conditions until dispatch for mass releases (Pizzol and Voegelé, 1988; Voegelé et al., 1988; Zaslavskii and Umarova, 1990). Storage of trichogrammatids as parasitized host eggs provides flexibility and efficiency in mass production and assures their availability in sufficient numbers at the time of release (Greenberg et al., 1996; Leopold, 1998; Tezze and Botto, 2004).

Temperature is also one of the important factors affecting dispersion, abundance, developmental rate, fecundity, and longevity of trichogrammatids (Cabello and Vargas, 1988; Maceda et al., 2003) and the knowledge of the thermal optimum and temperature amplitude of physiological or bodily activity must be evaluated before mass rearing and release of the wasps (Haile et al., 2002; Maceda et al., 2003).

The aim of this study was to investigate whether different strains of *Trichogramma oleae* Voegelé and *T. cacoeciae* Marchal are able to overwinter under the climatic conditions in southwestern Germany (Stuttgart-Hohenheim, state of Baden-Württemberg). Furthermore, the effect of diapause on the longevity, fecundity and fertility of the females emerged from parasitized eggs that passed the winter outdoors, was determined. Effects of low temperature on the performance of these strains, their parasitization potential and population growth parameters were assessed when reared on their factitious host, the Angoumois grain moth *Sitotroga cerealella* Olivier (Lep.: Gelechiidae).

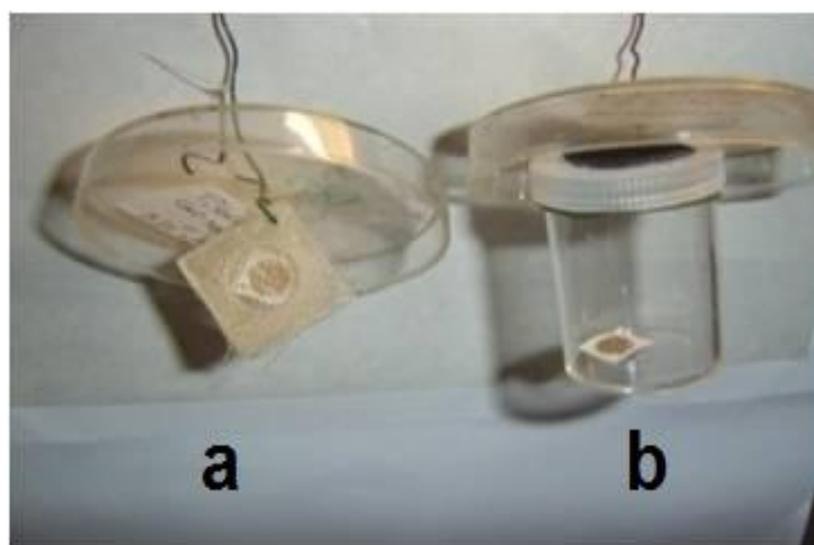


Figure 1(a) Plexiglas cage and (b) plastic tube, containing a paper with parasitized *S. cerealella* eggs, fixed with a wire and covered with a petri dish cover.

4.2. Materials and methods

4.2.1. *Trichogramma* rearing

Six strains of *Trichogramma* (*T. oleae* See2, *T. oleae* KIO4 TN, *T. cacoeciae* HOH 1 and HOH 2, *T. cacoeciae* KIO2 TN, and *T. cacoeciae* SY 98 01) (Hym.: Trichogrammatidae) were collected from parasitized eggs from different regions (Table 1). The strains were subsequently reared in the laboratory under standardized conditions (25 ± 1 °C, 16:8 L:D and R.H: 40-60% on eggs of the Angoumois grain moth for more than 65 generations. Eggs of *S. cerealella* were presented to wasps on egg paper cards in plastic tubes (PS Multipurpose Container, 15 ml, 38.80 * 23.00 mm, Cat.-No.: 203170, Greiner bio-one), closed with black nylon screen (0.7 mesh). The egg cards were prepared by spreading 24 h old host eggs on glued spots (9 mm diameter Arabic Gum on index cardboard) obtaining 100 ± 15 eggs per spot.

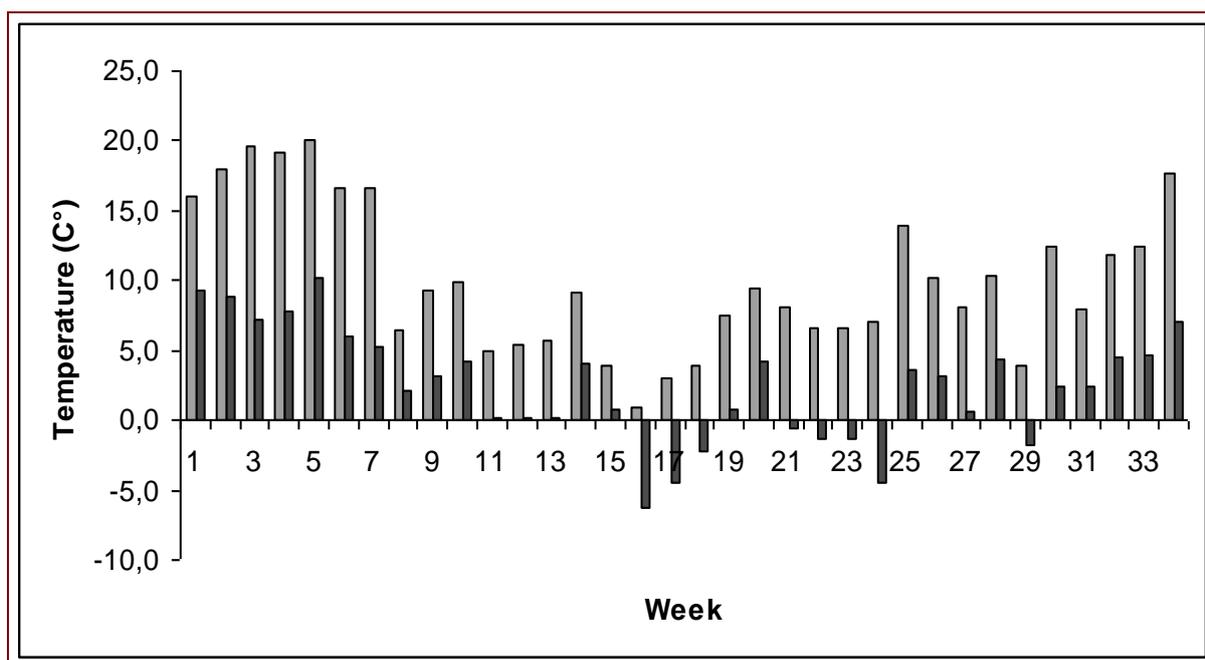


Figure 2 Weekly maximum and minimum temperatures of the first week of September 2007 until end of April 2008.

Effect of hibernation on different strains of *Trichogramma* (Hymenoptera:)

Table 1 List of the collected *T. cacoeciae* species and strains, their hosts, culture, location, and time of collection.

Species	Strain	Original host	Collected in culture	Country of origin	Latitude	Longitude	Collector	Year of collection
<i>T. cacoeciae</i> Marchal	HOH 1	<i>S. cerealella</i> Olivier ¹	Apple	Hohenheim Germany	48° 11' N	9° 13' E	H. Alkarrat	2006
<i>T. cacoeciae</i> Marchal	HOH 2	<i>S. cerealella</i> Olivier ¹	Apple	Hohenheim Germany	48° 11' N	48° 11' N	H. Alkarrat	2006
<i>T. cacoeciae</i> Marchal	KIO2 TN	<i>Ectomyelois</i> <i>ceratoniae</i> (Zeller)	Pomegranate	Tunisia	36° 50' N	10° 11' E	I. Ksentini	2004
<i>T. cacoeciae</i> Marchal	SY 98 01	<i>C. pomonella</i> L.	Apple	As Sweida Syria	32° 45' N	36° 45' E	W. Almatni	1998
<i>T. oleae</i> Voegelé	See 2	<i>S. cerealella</i> Olivier ¹	Weeds	Hohenheim Germany	48° 11' N	48° 11' N	C. P. W. Zebitz	2006
<i>T. oleae</i> Voegelé	KIO 4 TN	<i>E. ceratoniae</i> (Zeller)	Pomegranate	Tunisia	36° 50' N	10° 11' E	I. Ksentini	2004

¹ = Bait eggs.

4.2.2. Test procedure:

4.2.2.1. Overwintering ability

During overwintering in the field experiment, the temperature amplitude ranged between -8 and 24 °C (Fig. 2). Eight egg cards per *Trichogramma* strain were offered to females for 24 h parasitization opportunity time. The plastic tubes contained a droplet of a 10 % honey solution to provide the parasitoids with a carbohydrate source. After parasitization, the females were removed and the egg cards were transferred to Plexiglas® boxes (Fig.1a) (Sakr et al., 2004) on October 9th 2007, when the maximum daily temperature was assumed to decline below 15 - 17 °C (pilot tests at an earlier time in the year until end of September still resulted in adult emergence). Daily field weather data were obtained from the Institute of Physics and Meteorology of the University of Hohenheim.

Four of the eight boxes of every strain were taken into the laboratory after 60 days exposure to field conditions for daily observation until adult emergence. After adult emergence, 10 females of each box of each strain were separated individually into small plastic tubes (Fig. 1b). One *S. cerealella* egg card and a droplet of honey were offered to every individual female and replaced daily, until the female died. The parasitized eggs from each tube were transferred daily to another clean tube and kept in the laboratory for parasitoid development. After 4-5 days, when parasitized eggs turned black, the number of parasitized eggs and the number of emerged adults was assessed under a binocular microscope.

When the temperatures rised in April, the remaining egg cards were transferred from the plastic boxes to clean tubes with black net covers to prevent emerged adults to leave (Fig. 1b). Since these tubes were still kept in the field, they were covered with a Petri dish to prevent flooding by rain and attached to trees and bushes at a height of 1.5 m. Adult *Trichogramma* emergence was assessed twice daily at 10 a.m. and at 3 p.m. Time of emergence, number of females emerged, and adults with any morphological deformations was assessed before handling of the females to assess adult longevity and parasitization performance as described for the handling after 60 days exposure.

4.2.2.2. Effect low temperature on *Trichogramma* performances

To study the effect of low temperatures on the quality of the test strains, once the parasitoids reached the pupal stage inside their host eggs, these egg cards were stored at 4±1 °C in a

refrigerator at 70 % RH and in full darkness. The pupal stage was used in this study because it showed better tolerance to low temperature compared to other preimaginal stages in several *Trichogramma* spp. (Jalali and Singh 1992; Lopez and Morrison 1980). After 7, 15, 25, 40, 50, and 60 days, 15 randomly taken egg cards were removed from the cold storage and transferred to the climatic chamber and maintained at standard rearing conditions to monitor the emergence of adult parasitoids. Adult emergence before cold storage (0 days exposure) was taken as reference. To assess the quality of the cold-stored parasitoids, adult emergence time, proportion of emerged adults (no. of emerged adults/ no. of parasitized eggs), and deformed adults was assessed.

4.3. Statistical analysis:

All data were analysed using JMP[®] 9.0.0 software (2010, SAS Institute Inc., Cary, NC 27513, USA). All experimental data were normally distributed. Outliers were eliminated using Jackknife-procedure if necessary. Statistical procedures applied and necessary statistical information are given in the respective legends of tables and figures.

4.4. Results

4.4.1. Overwintering ability

During overwintering in the field experiment, the temperature amplitude ranged between -8 and 24 °C. Each strain tested was able to overwinter under Hohenheim field conditions (Stuttgart, Germany). The developmental period of all *Trichogramma* species and strains studied from egg to pupal stage as assessed by time until 50 % blackening at the beginning of the field experiments ranged between 7.25 ± 0.71 days for *T. oleae* See 2 to 12.00 ± 1.07 days for *T. cacoeciae* SY 98 01. Midterm and final evaluation revealed that the strains emerged successfully after 183 – 189 days showing no significant differences between the strains (Table 2).

Although percent adult emergence after hibernation differed significantly between strain HOH 1 and strain SY 98 01 of *T. cacoeciae*, these differences should not be overestimated because the respective of the other strains tested were rather homogenous. However, more serious differences could be observed in the ratio of deformed adults. Strain *T. cacoeciae* HOH 1 suffered from a significantly higher rate of deformed adults than strain *T. oleae* KIO 4 TN. The means of the other strains tested did not differ significantly (Table 2).

Effect of hibernation on different strains of *Trichogramma* (Hymenoptera:)

Table 2 Mean (\pm sd. respectively) time (days) to develop > 50% of eggs to pupal stage, diapause duration, total emergence rate (%) after hibernation, deformed adults (%) after hibernation, adult longevity, and net reproduction rate (R_0), of hibernated *Trichogramma* species / strains (one-way ANOVA **between strains**; for developmental time of > 50 % of eggs to pupal stage: $F = 21.8821$, $P < 0.0001$, d.f. = 5, 42; for diapause duration: $F = 1.0795$, $P = 0.3856$, d.f. = 5, 42; for emergence rate (%): $F = 2.9730$, $P = 0.0219$, d.f. = 5, 42; for deformed adults (%): $F = 2.3769$, $P = 0.0549$, d.f. = 5, 42; for longevity in the control: $F = 0.9863$, $P = 0.4347$, d.f. = 5, 54; for longevity after midterm exposure: $F = 4.1470$, $P = 0.0030$, d.f. = 5, 53; for longevity after complete hibernation: $F = 1.6751$, $P = 0.1564$, d.f. = 5, 54; one-way ANOVA **within strains**; longevity for: *T. cacoeciae* HOH 1: $F = 0.0125$, $P = 0.9875$, d.f. = 2, 27; *T. cacoeciae* HOH 2: $F = 1.2862$, $P < 0.2927$; d.f. = 2, 27; for *T. cacoeciae* KIO 2 TN: $F = 1.9837$, $P < 0.1571$; d.f. = 2, 27; for *T. cacoeciae* SY 98 01: $F = 5.5142$, $P < 0.0098$; d.f. = 2, 27; for *T. oleae* See 2: $F = 0.4126$, $P < 0.6660$; d.f. = 2, 27; for *T. oleae* KIO 4 TN: $F = 0.2762$, $P = 0.7608$; d.f. = 2, 27; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level).

	<i>T. oleae</i> See 2	<i>T. oleae</i> KIO 4 TN	<i>T. cacoeciae</i> HOH 1	<i>T. cacoeciae</i> HOH 2	<i>T. cacoeciae</i> KIO2 TN	<i>T. cacoeciae</i> SY 98 01	n
Time (d) to develop > 50% of eggs to pupal stage¹	7.25 \pm 0.71 ^b	7.88 \pm 0.99 ^b	8.13 \pm 1.13 ^b	8.50 \pm 0.76 ^b	8.50 \pm 1.31 ^b	12.00 \pm 1.07 ^a	8
Complete diapause duration (days)¹	183 \pm 4.34 ^a	184 \pm 4.50 ^a	185 \pm 6.30 ^a	186 \pm 4.72 ^a	185 \pm 6.59 ^a	189 \pm 6.72 ^a	8
Emergence rate (%) after hibernation¹	58.91 \pm 4.87 ^{ab}	56.63 \pm 4.49 ^{ab}	53.59 \pm 3.32 ^b	54.57 \pm 6.27 ^{ab}	53.80 \pm 4.75 ^{ab}	60.98 \pm 5.45 ^a	8
Deformed adults (%) after hibernation¹	20.78 \pm 7.31 ^{ab}	18.17 \pm 6.88 ^b	28.94 \pm 4.98 ^a	20.11 \pm 6.02 ^{ab}	22.38 \pm 6.60 ^{ab}	22.14 \pm 8.32 ^{ab}	8

Effect of hibernation on different strains of *Trichogramma* (Hymenoptera:)

Longevity (days)^{1/2}							
- control	7.90 ± 4.98 ^{a A}	9.10 ± 4.79 ^{a A}	10.20 ± 5.05 ^{a A}	10.90 ± 3.84 ^{a A}	11.00 ± 4.08 ^{a A}	11.90 ± 4.82 ^{a A}	10
- midterm	8.70 ± 2.75 ^{a B}	8.70 ± 2.75 ^{a B}	10.00 ± 2.05 ^{a AB}	10.60 ± 1.71 ^{a AB}	9.30 ± 2.98 ^{a AB}	12.20 ± 1.32 ^{a A}	10
- complete hibernation	7.20 ± 2.94 ^{a A}	8.00 ± 1.76 ^{a A}	10.20 ± 1.48 ^{a A}	8.90 ± 3.07 ^{a A}	8.10 ± 2.56 ^{a A}	7.50 ± 3.57 ^{b A}	10
Net reproductive rate (R₀)³							
- control	35.30	41.80	39.50	38.60	41.80	42.10	-
- midterm	32.60	32.10	27.20	20.80	26.30	24.10	-
- complete hibernation	26.50	29.32	39.10	36.80	32.10	37.10	-

¹Within a row, means with the same capital letter are not significantly different.

²Within a column, means with the same small letter are not significantly different.

³R₀ = (Σl_xm_x).

4.4.2. Life table of hibernated *Trichogramma* spp.

Only longevity of females of *T. cacoeciae* SY 98 01 taken from the field after complete hibernation (7.50 ± 3.57 days) was significantly shorter compared with the females taken after midterm (12.20 ± 1.32), whereas within all other species and strains tested no significant differences could be detected (Table 2).

In all strains, net reproduction rate (R_0) of control females was higher than that of females taken into the laboratory after midterm or full hibernation. R_0 of hibernated *T. cacoeciae* strains was higher than the R_0 of females taken into the laboratory after 60 days. In general, females of the *T. oleae* strains had lower R_0 -values after midterm and full hibernation than the *T. cacoeciae* strains (Table 2).

Total fecundity, total fertility and percent fertility after 60 days and/or after hibernation showed neither significant differences between strains nor within the strains studied. Total fertility of *T. cacoeciae* strains decreased significantly after midterm and/or after full hibernation compared with the control, while no significant differences were detected within *T. oleae* strains. Percent fertility of *T. oleae* strains after full hibernation was significantly higher compared with the control. Only absolute fertility of *T. cacoeciae* KIO 2 TN females after full hibernation was lower compared with the control (Table 3 and 5).

Table 3 Analysis of variance (ANOVA, GLM: General Linear Model, normal distribution, link: identity) of fecundity after three days, (n = 179, Chi-square = 137.5469, d.f. = 17, p < 0.0001); fertility after three days (n = 179, Chi-square = 167.5921, d.f. = 17, p < 0.0001); percent fertility after three days (n = 179, Chi-square = 100.8967, d.f. = 17, p < 0.0001); total fecundity (n = 179, Chi-square = 118.5307, d.f. = 17, p < 0.0001); total fertility (n = 179, Chi-square = 142.1936, d.f. = 17, p < 0.0001) and total percent fertility (n = 179, Chi-square = 71.1265, d.f. = 17, p < 0.0001) of six *Trichogramma* species/strains after midterm and full hibernation in the field.

Fecundity after 3 days			
Source	d.f.	Chi-square	P
Species / strains	5	9.0707	0.1063
Field exposure	2	104.2459	< 0.0001
Species / strains* field exposure	10	49.5026	< 0.0001

Fertility after 3 days			
Source	d.f.	Chi-square	P
Species / strains	5	106.2459	< 0.0001
Field exposure	2	7.0215	0.2190
Species / strains* field exposure	10	95.8873	< 0.0001

Fertility (%) after 3 days			
Source	d.f.	Chi-square	P
Species / strains	5	49.5077	< 0.0001
Field exposure	2	12.5349	0.0282
Species / strains* field exposure	10	54.4158	< 0.0001

Total fecundity			
Source	d.f.	Chi-square	P
Species / strains	5	11.4864	0.0425
Field exposure	2	87.2300	< 0.0001
Species / strains* field exposure	10	39.0278	< 0.0001

Total fertility			
Source	d.f.	Chi-square	P
Species/ strains	5	12.3901	0.0298
Field exposure	2	104.2856	< 0.0001
Species / strains* field exposure	10	53.5569	< 0.0001

Total fertility (%)			
Source	d.f.	Chi-square	P
Species / strains	5	1.9967	0.8496
Field exposure	2	35.0380	< 0.0001
Species / strains* field exposure	10	40.7559	< 0.0001

After midterm hibernation, maximum mean number of blackened eggs after three days was found for females of *T. oleae* See 2, in spite of the low mean fecundity of the respective control. The minimum mean fecundity was found in *T. cacoeciae* HOH 2. After complete hibernation, fecundity after three days of all strains showed no significant differences; however, all means differed significantly to their respective control. Fertility after midterm or complete hibernation was significantly lower compared to the respective control in *T. cacoeciae* strains, whereas there were no significant differences to the control in *T. oleae* strains. Fertility of completely hibernated females of *T. cacoeciae* KIO 2 TN was significantly lower compared with the females after 60 days and the control (Table 3 and 4).

After three days, total fertility of females of *T. cacoeciae* strains were lower than that of *T. oleae* strains after midterm and full hibernation, where in an overall comparison *T. cacoeciae* KIO 2 TN was affected strongest by hibernation (Table 3 and 4).

Comparing the relative change of total fecundity and absolute fertility after 60 days and full hibernation to their respective control, *T. cacoeciae* strains were suffering more than the *T. oleae* strains. Percent fertility was only slightly reduced or did not suffer from hibernation in the field (Table 6).

Effect of hibernation on different strains of *Trichogramma* (Hymenoptera:)

Table 4 Total fecundity, total fertility, and percent total fertility (mean \pm s.d. respectively) of six *Trichogramma* species / strains after 3 days before hibernation (control), after midterm, and full hibernation in the field. (**Between strains** one-way ANOVA; total fecundity for: *control*: F = 6.3920, P < 0.0001, d.f. = 5, 54; *midterm*: F = 2.2413, P= 0.0428; d.f. = 5, 54; *complete hibernation*: F = 1.1815, P = 0.3307; d.f. = 5, 53; For total fertility for: *control*: F = 12.6662, P < 0.0001; d.f. = 5, 54; *midterm*: F = 4.1591, P = 0.0029; d.f. = 5, 54; *complete hibernation*: F = 4.7490, P= 0.0012; d.f. = 5, 53), and for fertility (%) for: *control*: F = 9.3737, P < 0.0001, d.f. = 5, 54; *midterm*: F = 2.6988, P= 0.0301; d.f. = 5, 54; *complete hibernation*: F = 4.2979, P= 0.0024; d.f. = 5, 53); (**Within strains** one-way ANOVA; total fecundity for: *T. cacoeciae* HOH 1: F = 41.0759, P < 0.0001, d.f. = 2, 27; *T. cacoeciae* HOH 2: F = 16.2390, P < 0.0001; d.f. = 2, 27; *T. cacoeciae* KIO 2 TN: F = 28.5820, P < 0.0001, d.f. = 2, 26; *T. cacoeciae* SY 98 01: F = 7.5595, P = 0.0025, d.f. = 2, 27; *T. oleae* KIO 4 TN: F = 8.5385, P = 0.0013, d.f. = 2, 27; *T. oleae* See 2: F = 3.7877, P = 0.0355, d.f. = 2, 27; for total fertility for: *T. cacoeciae* HOH 1: F = 13.7043, P < 0.0001, d.f. = 2, 27; *T. cacoeciae* HOH 2: F = 27.9390, P < 0.0001; d.f. = 2, 27; *T. cacoeciae* KIO 2 TN: F = 48.9477, P < 0.0001, d.f. = 2, 26; *T. cacoeciae* SY 98 01: F = 8.4838, P = 0.014, d.f. = 2, 27; *T. oleae* KIO 4 TN: F = 1.5648, P = 0.2275, d.f. = 2, 27; *T. oleae* See 2: F = 2.6537, P = 0.0887, d.f. = 2, 27; total emergence rate for: *T. cacoeciae* HOH 1: F = 41.0759, P < 0.0001, d.f. = 2, 27; *T. cacoeciae* HOH 2: F = 15.7134, P < 0.0001; d.f. = 2, 27; *T. cacoeciae* KIO 2 TN: F = 3.4731, P = 0.0461, d.f. = 2, 26; *T. cacoeciae* SY 98 01: F = 3.2720, P = 0.0534, d.f. = 2, 27; *T. oleae* KIO 4 TN: F = 9.9374, P = 0.0006, d.f. = 2, 27; *T. oleae* See 2: F = 19.6468, P < 0.0001, d.f. = 2, 27; followed by Tukey-Kramer HSD-test, P \leq 0.05 level).

	<i>T. oleae</i> ¹ See 2	<i>T. oleae</i> ¹ KIO 4 TN	<i>T. cacoeciae</i> ¹ HOH 1	<i>T. cacoeciae</i> ¹ HOH 2	<i>T. cacoeciae</i> ¹ KIO2 TN	<i>T. cacoeciae</i> ¹ SY 98 01
Fecundity after 3 days²						
- control	28.10 \pm 9.40 ^{c AB} n = 10	35.80 \pm 7.30 ^{bc A} n = 10	46.90 \pm 6.84 ^{a A} n = 10	36.80 \pm 6.51 ^{bc A} n = 10	38.30 \pm 5.51 ^{ab A} n = 10	36.20 \pm 8.87 ^{bc A} n = 10
- midterm	35.30 \pm 9.06 ^{aA} n = 10	31.20 \pm 5.29 ^{ab AB} n = 10	28.20 \pm 7.39 ^{ab B} n = 10	26.20 \pm 5.61 ^{b B} n = 10	29.10 \pm 4.09 ^{ab C} n = 10	26.90 \pm 7.62 ^{ab B} n = 10
- complete hibernation	25.30 \pm 6.36 ^{aB} n = 10	25.90 \pm 2.33 ^{a B} n = 10	23.00 \pm 3.74 ^{a B} n = 10	23.10 \pm 4.63 ^{a B} n = 10	21.11 \pm 5.18 ^{a B} n = 10	23.30 \pm 6.25 ^{a B} n = 10

Effect of hibernation on different strains of *Trichogramma* (Hymenoptera:)

Fertility after 3 days²						
- control	19.10 ± 6.23 ^{c A} n = 10	24.40 ± 4.70 ^{bc A} n = 10	37.20 ± 3.61 ^{a A} n = 10	29.40 ± 4.17 ^{b A} n = 10	30.90 ± 3.96 ^{ab A} n = 10	29.70 ± 8.49 ^{b A} n = 10
- midterm	25.10 ± 5.69 ^{a A} n = 10	21.70 ± 5.01 ^{ab A} n = 10	18.50 ± 4.40 ^{b B} n = 10	16.40 ± 3.92 ^{b B} n = 10	22.10 ± 2.3 ^{ab B} n = 10	19.50 ± 6.19 ^{ab B} n = 10
-complete hibernation	22.70 ± 5.66 ^{a A} n = 10	21.30 ± 2.71 ^{a A} n = 10	18.00 ± 2.71 ^{ab B} n = 10	19.20 ± 4.18 ^{ab B} n = 10	14.78 ± 4.24 ^{b C} n = 10	19.80 ± 2.97 ^{ab B} n = 10
% Fertility after 3 days²						
- control	68.00 ± 5.10 ^{b B} n = 10	68.67 ± 7.77 ^{b B} n = 10	79.91 ± 5.43 ^{a A} n = 10	80.63 ± 6.97 ^{a A} n = 10	80.95 ± 4.92 ^{a A} n = 9	81.46 ± 8.71 ^{a AB} n = 10
- midterm	71.91 ± 10.46 ^{ab B} n = 10	69.16 ± 8.65 ^{ab B} n = 10	66.20 ± 4.78 ^{ab B} n = 10	62.96 ± 9.14 ^{b B} n = 10	77.05 ± 11.22 ^{a AB} n = 9	73.14 ± 12.33 ^{ab B} n = 10
- complete hibernation	90.60 ± 9.35 ^{a A} n = 10	82.06 ± 6.22 ^{ab A} n = 10	78.89 ± 8.69 ^{ab A} n = 10	83.43 ± 10.15 ^{ab A} n = 10	69.97 ± 10.19 ^{b B} n = 9	88.32 ± 17.38 ^{a AB} n = 10

¹ Within a column of a parameter, means with the same capital letter are not significantly different.

² Within a row, means with the same small letter are not significantly different.

Table 5 Total fecundity, total fertility, and percent total fertility (mean \pm s.d. respectively) of six *Trichogramma* species / strains before hibernation (control), after midterm, and full hibernation in the field. (**Between strains** one-way ANOVA; total fecundity for: *control*: $F = 4.5816$, $P = 0.0015$, d.f. = 5, 54; *midterm*: $F = 1.7272$, $P = 0.1441$; d.f. = 5, 54; *complete hibernation*: $F = 1.6977$, $P = 0.1513$; d.f. = 5, 53; total fertility for: *control*: $F = 5.5485$, $P = 0.0003$; d.f. = 5, 54; *midterm*: $F = 4.5608$, $P = 0.0015$; d.f. = 5, 54; *complete hibernation*: $F = 1.8291$, $P = 0.1230$; d.f. = 5, 53), and for percent total fertility for: *control*: $F = 4.8942$, $P = 0.0009$, d.f. = 5, 54; *midterm*: $F = 2.0808$, $P = 0.0820$; d.f. = 5, 54; *complete hibernation*: $F = 2.2874$, $P = 0.0590$; d.f. = 5, 53); (**Within strains** one-way ANOVA; total fecundity for: *T. cacoeciae* HOH 1: $F = 30.7267$, $P < 0.0001$, d.f. = 2, 27; *T. cacoeciae* HOH 2: $F = 9.5856$, $P = 0.0007$; d.f. = 2, 27; *T. cacoeciae* KIO 2 TN: $F = 25.5798$, $P < 0.0001$, d.f. = 2, 26; *T. cacoeciae* SY 98 01: $F = 6.3738$, $P = 0.0054$, d.f. = 2, 27; *T. oleae* KIO 4 TN: $F = 5.2557$, $P = 0.0118$, d.f. = 2, 27; *T. oleae* See 2: $F = 0.2818$, $P = 0.7566$, d.f. = 2, 27; total fertility for: *T. cacoeciae* HOH 1: $F = 31.5036$, $P < 0.0001$, d.f. = 2, 27; *T. cacoeciae* HOH 2: $F = 19.4577$, $P < 0.0001$; d.f. = 2, 27; *T. cacoeciae* KIO 2 TN: $F = 27.0801$, $P < 0.0001$, d.f. = 2, 26; *T. cacoeciae* SY 98 01: $F = 12.8985$, $P < 0.0001$, d.f. = 2, 27; *T. oleae* KIO 4 TN: $F = 2.5274$, $P = 0.0986$, d.f. = 2, 27; *T. oleae* See 2: $F = 0.8227$, $P = 0.4499$, d.f. = 2, 27; fertility (%) for: *T. cacoeciae* HOH 1: $F = 11.7433$, $P = 0.0002$, d.f. = 2, 27; *T. cacoeciae* HOH 2: $F = 10.3312$, $P = 0.0005$; d.f. = 2, 27; *T. cacoeciae* KIO 2 TN: $F = 4.0609$, $P = 0.0292$, d.f. = 2, 26; *T. cacoeciae* SY 98 01: $F = 2.5278$, $P = 0.0986$, d.f. = 2, 27; *T. oleae* KIO 4 TN: $F = 9.9130$, $P = 0.0006$, d.f. = 2, 27; *T. oleae* See 2: $F = 5.0926$, $P = 0.0133$, d.f. = 2, 27; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level).

Effect of hibernation on different strains of *Trichogramma* (Hymenoptera:)

	<i>T. oleae</i> ¹ See 2	<i>T. oleae</i> ¹ KIO 4 TN	<i>T. cacoeciae</i> ¹ HOH 1	<i>T. cacoeciae</i> ¹ HOH 2	<i>T. cacoeciae</i> ¹ KIO2 TN	<i>T. cacoeciae</i> ¹ SY 98 01
Total fecundity²						
- control	49.80 ± 19.66 ^{c A} n = 10	55.10 ± 16.11 ^{bc A} n = 10	78.40 ± 16.57 ^{a A} n = 10	57.00 ± 13.33 ^{abc A} n = 10	73.60 ± 16.40 ^{ab A} n = 9	58.90 ± 17.16 ^{abc A} n = 10
- midterm	44.90 ± 12.17 ^{a A} n = 10	42.40 ± 8.80 ^{a AB} n = 10	42.10 ± 7.80 ^{a B} n = 10	33.90 ± 10.48 ^{a B} n = 10	42.60 ± 6.35 ^{a B} n = 10	36.80 ± 13.01 ^{a B} n = 10
- complete hibernation	46.90 ± 10.56 ^{a A} n = 10	38.60 ± 9.45 ^{a B} n = 10	39.60 ± 11.18 ^{a B} n = 10	47.40 ± 11.57 ^{a A} n = 10	41.78 ± 7.90 ^{a B} n = 9	48.20 ± 10.55 ^{a AB} n = 10
Total fertility²						
- control	35.30 ± 16.21 ^{b A} n = 10	38.70 ± 12.71 ^{b A} n = 10	62.00 ± 15.12 ^{a A} n = 10	48.00 ± 12.36 ^{ab A} n = 10	59.40 ± 14.74 ^{a A} n = 9	49.10 ± 14.59 ^{ab A} n = 10
- midterm	32.60 ± 9.08 ^{a A} n = 10	28.90 ± 7.31 ^{ab A} n = 10	27.20 ± 3.74 ^{ab B} n = 10	20.80 ± 5.88 ^{b C} n = 10	32.10 ± 4.46 ^{a B} n = 10	24.70 ± 7.94 ^{ab C} n = 10
- complete hibernation	39.10 ± 6.61 ^{a A} n = 10	32.10 ± 9.02 ^{a A} n = 10	32.40 ± 9.64 ^{a B} n = 10	36.80 ± 10.03 ^{a B} n = 10	29.22 ± 7.64 ^{a B} n = 9	37.10 ± 8.37 ^{a B} n = 10

Effect of hibernation on different strains of *Trichogramma* (Hymenoptera:)

Fertility (%)²						
- control	70.00 ± 12.02 ^{b B} n = 10	70.29 ± 8.96 ^{b B} n = 10	79.06 ± 8.83 ^{ab A} n = 10	83.89 ± 5.93 ^{a A} n = 10	80.32 ± 5.09 ^{ab A} n = 9	83.13 ± 10.20 ^{a A} n = 10
- midterm	72.74 ± 11.29 ^{a AB} n = 10	67.85 ± 8.44 ^{a B} n = 10	65.42 ± 7.84 ^{a B} n = 10	62.56 ± 10.00 ^{a B} n = 10	76.06 ± 9.83 ^{a AB} n = 10	69.67 ± 15.30 ^{a A} n = 10
- complete hibernation	84.85 ± 9.82 ^{a A} n = 10	82.40 ± 5.67 ^{ab A} n = 10	81.77 ± 7.52 ^{ab A} n = 10	78.71 ± 14.97 ^{ab A} n = 10	69.48 ± 9.33 ^{b B} n = 9	78.01 ± 14.50 ^{ab A} n = 10

¹ Within a column of a parameter, means with the same capital letter are not significantly different

² Within a row, means with the same small letter are not significantly different.

Table 6 Relative effect of partial and complete hibernation on reproduction parameters of *Trichogramma* species / strains.

Species / strains	Field exposure treatments	Fecundity	Fertility	Fertility (%)
<i>T. cacoeciae</i> HOH 1	after 60 days	- 46.3 %	- 56.1 %	- 17.2 %
	complete hibernation	- 49.5 %	- 47.7 %	+ 3.4 %
<i>T. cacoeciae</i> HOH 2	after 60 days	- 40.5 %	- 56.7 %	- 25.4 %
	complete hibernation	- 16.8 %	- 23.3 %	- 6.2 %
<i>T. cacoeciae</i> KIO 2 TN	after 60 days	- 42.1 %	- 46.0 %	- 5.3 %
	complete hibernation	- 43.2 %	- 50.8 %	- 13.5 %
<i>T. cacoeciae</i> SY 98 01	after 60 days	- 37.5 %	- 49.7 %	- 16.2 %
	complete hibernation	- 18.2%	- 24.4 %	- 6.2 %
<i>T. oleae</i> KIO 4 TN	after 60 days	- 23.0 %	- 25.3 %	- 3.5 %
	complete hibernation	- 29.9 %	- 17.1 %	+ 17.2 %
<i>T. oleae</i> See 2	after 60 days	- 9.8 %	- 7.6 %	+ 3.9 %
	complete hibernation	- 5.8 %	+ 10.8%	+ 21.2 %

4.4.3. Survival after exposure to low temperature

The time elapsed until adult emergence increased significantly with increasing storage duration under low temperature. The maximal mean time span ranged between 3.79 days and 10.2 days after 7 and 60 days exposure respectively in *T. cacoeciae* KIO 2 TN, and the shortest mean time span ranged between 4.27 days and 8.73 days after 7 and 60 days exposure respectively in *T. cacoeciae* SY 98 01. Significant differences between strains and species could be detected when stored for different duration (Table 7 and Table 8).

Table 7 Analysis of variance of time until emergence (ANOVA, GLM: General Linear Model, normal distribution, link: identity; number of observations = 532, Chi-square = 676.0217, d.f. = 6, P < 0.0001), percent emerged adults (number of observations = 522, Chi-square = 677.0606, d.f. = 6, P < 0.0001), and percent deformed adults (number of observations = 521, Chi-square = 373.4952, d.f. = 6, P < 0.0001) of six *Trichogramma* species / strains as affected by low temperature storage for different duration.

Emergence time in days			
Source	d.f.	Chi-square	P
Species / strains	5	174.3279	< 0.0001
Storage duration	5	614.51125	< 0.0001
Emerged adults (%)			
Source	d.f.	Chi-square	P
Species / strains	5	76.338831	< 0.0001
Storage duration	5	654.16346	< 0.0001
Deformed adults (%)			
Source	d.f.	Chi-square	P
Species / strains	5	47.700429	< 0.0001
Storage duration	5	349.21633	0.2628

Also, when the cold storage duration increased, the proportion of emerged adults decreased significantly. The best emergence rates have been found for *T. oleae* KIO 4 TN after 7 – 25 days cold storage, however it decreased sharply when stored for a longer period. Strain *T. cacoeciae* HOH 1 was least affected after a 60 days cold storage compared to all other strains tested (Table 7 and 9). With increasing the duration of cold storage, the proportion of deformed adults increased significantly in all species/strains tested (Table 7 and 10).

Days until adult emergence (D) after terminated cold storage of all species and strains tested increased with duration of cold storage, following the equation $D = a + b \exp(c * \text{storage duration})$ (Table 11, Figure 3). However, the regression for *T. cacoeciae* HOH 1 and *T. oleae* KIO 4 TN followed an almost linear regression as indicated by the parameters a and b (Table 11).

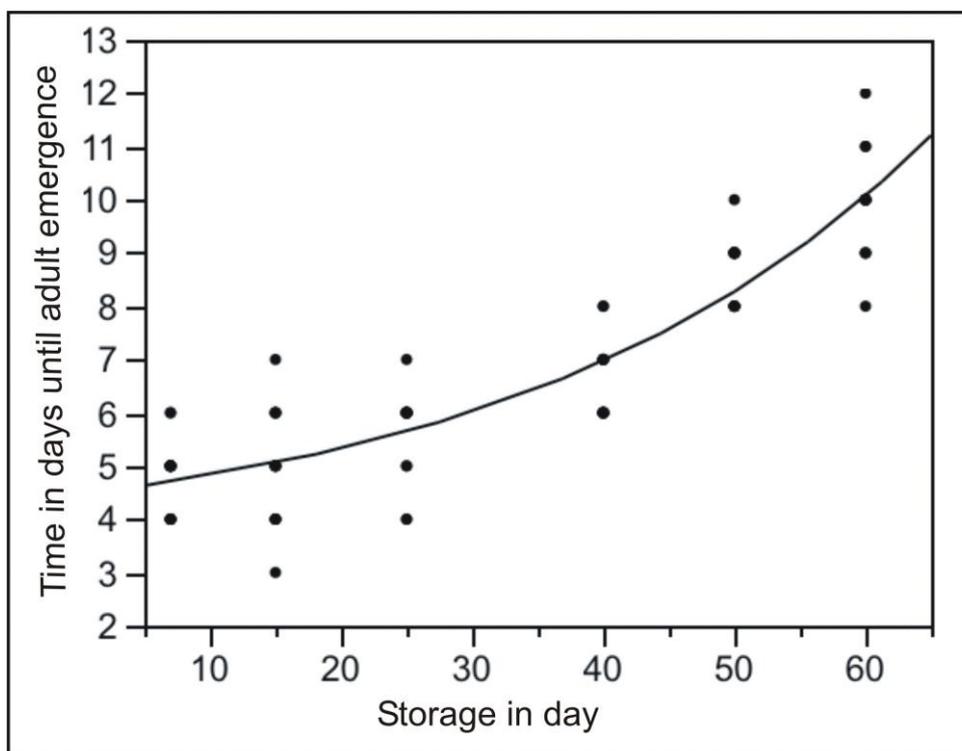


Figure 3 Non-linear correlation ($D = a + b \exp(c * \text{storage duration})$) between days until adult emergence after terminated cold storage and duration of cold storage, using strain *T. cacoeciae* HOH 2 as example.

Effect of hibernation on different strains of *Trichogramma* (Hymenoptera:)

Table 8 Time (days) until adult emergence of six *Trichogramma* species/ strains stored under 4±1°C for different duration (one-way ANOVA; for 7 days: F = 12.6797, P < 0.0001, d.f. = 5, 82; for 15 days: F = 22.7563, P < 0.0001; d.f. = 5, 84; for 25 days: F = 36.4108, P < 0.0001; d.f. = 5, 80; for 40 days: F = 24.2067, P < 0.0001; d.f. = 5, 77; for 50 days: F = 8.9786, P < 0.0001; d.f. = 5, 80; and for 60 days: F = 2.7094, P < 0.0256; d.f. = 5, 83; followed by Tukey-Kramer HSD-test, P ≤ 0.05 level).

Strains	Storage duration ¹					
	7 days	15 days	25 days	40 days	50 days	60 days
<i>T. cacoeciae</i> HOH 2	4.80 ± 0.68 ^{ab} n = 15	5.00 ± 1.07 ^b n = 15	5.64 ± 0.84 ^b n = 14	6.64 ± 0.74 ^b n = 14	8.67 ± 0.62 ^b n = 15	9.93 ± 1.00 ^{ab} n = 14
<i>T. cacoeciae</i> KIO 2 TN	3.79 ± 0.58 ^{cd} n = 14	4.80 ± 0.86 ^{bc} n = 15	5.93 ± 1.03 ^b n = 15	6.33 ± 0.82 ^{bc} n = 15	8.69 ± 0.48 ^{ab} n = 13	10.20 ± 0.94 ^a n = 15
<i>T. cacoeciae</i> SY 98 01	4.27 ± 1.10 ^{bc} n = 15	4.27 ± 1.10 ^{bcd} n = 15	3.86 ± 1.46 ^c n = 14	5.23 ± 0.83 ^c n = 13	7.27 ± 1.62 ^c n = 15	8.73 ± 1.67 ^b n = 15
<i>T. oleae</i> KIO 4 TN	3.07 ± 1.00 ^d n = 14	3.73 ± 1.33 ^{cd} n = 15	4.43 ± 0.76 ^c n = 14	7.29 ± 1.59 ^b n = 14	9.87 ± 1.36 ^a n = 15	9.67 ± 1.23 ^{ab} n = 15
<i>T. oleae</i> See 2	3.80 ± 0.86 ^{cd} n = 15	3.60 ± 1.35 ^d n = 15	3.47 ± 1.00 ^c n = 15	5.29 ± 1.54 ^c n = 14	8.38 ± 0.87 ^{bc} n = 13	9.67 ± 1.35 ^{ab} n = 15

¹Within a column, means with the same letter are not significantly different.

Effect of hibernation on different strains of *Trichogramma* (Hymenoptera:)

Table 9 Emerged adults (%) of six *Trichogramma* species/ strains stored under $4 \pm 1^\circ\text{C}$ at six different times (7, 15, 25,40, 50 and 60 days respectively) (one-way ANOVA; for 7 days: $F = 8.9101$, $P < 0.0001$, d.f. = 5, 82; for 15 days: $F = 68.1145$, $P < 0.0001$; d.f. = 5, 84; for 25 days: $F = 9.9687$, $P < 0.0001$; d.f. = 5, 80; for 40 days: $F = 12.4917$, $P < 0.0001$; d.f. = 5, 76; for 50 days: $F = 9.4204$, $P < 0.0001$; d.f. = 5, 80; and for 60 days: $F = 11.1638$, $P < 0.0001$; d.f. = 5, 83; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level).

Strains	Storage duration ¹					
	7 days	15 days	25 days	40 days	50 days	60 days
<i>T. cacoeciae</i> HOH 1	68.29 ± 3.32 ^{ab} n = 15	58.55 ± 5.35 ^b n = 15	49.57 ± 4.29 ^{ab} n = 14	43.48 ± 4.25 ^a n = 13	35.94 ± 7.68 ^{ab} n = 15	31.36 ± 8.01 ^a n = 15
<i>T. cacoeciae</i> HOH 2	70.03 ± 4.18 ^a n = 15	59.19 ± 6.43 ^b n = 15	54.60 ± 2.08 ^a n = 14	44.15 ± 12.70 ^a n = 13	28.58 ± 8.24 ^{bc} n = 15	23.60 ± 5.93 ^b n = 14
<i>T. cacoeciae</i> KIO 2 TN	66.96 ± 6.86 ^{ab} n = 14	56.87 ± 3.44 ^b n = 15	41.39 ± 4.06 ^c n = 15	27.94 ± 6.04 ^b n = 15	28.70 ± 2.22 ^{bc} n = 15	22.20 ± 10.41 ^{bc} n = 15
<i>T. cacoeciae</i> SY 98 01	54.55 ± 8.18 ^c n = 15	40.00 ± 4.58 ^d n = 15	43.60 ± 9.80 ^{bc} n = 14	49.57 ± 7.60 ^a n = 13	19.30 ± 9.24 ^c n = 15	16.87 ± 5.32 ^{bc} n = 15
<i>T. oleae</i> KIO 4 TN	72.61 ± 5.79 ^a n = 14	67.13 ± 2.89 ^a n = 15	55.16 ± 10.05 ^a n = 15	42.17 ± 8.06 ^a n = 14	23.19 ± 12.19 ^c n = 15	15.71 ± 2.38 ^c n = 15
<i>T. oleae</i> See 2	60.52 ± 16.36 ^{bc} n = 15	47.54 ± 3.30 ^c n = 15	52.17 ± 7.30 ^a n = 14	44.66 ± 6.40 ^a n = 14	40.80 ± 14.29 ^a n = 13	17.68 ± 5.76 ^{bc} n = 15

¹Within a column, means with the same letter are not significantly different.

Effect of hibernation on different strains of *Trichogramma* (Hymenoptera:)

Table 10 Deformed adults (%) of six *Trichogramma* species/ strains stored under $4 \pm 1^\circ\text{C}$ at six different times (7, 15, 25,40, 50 and 60 days respectively) (one-way ANOVA; for 7 days: $F = 11.4692$, $P < 0.0001$, d.f. = 5, 82; for 15 days: $F = 5.7900$, $P < 0.0001$; d.f. = 5, 84; for 25 days: $F = 10.0576$, $P < 0.0001$; d.f. = 5, 80; for 40 days: $F = 10.7731$, $P < 0.0001$; d.f. = 5, 77; for 50 days: $F = 17.5342$, $P < 0.0001$; d.f. = 5, 80; and for 60 days: $F = 1.8695$, $P < 0.1085$; d.f. = 5, 83; followed by Tukey-Kramer HSD-test, $P \leq 0.05$ level).

Strains	Storage duration ¹					
	7 days	15 days	25 days	40 days	50 days	60 days
<i>T. cacoeciae</i> HOH 1	2.93 ± 2.32^d n = 15	10.37 ± 2.56^{abc} n = 15	15.86 ± 3.35^a n = 14	22.64 ± 6.89^{ab} n = 13	30.69 ± 11.38^b n = 15	32.88 ± 13.79^a n = 15
<i>T. cacoeciae</i> HOH 2	5.81 ± 2.84^{cd} n = 15	10.07 ± 5.84^{ab} n = 15	16.58 ± 3.72^a n = 14	24.10 ± 14.01^a n = 14	48.49 ± 13.70^a n = 15	36.54 ± 10.84^a n = 14
<i>T. cacoeciae</i> KIO 2 TN	6.88 ± 3.87^{bc} n = 15	8.00 ± 1.85^{bc} n = 15	13.12 ± 3.83^{ab} n = 15	28.53 ± 6.60^a n = 15	15.67 ± 3.54^c n = 13	26.83 ± 11.90^a n = 15
<i>T. cacoeciae</i> SY 98 01	11.22 ± 4.02^a n = 14	13.57 ± 4.37^a n = 15	14.58 ± 4.01^a n = 14	14.66 ± 3.89^{bc} n = 13	45.52 ± 21.72^a n = 15	36.75 ± 17.09^a n = 15
<i>T. oleae</i> KIO 4 TN	7.40 ± 2.02^{bc} n = 15	6.53 ± 1.96^c n = 15	9.38 ± 3.39 n = 14	15.44 ± 3.65^{bc} n = 14	23.06 ± 12.67^{bc} n = 15	32.69 ± 8.61^a n = 15
<i>T. oleae</i> See 2	10.29 ± 4.76^{ab} n = 15	10.06 ± 4.91^{abc} n = 15	9.93 ± 3.21^b n = 15	12.09 ± 3.12^c n = 14	14.02 ± 6.60^c n = 13	40.04 ± 13.49^a n = 15

¹Within a column, means with the same letter are not significantly different.

Table 11 Parameters of non-linear regression ($D = a + b \exp(c * \text{storage duration})$); at the $P = 0.05$ level, d.f. for error for each strain = 84) between days until adult emergence (D) after terminated cold storage of six *Trichogramma* species/ strains and duration of cold storage.

Species / strain	a	b	C	Mean squared error
<i>T. cacoeciae</i> HOH 1	- 2706.8111	2712.537	0.000027	1.1047
<i>T. cacoeciae</i> HOH 2	3.4919	0.9693	0.031914	0.7304
<i>T. cacoeciae</i> KIO 2 TN	1.6811	2.0633	0.023621	0.8084
<i>T. cacoeciae</i> SY 98 01	3.6466	0.2196	0.052946	1.8805
<i>T. oleae</i> KIO 4 TN	- 53.4905	55.2815	0.002381	1.8848
<i>T. oleae</i> See 2	2.5677	0.5389	0.043706	1.7079

4.5. Discussion

Our data indicate that the six species / strains of *Trichogramma* were able to overwinter in Stuttgart-Hohenheim. Temperature did not drop below -9 °C during the winter which did not expose the trichogrammatids to extreme weather conditions. Adults of all six strains emerged successfully, and the proportion of emergence in our study was similar to other studies on Trichogrammatids in other places: *T. exiguum* Pinto and Platner in North Carolina (Keller, 1986), *T. pretiosum*, Riley in central Texas (López and Morrison, 1980), *T. brassicae* and *T. cacoeciae* in Turkey (Özder et al., 2005), *T. brassicae* in northern Switzerland (Babendreier et al., 2003a) and *T. evanescens* in Egypt (Zaki, 1996).

Duration of complete hibernation lasted between 183 ± 8.7 and 189 ± 10.1 days and did not differ between the six strains studied. During a 60 days or full field exposure, *Trichogramma* strains were able to complete their preimaginal development. This agrees with past research revealing the larval/pupal stage in the host egg being the main overwintering stage of *Trichogramma* sp. (Burbutis et al., 1976; Lopez and Morrison, 1980; Keller, 1986; Parker and

Pinnell, 1971; Reznik et al., 2008; Zaslavski et al., 1990). However, duration of hibernation may differ between species and environmental conditions, mainly winter temperatures and possibly here within the temperature minimum in the field. For example, overwintering of *T. evanescens* in Egypt was 99 days (Zaki, 1996), *T. brassicae* in Turkey was 132 days (Özder et al., 2005), *T. closterae* Pang et Chen in Huaibei-China was between 178 and 182 days (Tian, 1998), and *T. funiculatum* in Australia was 72.6 days (Bradley, 2003). The long winter period in southwestern Germany with low temperatures as well as day length may be responsible for the long hibernation duration (Reznik et al., 2008, 2011, 2012).

The longevity and fecundity of the overwintering female parasitoids decreased, compared with the control but the differences were not significant. These results correspond to past research which indicates that the longevity of females of *T. brassicae* kept under field conditions did not differ from that reared under laboratory conditions (Babendreier et al., 2003c; Özder, 2005), and thus, laboratory conditions are well suitable to test hibernation and cooling ability of trichogrammatids. Our strains seem to be well adapted to the field conditions even when subtropical species/strains (*T. oleae*, and *T. cacoeciae* SY 98 01) would be released in a new area. Similar results were reported by Özder and Sağlam (2005) for *T. cacoeciae* and *T. brassicae*, Tian (1998), for *T. closterae* and Bradely (2003), and for *T. funiculatum*. Given, *Trichogramma* females can survive after inundative release on alternative hosts in the field and parasitize hosts overwintering in the egg stage, they potentially can establish even in a new environment matching their preferred or suboptimal abiotic conditions.

This study also showed that the developmental time under outdoor conditions from egg to pupal stage occupied ~4 -7 % of the total duration of the pre-imaginal development under hibernation, while it took 43 -52 % under laboratory conditions at higher temperatures.

Storage experiments showed that percentage of emerged adults and the ratio of deformed adults was negatively correlated with duration of cold storage, corresponding with results reported by Cerutti and Bigler (1995) and Gross (1988). The decreasing percentage of emerged adults may be due to a reduction in fat body reserves during cold storage as explained by Couillien and Gregoire (1994) for the predator *Rhizophagus grandis* Gyllenhal. Increased numbers of deformed adults could result from the exposure of parasitized eggs to extremely suboptimal temperature and possible disturbance of adult ecdysis (Cerutti and Bigler, 1995; Gross, 1988).

Our study revealed different adaptations to cold storage where *T. cacoeciae* HOH 1 and *T. oleae* KIO 4 TN seemed to be less adapted to cold storage as percent emergence showed a negative linear correlation with increasing storage duration and the remaining species/strains suffered less from short cold storage as shown by the non-linear regression. Comparing the results between field and laboratory experiments, the present study showed that the proportion of emerged adults after hibernation was higher than that of parasitized eggs exposed to constant 4 °C for more than 25 days. Moreover the proportion of deformed adults of hibernated parasitoids was lower than that after cold storage for more than 25 days. Temperature is the limiting factor for parasitoid survival during diapause. Knowledge of temperature adaptation is necessary to know to conserve and store parasitoids in mass rearings until needed, by induction of diapause (Bigler, 1994), and also to select the best candidate species or strain to hibernate either in factitious or natural hosts.

4.6. Acknowledgments

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References

- Anunciada, L., 1983. A escolha de um oófito *Trichogramma* para o control biológico de *Mythimnauni puncta*. PhD Thesis. Universidade dos Açores, pp. 108.
- Babendreier, D., Schoch, D., Kuske, S., Dorn, S., Bigler, F., 2003b. Non-target habitat exploitation by *Trichogramma brassicae* (Hym.: Trichogrammatidae): what are the risks for endemic butterflies? *Agricultural and Forest Entomology* 5, 199-208.
- Babendreier, D., Kuske, S., Bigler, F., 2003c. Non-target host acceptance and parasitism by *Trichogramma brassicae* Bezdenko (Hym.: Trichogrammatidae) in the laboratory. *Biological Control* 26, 128-138.
- Babendreier, D., Kuske, S., Bigler, F., 2003a. Overwintering of the egg parasitoid *Trichogramma brassicae* in Northern Switzerland. *BioControl* 48, 261-273.

- Bigler, F., 1994. Quality control in *Trichogramma* production. In: Wajnberg, E., Hassan, S.A. (Eds.), Biological control with egg parasitoids. CAB International, Wallingford, UK, pp. 93-111.
- Blum, M.S., 1985. Fundamentals of insect physiology. Wiley, New York, pp. 616.
- Boivin, G., 1994. Overwintering strategies of egg parasitoids. In: Wajnberg, E., Hassan, S.A. (Eds.), Biological control with egg parasitoids. CAB International, Wallingford, UK, pp. 219-244.
- Bonnemaison, L., 1972. Diapause et superparasitisme chez *Trichogramma evanescens* Westwood. Bulletin de la Société Entomologique de France 77, 122-132.
- Bradley, J.R., Hoffmann, A.A., 2003. Overwintering of *Trichogramma funiculatum* Carver (Hym.: Trichogrammatidae) under semi-natural conditions. Environmental Entomology 32, 290-298.
- Burbutis, P.P., Curl, G.D., Davis, C.P., 1976. Overwintering of *Trichogramma nubilale* in Delaware. Environmental Entomology 5, 888-890.
- Cabello, T., Vargas, P., 1988. The effect of temperature on the bionomics of *Trichogramma cordubensis* (Hym.: Trichogrammatidae). Colloques de l' INRA 43, 155-164.
- Cerutti, F., Bigler, F., 1991. Methods for the quality evaluation of *Trichogramma evanescens* Westw. In: Bigler, F. (Eds.), used against the European corn borer. Proceedings, Fifth Workshop of the IOBC Working Group on Quality Control of Mass Reared Arthropods, March 25-28 1991, Wageningen, NL, pp. 119-126.
- Cerutti, F., Bigler, F., 1995. Quality assessment of *Trichogramma brassicae* in the laboratory. Entomologia Experimentalis et Applicata. 49, 283-290.
- Chapman, R.F., 1998. The Insects: Structure and function, 4th ed. Cambridge University Press, UK, pp.770.
- Couillien, D., Gregoire, J.C., 1994. Take-off capacity as criterion for quality control in mass produced predators, *Rhizophagus grandis* (Coleoptera: Rhizophagidae) for the biocontrol of bark beetles, *Dendroctonus micans* (Coleoptera: Scolytidae). Entomophaga 39, 385-395.

- Dutton, A., Bigler, F., 1995. Flight activity assessment of the egg parasitoid *Trichogramma brassicae* (Hym.: Trichogrammatidae) in laboratory and field conditions. *Entomophaga* 40, 223-233.
- Dutton, A., Cerutti, F., Bigler, F., 1996. Quality and environmental factors affecting *Trichogramma brassicae* efficiency under field conditions. *Entomologia Experimentalis et Applicata* 81, 71-79.
- Garcia, P.V., Wajnberg, E., Pizzol, J., Oliveira, M.L.M., 2002. Diapause in the egg parasitoid *Trichogramma cordubensis*: role of temperature. *Journal of Insect Physiology* 48, 349-355.
- Greenberg, S.M., 1991. Evaluation techniques for *Trichogramma* quality. In: Bigler, F. (Eds.), *Proceedings, Fifth Workshop of the IOBC Working Group on Quality Control of Mass Reared Arthropods, March 25-28 1991, Wageningen, NL*, pp. 138-145.
- Greenberg, S.M., Nordlund, D.A., King, E.G., 1996. Mass production of *Trichogramma* spp.: experience in the former Soviet Union, China, United States and western Europe. *Biocontrol News Inform* 17, 51-60.
- Gross, H.R., 1988. Effect of temperature, relative humidity, and free water on the number and normalcy of *Trichogramma pretiosum* Riley (Hym.: Trichogrammatidae) emerging from eggs of *Heliothis zea* (Boddie) (Lep.: Noctuidae). *Environmental Entomology* 17, 470-475.
- Haile, A.T., Hassan, S.A., Ogol, C.K.P.O., Baumgärtner, J., Sithanatham, S., Monje, J.C., Zebitz, C.P.W., 2002. Temperature-dependent development of four egg parasitoid *Trichogramma* species (Hym.: Trichogrammatidae). *Biocontrol Science and Technology*, 12, 555-567.
- Hassan, S.A., 1993. The mass rearing and utilization of *Trichogramma* to control lepidopterous pests: achievements and outlook. *Pesticide Science* 37, 387-391.
- Hunter, C.D., 1994. Suppliers of beneficial organisms in north America. California environmental protection agency, department of pesticide regulation, environmental monitoring and pest management branch, pp. 31.
- Jalali, S., Singh, P., 1992. Differential response of four *Trichogramma* species to low temperatures for short term storage. *Entomophaga* 37, 159-165.

- Keller, M.A., 1986. Overwintering by *Trichogramma exiguum* in North Carolina. *Environmental Entomology* 15, 659- 661.
- King, E.G., 1993. Augmentation of parasites and predators for suppression of arthropod pests. In: Lumsden, R.D., Vaughn, (Eds.), *Pest management: biologically based technologies, proceedings of the beltsville symposium 28, May 1993*. American Chemical Society, Maryland, Washington, DC, pp. 90-100.
- Kuske, S., Babendreier, D., Edwards, P.J., Turlings, T.C.J., Bigler, F., 2004. Parasitism of non-target lepidoptera by mass released *Trichogramma brassicae* and its implication for the larval parasitoid *Lydella thompsoni*. *BioControl* 49, 1-19.
- Laing, J.E., Corrigan, J.E., 1995. Diapause induction and post-diapause emergence in *Trichogramma minutum* Riley (Hym.: Trichogrammatidae): the role of host species, temperature and photoperiod. *Canadian Entomology* 127, 103-110.
- Lee, R.E., Denlinger, D.L., 1991. *Insects at low temperature*. Chapman and Hall, New York.
- Leopold RA (1998) Cold storage of insects: using cryopreservation and dormancy as an aid to mass rearing. In: *Area-Wide Control of Insect Pests Integrating the Sterile Insect and Related Nuclear and Other Techniques*. FAO/IAEA International Conference, Penang, Malaysia. June, 1998. IAEA-CN-71, 315-324.
- Li, L.Y., 1994. Worldwide use of *Trichogramma* for biological control on different crops: In: E Wajnberg, E., Hassan, S.A. (Eds.), *a survey. biological control with egg parasitoids*. CAB International, Wallingford, UK, pp. 37-53.
- Lopez, J.D., Morrison, R.K., 1980. Overwintering of *Trichogramma pretiosum* in central Texas. *Environmental Entomology* 9, 75-78.
- Ma, C.S., Chen, Y.W., 2006. Effects of constant temperature, exposure period, and age on diapause induction in *Trichogramma dendrolimi*. *Biological Control* 36, 267-273.
- Maceda, A., Hohmann, C.L., Santos, H.R., 2003. Temperature effects on *Trichogramma pretiosum* Riley and *Trichogrammatoidea annulata* De Santis. *Brazilian Archives of Biology and Technology* 46, 27-32.

- Maslennikova, V.A., 1959. On the problem of overwintering and diapause of Trichogrammatids (*Trichogramma evanescens* (Westw.)). Vestnik Leningradskogo Universiteta Biologiya 14, 91-96.
- Nafus, D.M., 1993. Movement of introduced biological control agents onto nontarget butterflies, *Hypolimnas* spp. (Lep.: Nymphalidae). Environmental Entomology 22, 265-272.
- Norris, K.R., 1994. Life history stages in a condition of arrested development. In: Naumann, I.D. (Eds.), Systematic and applied entomology an introduction. Melbourne University Press, Melbourne, Australia, pp. 60-100.
- Olkowski, W., Zhang, A., 1989. *Trichogramma*: a modern day frontier in biological control. In: least-toxic pest management in China: A collection of articles from the IPM practitioner. Berkeley, CA, Bio-Integral Resource Center China Program, pp. 38-52.
- Özder, N., Sağlam, Ö., 2005. Overwintering of the egg parasitoids *Trichogramma brassicae* and *T. cacoeciae* (Hym.: Trichogrammatidae) in the thrace region of Turkey. Journal of Pest Science 78, 129-132.
- Parker, F.D., Pinnel, R.E., 1971. Overwintering of *Trichogramma* spp. in Missouri. Journal of Economic Entomology 64, 80-91.
- Parra, J.R.P., Zucchi, R.A., 2004. *Trichogramma* in Brazil: feasibility of use after twenty years of research. Neotropical Entomology 33, 271-281.
- Pintureau, F., Daumal, J., 1995. Effects of diapause and host species on some morphometric characters in *Trichogramma* (Hym.: Trichogrammatidae). Experientia 51, 68-72.
- Pizzol, J., Voegelé, J., 1988. The diapause of *Trichogramma maidis* Pintureau and Voegelé in relation to some characteristics of its alternative host *Ephestia kuehniella* Zell. Colloques de l'INRA 48, 93-94.
- Pizzol, J., 1978. La diapause chez *Trichogramma evanescens* Westwood (Hym.: Trichogrammatidae), ecotype moldave parasite oophage de la Pyrale du maïs. Diplôme, de l'école Pratique des Hautes Études. Institut de Montpellier, pp. 78.

- Pizzol, J., Pintureau, B., Khoualdia, O., Desneux, N., 2010. Temperature dependent differences in biological traits between two strains of *Trichogramma cacoeciae* (Hym.: Trichogrammatidae). *Journal of Pest Science* 83, 447-452.
- Reznik, S.Y., Kats, T.S., Umarova, T.Y., Voinovich, N.D., 2002. Maternal age and endogenous variation in maternal influence on photoperiodic response in the progeny diapause in *Trichogramma embryophagum* (Hym.: Trichogrammatidae). *European Journal of Entomology* 99, 175-191.
- Reznik, S.Y., Kats, T.S., 2004. Exogenous and endogenous factors inducing diapause in *Trichogramma principium* Sug. et Sor. (Hym.: Trichogrammatidae). *Entomological Review* 84, 963-970.
- Reznik, S.Y., Vaghina, V.A., Voinovich, N.D., 2008. Diapause induction in *Trichogramma embryophagum* Htg. (Hym.: Trichogrammatidae): the dynamics of thermosensitivity. *Journal of Applied Entomology* 132, 502-509.
- Reznik, S.Y., Vaghina, V.A., Voinovich, N.D., 2010. Maternal influence on diapause induction in *Trichogramma* (Hym.: Trichogrammatidae): the dynamics of photosensitivity. *Journal of Applied Entomology* 135, 438-445.
- Reznik, S.Y., 2011. Ecological and evolutionary aspects of photothermal regulation of diapause in Trichogrammatidae. *Journal of Evolutionary Biochemistry and Physiology* 47, 512-523.
- Reznik, S.Y., Vaghina, N.P., Voinovich, N.D., 2012. Multigenerational maternal effect on diapause induction in *Trichogramma* species (Hym.: Trichogrammatidae). *Biocontrol Science and Technology* 22, 429-445.
- Rundle, B.J., Hoffmann, A.A., 2003. Overwintering of *Trichogramma funiculatum* Carver (Hym.: Trichogrammatidae) under semi-natural conditions. *Environmental Entomology* 32, 290-298.
- Sakr, H.E.A., Hassan, S.A., Zebitz, C.P.W., 2004. A new baiting device to capture and monitor the activity of the oophagous wasp *Trichogramma* in the field. *Egyptian Journal of Biological Pest Control* 14, 37-42.

- Saunders, D.S., 1982. The effects of ultra-short photoperiods on the seasonal clock in *Sarcophaga argyrostoma*. *Journal of Comparative Physiology* 145, 421-429.
- Smith, S.M., 1996. Biological control with *Trichogramma*: Advances, successes, and potential of their use. *Annual Review of Entomology* 41, 375-406.
- Sorokina, A.P., Maslennikova, V.A., 1988. Temperature optimum for diapause formation in species of *Trichogramma* (Hym.: Trichogrammatidae). *Entomologicheskoe Obozrenie* 67, 40-51.
- Tauber, M.J., Tauber, C.A., Masaki, S., 1984. Adaptations to hazardous seasonal conditions: dormancy, migration and polyphenism. In: Huffaker, C.B. (Eds.), *Ecological Entomology* Wiley, New York, USA, pp. 149-184
- Tauber, M.J., Tauber, C.A., Masaki, S., 1986. *Seasonal adaptations of insects*. Oxford University Press, New York, USA, pp. 411.
- Tauber, M.J., Tauber, C.A., Nechols, J.R., Obrycki, J.J., 1983. Seasonal activity of parasitoids: control by external, internal and genetic factors. In: Brown, V.K., Hodek, I. (Eds.), *Diapause and life cycle strategies in insects*. W. Junk, The Hague, NL, pp. 87-108.
- Tezze, A.A., Botto, E.N., 2004. Effects of cold storage on the quality of *Trichogramma nerudai* (Hym.: Trichogrammatidae). *Biological Control* 30, 11-16.
- Tian, K., Ruixiang, Y., Yongli, J., 1998. Study on the overwintering dynamics of *Trichogramma closterae* in nature. *Natural enemies of insects*. DOI: 1001-6155.0.1998-03-001 (Abstract only).
- van Lenteren, J.C., 1991. Quality control of natural enemies: hope or illusion? In: Bigler, F. (Eds.), *Proceedings, fifth workshop of the IOBC working group on quality control of mass reared arthropods, March 25-28 1991, Wageningen, NL*, pp. 1-14.
- van Lenteren, J.C., 2000. Success in biological control of arthropods by augmentation of natural enemies. In: Gurrand, G., Wratten, S. (Eds.), *Biological Control: measures of success*. Kluwer Academic Publishers, Hingham, USA, pp. 77-103.

- Voegelé, J., Pizzol, J., Babi, A., 1988. The overwintering of some *Trichogramma* species. Colloques de l'INRA 43, 275-282.
- Voegelé, J., Pizzol, J., Raynaud, B., Hawlitzky, N., 1986. La diapause chez les trichogrammes et ses avantages pour la production de masse et la lutte biologique. Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent 51, 1033-1039.
- Yong, T.H., Hoffmann, M.P., 2006. Habitat selection by the introduced biological control agent *Trichogramma ostrinae* (Hym.: Trichogrammatidae) and implications for non-target effects. Environmental Entomology 35, 725-732.
- Zaki, F.N., 1996. Overwintering of *Trichogramma evanescens* Westw. in Egypt (Hym.: Trichogrammatidae). Anzeiger für Schädlingkunde, Pflanzenschutz, Umweltschutz 69, 44-45.
- Zaslavski, V.A., Umarova, T.Y., 1990. Environmental and endogenous control of diapause in *Trichogramma* species. Entomophaga 35, 23-29.

GENERAL DISCUSSION

5. General discussion

5.1 Biological control

Biological control is the use of natural enemies to suppress the population of a specific pest organism, making it less abundant or less damaging than it would otherwise be (Eilenberg et al., 2001). The term “natural enemies” includes parasitoids, predators and pathogens. Biological control practice comprises three general approaches: importation, augmentation and conservation of natural enemies (Gordh et al., 1999).

The first major successful examples of importation of natural enemies occurred over 100 years ago and involved the control of many invasive pest species, such as: Cottony cushion scale (*Icerya purchasi* Maskell), Gypsy Moth (*Lymantria dispar* L.), Sugarcane Leafhopper (*Pyrilla perpusilla* Walk), Mediterranean flour moth (*Ephestia kuehniella*) (Greathead, 1995; van den Bosch et al., 1982; van Driesche and Bellows, 1996; van Lenteren, 2000). Importing new natural enemies from their original area of distribution and releasing them in a new environment can reduce exotic introduced pests in this area, and is also named as “classical biological control”. Compared to the “classical biological control”, augmentative release of natural enemies is the key technology in biological control programs (van Lenteren, 2000). In this approach, natural enemies are produced on a large scale and released artificially in order to colonize the crop for long term pest reduction (DeBach and Rosen, 1991). Conservation and enhancement of natural enemies, either after release for establishment or after inundative release is the most important concept in practiced biological control programs. This means: habitat management to create favourable environmental conditions for natural antagonists or crop protection practices being not harmful to beneficial (Barbosa, 1998).

5.2 Biological control using *Trichogramma* species

The often high degree of host specificity of parasitoid species makes these natural enemies first choice for classical biological control introductions. The egg parasitoids of the genus *Trichogramma* are the most important natural enemies used in these programmes (Hajek, 2004). Over 32 million ha were treated each year worldwide with *Trichogramma* species, mainly to control lepidopterous pests of corn, sugar cane, cotton, fruit and vegetables (Li, 1994) and also in stored products (Grieshop, 2005). The principal countries using trichogrammatids are the former USSR, China and Mexico with over two million ha treated every year (Li, 1994; Hoffmann et al., 2006; Mills et al., 2000).

Developing successful biological control methods requires sound knowledge of the pest biology and of the biology, ecology and behaviour of the antagonist. Basing on that knowledge, it is important to select the optimal species or strain of a species to be used against a specific pest. This research has to be done before using the antagonist, because choice of an inappropriate antagonist can result in non-efficient control (Li, 1994). In addition, indigenous species should be preferred, because they are often more effective than exotic species and adapted to the respective environmental conditions (Herz and Hassan, 2006). Laboratory studies, field and semi field studies can provide extensive information to predict the effectiveness of a parasitoid to control a pest. Using effective *Trichogramma* species in biological control programmes should follow defined selection criteria (Hassan, 1994). The major criterion includes environmental risks, tolerance of climatic extremes, host plant adaptation, host selection, host suitability, seasonal synchronization, reproductive capacity, host finding capacity, and culture methods. Selected topics of these criteria are discussed in the present studies in different chapters.

5.3 Biological characteristics of *Trichogramma* species

Important life table parameters include longevity, fecundity, and fertility, suitable to evaluate the effectiveness of a candidate parasitoid, and also to compare the efficacy of different parasitoid species or strain, exposed to different biotic and abiotic factors such as diets, temperature, and adaptability to different hosts (Maceda et al., 1994, Zhang et al., 2001; Pratisoli and Parra, 2000). In the present study, life table parameters of different species and strains of *Trichogramma* were assessed and compared under laboratory conditions. Our results indicated significant differences in life-table parameters between the four strains of *Trichogramma cacoeciae* (HOH 1, HOH 2 and SY 98 01). Longevity, fecundity, and fertility differed between the strains when alimented with either water, or honey, pollen, or combinations of pollen with water or honey (Chapter II).

Many factors may affect the biology of *Trichogramma* species. One of the most important factors is the availability and accessibility of food sources. Food could be provided from naturally occurring sources in the field, such as honeydew produced by herbivorous phloem-feeders, flower nectar and pollen, or provided as supplements either in the release container before, or applied as a foliage spray in conjunction with releases. The influence of several species of pollen as food source on biological traits of different strains of *Trichogramma* was studied. This study demonstrated that honey has positive effects on longevity, fecundity, and fertility of all *Trichogramma* strains and species studied. It may be assumed that the

carbohydrates serve as energy source resulting in a higher longevity, but does not necessarily explain a higher fertility (Romeis and Wäckers, 2002; Mevi-Schutz and Erhardt, 2005; Vrzal et al., 2010). Pollen may improve *Trichogramma* performance as well; however, differences in honey and pollen utilization as food source occur between *Trichogramma* species and pollen sources. Carbohydrates and pollen, when taken up either opportunistically or after directed search may provide a better control efficacy after augmentative release of *Trichogramma*. Furthermore honey-pollen mixtures may improve mass-rearing. A reliable interpretation of the differences in food quality may be difficult because of lacking information on the food constituents of pollen or honey and requires a skilful and thorough separate investigation. Only with this detailed knowledge, a correlation between life table parameters and food constituents will be possible. On the other hand this study revealed significant intraspecific variation in food adaptation and performance which has to be considered for selection of an appropriate strain for biocontrol or for commercial mass-rearing of a strain.

5.4 Searching behaviour

Searching behaviour and parasitization efficiency of the minute adults of *Trichogramma* was affected by several characters of the host plant (shape, size, colour and physical characteristics of the host plant surface) (Rabb and Bradley, 1968; Treacy et al., 1985, 1986; Keller, 1987; Kauffman and Kennedy, 1989; Kashyap et al., 1991).

In chapter III, the impact of leaf and fruit surfaces of three apple varieties on searching behaviour of three strains of *T. cacoeciae* was assessed. Searching behaviour of *T. cacoeciae* (foraging, host handling, and leaving the host plant after unsuccessful search) differed significantly between the strains but was not affected by the leaf surfaces of the apple cultivars tested. The differences of leaf surfaces between apple cultivars did not affect foraging time until host-egg finding, handling time or post-parasitization time until leaving the leaf by *T. cacoeciae*. Only leaving time on leaves with no stimulus (blank) or adult stimuli without host eggs was significantly affected by the factor apple cultivar. These results may be explained by the different number of trichomes and the trichome-covered area on the upper leaf side of apple leaves, where “Golden Delicious”-leaves have a relative small trichome-covered area of only 3.4 % compared to the almost doubled trichome-covered area of leaves of the cultivars “Boskoop” and “Topaz” with 7.1 and 6.3 %, respectively (Al Bitar et al., 2012).

Our results show significant differences between the *Trichogramma* strains in searching behaviour, where strain *T. cacoeciae* HOH 1 performs best among all strains studied, because they needed the shortest time to find the host. Moreover, host egg location on fruits (as shown by the parasitization rate) differed between *Trichogramma* strains, apple varieties, and position of apple fruits above the cage bottom. Also, the parasitization rates differed significantly between apple cultivars tested, however, being not consistent and, thus, should not be overestimated. It may be concluded that leaf-hairiness (number of trichomes and trichome-covered area) should be considered for *Trichogramma*-releases in apple orchards by increased number of egg parasitoids released per area and crown height to achieve a sufficient control efficacy.

5.5 Diapause and weather adaptation

Overwintering of Trichogrammatids is affected by several factors such as photoperiod, temperature and species of *Trichogramma* (Pizzol and Pintureau, 2008; Reznik et al., 2008; Zaslavski and Umarova, 1981) and adaptation to winter weather conditions may differ between *Trichogramma* species or strains of species. *Trichogramma* species from different areas varied in their ability to survive; while others overwintered successfully via quiescence, diapause or both (Voegelé et al., 2008). In Chapter IV all experiments were conducted to find out whether *Trichogramma* is able to hibernate in Hohenheim in southern Germany on eggs of *S. cerealella* as alternative host, and whether allochthonous strains from Syria or Tunisia are adapted to the environmental conditions in a new release area.

The present study proved that all *Trichogramma* strains studied can survive after inundative release on alternative hosts in the field. When successfully hibernating in parasitized host eggs, they have the potential to establish even in a new environment matching in part their preferred abiotic conditions as given in the environment they came from. However, the longevity and fecundity of the overwintering female parasitoids decreased, compared with the non-hibernated control. In addition, storage of *Trichogramma* species and strains at 4 °C for different duration confirmed their hibernation capability, although vitality and life table parameters perished with a storage duration longer than 25 days compared with the parasitoids kept under laboratory conditions. The results showed that percentage of emerged adults and the ratio of deformed adults was negatively correlated with duration of cold storage. These results are valuable information for commercial *Trichogramma*-producers to optimize their production process.

References

- Al Bitar, L., Gorb, S. N., Zebitz, C. P. W., Voigt, D., 2012. Egg adhesion of the Codling moth *Cydia pomonella* L. (Lep.: Tortricidae) to various substrates: I. Leaf surfaces of different apple cultivars. *Arthropod-Plant-Interactions* 6, 471-488.
- Barbosa, P. (Eds.), 1980. Conservation biological control. San Diego, CA: Academic Press, pp. 96-110.
- DeBach, P., Rosen, D., 1991. Biological control by natural enemies, 2nd ed. Cambridge: Cambridge University Press, pp. 440.
- Eilenberg, J., Hajek, A., Lomer, C., 2001. Suggestions for unifying the terminology in biological control. *BioControl* 46, 387-400.
- Gordh, L., G., Caltagirone, L.E., 1999. Biology of Parasitic Hymenoptera. In: T.S. Bellows jr., T.S., Fisher, T.W. (Eds.), *Handbook of Biological control: Principles and Application*. Academic Press. San Diego, CA, pp. 355-381.
- Greathead, D.J., 1995. Benefits and risks of classical biological control. In: Hokkanen, H.M.T. Lynch, J.M. (Eds.), *Biological Control: Benefits and Risks*. Cambridge University Press, Cambridge. pp. 53-63.
- Grieshop, M.J., 2005. Evaluation of three species of *Trichogramma* egg parasitoids for the biological control of the Indian meal moth in retail stores and warehouses. Ph.D. dissertation, Kansas State University, Manhattan, pp. 188.
- Hajek, E.A., 2004. *Natural enemies: An introduction to biological control*. Cambridge University Press. Cambridge, pp. 107-115.
- Herz, A. and Hassan, S.A., 2006. Are indigenous strains of *Trichogramma* sp. (Hym., Trichogrammatidae) better candidates for biological control of lepidopterous pests of the olive tree? *Biocontrol Science and Technology* 16, 841-857.
- Hoffmann, M.P., Pitcher, S.A., Cheever, S.A., Gardner, J., Losey, J.E., Kuhar, T.P., Laub, C.A., Youngman, R.R., 2006. Efficacy of inoculative releases of *Trichogramma ostrinia* (Hym.: Trichogrammatidae) against European corn borer *Ostrinia nubilalis* (Lep.: Crambidae) in field corn. *Biological Control* 36, 345-349.

- Kashyap, R.K., Kennedy, G.G., Farrar, Jr.R., 1991. Behavioural response of *Trichogramma pretiosum* Riley and *Telenomus sphingis* (Ashmead) to trichome/methyl ketone mediated resistance in tomato. *Journal of Chemical Ecology* 17, 543-556.
- Kauffman, W.C., Kennedy, G.G., 1989. Relationship between trichome density in tomato and parasitism of *Heliothis* spp. (Lepidoptera: Noctuidae) eggs by *Trichogramma* spp. (Hym.: Trichogrammatidae). *Environmental Entomology* 18, 698-704.
- Keller, M.A., 1987. Influence of leaf surfaces on movements by the hymenopterous parasitoid *Trichogramma exiguum*. *Entomologia Experimentalis et Applicata* 43, 55-59.
- Lenteren, J.C., van, 2000. Measures of success in biological control of arthropods by augmentation of natural enemies. In: Wratten, S., Gurr, G.S. (Eds.), *Measures of success in biological control*. Kluwer Academic Publishers, Dordrecht, pp. 77-103.
- Li, L.Y., 1994. Worldwide use of *Trichogramma* for biological control on different crops: a survey, In: Wajnberg, E., Hassan, S.A. (Eds.), *Biological control with egg parasitoids*, Wallingford: CAB International, pp. 37-53.
- Maceda, A., Hohmann, C.L., dos Santos, H.R., 1994. Comparative life table for *Trichogramma pretiosum* Riley and *Trichogramma annulata* De Santis (Hym.: Trichogrammatidae). *Revista do setor de Ciências Agrárias* 13, 279-281.
- Mevi-Schutz, J., Erhardt, A., 2005. Amino acids in nectar enhance butterfly fecundity: a long-awaited link. *American Naturalist* 165, 411-419.
- Mills, N., Pickel, C., Mansfield, S., McDougall, S., Buchner, R., Caprile, J., Edstrom, J., Elkins, R., Hasey, J., Kelley, K., Krueger, B., Olson, B., Stocker, R., 2000. Mass releases of *Trichogramma* wasps can reduce damage from Codling moth. *California Agriculture* 54, 22-25.
- Pizzol, J., Pintureau, B., 2008. Effect of photoperiod experienced by parents on diapause induction in *Trichogramma cacoeciae*. *Entomologia Experimentalis et Applicata* 127, 72-77.
- Rabb, R. L., Bradley, R.J., 1968. The influence of host plants on parasitism of the eggs of the tobacco budworm. *Journal of Economic Entomology* 61, 1249-1252.

- Reznik, S.Ya., Vaghina, N.P., Voinovich, N.D., 2008., Diapause induction in *Trichogramma embryophagum* Htg. (Hym.: Trichogrammatidae): the dynamics of thermosensitivity. Journal of Applied Entomology 132, 502-509.
- Romeis, J., Wäckers, F., 2002. Nutritional suitability of individual carbohydrates and amino acids for *Pieris brassicae*. Physiological Entomology 27, 148-156.
- Treacy, M. F., Benedict, J. H., Segers, J. C., Morrison, R. K., Lopez, D.J., 1986. Role of cotton trichome density in bollworm (Lep.: Noctuidae) egg parasitism. Environmental Entomology 15, 365-368.
- Treacy, M.F., Zummo, G.R., Benedict, R.H., 1985. Interactions of host-plant resistance in cotton with predators and parasites. Agriculture, Ecosystems and Environment 13, 151-157.
- van den Bosch, R., Messenger, P. S., Gutierrez, A.P., 1982. An introduction to biological control. Plenum Press, New York and London, pp. 247.
- van Driesche, R.G., Bellows, T.S., 1996. Biological control. Chapman and Hall, New York. pp. 3-20.
- Voegelé, J., Pizzol, J., Babi, A., 1988. The overwintering of some *Trichogramma* species. In Voegelé, J., Waage, J., van Lenteren, J. (Eds.), *Trichogramma* and other egg parasites. Les Colloques de l'Institut National de la Recherche Agronomique, No. 43, pp. 275-282.
- Vrzal, E.M., Allan, S.A., Hahn, D.A., 2010. Amino acids in nectar enhance longevity of female *Culex quinquefasciatus* mosquitoes. Journal of Insect Physiology 56, 1659-1664
- Zaslavskii, V.A., Umarova, T.Ya., 1981. Photoperiodic and temperature control of diapause in *Trichogramma evanescens* Westw. (Hym.: Trichogrammatidae). Entomologicheskoe Obozrenie 60, 721-731.
- Zhang, W.Q., Agamy, E., Hassan, S.A., 2001. Life table characteristics of four candidate species of the genus *Trichogramma* to control the diamond back moth *Plutella xylostella*. Journal of Plant Diseases and Protection 108, 413-418.

Summary/ Zusammenfassung

Summary

The biology and the use of egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) as a biological control agent has been studied since 1904 and more than one thousand scientific papers on *Trichogramma* have been published. However, there are still open questions about the impact of biotic and abiotic environmental factors on the biology of these beneficials, such as nutritional ecology, diapause, cold storage, plant-pest-*Trichogramma*-interactions, and, particularly, the intraspecific variation of environmental adaptations. These parameters can seriously affect the effectiveness and the practical application of these parasitoids in many crop production systems. In this study, we have investigated the effect of: (i) different of diets, (ii) properties of pest's host-plant surfaces and (iii) overwintering and cold storage on the performance of different species and strains of *Trichogramma* to select the best candidate for biocontrol of the Codling moth, taken here as a model target.

Experiments with different diets (honey, pollen and honey with pollen) were carried out and its effects on life-table parameters of four strains of *Trichogramma cacoeciae* Marchal (HOH 1, HOH 2, KIO 2 TN and SY 98 01), and one strain of *T. evanescens* Westwood (195 DE 06) and *T. oleae* Voegelé (See 2) was evaluated under laboratory conditions. Honey diet improved significantly adult mean longevity, mean fecundity, mean fertility, and net reproduction rate (R_0). Significant intraspecific variability was shown only in mean fecundity and mean fertility, but not in longevity. The best performing strain of *T. cacoeciae* (HOH 1) in these experiments has been taken to assess the nutritional value of pollen collected from birch, maize and sunflower. Pollen as honey additive had a significant positive effect on female longevity as compared to honey alone, but longevity did not differ significantly between the honey-pollen diets. Pollen added to water had no ameliorative effect on longevity except sunflower pollen, which had a significant detrimental effect. Mean total fecundity and fertility of females was not improved when fed honey and birch or maize pollen. Sunflower pollen added to honey, however, had a pronounced detrimental effect on these parameters. When these three pollens were offered with water, all parameters were not or negatively affected, revealing sunflower pollen having the worst nutritional value. Tests on the suitability of bee-pollen to ameliorate *Trichogramma* mass-rearing were done with *T. cacoeciae*, *T. evanescens* and *T. oleae*. Except fertility, longevity and fecundity differed significantly between the species. Basic diet (water or honey) and pollen added showed interactive effects on the performance of *Trichogramma* species. Interactions between species and added pollen only revealed significant differences in fecundity and fertility, whereas longevity was only slightly affected. Species -

basic diet - pollen interactions could only be shown for fertility. *Trichogramma oleae* had a shorter adult lifespan and the lowest number of parasitized eggs compared with the other two species, but it had the highest rate of emergence when fed on honey mixed with bee pollen. The results indicate that (i) *Trichogramma* adults are feeding upon pollen, and (ii) nutritional quality of pollen differs significantly between plant species, and (iii) honeybee-pollen may be used to improve mass-rearing of *Trichogramma* species.

Properties of the pest's host-plant surfaces (apple, cv. "Golden Delicious", "Boskoop" and "Topaz") were evaluated in their effect upon searching behaviour and foraging success of three strains of *T. cacoeciae* (HOH 1, KIO 2 TN and SY 98 01) when eggs of the Codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae) were offered as hosts. Foraging time differed significantly between the strains tested, showing strain HOH 1 performing best. Host handling time and time spent post-parasitization did not differ between the strains. The apple cultivar did not affect any of these parameters. Leaving time was significantly affected by chemical cues comprising lepidopteran egg and scale volatiles or contact chemicals, host pheromones, host frass or female moth accessory gland secretions abundant on the leaves, where giving up time was shortest on blank leaves and, compared to blank leaves, significantly longer on leaves with adult stimuli only but no host eggs. Leaving time was further affected by apple cultivar and *T. cacoeciae* strain tested. Compared with the other cv. tested, time spent on leaves of cv. "Golden Delicious" was longest for any treatment. *Trichogramma cacoeciae* strain HOH 1 always spent the shortest on any cultivar and treatment. In another experiment, parasitization of eggs on apples placed at different heights (0, 25, and 50 cm above cage ground) was assessed. The parasitization rate of *Cydia* eggs was slightly affected by apple varieties; however the differences were not consistent. Parasitization rates decreased with height of apples above ground, with the highest rate on apples placed on ground, near the releasing point of *Trichogramma* females. Significant differences in parasitization rates between strains could be detected, but they were not consistent also.

One German and one Tunisian strain of *Trichogramma oleae* and two German, one Tunisian, and one Syrian strain of *T. cacoeciae* were hibernated from October 10th 2007, up to parasitoid emergence in 2008 under field conditions in Stuttgart-Hohenheim, Germany. 60 days after exposure to outdoor conditions and after complete hibernation life table parameters of survivors was assessed. Our results demonstrate that all strains studied were able to overwinter successfully on eggs of the factitious host *Sitotroga cerealella* Olivier (Lep.: Gelechiidae). Mean development time of trichogrammatids until adult emergence after full hibernation ranged between 183 and 189 days. Emergence rate ranged between 53.59 and 60.98 %. Net

reproduction rate (R_0) of *T. oleae* strains decreased with duration of field exposure, whereas in *T. cacoeciae* strains R_0 was lowest after 60 days field exposure. Longevity of all adults emerged from parasitized eggs was affected by field exposure compared to the control. Total fecundity, total fertility and percent fertility after 60 days and/or after hibernation showed neither significant difference between strains nor within the strains studied. Total fertility of *T. cacoeciae* strains decreased significantly after midterm and/or after full hibernation compared with the control, while no significant differences were detected within *T. oleae* strains. Time until adult emergence, mortality, and percentage of deformed adults increased with storage duration at 4 °C. *Trichogramma oleae* (German strain) showed best adaptation to cold storage for 50 days.

Finally and based on this study, our data suggest that the diets of honeybee-pollen could be used as food to improve *Trichogramma* mass-rearing, and also the German candidate *T. cacoeciae* HOH 1 could be a potential candidate for future mass rearing and field release programs for biocontrol of different lepidopteran pests in orchards. Furthermore, it could be proved that even allochthonous *Trichogramma* species / strains originated from countries with a higher annual average temperature (Syria and Tunisia) are theoretically able to establish and survive winter conditions in southwestern Germany. At least this possibility requires an environmental risk-analysis for these species/strains or any potential other *Trichogramma* species intended to be used as biocontrol agent in Germany.

Zusammenfassung

Die Biologie und der Einsatz von Eiparasitoiden der Gattung *Trichogramma* (Hymenoptera: Trichogrammatidae) als Mittel zur biologischen Kontrolle werden seit 1904 untersucht, und mehr als tausend wissenschaftliche Arbeiten über diese Gattung wurden bereits veröffentlicht. Es sind jedoch noch immer Fragen offen über die Auswirkungen von biotischen und abiotischen Umweltfaktoren auf die Biologie dieser Nützlinge, wie Ernährungsökologie, Diapause, Kaltlagerung, Pflanzen-Schädlings-Trichogramma-Interaktionen, und besonders auf intraspezifische Variation von Umwelthanpassungen. Diese Faktoren können die Wirksamkeit und die praktische Anwendung dieser Parasitoide in vielen Pflanzenbau-Systemen erheblich beeinflussen.

In dieser Studie haben wir die Wirkung von: (i) unterschiedlichen Diäten, (ii) Oberflächeneigenschaften von Schädlingswirtspflanzen und (iii) Überwinterung und Kaltlagerung auf die Leistung der verschiedenen Arten und Stämme von *Trichogramma* untersucht, um den besten Kandidaten für die biologische Bekämpfung des Apfelwicklers, der hier als Modell-Ziel benutzt wird, auszuwählen. Es wurden Versuche mit verschiedenen Nahrungszusammensetzungen (Honig, Pollen sowie Honig und Pollen) bei vier Stämmen von *Trichogramma cacoeciae* Marchal (HOH 1, HOH 2, KIO 2, TN und SY 98 01), und jeweils einem Stamm von *T. evanescens* Westwood (195 DE 06) und *T. oleae* Voegelé (See 2) unter Laborbedingungen durchgeführt und die Auswirkungen auf Lebensstafelparameter erfaßt.

Ernährung mit Honig verbesserte signifikant die Durchschnittswerte für Lebensdauer, Fekundität, Fertilität und die Nettoerproduktionsrate (R_0) von Adulten. Signifikante intraspezifische Variabilität zeigten nur die Durchschnittswerte für Fekundität und Fertilität, aber nicht die für Lebensdauer. Der leistungsfähigste Stamm von *T. cacoeciae* (HOH 1) aus diesem Experiment wurde verwendet, um den Nährwert gesammelten Pollens von Birke, Mais und Sonnenblume zu bewerten. Dem Honig zugesetzter Pollen hatte signifikant positive Auswirkungen auf die Lebensdauer der Weibchen, verglichen mit Honig allein, aber die Unterschiede in der Lebensdauer zwischen den Honig-Pollen-Diäten waren nicht signifikant. In Wasser aufgerührter Pollen hatte keinen verlängernden Effekt auf die Lebensdauer, und Sonnenblumenpollen wies sogar einen erheblichen schädlichen Effekt auf. Die durchschnittliche Fekundität und Fertilität von Weibchen wurde durch Fütterung mit Honig und Birken- oder Maispollen nicht verbessert. Dem Honig zugegebener Sonnenblumenpollen hatte einen ausgesprochen schädlichen Effekt auf diese Parameter. Wurden diese drei Pollen in Wasser

angeboten, beeinflusste dies alle Parameter nicht oder negativ, was verdeutlichte, dass Sonnenblumenpollen den schlechtesten Nährwert aufweisen.

Versuche zur Eignung von Bienen-Pollen für die Verbesserung von *Trichogramma*-Massenaufzuchten wurden mit *T. cacoeciae*, *T. evanescens* und *T. oleae* durchgeführt. Im Gegensatz zur Fertilität unterschieden sich Lebensdauer und Fekundität signifikant zwischen den Arten. Die Ernährungsgrundlage (Wasser oder Honig) und zugefügter Pollen zeigten voneinander abhängige Auswirkungen auf die Leistung der *Trichogramma* Stämme. Wechselwirkungen zwischen den *Trichogramma*-Arten und der zugefügten Pollenart wiesen lediglich bei Fekundität und Fertilität signifikante Unterschiede auf, während die Lebensdauer nur leicht beeinflusst wurde. Wechselwirkungen zwischen Stämmen, Ernährungsgrundlage und Pollenart konnten nur für Fertilität nachgewiesen werden. *Trichogramma oleae* hatte, verglichen mit den zwei anderen Stämmen, eine kürzere Lebensdauer der Adulten und die niedrigste Anzahl parasitierter Eier, aber die höchste Schlupfrate, wenn mit einer Mischung aus Honig und Bienenpollen gefüttert wurde. Die Ergebnisse weisen darauf hin, dass (i) adulte *Trichogramma* Pollen fressen, (ii) sich die Ernährungsqualität von Pollen zwischen den Pflanzenarten erheblich unterscheidet, und (iii) Honigbienen-Pollen verwendet werden könnte, um die *Trichogramma* Massenzucht zu verbessern.

Die Eigenschaften von Blatt- und Fruchtoberflächen des Apfels (cv. "Golden Delicious", "Boskoop" und "Topaz"), auf denen sich als Wirt angebotene Eier des Apfelwicklers, *Cydia pomonella* (Lepidoptera: Tortricidae) befanden, wurden bezüglich ihrer Auswirkungen auf Suchverhalten und -erfolg bei drei Stämmen von *T. cacoeciae* (HOH 1, KIO 2 TN und SY 98 01) untersucht. Die Zeit zur Nahrungssuche unterschied sich erheblich zwischen ihnen, wobei der Stamm HOH 1 die beste Leistung zeigte. Die auf das einzelne Wirtsei verwendete Zeit („host handling“ und „post parasitization“) variierte nicht zwischen den Stämmen. Auch die Apfelsorte hatte darauf keinen Einfluß. Die Zeit zum Verlassen des Blattes („leaving time“) wurde erheblich von chemischen Reizen beeinflusst, welche aus von den Schmetterlingseiern und -schuppen ausgehenden Duftstoffen oder Kontaktsubstanzen, Wirtspheomonen, Wirtskot, oder den auf den Blättern reichlich vorhandenen Drüsensekreten der weiblichen Motten bestehen. Die Zeit bis zum Abbruch der Suche („giving up“) war am kürzesten auf leeren Blättern, aber signifikant länger auf zwar leeren, aber mit den beschriebenen Reizen versehenen. Die „leaving time“ wurde außerdem auch durch die Apfelsorte und den getesteten Stamm von *T. cacoeciae* beeinflusst.

Verglichen mit den anderen getesteten Apfelsorten war auf den Blättern der Sorte „Golden Delicious“ die verbrachte Zeit in allen Varianten am längsten. *Trichogramma cacoeciae* HOH 1 verbrachte bei jeder Behandlungsvariante die kürzeste Zeit auf allen Apfelsorten.

In einem anderen Experiment wurde die Parasitierung der Eier auf in unterschiedlichen Höhen (direkt auf, sowie 25 und 50 cm über dem Käfigboden) platzierten Äpfeln erfaßt. Die Unterschiede in der Parasitierungsrate der *Cydia*- Eier zwischen den Apfelsorten waren dabei gering und nicht eindeutig. Sie nahm mit zunehmender Höhe der Äpfel über dem Boden ab und war am höchsten bei Äpfeln, die auf dem Boden lagen, also nahe an dem Punkt, wo die *Trichogramma*-Weibchen ausgesetzt wurden. Zwischen den Stämmen waren deutliche Unterschiede in der Parasitierungsrate erkennbar, aber auch sie waren nicht eindeutig.

Ein deutscher und ein tunesischer Stamm von *Trichogramma oleae* sowie zwei deutsche, ein tunesischer und ein syrischer Stamm von *T. cacoeciae* wurden ab 10. Oktober 2007 in parasitierten Eiern im Freiland (Stuttgart-Hohenheim, Deutschland) überwintert bis 2008 die ersten Parasitoide schlüpften. Nach 60 Tagen unter Freilandbedingungen sowie nach der gesamten Überwinterung, wurden jeweils die Lebensstafelparameter der geschlüpften Tiere erhoben. Unsere Ergebnisse zeigen, dass alle untersuchten Stämme in der Lage waren, erfolgreich in Eiern des Ersatzwirts *Sitotroga cerealella* Olivier (Lep., Gelechiidae) zu überwintern. Die durchschnittliche Entwicklungszeit der Trichogrammatiden bis zum Schlupf der Adulten nach vollständiger Überwinterung lag zwischen 183 und 189 Tagen. Die Schlupfrate lag zwischen 53,59 und 60,98 %. Die Nettoerproduktionsrate (R_0) der *T. oleae* Stämme nahm mit der Dauer der Exposition ab, wobei in *T.cacoeciae*-Stämmen nach 60 Tagen unter Freilandbedingungen R_0 am niedrigsten war.

Die Lebensdauer der aus unter Freilandbedingungen überwinterten Puppen geschlüpften Adulten unterschied sich im Vergleich zur Kontrolle. Die Gesamtfekundität und fertilität sowie relative Fertilität nach 60 Tagen beziehungsweise nach der Überwinterung zeigten weder zwischen den Arten noch zwischen den Stämmen Unterschiede. Die Gesamtfertilität der *T.cacoeciae*-Stämme nahm im Vergleich mit der Kontrolle ab, während zwischen den Stämmen von *T.oleae* keine signifikanten Unterschiede erkennbar waren. Die Zeit bis zum Schlupf der Adulten, die Mortalität und der Anteil deformierter Adulter nahm mit der Lagerdauer bei 4 °C zu. *Trichogramma oleae* deutscher Herkunft zeigte die beste Anpassung an Kaltlagerung für 50 Tage.

Abschliessend, und basierend auf dieser Studie, deuten unsere Daten darauf hin, dass Diäten mit Honigbienen-Pollen als Futter verwendet werden können, um die Massenzucht von *Trichogramma* zu verbessern. Der deutsche Stamm von *T.cacoeciae*, HOH 1, könnte ein potentieller Kandidat für zukünftige Massenzüchtungen und Feldfreilassungsprogramme im Biologischen Pflanzenschutz gegen verschiedene Schadlepidopteren in Obstanlagen sein. Weiterhin konnte belegt werden, dass selbst allochthone *Trichogramma*-Arten und -Stämme aus Ländern mit höheren jährlichen Durchschnittstemperaturen (Syrien und Tunesien) theoretisch in der Lage sind, sich zu etablieren und die winterlichen Bedingungen in Südwestdeutschland zu überleben. Zumindest macht diese Fähigkeit eine Analyse des Umweltrisikos für diese oder alle anderen *Trichogramma*-Arten und -stämme erforderlich, die zukünftig als Mittel des Biologischen Pflanzenschutzes in Deutschland eingesetzt werden sollen.

APPENDIX

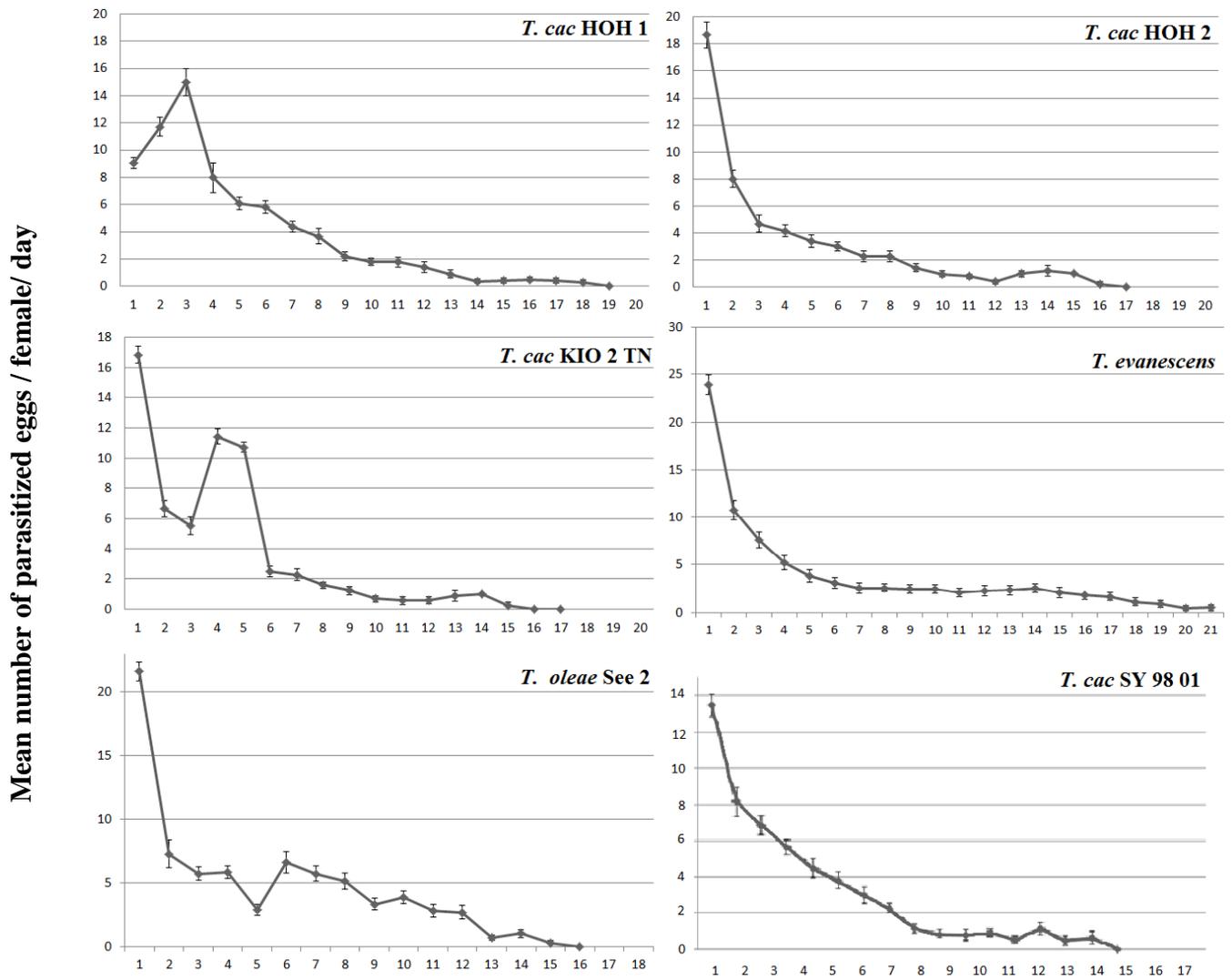


Figure 1 Longevity and fecundity of different species of *Trichogramma* fed with honey alone.

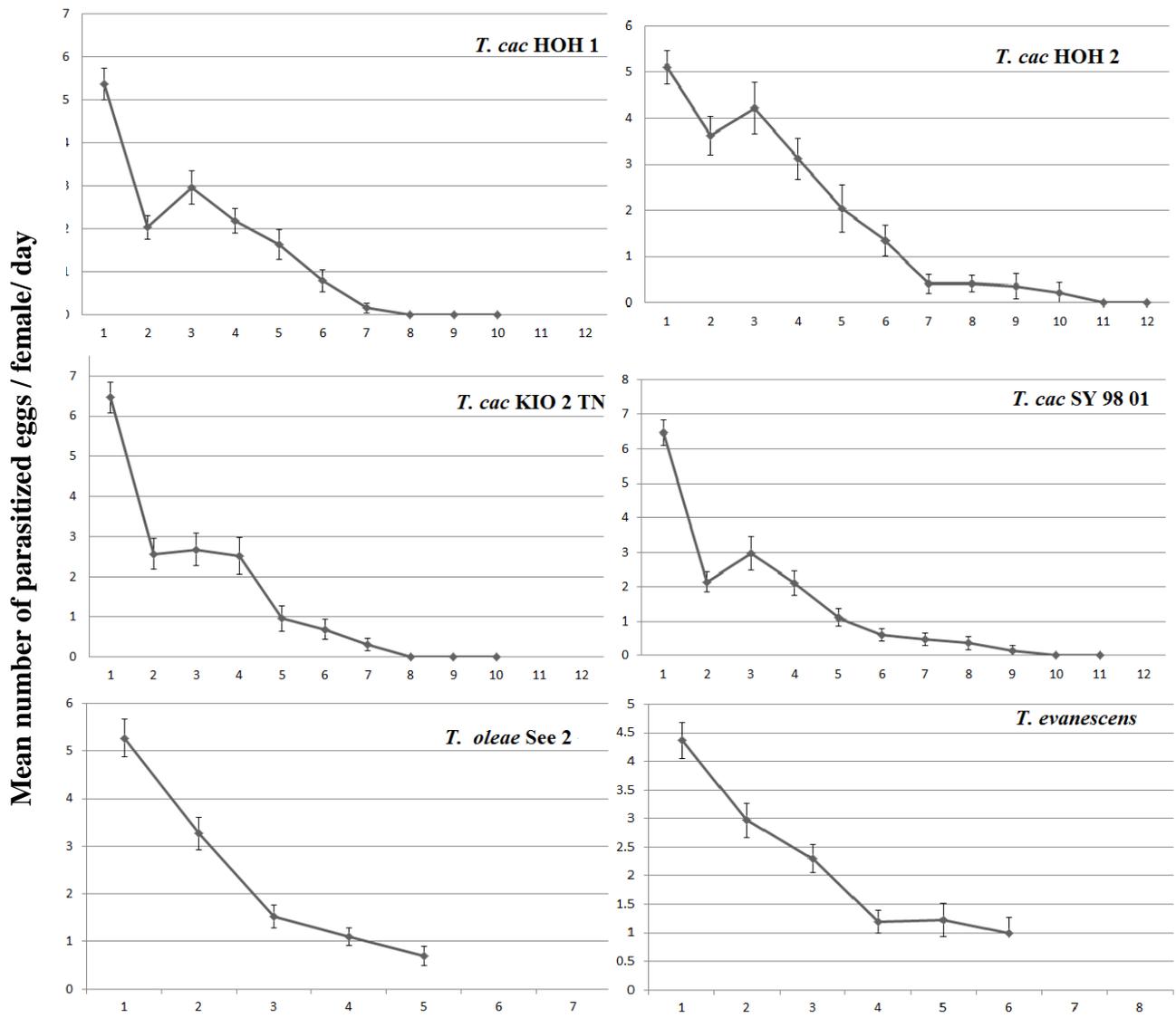


Figure 2 Longevity and fecundity of different species of *Trichogramma* supplied with water alone.

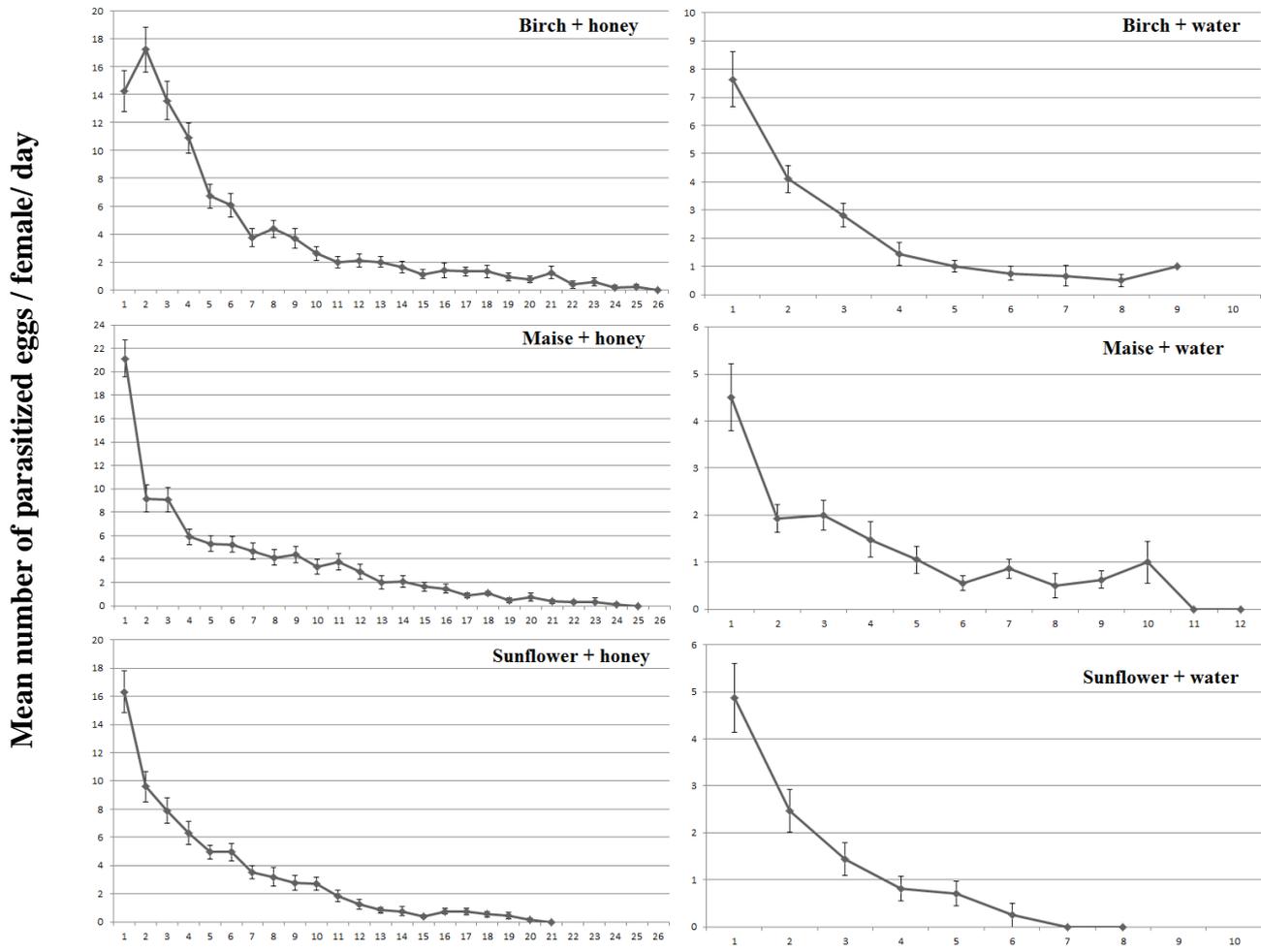


Figure 3 Longevity and fecundity of *T. cacaoeciae* HOH 1 fed with different sorts of pollen mixed with honey and/ or with water. .

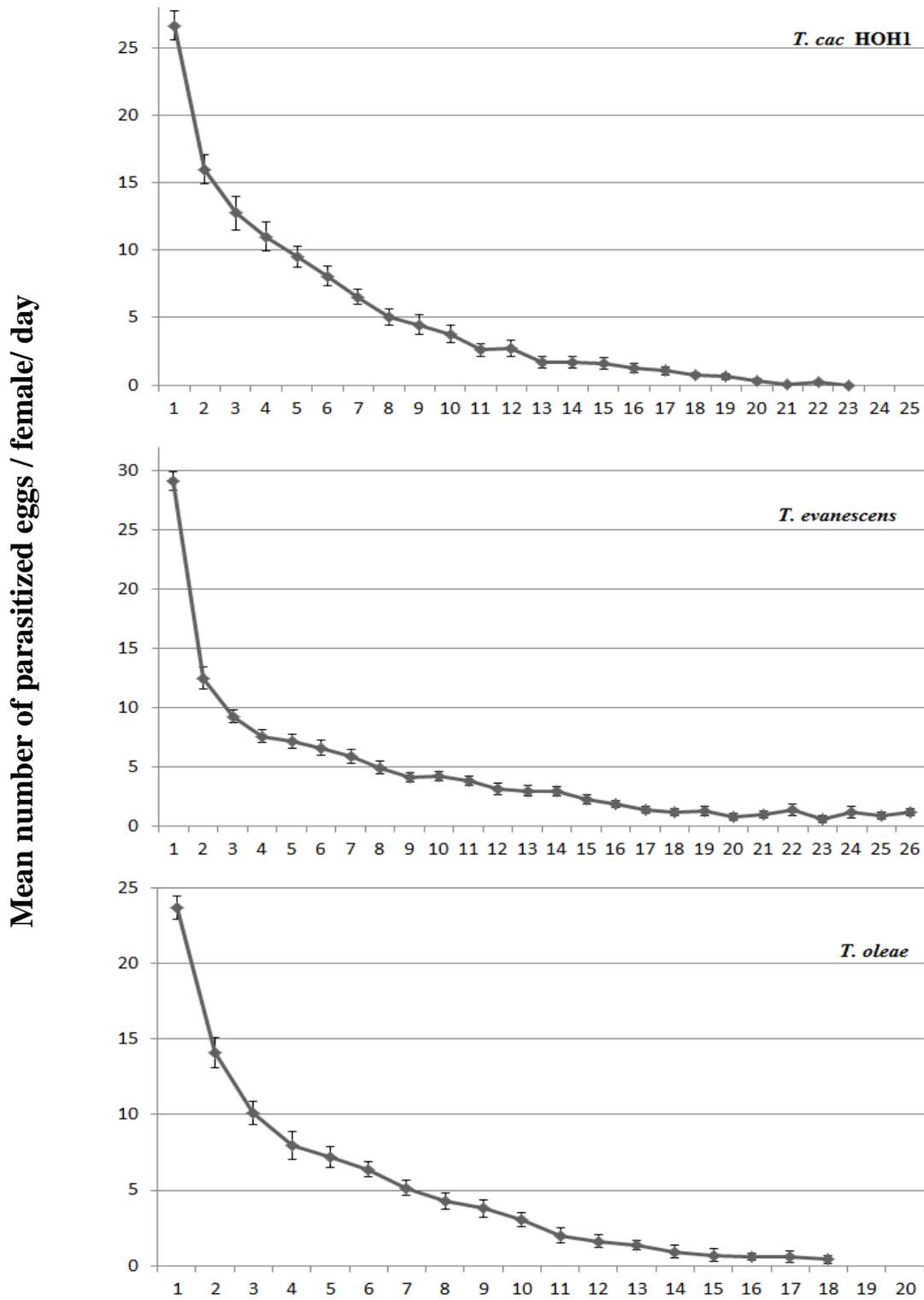


Figure 4 Longevity and fecundity of different species of *Trichogramma* fed with honey mixed with honeybee pollen.



Figure 5 Three varieties of pollen used as alternative food with honey and with water for *Trichogramma cacoeciae* females.

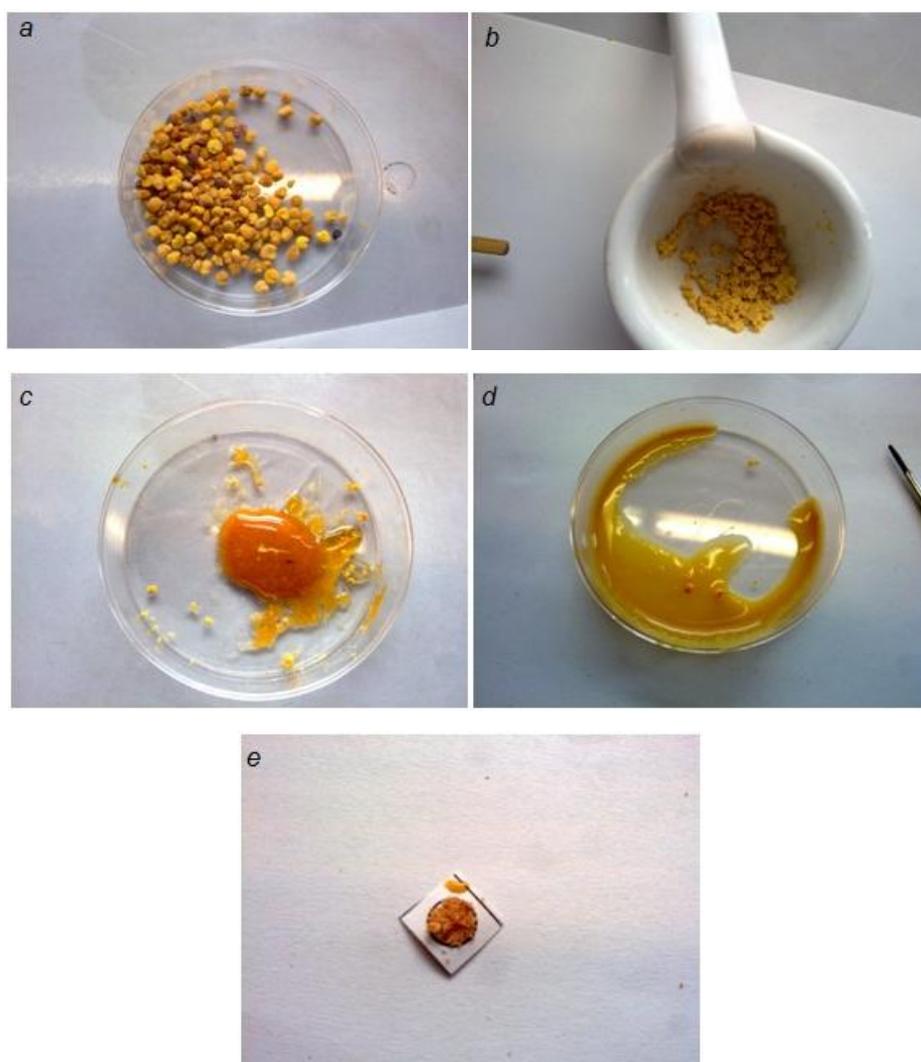


Figure 6 Bee pollen photos (a: Bee pollen, b: pollen milling, c: pollen powder mixed with honey, d: pollen powder mixed with water and e: *Sitotroga cerealella* eggs cards with dropped of pollen diet.