Combatting whiteflies: predatory mites as a novel weapon

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INTRODUCTION AND OUTLINE

Biological control is a crop protection method that aims at the reduction of pests below the level of the economic threshold by utilizing their natural enemies. This method poses low environmental risks and it is relatively safe for growers and consumers, because chemical pesticides are not used in crops. Biological control has a long history of success (van Driesche and Bellows 1996; Albajes et al. 1999) and it employs introductions of native and/or exotic natural enemies (van Lenteren and Manzaroli 1999).

While selecting among natural enemies for biological control agents of pests, a wide range of criteria has been considered (McMurtry 1982; van Lenteren and Manzaroli 1999). All criteria can be translated in measurable traits of natural enemies and aim at recognizing efficiency of natural enemies in controlling pest populations. Due to high numbers of natural enemies and to limited time and expenses for research, only a few traits of the candidate species has quantified in lab experiments. Based on comparisons of these traits, candidate natural enemies are selected for biological control agents. The advantage of this approach lies in the general use of a short list of criteria for all systems, but there is the danger that the most desirable traits differ among systems. Moreover, results from lab experiments should be cautiously extrapolated to larger scales, as natural enemies in the lab are usually restricted on experimental arenas where they cannot display the full range of their behaviour (Kareiva 1989).

The design of successful biological control programs can only emerge from knowledge on the ecological interactions of system (Waage 1989). In this way, traits of natural enemies that are key-factors in prey suppression under crop conditions can be recognized and manipulated as to have to desirable results at levels below the economic threshold. Both theoretical and experimental studies of the system can contribute to identify properties of the system that result in prey elimination or suppression (Murdoch et al. 1985). Hence, for different agricultural systems different solutions can be proposed concerning the types of natural enemies as well as the quantities and timing of their release. An advantage in performing such ecological exercises resides in the value of the information obtained. The knowledge that is gained on how ecological interactions operate, apart from recognizing specific conditions that yield successful control, is useful in applying the biological control under different conditions but also in understanding other systems.

1It has recently been claimed that some species of predatory mites that are used for biological control are allergenic (van Hage-Hamsten 2000; Groenewoud 2002)
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In the present thesis, I follow this approach by studying the ecological interactions between phytoseiid predators and the silverleaf whitefly, a pest that can as yet not be controlled sufficiently by natural enemies.

A NEW GREENHOUSE PEST: THE SILVERLEAF WHITEFLY

Outbreaks of *Bemisia tabaci* (Gennadius), occur worldwide, resulting in yield loss and economic injury in many crop species (Gerling and Mayer 1996; Oliveira *et al.* 2001). Research on the systematics of *B. tabaci* with a variety of morphological, behaviour and molecular tools showed that there are seven different groups within this species, supporting the hypothesis that *B. tabaci* is a species complex (Perring 2001). The recent geographic expansion of *B. tabaci* has been closely associated with a new and more virulent biotype B (Naranjo and Ellsworth 2001), also described as *Bemisia argentifolii*, and commonly known as silverleaf whitefly. It was accidentally imported into Europe where it infested several greenhouse and outdoor crops such as poinsettia, begonia, bouvardia, gerbera, hibiscus, tomato, pepper and melon (Enkegaard 1990; Baker and Cheek 1993; Arnó and Gaborra 1994; Cebrián *et al.* 1994). It first occurred in greenhouses in The Netherlands on poinsettia in 1987 (Fransen 1994).

*Bemisia tabaci* causes damage to crops directly due to its feeding and indirectly due to the production of honeydew, a sticky sugar-rich excretion on which black mould develops. As a result of this, the photosynthesis of the plant as well as the commercial value of fruits and plants are reduced. The biotype B of *B. tabaci* also causes phytotoxic disorder on several crops (Brown *et al.* 1995). Moreover, *B. tabaci* is the most important vector of plant-pathogenic viruses among whitefly species (Duffus 1987, 1996). The invasion of new genotypes in the New World resulted in a pandemic of these viruses (Brown *et al.* 1995). The economic injury level for virus infestation is low. As some viruses cannot be controlled directly and breeding for virus resistance is not yet sufficiently advanced, preventing virus infections relies mainly in the control of its vector (Berlinger *et al.* 1990).

Due to its polyphagy (Greathead 1986) and its high level of resistance to organophosphates, pyrethroids and cyclodiene insecticides as well as to insect growth regulators (Palumbo *et al.* 2001), *B. tabaci* poses a particular threat to growers. Most attempts of controlling *B. tabaci* with parasitoids, insect predators or fungi did not yield satisfactory results (see reviews of Gerling *et al.* 2001 and of Faria and Wright 2001). This prompted a quest for natural enemies of *B. tabaci* with special emphasis on parasitoids and insect predators (van Lenteren and Martin 1999; De Barro *et al.* 2000; Drost *et al.* 2000; Castañé *et al.* 2001; Hoelmer *et al.* 2001; Oliveira *et al.* 2001).
PREDATORY MITES AS BIOLOGICAL CONTROL AGENTS

Predatory mites (family Phytoseiidae) are small wingless arthropods that are currently used in protected and outdoor crops as biological control agents against herbivorous mites such as spider mites, but also against insect pests such as thrips. *Neoseiulus cucumeris* (Oudemans), *Neoseiulus barkeri* (Hughes) and *Iphiseius degenerans* (Berlese) are predatory mites used for controlling the thrips *Frankliniella occidentalis* (Pergande) and *Thrips tabaci* Lind. (Thysanoptera). They can effectively attack only the first and early second instar thrips larvae (Bakker and Sabelis 1989; van der Hoeven and van Rijn 1990). These predatory mites have smaller size than thrips, when comparing adults, and shorter life cycle than their thrips prey (Sabelis and van Rijn 1997). The predation and oviposition rates of phytoseiids on thrips prey are relatively low (van Houten *et al.* 1995). However, in presence of supplementary food such as pollen, the population growth of phytoseiids is enhanced and consequently the population of thrips and the related levels of plant damage are reduced (van Rijn *et al.* 2002).

The predatory mite-thrips system provides a landmark for the research reported in this thesis. Thrips and whiteflies show several similarities: the adults of both species are larger than the adult predatory mites, the generation time of both pests is longer than that of phytoseiids, and both pest species have stages that are invulnerable to predators (Figure 1; Byrne and Belows 1991; Sabelis and van Rijn 1997; Tsai and Wang 1996). The successful biological control of thrips by various species of predatory mites, as well as the reported co-occurrence and predation of several phytoseiid species with whiteflies inspired me to investigate the possibility of using predatory mites as biological control agents of whiteflies.

PREDATORY MITES AS WHITEFLY PREDATORS

The predatory mite species used currently against other greenhouse pests [*Phytoseiulus persimilis* (Athias-Henriot), *Neoseiulus californicus* (McGregor), *N. cucumeris* and *I. degenerans*], were the first candidates that I studied. Laboratory experiments with these species on whitefly prey showed no or low predation and oviposition on *B. tabaci* (Nomikou, unpublished data). Hence, exploration and collection of other phytoseiid whitefly predators was necessary. Several predatory mite species have been found in association with whiteflies mainly in the Middle East and in India. These species have been recorded to feed, develop and oviposit on whiteflies but they have never been evaluated or used in biological control programmes for *B. tabaci* (Chapter 1, Table 1).
The aim of this thesis is the collection of phytoseiid whitefly predators and their study and evaluation as biological control agents for *B. tabaci* in greenhouses. For this evaluation, I considered a set of criteria, rather than single-best-traitss of a natural enemy, taking into account the different spatial scales at which the natural enemy and the pest interact (Waage 1989). I chose to study the predatory mite-whitefly system on cucumber because this plant species is a good host for *B. tabaci*. Moreover, most other cucumber pests such as spider mites, thrips, aphids, etc. can be controlled biologically; hence, biological control of *B. tabaci* will not be hampered by the use of pesticides against other pests than whiteflies. Biological control is widely applied as a crop protection method on other crops such as tomato, sweet pepper, strawberry, gerbera, poinsettia, rose, etc. and thus phytoseiids could be useful for controlling whiteflies in these crops as well. Hence, results from this research can lead to biological control of whiteflies in other crops as well.
OUTLINE OF THIS THESIS AND SUMMARY OF RESULTS

This thesis is divided into three parts, corresponding to the different spatial scales at which the research was carried out:

Part 1 describes laboratory experiments with predators that were collected from the field and established in cultures. Predator traits, such as oviposition, juvenile survival, developmental rate and sex ratio are measured on whitefly prey and on non-prey diet e.g. pollen, whitefly honeydew and associated microbes, and on plant tissue alone.

In Part 2, I scale up from the individual to the population level on single cucumber plants and I test whether predators are able to control whitefly populations. Further, I investigate the effect of pollen, an alternative food for the predators, on the dynamics of the predatory mite-whitefly populations.

Part 3 describes the between-plant mobility of predators and prey. Here, I study the searching efficiency of predators and the foraging decisions of adult whiteflies in a group of plants in a greenhouse and I conclude with a discussion on the spatial predator-prey dynamics at a metapopulation level.

Part 1: Screening predator traits in the lab

After considering literature reports, the Middle-East area was chosen for exploration of phytoseiid-whitefly associations and collection of predatory mites (Chapter 1). I selected Israel and Jordan as collection sites because B. tabaci is a major agricultural pest in field crops in these countries. Cultures of five collected predatory mite species were established in the laboratory (UvA, The Netherlands) and for most species, I followed the method on plastic arenas. Only Euseius scutalis (Athias-Henriot) could not be cultured using this method, but had to be reared on detached leaves. Both methods are described by Overmeer (1985).

To exclude species that co-occurred in the field with whiteflies for reasons other than predation, I screened predator oviposition on a diet of whiteflies (Chapter 1). Out of the 5 species tested, only Phytoseius finitimus Ribaga was not able to reproduce on a diet of whitefly prey and it was excluded from further research. The population growth rates of the other 4 predatory mite species were evaluated on B. tabaci by estimating life-history traits such as juvenile survival, oviposition rate and sex ratio. These characteristics were measured with ample prey supply, thus representing the potential numerical response of predators on B. tabaci. Out of the 4 species tested, N. barkeri showed high juvenile mortality and Typhlodromus athiasae (Porath and Swirski) had the lowest growth rate, therefore, both species were excluded from further research. Typhlodromips swirskii (Athias-Henriot) and E. scutalis showed high juvenile survival and oviposition on whitefly prey.
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Since most phytoseiids are polyphagous predators, I studied whether pollen, a plant-produced food, could be used as alternative food for predators. Pilot experiments showed that all predators were able to reproduce and develop on a diet of bean pollen (Nomikou, pers. obs.) and it was used as food for the cultures. Oviposition of predators on broad bean pollen was measured in the lab. *Euseius scutalis* showed a higher oviposition rate on pollen than on whiteflies. From the two strains of *T. swirskii* that I collected, one showed similar oviposition rates while the other showed higher oviposition rate on pollen that on whiteflies.

In Chapter 2, I continued the evaluation with *T. swirskii* and *E. scutalis*, the two species that performed best in the previous laboratory experiments. I measured their predation rate on each of the various immature whitefly stages (Figure 1). Both phytoseiid species fed mainly on whitefly eggs and crawlers and hardly killed later whitefly immature stages. I also measured oviposition rates in the same experiments to assess the consequences of differential prey vulnerability for the reproduction of predatory mites. The percentage of predators ovipositing on a diet of less vulnerable prey stages was lower than on a diet of vulnerable young stages. Strikingly, few female predators reproduced on arenas with less vulnerable whitefly stages without killing prey. These predators either retained eggs produced when feeding on their previous food source, or they fed on whitefly-produced honeydew.

In a similar biological control system to the one under study, e.g. thrips with phytoseiid predators, pest control is improved when pollen is present in the crop because in the long term, this supplementary food enhances the population growth of predators and subsequently, the population level of prey is reduced (van Rijn et al. 2002). However, feeding on pollen might increase satiation of predators beyond the level where they stop attacking prey, thus, they will feed less on prey. Therefore, I tested whether the presence of pollen altered predation and oviposition rates of predators on whitefly crawlers (Figure 1), when both food sources are offered in ample supply. On a mixed diet of crawlers and pollen, the predation rate of *E. scutalis*, but not that of *T. swirskii*, was reduced compared to the predation rate that was observed on crawlers alone. For both species, the oviposition rates on these two diets were similar.

In Chapters 3 and 4, I tested whether the two species under study were able to feed on alternative food sources, such as whitefly-produced honeydew and associated microbes and/or plant tissue, which are likely to be found in crops where the two phytoseiids will be released for biological control of whitefly. Utilisation of non-prey food sources by phytoseiids is significant for biological control since it allows populations of predators to persist in the crop in periods when prey is scarce or absent (Bakker 1993; van Rijn et al. 2002). A diet of honeydew and associated microbes enhanced adult survival and oviposition of *E. scutalis* when compared to a diet of plant leaf tissue alone. This explained my previous results, where I found that
some predators reproduced without feeding on less vulnerable whitefly prey, i.e. pupae (Figure 1; Chapter 2). For *T. swirskii*, I did not find any differences between the two diets in terms of adult survival for a week period that the experiment lasted, but there was very low oviposition on a diet of honeydew and associated microbes, while none was observed on plant leaf tissue. *Euseius scutalis* was able to develop and reach adulthood on a diet of honeydew and associated microbes, whereas *T. swirskii* showed high juvenile mortality on this diet. In Chapter 3, I also measured juvenile survival and developmental rate of predators on a diet of pollen. The two predatory mite species were found to have similar developmental rates on a diet of cattail pollen and on a diet of immature whiteflies (measured in Chapter 1).

In Chapter 4, I tested the ability of predators to feed on plant leaf tissue by assessing adult survival of predators on leaf substrate from cucumber plants treated with a systemic insecticide. *Euseius scutalis* showed high mortality, whereas *T. swirskii* did not. Apparently, *E. scutalis* ingested insecticide through feeding on the leaf tissue but whether plant feeding results in nutrient uptake and utilization by *E. scutalis* requires further analysis.

**Part 2: Biological control of whitefly populations on single plants**

Although life-history traits and behaviour of individual predators can elucidate the mechanisms underling population dynamics, they are only indicative for the outcome of population interactions. Eradication or suppression of prey cannot be achieved at a large spatial scale, if it cannot be attained at the spatial scale of one plant. Therefore, as a first step, I studied the local predator-prey dynamics on isolated cucumber plants. In Chapter 5, I investigated population dynamics of each of the two predatory mite species and their whitefly prey. Predatory mites were released on the plants 2 weeks before the introduction of *B. tabaci*. To enable survival and promote reproduction of phytoseiids, they were provided weekly with *Typha* sp. pollen. Both predators were capable of suppressing local whitefly populations to a large extent within a period of 2 months (more than 90% decrease compared with the control). This suppression may be affected by (1) the choice of initial predator numbers, and/or (2) the effect of pollen on the numerical response of the predators before and after pest introduction.

In order to discriminate between these two effects, I conducted a separate experiment with *T. swirskii* (Chapter 6) where I studied the predator-prey dynamics in presence and absence of pollen. In absence of predators, whitefly populations increased exponentially over a period of two months, whereas their growth was much slower in presence of predators (more than 90% lower compared with the control). When predators were present, whitefly populations were suppressed most when pollen was also present (more than 80% decrease in presence of pollen). The number of
predators per plant was higher on plants with pollen than on pollen-free plants (more than 70% higher in presence of pollen). Hence, the positive effect of pollen on whitefly control can only arise from its impact on the numerical response of predators. Since predatory mites tend to aggregate on leaves near pollen, it is striking to find that their impact on whitefly populations is more pronounced in presence of pollen. The same phenomenon was observed in the predatory mite-thrips system (van Rijn et al. 2002). This suggests that local supply of pollen on plants does not arrest predatory mites to such an extent that they fail to find and feed on prey in other plant strata.

**Part 3: Between-plant mobility of predator and prey**

The relative mobility of predator and prey, as well as the probability of ending up on the same plant will determine spatial distribution of phytoseiids and whiteflies in the crop. In Chapter 8, I studied the searching behaviour of predators in a group of plants consisting of clean plants and plants infested with whiteflies. Both predatory mite species, took a few days to find the plants. The numbers of *E. scutalis* that were recaptured on clean plants or plants infested with whiteflies did not differ much, suggesting that this species does not discriminate between the two types of plants. However, *T. swirskii* were recaptured significantly more frequently on plants with whiteflies than on prey-free plants. This was apparent from the beginning of the experiment, suggesting that these mites are able to discriminate between plants with and without whiteflies from a distance. Since predatory mites have no eyes, they possibly use volatile chemicals to discriminate between plants from a distance, as in other phytoseiid species (Sabelis and van de Baan 1983; Sabelis and Dicke 1985). Therefore, I used a Y-tube olfactometer to test the response of the predatory mite *T. swirskii* to odours emitted by whitefly infestations on cucumber as well as on cotton plants, the host plant on which I collected this predatory mite species. *Typhlodromips swirskii* was not attracted to the odours of either plant species with whiteflies suggesting that mites do not use volatiles to discriminate between infested and clean plants, or that the set-up as used here is not suitable to detect the response of this species to odours.

In Chapter 9, I tested whether the spatial distribution of whiteflies is affected by the presence of predators. As in many arthropod species, the younger stages of *B. tabaci* that are vulnerable to predation, are not efficient in escaping predation themselves, whereas the older, adult stages have a low vulnerability. In these cases, the adult females should avoid ovipositing at places where their offspring would suffer from high risks of predation (Ohsaki and Sato 1994; Mappes and Kaitala 1995; Ballabeni et al. 2001). I tested the host plant selection behaviour of female whiteflies that had either experience with predatory mites in their adulthood prior to the experiment, or were naïve with respect to predators. I conducted this
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experiment in a group of plants that either harboured predators and whiteflies or whiteflies only. Adult female whiteflies avoided plants with predators after having experienced predators prior to the experiment, but much less when not having had such experience.

Taking into account the foraging behaviour of both prey and predator, I close with a discussion on the spatial predator-prey dynamics at a metapopulation level. The long-range searching efficiency of predatory mites is low compared to that of adult whiteflies (86.7% of the released whiteflies was recaptured on plants in one day, and 26% of the released predators was recaptured in 4 days). Adult whiteflies learn to discriminate between plants with predators and whiteflies and plants with only whiteflies and the first offspring that whitefly produce on plants without predators may reach an invulnerable stage before predators arrive. Hence, local whitefly populations can be founded that temporarily escape from their predators. I expect this to lead to spatial dynamics with prey moving away from plants with predators and predators slowly following prey, resulting in a front of prey-infested plants that moves outward from the focus of infestation.

COULD PREDATORY MITES BE USED FOR BIOLOGICAL CONTROL OF THE SILVERLEAF WHITEFLY?

Our results show that phytoseiids could be of use in biological control programmes for the silverleaf whitefly. Further studies are needed for the actual implementation of the system.

Initial predator-prey ratios will strongly affect the fluctuations of the population dynamics. In short-lived crops such as greenhouse vegetables, the success of biological control will depend on the height of peaks of whitefly populations relative to the level of the economic threshold. The presence of pollen in the crop will strongly affect the success of biological control. First, it will increase the predator-prey ratio due to its positive effect on the numerical response of predators and this will lead to lower prey populations. With pollen supply, predators can also be released into the crop preventively, i.e. before prey occurs, and they will persist during the growing season. Second, since immature prey can find a temporal refuge in the predator-free plants due to the anti-predator behaviour of adult whiteflies, infestations can occur within a crop. In the presence of pollen, predators can get established in the crop even on the non-infested plants and thus post-invasions of whiteflies will be controlled rapidly. Addition of alternative food for the predators will be indispensable for parthenocarpic crops, such as several cucumber varieties. For other crops, it will be necessary only if their pollen cannot be utilized by phytoseiids or during non-flowering periods. Preliminary results have shown that pollen from gerbera was a suitable food source for oviposition of the two phytoseiids tested here.
In this thesis, the dynamics of the predatory mite-whitefly system were studied in a glasshouse. In this environment, seasonal inoculative releases of phytoseiids could be employed for whitefly control and inundative releases could also be considered for crops with a low damage threshold (van Lenteren and Manzaroli 1999). The predators studied in this thesis were collected in Israel and Jordan but they have also been reported in other countries in the Mediterranean area, as well as in India and Pakistan (de Moraes et al. 1986). Hence, the two phytoseiids can also be used in biological control programmes for whiteflies in these areas. In this case, native strains should be considered first and their conservation can be enhanced by manipulating all factors that directly or indirectly constrain or increase their abundance (De Bach 1974; Naranjo 2001).

Introduction of exotic biological control agents poses high risks for non-target species and it may result in undesirable side-effects (van Lenteren 1997). However, these risks are minimized if the introduced agent originates from tropical and sub-tropical climates and is unable to survive outdoors in cooler climates, which is the case for the predators that I tested. Direct and indirect interactions with other natural enemies currently released in greenhouses need to be studied in order to test whether the two phytoseiids studied here are compatible with biological control agents of other pests. Preliminary results show that both phytoseiids feed and oviposit on other pests such as spider mites and thrips but to a lesser extent than on whiteflies. Although further study of interactions between various biological control agents is still needed, there are no indications for negative side-effects so far.

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