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Effects of temperature regime and food supplementation on the performance of phytoseiid mites as biological control agents

by

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Thesis submitted in the fulfillment of the requirements for the Degree of Doctor (PhD)
in Applied Biological Sciences

Dutch translation:

Effecten van temperatuurregime en voedingssupplementen op de prestaties van Phytoseiidae roofmijten als biologische bestrijders

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List of abbreviations

ANOVA	analysis of variance
ARC	Artemia Reference Center, GhentUniversity
df	degrees of freedom
DTR	diurnal temperature range
DTV	dagelijkse temperatuurvariatie
EFN	extrafloral nectar
HIPV	herbivore-induced plant volatiles
IPM	integrated pest management
K	thermal constant (degree-days)
L:D	light: dark cycle expressed in hours
n	number of tested replicates
RH	relative humidity (in %)
r_m	intrinsic rate of increase (females/female/day)
R^2	coefficient of determination
RSS	residual sum of squares
SE	standard error
T	temperature (°C)
T_0	lower developmental threshold temperature (°C)
T_L	lethal temperature (°C)
T_{opt}	optimal temperature (°C)
WFT	Western flower thrips

Scope and thesis outline

Ever since the early start of agriculture about 10,000 years ago, crops have been under the continuous threat of a variety of pests, diseases and weeds, accounting for yield losses of 50 up to 82% in the absence of management practices (Oerke 2006). For the past 70 years, chemical treatments have been the prevailing control strategy and were initially considered as the "silver bullet" for pest control. However, repeated and excessive use of pesticides gave rise to resistance of target organisms, which strongly reduced the efficiency of these chemicals. In 1962, Rachel Carson published her pioneering book "Silent Spring", which triggered the change in public awareness and perception towards an aversion of pesticide-residues in food products and the environment (Cock et al. 2010). As a result, more sustainable and environmentally-friendly pest management strategies arose, like biological and physical control and breeding for resistance. This new approach soon resulted in the concept of **integrated pest management (IPM)**, a technique aiming at only applying pesticides when other non-chemical control practices fail. In 2009, the European commission launched directive 2009/128/EC that obliged professional users of pesticides to implement the principles of integrated pest management and thus to restrain the use of pesticides, which is expected to further increase demand for biological control agents.

Biological control is a key component of integrated pest management and has been defined as "the use of an organism to reduce the population density of another organism and thus includes the control of animals, weeds and diseases" (Bale et al. 2008). In this study, we will focus on the biological control of arthropod plant pests which De Bach (1964) defined as "the study and uses of parasites, predators and pathogens for the regulation of host (pest) densities". The so-called 'macrobial' biological control agents represent predatory and parasitizing insects and mites used for the control of insect and mite pests (Bale et al. 2008). Predatory mites of the family **Phytoseiidae** are among the most used biological control agents worldwide (van Lenteren 2012). These predators are being introduced in vegetable and ornamental crops to control a wide range of key pests, such as mites, thrips and whiteflies (McMurtry and Croft 1997).

Nowadays, many vegetable and ornamental crops are being produced in protected environments, such as greenhouses. In this study, we will focus on two challenges that greenhouse growers are facing in modern protected cultivation.

Firstly, greenhouse growers are forced to use several strategies to counterbalance the ever-increasing energy costs (Bolla et al. 2011). One such strategy is **temperature integration** in which temperatures are allowed to vary within limits tolerated by the crop. Hence, less ventilation during the day and less heating during the night is required, thus reducing the energy inputs. However, this strategy results in large diurnal temperature variations (DTR= diurnal temperature range). Insect and mites living in a greenhouse environment –pest or biocontrol agents- are ectothermic (i.e. cold-blooded) organisms. As a result their activity and developmental and reproductive performance are closely associated with the ambient temperature. Although there is a plethora of studies on the effects of constant temperatures on ectothermic thermal biology, little is known about the effects of temperature variations on life history traits of pest and predator present in an environment with dynamically changing temperatures, like in today's greenhouses. Arguably, the performances of pests and predators as affected by climatic conditions is likely to affect the outcome of biological control programs.

Secondly, greenhouse growers are often confronted with the necessity to re-introduce natural enemies in order to deal with poor establishment of these organisms due to low prey levels and/or absence of alternative foods (e.g. pollen). This is especially problematic in ornamental crop production, which is characterized by its short production cycles and its lack of alternative food sources, resulting in less chances for natural enemies to establish. As a result, multiple releases most often remain necessary for pest control. Additionally, there is zero-tolerance for crop damage as ornamentals are being sold for their aesthetic value (Messelink et al. 2014; Buitenhuis et al. 2015). One of the emerging approaches gaining attention is to provide natural enemies with **food supplements** to overcome times of limited food availability. For instance, recently, a commercial pollen-based product was launched for the support of generalist predatory mites (Nutrimite^{TM}, Biobest N.V., Westerlo, Belgium). Possibly, other types of food supplements can be used for the enhancement of predator populations in the crop. Food supplements might have an influence on omnivorous prey too. In a number of studies it was shown that the western flower thrips *Frankliniella occidentalis* showed a significant increase in its fecundity due to pollen supplementation (Hulshof et al. 2003; Zhi et al. 2005; Leman and Messelink 2015). A beneficial effect of supplements on the prey should be avoided.

The main research questions of this thesis are:

1. What are the effects of diurnal temperature ranges on life history traits of predatory mites and their prey?
2. Can a model be constructed that allows the prediction of developmental rates at variable temperatures based on constant temperature data?
3. How do relative performances of predatory mites and their target pests under these temperature variations affect biological control?
4. Which food supplements are suitable for predatory mites, but less suitable for omnivorous pests?
5. How do food supplements affect tritrophic interactions in the crop environment (plant-prey-predator)?
6. Which food supplements allow the pre-establishment of generalist phytoseiids in the crop?

In **Chapter 1**, we will provide a concise overview of the bio-ecology and use of phytoseiid predatory mites in biological control programs. In this dissertation, we will primarily focus on four commercially important phytoseiids: the spider mite predators *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* McGregor and the generalist predators *Amblyseius swirskii* Athias-Henriot and *Amblydromalus limonicus* Garman & McGregor. The former three species are listed among the most important biological control agents worldwide (van Lenteren 2012), whereas *A. limonicus* is a newly-launched predator for the control of mainly thrips and whiteflies (Knapp et al. 2013). In a first part, general biology, ecology and use of Phytoseiidae in biological control programs will be discussed, whereas the second part will focus on conservation techniques in the crop.

The development of the predators *P. persimilis, N.californicus* as well as their prey *T. urticae* over a wide temperature range (11.7°C to 40°C) at both constant (DTR0) and alternating (DTR+5, +10 and +15°C) temperature regimes was examined in **Chapter 2**. The obtained developmental rates were fitted against a linear and two nonlinear models. In addition, we compared several life history traits at a constant temperature of 15°C and its corresponding alternating temperature regime with a DTR of 15°C (20°C/5°C).

The effects of an alternating temperature regime (DTR+15) on spider mite consumption of *P. persimilis* and *N. californicus* were assessed in **Chapter 3**. We compared the predation capacity of female phytoseiids reared at 5 different temperatures, both at a constant and alternating temperature regime. Additionally, females were measured to assess the effect of temperature and temperature variation on the body size of female *P. persimilis* and *N. californicus*.

In **Chapters 4** and **5**, we studied the impact of food supplements (pollen of *Typha* spp., *Ephestia kuehniella* eggs and *Artemia* cysts) on tritrophic interactions between the plant (bean), the target prey (*F. occidentalis*) and its predators (*A. limonicus* and *A. swirskii*, respectively).

Two greenhouse experiments conducted with chrysanthemum and ivy are reported in the final research chapter (**Chapter 6**). Here, we tested a commercial pollen product of *T. angustifolia*, a commercial and non-commercial strain of *Artemia* cysts and a powdered artificial diet for the establishment of *A. swirskii*.

Finally, in **Chapter 7**, we critically discuss the results obtained in this study and provide perspectives for future research.

1

Phytoseiidae as biological control agents

1.1. The diversity of the Acari

Mites[1] are minute creatures barely visible to the naked eye. They are therefore often overlooked. However, these tiny organisms are omnipresent and can be encountered in the most extraordinary habitats. There are mites that live on or under human skin (*Demodex* sp. and *Sarcoptes scabiei* De Geer, respectively, Arlian 1989; Grice and Segre 2011), while others are used to season cheese (*Acarus siro* (L.), Melnyk et al. 2010). Mites can colonize the ears of moths (*Dicrocheles phalaenodectes* (Treat), Treat 1975), are found sucking blood from poultry (*Dermanyssus gallinae* (De Geer), Chauve 1998), or infest the cloacae of turtles (Cloacardiae, Camin et al. 1967). In apiculture, they are found in tracheae of bees (*Acarapis woodi* (Rennie), Sammataro et al. 2000) or infesting the brood and transmitting viruses in honeybees (*Varroa destructor* (Anderson & Trueman), Tentcheva et al. 2004). Mites can be found in practically all terrestrial and aquatic habitats on all continents, including Antarctica. As a result of their small size and the lack of adequate tools for identification, the taxonomy of mites is lagging 50 to 100 years behind that of insects. Nevertheless, up to date about 55,000 mite species are described, though it has been suggested that over 90% of the acarofauna thriving on this planet is yet to be discovered (Krantz 1978; Walter and Proctor 1999; Hoy 2011; Gerson and Weintraub 2012).

1.2. Adoption of phytoseiid predatory mites as biological control agents in covered crops

In agricultural habitats, mites can be encountered both as pest and predator. For the chemical control of pest mites, Van Leeuwen et al. (2014) recently estimated an annual cost of about 900 million euros on the expenditures for acaricides worldwide, with about 80% being used for the control of spider mite species (*Tetranychus* spp.). Within this genus, the two-spotted spider mite, *Tetranychus urticae* Koch, is by far the most problematic species (Gerson and Weintraub 2012), because of its wide host range, which comprises over 1100 plant species (Grbić et al. 2011), in combination with a fast generation time (Sabelis 1991). In addition, *T. urticae* has an unrivalled capacity to develop resistance against pesticides (Van Leeuwen et al. 2010a). In 1968, the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) was one of the first natural enemies to become available (van Lenteren 2012).

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[1] The word “mite” is the Old English word for “very small creature”.

Even today, this predator is still one of the most used biocontrol agents to control *T. urticae* in protected cultivation (van Lenteren 2012; Calvo et al. 2015), and is therefore sometimes called the “acaricide on legs” (Hoy 2011). In the 1980’s the phytoseiid predatory mites *Neoseiulus barkeri* Hughes and *Neoseiulus cucumeris* (Oudemans) were marketed for the control of thrips species, such as *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) (De Klerk and Ramakers 1986). From 1990 onwards, the European invasion of the western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), along with its potential to develop resistance against a wide range of pesticides triggered researchers to further investigate the potential of phytoseiid predators (Bielza 2008; Messelink et al. 2006; van Lenteren 2012). In 2001, Nomikou et al. demonstrated the potential of predatory mites to control the tobacco whitefly *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) and subsequently the greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Bolckmans et al. 2005). In 2006, the detection of pesticide residues on vegetables from Spain initiated the adoption of *Amblyseius swirskii* Athias-Henriot. Since then, phytoseiid mites have become a cornerstone in the integrated management of a wide range of pests (Gerson and Weintraub 2007; Calvo et al. 2015; Buitenhuis et al. 2015), especially in protected crops.

1.3. Taxonomy

The taxonomic classification is as follows:

Kingdom	Animalia
Phylum	Arthropoda
Subphylum	Chelicerata
Class	Arachnida
Subclass	Micrura
Infraclass	Acari
Superorder	Anactinotrichidae
Order	Mesostigmata
Suborder	Dermanyssina
Superfamily	Ascoidea
Family	Phytoseiidae

Mites belong to the Chelicerata, which is the second largest group in the animal kingdom after the Insecta. Mites and ticks (assembled in the infraclass of the Acari or Acarina) belong to the class of the Arachnida, together with the order Araneae (spiders) and Scorpiones (scorpions). The group of mites is further subdivided in the Mesostigmata, Prostigmata and Cryptostigmata. Most beneficial predatory mites belong to the first group, whereas pest mites usually belong to the Prostigmata or, to a much lesser extent, to the Astigmata. Within the Mesostigmata, Phytoseiidae (or leaf-inhabiting predatory mites) are an important family of predatory mites in agricultural crops which currently comprises over 2700 described species (Demite et al. 2014). Members of this family can be further subdivided in three subfamilies: Amblyseiinae, Phytoseiinae and Typhlodrominae (Chant and McMurtry 1994; Demite et al. 2014). The vast majority of economically important and commercially available predatory mite species belongs to the subfamily of Amblyseiinae (Zhang 2003).

1.4. Origin and distribution

Phytoseiidae are cosmopolitan, and some species have spread worldwide due to human activities, either unintentionally or deliberately. As a result, the origin and distribution of its species is not always clear. For example, *P. persimilis* is believed to originate from Chile (commonly named “the Chilean predatory mite” (McMurtry et al. 1978; Griffiths 1999)), but was accidentally introduced in Europe, more specifically in Germany in the late 1950s. From then on, *P. persimilis* has spread to the Mediterranean area and established there. The origin of *Neoseiulus californicus* McGregor and *Amblydromalus limonicus* Garman & McGregor is found in California (USA), and much like *P. persimilis* this former species has spread worldwide with human assistance. *Amblydromalus limonicus* is presently distributed in the temperate to subtropical areas on the American continent, New Zealand and Australia (Knapp et al. 2013) and has not been reported in nature in Europe until 2012 in Spain (Escudero-Colomar and Chorąży 2012). *Amblyseius swirskii* is a subtropical species that originates from the Mediterranean area but is now mainly found in orchards in Israel (Demite et al. 2014), yet this species has also been reported to occur in North America (Denmark and Evans 2011).

1.5. Morphological characteristics

Mites can be distinguished from insects by the lack of antennae, mandibles and maxillae. Unlike spiders, which have two major body parts (i.e. cephalothorax and abdomen), mites have only one major body part (i.e. idiosoma) with an anterior section bearing the mouthparts (i.e. gnathosoma) (Zhang 2003). Mite species can be further separated from spiders in that mites have a six-legged larval stage, followed by one or more eight-legged nymphal stages. The family of Phytoseiidae comprises medium-sized mites (between 250-500 µm). The gnathosoma bears the mouthparts, i.e. one pair of pedipalps, one pair of chelicerae and one pair of stylets (Figure 1.1 and 1.4). The chelicerae consist of a fixed and movable digit with small teeth used for capturing and subduing prey (Figure 1.1). In male phytoseiids, each chelicera carries a spermatodactyl (Figure 1.2) that is used to transfer spermatophores to sperm induction pores of females. The shape and structure of the male aedeagus is often applied as a diagnostic tool (Zhang 2003). In females, induction pores (situated between between coxae III and IV) lead to the spermatheca, the storage organs of spermatophores. The shape of the spermatheca, either flask, bell or tube shaped, is another morphological trait used for identification of phytoseiid species (Figure 1.3).

Figure 1.1: Close-up of a chelicera of a female *Amblydromalus limonicus* (Photo: Author)

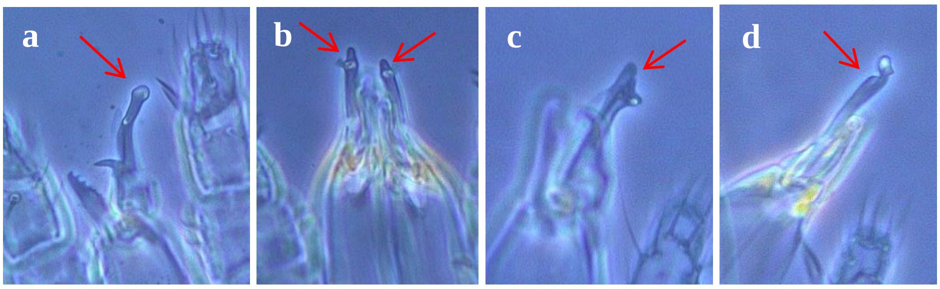

Figure 1.2: Close-up of chelicerae and spermatodactyl (arrows) of a male *Phytoseiulus persimilis* (a), *Neoseiulus californicus* (b), *Amblyseius swirskii* (c) and *Amblydromalus limonicus* (d) (Photo: Author)

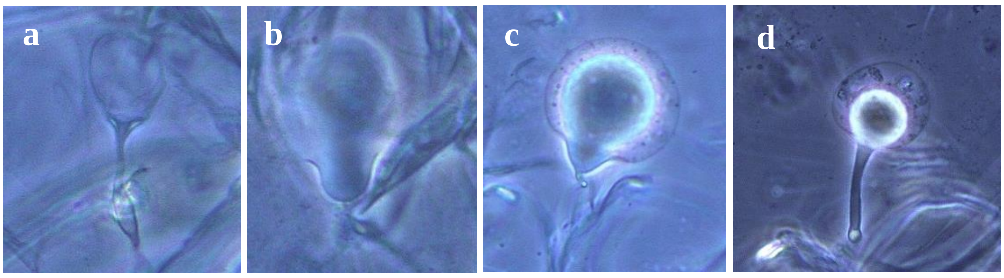

Figure 1.3: Spermatheca of females of *Phytoseiulus persimilis* (a), *Neoseiulus californicus* (b), *Amblyseius swirskii* (c) and *Amblydromalus limonicus* (d) (Photo: Author)

The idiosoma is the body part to which the legs are attached (Figure 1.4). The dorsal surface is covered with a single sclerotized dorsal shield with no more than 24 pairs of setae. This characteristic is used to separate Phytoseiidae from other mesostigmatid families. The location and length of these setae are often used as characteristics for identifying and classifying phytoseiid species (Chant 1993).

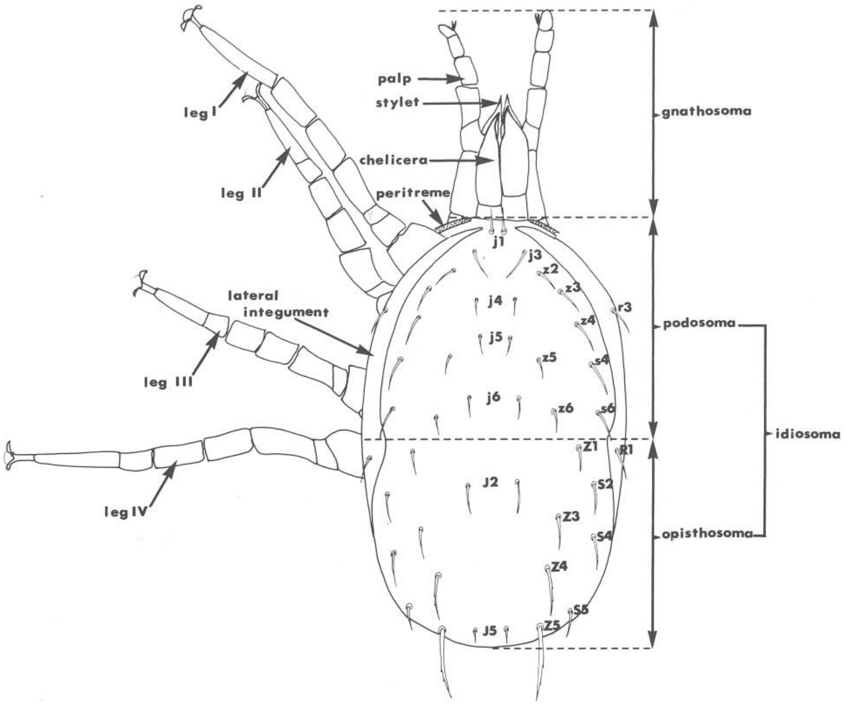

Figure 1.4: Dorsal view a phytoseiid mite with corresponding setae nomenclature (Helle and Sabelis 1985)

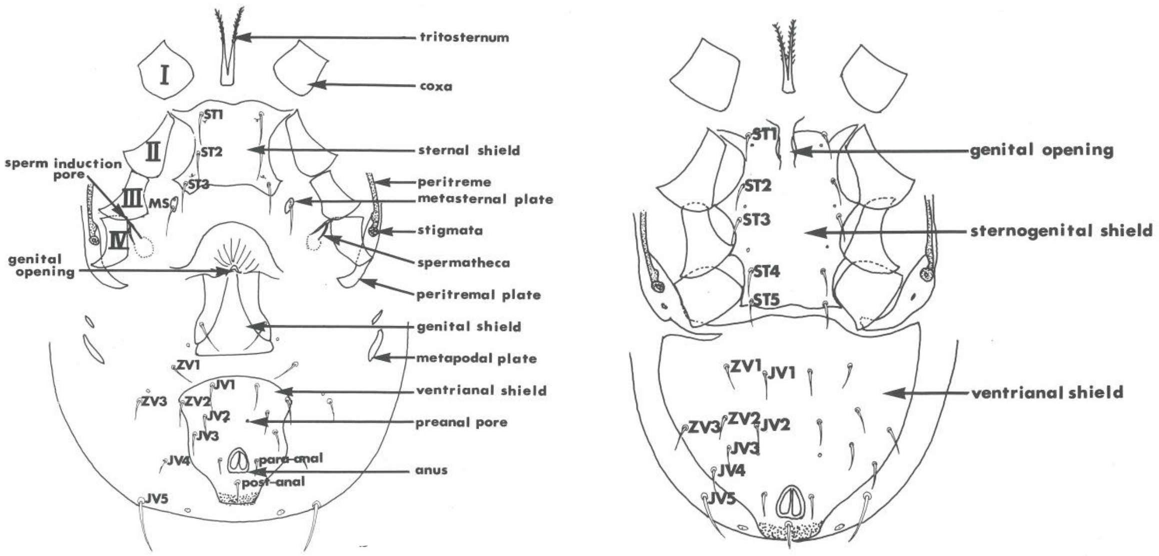

Figure 1.5: Ventral view of a female (a) and male (b) phytoseiid with corresponding setae nomenclature (Helle and Sabelis 1985)

Ventrally, female mites possess three ventral shields (Figure 1.5a), whereas males have only two (Figure 1.5b). The sternal shield of female phytoseiids bears three pairs of setae and two pairs of pores. The truncate genital shield, with a single pair of setae, has a central genital opening through which the eggs are laid. The ventrianal shield comprises 1 to 4 pairs of preanal setae and one pair of para-anal setae (one on each side of the anus). Usually there is a single seta located post-anally. In males, the sternal and genital shield are comprised into one sternogenital shield, from which –at the anterior margin– spermatophores are being inserted via the genital opening. Five pairs of setae are located on the male's sternogenital shield (Krantz 1978; Chant 1985; Zhang 2003)

1.6. Bionomics

The life cycle of Phytoseiidae consists of five developmental stages: the egg-stage, the larval stage, the two nymphal stages (i.e. proto-and deutonymphs) and the adult stage (Figure 1.6). The mobile stages bear 4 pairs of legs, except for the six-legged larvae.

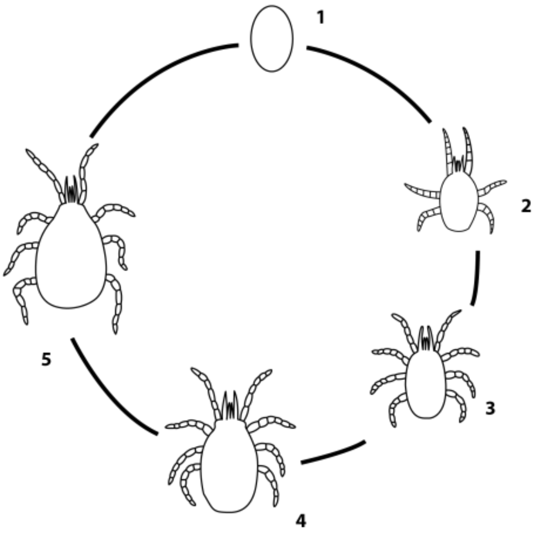

Figure 1.6: Life cycle of Phytoseiidae; 1: egg; 2: larva; 3: protonymph; 4: deutonymph; 5: adult (Figure: M. Goeminne)

1.6.1. Development

1.6.1.1. Egg stage

Phytoseiid eggs are oval-shaped, with a white to creamy color (Figure 1.7). Male eggs are usually smaller than female eggs. A female mite deposits a male egg first, followed by a female egg. Thereafter, the sequence of the eggs' sex depends on several biotic and abiotic factors. For example, in *P. persimilis* and *Amblyseius womersleyi* (Schicha), low prey densities have been shown to lower sex ratio to 0.5 with male and female eggs deposited in an alternating sequence, whereas a female-biased sex ratio (0.8) was observed at higher prey densities (Toyoshima and Amano 1998). Similar observations were made for other phytoseiid species, such as *N. californicus, Metaseiulus occidentalis* (Nesbitt) and *N. barkeri* (Friese and Gilstrap 1982; Momen 1996). Sex ratio was also found to be unbiased when several females were allowed to deposit their eggs on the same prey patch, whereas a biased sex ratio was observed for an isolated female (Van Dinh et al. 1988; Nagelkerke and Sabelis 1998). Phytoseiid mothers invest a large amount of the ingested food into their eggs; up to 25% of their own body weight (Helle and Sabelis 1985). A smaller egg size can be a result of low prey density (Toyoshima and Amano 1998; Walzer and Schausberger 2013). Usually, larger eggs are more viable, indicating that females do not invest more energy in their eggs to secure a higher offspring survival rate under low prey conditions.

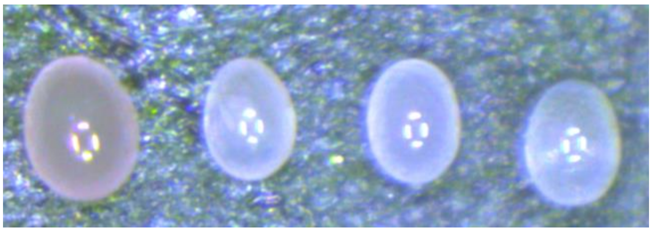

Figure 1.7: Eggs of *P. persimilis, N. californicus, A. swirskii* and *A. limonicus* (fltr) (Photo: Author)

The hatching rate of phytoseiid eggs is influenced by several abiotic factors, such as temperature and relative humidity. Moreover, the egg stage is more susceptible to unfavorable environmental conditions than the mobile stages in which it is possible to move to a more favorable site or to compensate for water loss by ingesting free water or plant fluids (Croft et al. 1993; Schausberger 1998). Therefore, it is crucial for females to deposit their eggs at optimal oviposition sites. Depending on the species, female phytoseiids prefer to deposit their eggs inside so-called acarodomatia. These leaf domatia are small hairy structures located in vein axils at the abaxial surface of leaves of over 1000 plant species (Walter and O'Dowd 1992; Walter 1996; Agrawal 1997). Plants accommodated with these domatia usually harbor more carnivorous and fungivorous mites, a trait believed to be evolved as a plant-mite mutualism. Plants provide shelter (for hiding and ovipositing eggs), and in exchange they benefit from reduced attacks by herbivores and pathogens (Agrawal and Karban 1997). Phytoseiid eggs deposited in such domatia are better protected against abiotic and biotic stresses (Walter and O'Dowd 1992; Faraji et al. 2002), including cannibalism (Ferreira et al. 2008a).

For *P. persimilis* and *N. californicus,* the duration of the egg stage increased significantly at a relative humidity of 60 to 70% compared to 82 and 90% at 20°C. At 50% RH only 3% of the *P. persimilis* eggs and none of the *N. californicus* eggs hatched (De Courcy Williams et al. 2004). For *A. swirskii*, Ferrero et al. (2010) reported 50% egg mortality at a relative humidity of 63% and classified *A. swirskii* together with *P. persimilis* as drought-sensitive. Likewise, eggs of *A. limonicus* were reported to be highly drought-sensitive with 50% egg mortality at a relative humidity of 60%. At a relative humidity below 50%, none of the eggs hatched (McMurtry and Scriven 1965). It has been argued that when describing the effects of humidity on biological features, such as egg viability, “vapor pressure deficit” might be a more suitable parameter as it is more closely related to evaporation irrespective of the actual temperature (Ferro and Chapman 1979; van Houten et al. 1995a).

1.6.1.2. Larval and nymphal stage

Based on their feeding behavior, larvae can be divided into three groups: non-feeding, facultative feeding, and obligatory feeding larvae (Table 1.1). Non-feeding larvae do not need to feed to develop into the protonymphal stage, whereas obligatory feeding larvae require food for further development. Facultatively feeding larvae do not need to feed, but will feed when suitable food is present. *Phytoseiulus persimilis* larvae belong to the non-feeding type, whereas *N. californicus* larvae were found to be facultatively feeding, with ca. 60% of the larvae feeding (Chittenden and Saito 2001). Wimmer et al. (2008) reported *A. swirskii* larvae to be facultative feeders as well. It has been suggested that species with non-feeding larvae oviposit larger eggs to provide the larvae with more nutrients (Zhang and Croft 1994).

Table 1.1: Some phytoseiid larvae and their respective feeding type (Chittenden and Saito 2001; Palevsky et al. 1999; Schausberger and Croft 1999a; Wimmer et al. 2008)

Non-feeding	Facultative feeding	Obligatory feeding
Phytoseiulus persimilis	*Neoseiulus fallacis*	*Galendromus occidentalis*
Phytoseiulus macropilis	*Neoseiulus californicus*	*Euseius finlandicus*
Kampidromus aberrans	*Amblyseius andersoni*	*Euseius hibisci*
Neoseiulus longispinosus	*Amblyseius swirskii*	*Amblyseius orientalis*
Neoseiulus cucumeris	*Iphiseius degenerans*	*Amblyseius eharai*
Neoseiulus barkeri		
Typhlodromus pyri		
Typhlodromus bambusae		
Typhlodromus athiasae		
Phytoseius tenuiformis		
Amblyseius womersleyi		

The duration of the developmental period of juvenile mites is highly dependent on abiotic factors (see section 1.6.1.1.). Temperature is a key factor affecting development of all ectothermic organisms (e.g. Wagner et al. 1984; Ratte 1985; Huey and Kingsolver 1989; Lactin et al. 1995). Usually, there is a typically nonlinear relationship between an ectotherm's developmental rate (i.e. reciprocal of developmental time) and temperature, as demonstrated in Figure 1.8.

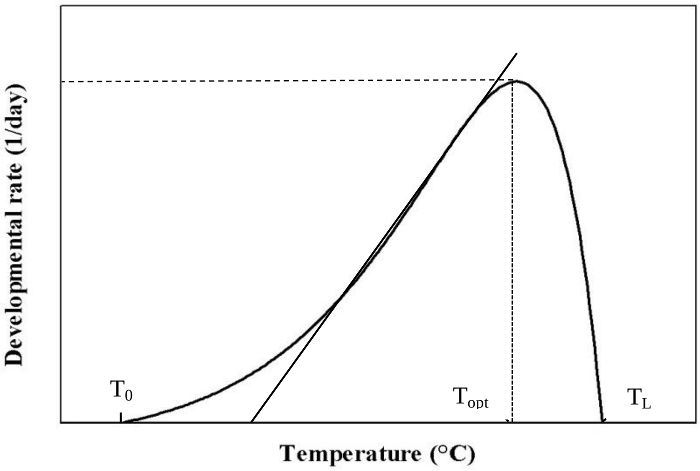

Figure 1.8: Linear and nonlinear relationship between constant temperatures and an ectotherm's developmental rate

Development does not occur below a lower threshold temperature, T_0. With temperature rising, developmental rate increases until a maximum rate is reached at the optimal temperature for development (T_{opt}). When temperatures exceed T_{opt}, there is a steep decrease in developmental rate until the upper developmental threshold (at lethal temperature, T_L) is reached (Brière et al. 1999). Both linear and non-linear models have been developed to predict the developmental rate as a function of temperature (for review a see Roy et al. 2002 and Kontodimas et al. 2004). The linear model approximation is a widely used tool to predict developmental rates at the intermediate temperature range enabling the estimation of lower developmental threshold and thermal constant (Arnold 1959; Campbell et al. 1974; Lamb 1992). The lower developmental threshold is estimated as the intercept between the linear curve and the x-axis. However, the linear model underestimates the developmental rate at low temperatures as shown in Figure 1.8 (Worner 1992). Similarly, developmental rates estimated using the linear model are overestimated in the higher temperature range. Nevertheless, the user-friendly linear model is still widely used because it accurately predicts development at temperatures that are relevant in the field (Campbell et al. 1974; Kontodimas et al. 2004). In this more linear part of the relationship, rates estimated by either the linear or non-linear models are broadly similar. The thermal constant, expressed in degree-days, is defined as the accumulated degrees of temperature above the lower threshold temperature needed for development of a given stage. The lower threshold value of a life stage is easily derived from the linear model as it intersects with the x-axis. Developmental thresholds and thermal constants of several phytoseiids are listed in Table 1.2.

Table 1.2: Lower developmental thresholds (T_0, in °C) and thermal constants (K, in degree-days) for some phytoseiid predatory mites

Species	Prey/food source	T_0 (°C)	K (degree-days)	Reference	Model
Amblyseius andersoni	*Tetranychus urticae*	10.6	84.2	Genini et al. 1991	Linear
Amblyseius swirskii	*Typha latifolia*	11.3	117.6[b]	Lee & Gillespie 2011	Linear
	Typha latifolia	15.5	-[a]	Lee & Gillespie 2011	Non-linear (Brière-2)
Amblyseius womersleyi	*Tetranychus urticae*	11.6	52.6[b]	Lee & Ahn 2000	Linear
	Tetranychus urticae	13.4	91.8	Kadono et al. 1975	Linear
	Tetranychus kanzawai	11.5	80.5	Nakagawa 1984	Linear
Euseius finlandicus	*Typha sp.*	8.9	93.5	Broufas and Koveos 2001	Linear (females)
		6.4	100	Broufas and Koveos 2001	Linear (males)
Galendromus longipilus	*Tetranychusurticae*	10.3	82.7	Genini et al. 1991	Linear
Iphiseius degenerans	*Tetranychus urticae*	10.4	76.9	Tsoukanas et al. 2006	Linear

		9.5	-[a]	Tsoukanas et al. 2006	Non-linear (Lactin-2)
Kampimodromus aberrans	*Typha* sp.	10.7	103.1b	Broufas et al. 2007	Linear
		10.7	-[a]	Broufas et al. 2007	Non-linear (Lactin)
Neoseiulus baraki	*Aceria guerreronis*	15.8	84.2	Domingos et al. 2010	Linear
Neoseiulus barkeri	*Tetranychus urticae*	12.1	86.2	Jafari et al. 2011	Linear
		11.9	-[a]	Jafari et al. 2011	Non-linear (SSI)[d]
Neoseiulus barkeri	*Aleuroglyphus ovatus*	9.7	111.1	Xia et al. 2012	Linear
Neoseiulus californicus	*Tetranychus urticae*	9.9	123.5	Hart et al. 2002	Linear
	Tetranychus urticae	8.6	142.9	Hart et al. 2002	Weighted linear
	Tetranychus urticae	9	90	Castagnoli & Simoni 1991	Linear
	Tetranychus urticae	8.3	100.1	Rencken & Pringle 1998	Linear
	Tetranychus urticae	10.9	59.2	Gotoh et al. 2004	Linear
	Tetranychus urticae	10.6	71.4	Canlas et al. 2006	Linear
	Panonychus ulmi	11	57.5	Taj & Jung 2012	Linear
Neoseiulus fallacis	*Tetranychus urticae*	11.8	67.3	Genini et al. 1991	Linear [c]
Neoseiulus longispinosus	*Oligonychus coffeae*	10	84	Rahman et al. 2013	Linear
Proprioseiopsis asetus	*Thrips tabaci*	15.2	75.8	Huang et al. 2014	Linear
Phytoseiulus longipes	*Tetranychus evansi*	12	58.5[b]	Ferrero et al. 2007	Linear [e]
Phytoseiulus persimilis (strain SI)[f]	*Tetranychus urticae*	7.8	82.1[b]	Galazzi & Nicoli 1996	Linear
Phytoseiulus persimilis (strain NE)[f]	*Tetranychus urticae*	10	62.6[b]	Galazzi & Nicoli 1996	Linear
Phytoseiulus persimilis (strain NI)[f]	*Tetranychus urticae*	9	68.9[b]	Galazzi & Nicoli 1996	Linear
Phytoseiulus persimilis	*Tetranychus urticae*	10.6	68.5	Davies et al. 2009	Linear
Typhlodromus bagdasarjani	*Tetranychus urticae*	9.2	162	Ganjisaffer et al. 2011	Linear
Typhlodromus pyri	*Calepitrimerus vitis*	7.8	135.1	Gadino & Walten 2012	Linear
Typhlodromus pyri	*Tetranychus urticae*	9.8	119.7	Genini et al. 1991	Linear

[a] thermal constant cannot be calculated using nonlinear regression
[b] calculated from data provided by the authors
[c] data from mobile stages only
[d] Sharpe-Schoolfield-Ikemoto model
[e] calculated from data
[f] strain SI: Sicily; strain NE: northern European; strain NI: northern Italy

Non-linear models have been shown to more accurately predict the developmental rate over a wider range of temperature. Some non-linear models also enable prediction and/or estimation of several biologically relevant parameters such as lower and upper developmental thresholds and optimal temperature (Roy et al. 2002). Several studies have described the relationship between a phytoseiid's immature developmental time and temperature (e.g. Stenseth 1979; Lee and Ahn 2000; Hart et al. 2002; Gotoh et al. 2004; Lee and Gillespie 2011). However, only few studies have fitted developmental data of predatory mites to non-linear models (Lee and Ahn 2000; Broufas and Koveos 2001; Tsoukanas et al. 2006; Lee and Gillespie 2011).

A plethora of studies has been conducted on the effects of constant temperatures, but relatively little is known about the effects of temperature variations. This is surprising as natural temperature regimes usually undergo diurnal cycles (Hagstrum and Hagstrum 1970; Beck 1983). Temperature variations can be either smoothly changing, sinusoid-waved pattern (fluctuating temperatures), or block-waved with an abrupt transition from the higher to the lower temperature (and vice versa) (alternating temperatures) (Liu et al. 1995; Worner 1992). Usually, arthropods tend to develop faster at varying temperatures in the lower temperature range, whereas the opposite is observed in the higher temperature range. At intermediate temperatures, little to no differences are observed. These effects have been attributed to the typically non-linear relationship between an ectotherm's developmental rate and temperature (see also section 1.6.1.2. and Figure 1.8) and have been referred to as the rate summation effect or Kaufmann effect (Ratte 1985; Worner 1992). More generally, this phenomenon is a consequence of Jensen's inequality, which dictates that the average value of a nonlinear function (E[f(x)]) of two values of x does not necessarily equal the value of the nonlinear function evaluated at the average variable (f(E[x]) (see Figure 1.9). This mathematical property may, at least partly, explain the variation in arthropod developmental rates between constant and varying temperature regimes.

Figure 1.9 illustrates Jensen's inequality for a hypothetical developmental rate-temperature curve of an ectothermic organism. At temperature x, the corresponding developmental rate derived from the nonlinear function equals f(x).

- Temperature range a_1-a_2, with an average temperature of a_{1-2}:
  Mean developmental rate $a^* > f(a_{1-2})$
- Temperature range b_1-b_2, with an average temperature of b_{1-2}:
  Mean developmental rate $b^* = f(b_{1-2})$
- Temperature range c_1-c_2, with an average temperature of c_{1-2}:
  Mean developmental rate $c^* < f(c_{1-2})$

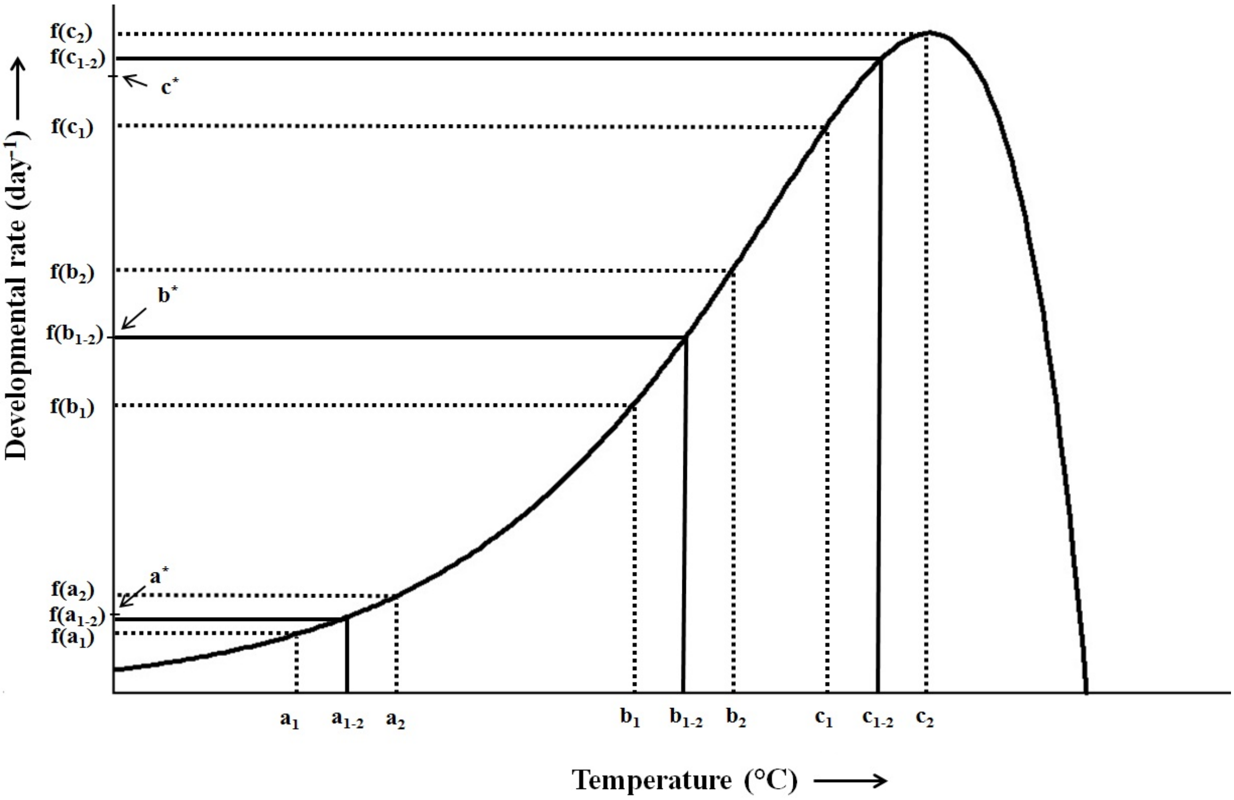

Figure 1.9: Developmental rate-temperature curve (after Pradhan 1945 and Tanigoshi et al. 1976). a_{1-2} is the mean temperature of a_1 and a_2 with a corresponding developmental rate of $f(a_{1-2})$. a* is the mean developmental rate $[f(a_1)+f(a_2)]/2$

1.6.2. Reproduction

Males locate females and female deutonymphs by means of a sex pheromone (Hoy and Smilanick 1979). Males often wait on the back of a deutonymphal female to secure immediate copulation after the female molts to adulthood (Figure 1.10). Phytoseiid females require mating to commence egg formation. Depending on the species, single or multiple matings are necessary to obtain full reproduction (Pappas et al. 2007). For *P. persimilis*, a single mating suffices to maximize the reproductive potential (Amano and Chant 1978). For *N. californicus* and *A. swirskii*, more than one mating is necessary to attain the full egg deposition (Gotoh et al. 2006; Zaher et al. 2007). The reproductive system of phytoseiid mites, called pseudo-arrhenotoky, is unique. Under arrhenotoky, haploid males arise from unfertilized eggs, whereas diploid females develop from fertilized eggs. Hence, arrhenotokous females can adjust the sex of the offspring by controlling the fertilization process. In pseudo-arrhenotokous phytoseiids, males are also haploid, but hatch from fertilized eggs. Somewhere in early embryogenesis, the paternal set of chromosomes is inactivated (heterochromatization) and/or eliminated (Sabelis and Nagelkerke 1988; Nagelkerke and Sabelis 1998).

Figure 1.10: Male *Neoseiulus californicus* waiting on a deutonymph female to molt (Photo: Author)

Figure 1.11: Mating adult pair of *Amblydromalus limonicus* (Photo: Author)

Reproduction is also strongly affected by several biotic and abiotic factors. Again, temperature is an important factor since it affects the oviposition rate and lifetime fecundity of phytoseiid mites. Kim et al. (2013) demonstrated a higher fecundity in *N. californicus* at 24°C than at higher and lower temperatures. Other studies also reported a Gaussian relationship between fecundity and temperature, with a higher fecundity at intermediate to high temperatures and a decreasing fecundity at the lower and higher temperatures tested (e.g. Ali 1998; Lee and Ahn 2000; Gotoh et al. 2004; Broufas et al. 2007; Lee and Gillespie 2011). At 25°C, *P. persimilis* and *N. californicus* had an average lifetime fecundity of 61.7 and 56.7 eggs/female, respectively (Escudero and Ferragut 2005). For *A. swirskii*, Lee and Gillespie (2011) reported an average lifetime fecundity of only 1.3 eggs/female at 15°C and a maximum number of eggs (16.1 eggs/female) deposited at 25°C. But other factors also may affect reproduction. For example, reproduction of the leaf-feeding predatory mite *Euseius* *tularensis* (Congdon) was affected by the level of fertilization of citrus trees (*Citrus sinensis*) (Grafton-Cardwell and Ouyang 1996).

1.6.3. Diapause induction

In order to survive winter times, phytoseiid predatory mites are either resistant against low temperatures (e.g. accumulation of cryoprotectants such as glycerol, Lee et al. (1987)) or go into a reproductive diapause (Danks 1987; Veerman 1992). Tauber et al. (1986) defined diapause as “a neurohormonally mediated, dynamic state of low metabolic activity, which is associated with a reduced morphogenesis, increased resistance to environmental extremes and altered or reduced behavioral activity”. It is induced by a series of environmental stimuli that precede unfavorable conditions such as short daylight and/or low temperatures. Usually, diapause occurs during a genetically determined developmental stage. In phytoseiids, diapause occurs in gravid females with a reproductive arrest caused by prolonging the preoviposition period (Veerman 1992). As a result, using diapause sensitive species during winter, biological control in greenhouses is less effective. Selecting and introducing non-diapausing strains is therefore preferred. For example, strains of *A. andersoni* collected in the Netherlands entered diapause when reared under short-day conditions of 8:16 h (L:D), whereas strains collected from Italy did not (McMurtry et al. 1976). For *N. californicus,* depending on the geographical distribution, some strains enter diapause under short-day photoperiods, whereas others do not (Castagnoli et al. 1996; Jolly 2000; Hart et al. 2002, Gotoh et al. 2005). Selecting for non-diapausing phytoseiid strains can be done by rearing the mites under short-day conditions. In a commercial strain of *N. cucumeris* from Canada, Morewood and Gilkeson (1991) were able to reduce diapause incidence from 88% in the parent generation to 33% in the 14th generation. Similarly, van Houten et al. (1995b) successfully selected for non-diapausing populations of *N. barkeri* and *N. cucumeris* using a short-day photoperiod (10h light at 23°C and 14h dark at 16°C). For *M. occidentalis,* only four generations needed to be reared at a photoperiod of 8:16 h (L:D) (at 19°C) to reduce diapause incidence from 99% to 11% (Hoy 1984).

1.6.4. Feeding habits

McMurtry and Croft (1997) categorized Phytoseiidae into four types according to their feeding habits (Table 1.3). The first group (Type I) assembles the specialist predators of

Tetranychus spp. with *P. persimilis* and *Phytoseiulus macropilis* (Banks) as the most important members. Type II predators comprise oligophagous phytoseiids that prefer to prey on spider mites, such as *N. californicus* and *Neoseiulus fallacis* (Garman). The third group (Type III) represents generalist predators that have a wide range of prey and are able to reproduce on pollen. *Amblyseius swirskii* and *A. limonicus* belong to this group. To prolong survival, they can also feed on plant exudates and honeydew. In addition, there is some evidence that certain species in this group can feed on plant sap. The fourth group (Type IV) includes generalist predators that have a higher reproductive output on pollen than on animal prey, and is mainly represented by mites of the genus *Euseius*.

In 2013, McMurtry et al. revised these categories by including a number of subtypes based on the phytoseiids' prey specificity (for Type I) and preferred microhabitat and morphology (Type III) (see Table 1.3). Type II and IV largely remained the same.

Table 1.3: Classification of Phytoseiidae based on their feeding habitats and preferred microhabitat (after McMurtry and Croft 1997 and McMurtry et al. 2013)

Type		**Sub-type**		**Species example**
I	Specific predators of *Tetranychus* spp.	I-a	Specialized predators of *Tetranychus* (Tetranychidae) that produce a complex web	*Phytoseiulus persimilis*
		I-b	Specialized predators of web-nest producing mites (Tetanychidae)	*Typhlodromus bambusae*
		I-c	Specialized predators of tydeoids (Tydeoidea)	*Paraseiulus* sp.
II	Tetranychid predators - broadly specific			*Neoseiulus californicus*
III	Generalist predators	III-a	Generalists living on pubescent leaves	*Kampimodromus aberrans*
		III-b	Generalists living on glabrous leaves	*Amblyseius swirskii*
		III-c	Generalists living in confined space on dicotylednous plants	*Iphiseius degenerans*
		III-d	Generalists living in confined spaces on monocotyledonous plants	*Neoseiulus baraki*
		III-e	Generalists living in soil/litter habitats	*Neoseiulus cucumeris*
IV	Specialized pollen feeders - generalist predators			*Euseius* sp.

Few studies have focused on how predatory mites feed. When feeding on spider mite prey, chelicerae of phytoseiids (of the genera *Galendromus*, *Neoseiulus*, *Phytoseiulus* and *Typhlodromus* in the study of Flechtmann and McMurtry (1992)) are protracted alternately beyond the apex of the corniculi and this for a distance at least similar to the movable digit's length. While the movable digit cuts into the cuticle, the fixed digit is located on the outside of the prey. The tip of the corniculi enter the body of the prey, but do not seem to play a part in piercing the cuticle. The corniculi seem to be functioning rather as a supporting and guiding device for the salivary styli. Proteolytic enzymes are believed to be injected in the prey for pre-ingestional digestion. Once the prey cuticle has been ruptured, prey fluids are imbibed by contraction and expansion of pharyngeal muscles of the predatory mite (Evans and Till 1965; Flechtmann and McMurtry 1992). Some attempts have been made to correlate the cheliceral morphology with the predatory mite's diet. Buryn and Brandl (1992) reported that predatory mites that mainly feed on arthropod prey possess larger chelicerae than the ones feeding on nematodes. On the other hand, the latter predators tend to have more teeth on their chelicerae. The ability of phytoseiids to feed on plant tissue has been attributed to their cheliceral morphology (Adar et al. 2012). Apparently, plant feeders possess a larger ratio of the dorsal perimeter length of the fixed digit to the ventral perimeter length of the movable digit, than non-plant feeders.

1.6.4.1. Prey food

1.6.4.1.1. Mite prey

Predatory mites of the Phytoseiidae are recognized to be the most important natural enemies of phytophagous mites, of which the two-spotted spider mite, *T. urticae*, is the most important one (Gerson and Weintraub 2012). Phytoseiids also demonstrated to be effective natural enemies of other phytophagous mites, including species belonging to the Tetranychidae, Eriophyoidea, Tenuipalpidae, Tarsonemidae and Tydeidae (Kostiainen and Hoy 1996; Van Leeuwen et al. 2010b; Momen 2011; van Maanen et al. 2010).

1.6.4.1.1.1. Tetranychidae

Many phytoseiids, both specialists and generalists, demonstrate a high reproductive potential on spider mite prey (Gillespie and Quiring 1994; Pratt et al. 1999; Xu and Enkegaard 2010), but few species can cope with the spider mites' dense webbing (van Houten et al. 2008; Messelink et al. 2010). According to the "chaetotaxy hypothesis" formulated by Sabelis and

Bakker (1992), the way a predatory mite can cope with spider mite webbing is related to the length of setae located on the middorsal and marginodorsal regions of its body. These setae avoid contact of the body with sticky silk strands, thereby preventing phytoseiids like *P. persimilis*, from getting entangled in the spider mite webs (Sabelis and Bakker 1992). Over the past 50 years, *P. persimilis* has proven to be a reliable biocontrol agent of *Tetranychus* spider mites in vegetable and ornamental crops (e.g. Chant 1961; Gould and Light 1971; French et al. 1976; Kropczynska et al. 1999; Alatawi et al. 2011). Various studies have shown that *P. persimilis* possesses the highest population growth rates compared to other phytoseiid predators such as *N. californicus* (Sabelis 1981; Takahashi and Chant 1994; Escudero and Ferragut 2005; van Houten et al. 2007). Krips et al. (1999) reported intrinsic rates of increase (r_m) ranging from 0.424 to 0.451 females/female/day when feeding on *T. urticae* on different cultivars of gerbera. *Phytoseiulus persimilis* can complete a generation within 1 week at 25°C and each female is capable of producing 80 eggs (Gerson and Weintraub 2007). However, when temperature exceeds 30°C and humidity is low, this species is not capable of fully controlling spider mites (Lindquist and Short 2004; Weintraub et al. 2006). In these climatic conditions, *N. californicus* has proven to be a more effective predator capable of developing a population at temperatures ranging from 15 to 35°C (Rott and Ponsonby 2000; Gotoh et al. 2004; Palevsky et al. 2008). A combined release of *P. persimilis* and *N. californicus* has been shown to act synergistically for controlling *T. urticae* (Blumel and Walzer 2002; Schausberger and Walzer 2001). However, at spider mite prey densities that are too low, intraguild predation of *N. californicus* feeding on *P. persimilis* has been shown to ultimately result in a depletion of the latter (Walzer et al. 2001). *Neoseiulus fallacis* is another type II spider mite predator, originating from humid regions in North America (Coop and Croft 1995; Strong et al. 1997), which has been demonstrated to suppress spider mite populations in a wide range of crops, such as apple, peppermint, hops and strawberry (Croft and Croft 1993 Strong et al. 1997; Morris et al. 1999).

Although *A. swirskii* has difficulties penetrating the webbing of *T. urticae* (Messelink et al. 2010), this predator will feed on spider mite eggs and nymphs (El-Laithy and Fouly 1992; Rasmy et al. 2004; van Houten et al. 2007; Xu and Enkegaard 2010). A similar result was found for *A. limonicus*, with an oviposition rate of only 0.4 eggs/female when fed *T. urticae* with webbing, whereas the oviposition rate increased to 2.8 eggs/female in the absence of webbing (van Houten et al. 2007).

1.6.4.1.1.2. Eriophyoidea

Worm-like mites belonging to the superfamily of Eriophyoidea comprise the second largest pest mite species complex after tetranychids. Biological control of this group using phytoseiids was reviewed by Van Leeuwen et al. (2010b). Being very minute (40-100µm), eriophyoids can reach places that are small enough to hinder access to predators (Sabelis and Bruin 1996; Aratchige et al. 2007; Lesna et al. 2014). For example, the dry bulb mite *Aceria tulipae* Keifer can move in between the scales of bulbs. Similarly, the coconut mite *Aceria guerreronis* Keifer lives under the perianths of coconut fruits (Aratchige et al. 2007). In both cases, plants under eriophyoid attack respond by changing their internal structure, either by increasing the distance between bulb scales or increasing the gaps between perianth and coconut fruit, allowing entrance of the phytoseiid predators *N. cucumeris* and *N. baraki*, respectively (Lesna et al. 2004; Aratchige et al. 2007). Lesna et al. (2014) reported that the coconut mite predator *Neoseiulus paspalivorus* (De Leon) was a better biocontrol agent of *A. tulipae* inside the bulbs as its smaller size allowed the predator to more easily enter between the scales than *N. cucumeris*.

Amblyseius swirskii has been reported to be an excellent predator of eriophyoid mites such as the tomato russet mite *Aculops lycopersici* (Massee), with a higher intrinsic rate of increase than when fed on *T. latifolia* pollen (Park et al. 2010; Park et al. 2011). Also *N. californicus* (Castagnoli et al. 2003) and *A. limonicus* were found to feed and reproduce on *A. lycopersici*, but only when densities of glandular trichomes on tomato leaves were low (van Houten et al. 2013). These glandular trichomes on tomato have demonstrated to also hinder other phytoseiids such as *P. persimilis* (Vanharen et al. 1987).

Other important eriophyoids on the Phytoseiidae's menu are the citrus russet mite, *Phyllocoptreta oleivora* (Ashmead) (attacked by *Amblyseius victoriensis* (Womersley) (Smith and Papacek 1991) and *Typhlodromus rickeri* Chant (McMurtry and Scriven 1964a)), and the apple rust mite *Aculus schlechtendali* (Nalepa) (attacked by *A. andersoni* (Strapazzon and Monta 1988; Easterbrook 1996) and *N. fallacis* (Johnson and Croft 1981)).

1.6.4.1.1.3. Tarsonemidae

The broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) is a destructive greenhouse pest, both in ornamentals and vegetables (Gerson 1992; Gerson and Weintraub 2012; Luypaert et al. 2014). Several phytoseiids have shown to be good predators and effective control agents of *P. latus*, including *N. californicus* (Peña and Osborne 1996), *N. barkeri* (Fan and Petitt 1994), *N. cucumeris* (Weintraub et al. 2003), *Euseius stipulatus* (Athias-Henriot) (McMurtry et al. 1984) and *Amblyseius largoensis* (Muma) (Rodríguez and Ramos 2004). Several authors demonstrated the potential of *A. swirskii* for the control of *P. latus* (Tal et al. 2007; Audenaert et al. 2009; van Maanen et al. 2010; Onzo et al. 2012). Another important tarsonemid pest mite is the strawberry mite (or cyclamen mite), *Phytonemus pallidus* (Zimmerman). The predatory mites *N. californicus* and *N. cucumeris* have been reported to significantly reduce *P. pallidus* numbers on potted strawberry plants (Easterbrook et al. 2001). Petrova et al. (2000) reported a satisfactory control of the strawberry mite using *N. cucumeris*. In a study on strawberry performed by Croft et al. (1998), *N. fallacis* demonstrated to yield a more rapid control, but *N. cucumeris* provided a more long-lasting control of *P. pallidus*.

1.6.4.1.2. Thripidae

The most important insect prey of phytoseiid predatory mites are thrips (Thysanoptera: Thripidae). Thrips are a major group of destructive virus-vectoring pests in vegetables, fruit, and ornamental crops (Sakimura 1962; Lewis 1997). The first records of phytoseiid mites preying on thrips larvae were reported by MacGill (1939), who found *Typhlodromus thripsi* (now *Neoseiulus cucumeris*, Messelink et al. (2006)) killing *T. tabaci* immatures. After first attempts to control thrips with *N. barkeri* (Ramakers 1980), better success was obtained when *N. cucumeris* was introduced into greenhouses. Especially in sweet pepper (due to the presence of pollen) *N. cucumeris* was found to successfully control *T. tabaci* (De Klerk and Ramakers 1986).

Ever since its establishment in Europe during the late 1970s (Kirk and Terry 2003), the western flower thrips (WFT) *F. occidentals* has been a destructive pest in various ornamental and vegetable crops (Lewis 1997; Kirk 2002). In the Netherlands, *F. occidentalis* was recently considered to be the most problematic greenhouse pest in ornamental crops (Messelink 2014). This pest is a vector of several viruses, including *Tomato Spotted Wilt Virus* (Sakimura 1962; Allen and Broadbent 1986) and rapidly develops resistance against pesticides (Bielza 2008). Besides leaf tissue, this omnivorous pest can also feed on pollen (Kirk 1984) and (extra-floral) nectar (Yokoyama 1978) as well as on animal foods such as spider mite eggs (Trichilo and Leigh 1986) and whitefly crawlers (van Maanen et al. 2012). In addition, *F. occidentalis* is capable of killing eggs of its phytoseiid predators (Janssen et al. 2002). On the other hand, many phytoseiids are predators of western flower thrips, both specialists and generalists. Juvenile *P. persimilis* are able to kill *F. occidentalis* larvae, whereas adult *P. persimilis* were not observed to do so (Walzer et al. 2004). The first successful introduction of a phytoseiid in the control of *F. occidentalis* came with *N. cucumeris* in various greenhouse crops (De Klerk and Ramakers 1986; Ramakers 1988; Gillespie 1989; Shipp and Whitfield 1991), including tomato (Shipp and Wang 2003). The latter phytoseiid can be easily produced in large numbers on the cheese mite, *Tyrophagus putrescentiae* (Schrank) (Kim et al. 2001). However, *N. cucumeris* demonstrates a weak numerical response when prey is present at low densities and has difficulties with establishing in the absence of pollen (Gillespie 1989; Brodsgaard and Hansen 1992).

In a screening performed by Messelink et al. (2006), 9 phytoseiid predatory mites were compared with *N. cucumeris* for the control of western flower thrips on cucumber in greenhouses. A significantly better mite establishment and thrips control was obtained using *A. limonicus, A. swirskii* and *E. ovalis,* with the *A. limonicus* demonstrating the highest predation capacity. Similar results were reported by van Houten et al. (1995a). *Iphiseius degenerans, N. barkeri, E. finlandicus* and *T. pyri* did not establish better than *N. cucumeris*. *Euseius scutalis* also reached high densities on the lower leaves of the cucumber crop, but without controlling *F. occidentalis,* as this pest mainly colonizes the higher leaves of the plant. Since the commercial introduction of *A. swirskii*, this predator quickly became one of the most important natural enemies of WFT (Calvo et al. 2015). Together with its ability to feed on other prey and non-prey foods (e.g. pollen) and the availability of a cost-effective mass-production system, this mite is a key component in current integrated pest management strategies.

An emerging thrips pest in European horticulture is *Echinothrips americanus* (Morgan), which originates from the eastern part of North-America and established in Europe in 1989 (Vierbergen et al. 2006). Biocontrol of this thrips was found to be extremely difficult and non-selective pesticides were deemed to be necessary for control. Opit et al. (1997) reported that

N. cucumeris and *I. degenerans* were incapable of controlling *E. americanus* on pepper plants. In an attempt to explain this, these authors blamed the large body size of this thrips pest -as compared with *F. occidentalis* - as a reason for the failed control. Better results were obtained by Hoogerbrugge et al. (2014) when using *A. swirskii* and *A. limonicus*, but not with *Euseius gallicus* Kreiter and Tixier both in the laboratory and in field tests. Both *A. swirskii* and *A. limonicus* were capable of preying and laying eggs when provided with first instars of *E. americanus*. On rose plants, both predators were capable of reducing population growth of *E. americanus*, whereas on sweet pepper better results were obtained with *A. limonicus*.

1.6.4.1.3. Aleyrodidae

The greenhouse whitefly *Trialeurodes vaporarirum* (Westwood) and the tobacco whitefly *Bemisia tabaci* (Gennadius) are widespread, extremely polyphagous greenhouse pests (Gerling 1990; Byrne and Bellows Jr 1991). These phloem-sucking pests are capable of transmitting viruses and produce honeydew on which sooty molds can develop (Byrne and Miller 1990; Berlinger et al. 1996), thereby reducing the plant's photosynthetic capacity (Yee et al. 1996). The parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) is well-known for its efficacy in the control of whitefly outbreaks (Hoddle et al. 1998). Also several phytoseiid predatory mite species were reported to be predators of both eggs and crawlers of whiteflies (e.g. Teich 1966; El-Badry 1967; Gerling 1990). In 1968, Elbadry reported that *Amblyseius aleyrodis* Elbadry was a good predator of *B. tabaci* eggs and crawlers. Later on, several other phytoseiids were reported as predators of *B. tabaci*, such as *A. limonicus* (Swirski and Dorzia 1968), *Euseius hibisci* (Chant) (Meyerdirk and Coudriet 1985) and *E. scutalis* (Meyerdirk and Coudriet 1986). Nomikou et al. (2001) tested several phytoseiids for their appreciation of whiteflies: *Typhlodromus athiasae* (Porath and Swirski), *N. barkeri*, *A. swirskii*, *E. scutalis* and *Phytoseius finitimus* Ribaga; *E. scutalis* and *A. swirskii* feeding on *B. tabaci* resulted in the highest intrinsic rates of increase (r_m). The good performance of *A. swirskii* on whiteflies was also confirmed when feeding on greenhouse whitefly, *T. vaporariorum* (Messelink et al. 2008). Control of whiteflies in the latter study was further improved when also *F. occidentalis* was present in the system, demonstrating that a combined diet of two pests may result in such numerical response of the predator leading to an overall better control.

1.6.4.1.4. **Cannibalism**

Cannibalism is an ubiquitous trait in Phytoseiidae (reviewed by Schausberger 2003). As a result, it is a well-studied topic in phytoseiid predatory mites. However, only few studies report on possible effects of cannibalism on the population levels of phytoseiids, or on biological control success (Walde et al. 1992; Schausberger and Walzer 2001; Walzer et al. 2001). Both costs and benefits are associated with cannibalism. The benefits of cannibalism can be (1) obtaining nutrients in times of food scarcity, (2) eliminating intraspecific competition for food, (3) decreasing intraspecific potential in competition for oviposition sites and/or shelter, (4) enhancing survival of related individuals by killing/eliminating unrelated individuals, or (5) reducing fitness of other individuals of the same sex to reach superiority in reproductive competition (Elgar and Crespi 1992; Schausberger 2003). Potential costs may entail (1) risks that the attacked prey is a counterattacking predator, (2) potential of transmitting parasites and diseases, or (3) loss of inclusive fitness when a genetically related individual is consumed (Elgar and Crespi 1992; Pfennig et al. 1998). A key player in the evolution of cannibalism is kin-recognition (Hepper 1986; Waldman 1988; Schausberger 2007). In Phytoseiidae, several species have been demonstrated to discriminate not only between con- and heterospecific eggs, but were also able to distuingish the level of relatedness between conspecific eggs. Female *I. degenerans* preferred to deposit their eggs near conspecific eggs over depositing near heterospecific eggs (Faraji et al. 2000). Adult *P. persimilis* and *P. macropilis* preferred consuming unrelated larvae over related larvae (Schausberger and Croft 2001). Protonymphs of *P. persimilis* preferred cannibalizing non-kin over sibling larvae (Schausberger 2007). In a study performed by Schausberger and Croft (2000), the nutritional benefits of cannibalistic behavior in both specialist (*P. macropilis, G. occidentalis* and *N. longispinosus*) and generalist (*A. andersoni, N. cucumeris* and *N. fallacis*) phytoseiids were assessed. The tested generalists and *P. macropilis* were able to complete their juvenile development on conspecific larvae and eggs. Only a few individuals of the specialists *N. longispinosus* and *M. occidentalis* were able to reach adulthood. In terms of oviposition, generalists were able to produce and deposit eggs when fed on conspecific prey, whereas specialist phytoseiids were not able to produce eggs. Female *A. swirskii* were reported to feed on conspecific protonymphs (Rasmy et al. 2004), but no cannibalism was observed when conspecific eggs were presented (Momen and Abdel-Khalek 2009). Although eggs would be the ideal victims to cannibalize - as eggs cannot counterattack - some species have difficulty penetrating the egg chorion. Schausberger (2003) discussed that this was due to either non-adapted mouthparts that are unable to pierce the chorion or to a predator-deterring substance on the chorion. The latter hypothesis was also put forward by Palevsky et al. (1999), who found that levels of egg cannibalism in *T. athiasae* and *N. californicus* were significantly increased by soaking the eggs in deionized water. Larvae are the preferred victim's developmental stage to cannibalize, as they are slower and cannot easily escape from larger individuals (Schausberger 2003).

1.6.4.1.5. Intraguild predation

Phytoseiidae are also well-known to kill and consume heterospecific predatory mites. Walzer and Schausberger (1999a) showed that *N. californicus* was a strong intraguild predator of *P. persimilis*, able to reproduce when fed eggs of *P. persimilis*. Vice versa, *P. persimilis* was able to kill eggs and larvae of *N. californicus,* but no oviposition was observed. In a study performed by Buitenhuis et al. (2010) *A. swirskii* even preferred to kill *N. cucumeris* larvae over first instar larvae of *F. occidentalis*. Other studies demonstrated that a combined use of *P. persimilis* with more generalist phytoseiids ultimately results in the reduction of the *P. persimilis* populations (Pruszynski and Cone 1972; Yao and Chant 1989). Many studies have demonstrated that phytoseiids can survive, develop and reproduce well on heterospecific phytoseiid prey (Schausberger and Croft 2000; Rasmy et al. 2004; Momen and Abdel-Khalek 2009). As was also mentioned in the previous section on cannibalism, the ability to discriminate between con-and heterospecific prey is an important trait determining the outcome of intraguild interactions. The polyphagous predatory mites *T. pyri* and *K. aberrans* were able to discriminate between individuals of their own and other species, subsequently preferring the latter as prey (Schausberger 1999). For other polyphagous phytoseiids, *N. californicus*, *A. andersoni*, *N. fallacis* and *N. cucumeris*, Schausberger and Croft (1999b) reported that juveniles were able to develop, and adults to maintain reproduction by feeding on heterospecific eggs. In general, the ability to feed, develop and reproduce on heterospecific prey increases along with an increasing level of polyphagy. Relative body size is another important trait that determined the outcome of IGP. Zhang and Croft (1995) found that the relatively large *A. andersoni* outcompeted other smaller and less polyphagous phytoseiids, such as *T. occidentalis*, *N. fallacis* and *T. pyri*.

The most vulnerable developmental stages in intraguild interactions between phytoseiid predatory mites appear to be larvae and nymphs and to a lesser extent the eggs (see also section 1.6.4.1.4.). In a study performed by Walzer and Schausberger (1999b), adult females of *P. persimilis* and *N. californicus* preferred heterospecific larvae over heterospecific eggs.

1.6.4.2. Alternative food

1.6.4.2.1. Plant tissue

Several phytoseiids, mainly species belonging to type IV, have the capacity to feed on leaf tissue (McMurtry and Croft 1997a). In the first report on leaf feeding by phytoseiids, *Typhlodromus rhenanus* (Oudemans) and *Euseius finlandicus* (Oudemans) proved to be leaf feeders as their digestive tracts were colored after exposure to leaves treated with systemic dyes (Chant 1959). Using plants labeled with radioactive phosphoric acid, Porres et al. (1975) found that *E. hibisci* was able to feed on avocado leaves. In contrast, *E. stipulatus* and *E. fructicolus* (Gonzales and Schuster) were not able to feed on leaves of lemon or avocado. Kreiter et al. (2002) demonstrated leaf feeding by *K. aberrans* on *Celtis australis* plants (European nettle tree) marked with rubidium. Similary, Magalhaes and Bakker (2002) reported evidence of *Typhlodromalus aripo* DeLeon feeding and subsequently dying on cassava plants treated with the systemic insecticide aldicarb, whereas no increased mortality was observed for the spider mite predators *Neoseiulus idaeus* Denmark and Muma and *P. persimilis*. Using the same experimental set up, Nomikou et al. (2003b) demonstrated leaf feeding on cucumber by *E. scutalis*, but not by *A. swirskii*. Leaf feeding in *E. scutalis* was also observed in the presence of pollen. Damage through phytoseiid feeding was found for *T. pyri* with minute feeding scars on apple fruits (Sengonca et al. 2004) and for *E. scutalis* on young pepper plants when present in unrealistically large numbers (Adar et al. 2015). The ability of phytoseiids to feed on plants has been attributed to their cheliceral morphology (Adar et al. 2012).

1.6.4.2.2. Pollen

The ability of phytoseiids to feed on pollen has been documented extensively (e.g. McMurtry and Scriven 1965; van Rijn and Tanigoshi 1999b; Goleva and Zebitz 2013). Although not all phytoseiid mite species feed on pollen (i.e. type I specialists), type II (oligophagous spider mite predators) and type III (generalist) predators do feed on pollen, although there is a preference for, or better performance on, a diet of animal prey (McMurtry and Croft 1997a). Type IV comprises specialized pollen feeders showing the best performance on pollen. Not all pollen species are suitable as a food source for phytoseiids. This was clearly demonstrated by van Rijn and Tanigoshi (1999b) who tested 25 species of pollen for *N. cucumeris* and *I.*

degenerans. Clear intra- and interspecific differences emerged in the appreciation of the tested pollen. A low oviposition rate was observed when both mites were fed on *Pinus sylvestris* pollen, whereas pollen of for example *Vicia faba* resulted in high oviposition rates for both species. When fed on pollen of common hazel, *Corylus avellana*, oviposition rates of *I. degenerans* were similar to that on *V. faba* pollen, whereas *N. cucumeris* did not lay eggs on hazel pollen. Some pollen were found to be toxic, such as pollens of the martagon lily, *Lilium martagon* and *Hippeastrum* sp. which were found to cause 100% juvenile mortality, probably due to secondary plant metabolites (Goleva and Zebitz 2013). Pollen contain proteins, free amino acids, lipids, carbohydrates and vitamins in various proportions (Pacini et al. 2006; Lundgren 2009). For example protein content can range from 2.5 up to 60% depending on the pollen species (Roulston and Cane 2000). Although Lundgren (2009) noted that protein intake is a requisite for reproduction, Delisle et al. (2014) found that the amount of soluble protein was not a key determinant of pollen quality for *N. cucumeris* and *A. swirskii*. One could postulate that entomophilous (insect-pollinated) plants reward their pollinators with high quality pollen that more closely meets their nutritional needs than anemophilous (wind-pollinated) plants do (Roulston and Cane 2000). However, several studies show conflicting results. Whereas Petanidou and Vokou (1990) stated that entomophilous pollen has a higher energetic content than anemophilous pollen, some studies reported no significant nutritional differences between pollen from the different pollination types (Colin and Jones 1980; Solberg and Remedios 1980; Goleva and Zebitz 2013).

Bee-collected pollen are commercially available and could thus be an easily available food source for the rearing of Phytoseiidae. Moreover, using bee pollen may reduce labor costs for collecting the pollen, as compared to the labor-intensive collection of fresh plant pollen (van Rijn and Tanigoshi 1999b). However, dietary tests using bee pollen are inconsistent. A drawback of bee pollen is that it needs to be refreshed daily, as the pollen granules absorb water from the air, thereby affecting their nutritional value and enhancing fungus growth. Bees regurgitate nectar to mix with the collected pollen for transport on their legs (Roulston and Cane 2000). Additionally, more than one pollen species may be present in the pollen pellets. Goleva and Zebitz (2013) found that commercial bee pollen could not improve life table parameters compared to fresh plant pollen. For *I. degenerans* and *N. cucumeris*, some bee pollen resulted in high oviposition rates, such as bee-collected pollen from *Eucalyptus* sp., whereas bee pollen from other plants resulted in much lower oviposition rates (van Rijn and Tanigoshi 1999b). Kolokytha et al. (2011) found a slow development and low fecundity for *Typhlodromus athenas* Swirski & Ragusa fed on bee pollen compared to plant collected pollen. For *E. stipulatus*, a suspension of bee pollen in tap water resulted in an equally high oviposition rate as maize pollen (0.96 and 1.15 eggs/day, respectively) (Montserrat et al. 2013).

1.6.4.2.3. Extrafloral nectar

Extrafloral nectaries are believed to be evolved as a plant-predator mutualism (Wäckers 2005; Heil 2015) and have been described in over 1000 plant species (Koptur 1992). Extrafloral nectar (EFN) comprises an aqueous solution of mainly mono- and disaccharide sugars such as glucose, fructose, and sucrose, and to a lesser extent amino acids, lipids and enzymes (Heil 2015). Plants provide nectar to their bodyguards in exchange for protection against herbivores and are able to increase their nectar production upon herbivory, even actively. For example, leaf feeding by *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) larvae significantly increased the EFN production in cotton (*Gossypium herbaceum*) and castor (*Ricinus communis*) (Wäckers et al. 2001). Lima bean plants (*Phaseolus lunatus*) attacked by *T. urticae* demonstrated higher levels of EFN than uninfested plants (Choh and Takabayashi 2006).

Nectar-feeding in predatory mites has been reported repeatedly. Walter et al. (1995) found that the survival of *M. occidentalis* significantly decreased when nectaries on shoots of *Viburnum tinus* L. were excised. Bakker and Klein (1992) showed that EFN that was exuded from cassava (*Manihot esculenta*) petioles increased both juvenile and adult survival of *A. limonicus*. Later, van Rijn and Tanigoshi (1999a) reported that EFN of *R. communis* substantially increased the survival of *I. degenerans*. When EFN was added to a diet of castor pollen oviposition rate of *I. degenerans* increased from 1.7 to 2.2 eggs/female/day. Even the specialist spider mite predator *P. persimilis* showed a higher survival on and a lower dispersal rate from lima beans (*P. lunatus*) that were exposed to *T. urticae*-induced plant volatiles, increasing the secretion of EFN (Choh et al. 2006).

1.6.4.2.4. Honeydew

Phloem-feeders, such as aphids and whiteflies, excrete sugar-rich waste products as a result of the nutritional imbalance of the phloem sap they imbibe. Usually, when the ratio of carbohydrates over amino acids exceeds the nutritional requirements of phloem-feeders, the excess of carbohydrates is excreted as honeydew (Sandström and Moran 2001; Wäckers 2005). Although the primary function is to discard waste products, honeydew also functions as a sugary reward for ants, which in turn protect aphids from their natural enemies (Way 1963; Völkl et al. 1999; Styrsky and Eubanks 2007). Besides ants, about 250 other insect species were reported to consume honeydew (in a natural environment) (Zoebelein 1956). Also phytoseiid predatory mites are known to feed on honeydew (van Rijn and Tanigoshi 1999a and references therein), although its nutritional value can be rather low (Ragusa and Swirski 1977). Honeydew is considered to be an alternative food source that enhances survival and oviposition when combined with natural prey. For example, Chant and Fleschner (1960) reported that aphid honeydew enabled *A. hibisci* and *A. limonicus* to live longer and even reproduce, whereas this was not the case for honey and leaf-only experiments. An increased oviposition and higher proportion of juveniles reaching adulthood was demonstrated for *A. hibisci* when honeydew of *Pseudococcus citri* Risso (Hemiptera: Pseudococcidae) was added to a diet of *Panonychus citri* (McGregor) mites (McMurtry and Scriven 1964b). McMurtry and Scriven (1964a) showed an increased survival for *T. rickeri* feeding on mealybug honeydew (*Pseudococcus adonidum* L.), however, egg laying was negligible. Similarly, Swirski et al. (1967) reported a low level of egg laying by *A. rubini* and *A. swirskii* on the honeydew of another mealybug, *Pseudococcus citriculus* Green. For three *Euseius* species (*E. tularensis*, *E. stipulatus* and *E. hibisci*), Zhimo and McMurtry (1990) demonstrated an increased oviposition rate when either aphid (*Aphis fabae* Scopoli) or whitefly (*Aleurothrixus floccosus* (Maskell)) honeydew was supplemented to a pollen or mite prey diet. In *E. scutalis*, *B. tabaci* honeydew increased survival and allowed some development and egg laying (Nomikou et al. 2003b). In the latter study, *A. swirskii* showed a high juvenile mortality when provided with whitefly-produced honeydew, but only few eggs were laid by the female mites.

1.6.4.3. Factitious prey

Many commercially available phytoseiid predatory mites are being mass-produced on so-called factitious or unnatural prey. This can be defined as a prey that would normally not be encountered in a natural habitat (Zhang 2003). Many generalist phytoseiids feed, develop, and reproduce well on factitious prey mites (Ramakers 1983; Bolckmans and van Houten 2006). The success of phytoseiid predatory mites as biological control agents is partly due to the possibility to cost-effectively mass rear them on prey mites (Ramakers et al. 1989; Midthassel et al. 2013). The dust mite *Dermatophagoides farina* Hughes was found to be a suitable alternative prey for *N. cucumeris* and *N. californicus* (Castagnoli 1989; Castagnoli et al. 1999). Although this prey mite showed a good developmental and reproductive performance for the predators and could be reared on a cheap artificial diet, dust mites can provoke serious allergic reactions in humans (Stewart 1995). In a screening of astigmatid mites for mass-rearing *N. californicus* performed by Castagnoli et al. (2006*)*, *Lepidopglyphus destructor* (Schrank) was found the most suitable prey mite. Other promising prey mites were *Glycophagus domesticus* (De Geer) and *Acarus siro* L., although to a lesser extent than *L. destructor*. Simoni et al. (2006) reported higher intrinsic rates of increase when *N. californicus* was fed on *L. destructor* than on *A. siro*, in the first generation as well as after 18 months of consecutive rearing on the same prey mite. The dried-fruit mite *Carpoglyphus lactis* L. and *Suidasia medanensis* (Oudemans) were found to be a good food source for *A. swirskii* (Bolckmans and van Houten 2006; Hoogerbrugge et al. 2008; Smytheman 2011; Midthassel et al. 2013; Nguyen et al. 2013).

Vantornhout et al. (2004) reported that eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and decapsulated cysts of the brine shrimp *Artemia franciscana* Kellogg (Branchiopoda: Artemiidae) were a suitable food source for the development of the predatory mite *I. degenerans*, although development was slower than on natural prey. If the cysts were not decapsulated, development was not possible beyond the protonymphal stage. Possibly, the outer alveolar layer of encapsulated cysts was too thick for *I. degenerans* to pierce (Van Stappen 1996). Nguyen et al. (2014b) showed that both *E. kuehniella* eggs and decapsulated *A. franciscana* cysts were a good food source to support development and reproduction of *A. swirskii*. Leman and Messelink (2015) found similar results for *A. swirskii* when fed on *E. kuehniella*, but oviposition rates were substantially lower on *Artemia* cysts. For *A. limonicus* both *E. kuehniella* eggs and *Artem*ia cysts resulted in significantly lower oviposition rates when compared to those on pollen. In the experiments of Nguyen et al. (2014b), *A. franciscana* cysts were not hydrated before use, whereas this was the case in the study of Leman and Messelink (2015). The suitability of *E. kuehniella* eggs was tested for *N. barkeri*, *Typhlodromus balanites* El-Badry and *Amblyseius zaheri* Yousef & El-Borolossy by Momen and El-Laithy (2007). The flour moth eggs were found to be an excellent food for *N. barkeri* and *A. zaheri*, whereas *T. balanites* failed to develop to adulthood.

1.6.4.4. Artificial diets

Whereas many artificial diets have been developed for predatory arthropods (Cohen 2003; Riddick 2009), research on artificial diets for predatory mites is relatively scant (Singh 1977). One of the earliest attempts to create an artificial diet for phytoseiids was that by McMurtry and Scriven in 1966. Increased developmental times and lower oviposition rates were observed for *A. limonicus*, *A. hibisci*, *M. occidentalis* and *T. rickeri* when they were fed on several diets composed of sucrose, molasses, yeast mixed with sucrose and yeast mixed with molasses, as compared to being fed a diet of pollen and prey mites. Later, Kennett and Hamai (1980) tested a liquid artificial diet that consisted of honey, yeast, sugars, egg yolk and casein hydrolysate for several phytoseiids. For *A. limonicus*, *E. hibisci* and *I. degenerans* immature survival rates were >75%; however, oviposition rates were under 50% of those on a reference diet of pollen or mite prey. The specialist spider mite predator *P. persimilis* was unable to survive and develop on the artificial diet. In another study, *P. persimilis* could develop to adulthood on an artificial diet, but failed to produce offspring (Shehata and Weismann 1972). Ochieng et al. (1987) was able to produce the predatory mite *Amblyseius teke* Pritchard and Baker for over 25 generations when fed on an artificial diet consisting of milk powder, bee honey, egg yolk and Wesson's salt. For *A. swirskii* and *Amblyseius gossipi* El-Badry, Abou-Awad et al. (1992) developed a diet composed of yeast, milk, cysteine, proline, arginine, sucrose, glucose, sorbic acid and streptomycin sulphate. Longevity of *A. swirskii* was found to be twice as long, with an oviposition rate of 75% of that of a natural diet. For *A. gossipi*, oviposition rate on the artificial diet was about 50% lower as compared to the natural diet. The spider mite predator *N. californicus* was able to develop on a diet that consisted of honey, sucrose, tryptone, yeast extract, fresh egg yolk and water, but again a substantially lower fecundity was obtained (Kennett and Hamai 1980).

Recently, better success was achieved by Nguyen et al. (2013) who developed a liquid artificial diet for *A. swirskii* that consisted of sucrose, honey, yeast extract, tryptone, egg yolk and water. This diet allowed *A. swirskii* to successfully develop to adulthood, but reproduction was significantly lower than on control diets of *Typha latifolia* L. pollen and *C. lactis*. This basic diet could be improved substantially by adding pupal hemolymph of the oak silk worm, *Antheraea pernyi* (Guérin-Méneville) (Lepidoptera: Saturniidae), yielding an oviposition rate and total fecundity higher than that on pollen or *C. lactis*. Further study showed that this pupal hemolymph could be replaced with an extract of brine shrimp cysts, *A.*

franciscana, to further reduce the production cost of the diet (Nguyen et al. 2014b). The diet containing *Artemia* cysts also supported the development and reproduction of *A. limonicus*, *A. andersoni* and *N. cucumeris* (Nguyen et al. 2015). Hemolymph of the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomydiae) was also found to be a nutritionally valuable additive to improve the basic artificial diet for *A. swirskii* (Nguyen et al. 2014a). In another study, Nguyen et al. (2014c) demonstrated that lyophilizing liquid diets did not negatively affect development and reproduction for *A. swirskii*. Such dry diets may have several advantages in terms of storage and application to serve as a supporting food source in the crop after release.

1.6.5. Phytoseiids as biological control agents

From 2014 onwards, professional users of chemical pesticides in Europe are obliged to implement the principles of integrated pest management (IPM) imposed by European Directive 2009/128/EC. This directive aims at a strong reduction in the use of chemical pesticides. At the same time, the increasing number of cases of pesticide resistance in greenhouse pests have forced growers to use alternative pest control measures. Further, there is an ever-increasing consumer demand for pesticide-free agricultural products (Dabbert et al. 2004; Gerson and Weintraub 2007). Biological control is a key component in IPM strategies (Pilkington et al. 2010) and predatory mites represent the second largest group of biological control agents after hymenopteran parasitoids since 1990. Six predatory mite species are ranked among the most used biological control agents in augmentative biological control, namely the phytoseiids *A. swirskii*, *N. cucumeris*, *P. persimilis*, *N. californicus* and the laelapid soil predatory mites *Gaeolaelaps aculeifer* (Canestrini) and *Stratiolaelaps miles* (Berlese) (van Lenteren 2012; Gerson 2014; Buitenhuis et al. 2015). Worldwide 2709 species in 91 genera of Phytoseiidae have been described (Demite et al. 2014) and the potential as biocontrol agents of the majority of species still needs to be assessed.

Several characteristics contributing to the success of phytoseiid biocontrol agents are discussed below.

1. Mass production

Cost effectiveness of augmentative releases of natural enemies is quintessential for the adoption of biological control (Collier and Van Steenwyk 2004), especially in comparison to the use of relatively inexpensive pesticides. The ability to mass rear predacious mites on prey mites substantially contributes to their economic value as pest control agents. Generalist predatory mites can easily be produced in large numbers and at high densities on astigmatid prey mites (Zhang 2003; Midthassel et al. 2013) (see also section 1.6.4.3.). However, not all Phytoseiidae can be produced on prey mites. In some cases, a tritrophic system consisting of host plant, herbivorous prey, and predator is required, which usually entails a higher production cost due to requirements for labor and space (e.g. *P. persimilis* reared on bean plants infested with *T. urticae* in greenhouses).

2. Searching capacity

For any natural enemy, searching capacity is one of the most important traits underlying its success (Hokkanen 1989; Gerson et al. 2008). Like other carnivores, phytoseiids are attracted by blends of herbivore-induced plant volatiles (HIPV) emitted by plants as an indirect defense mechanism (Dicke and Sabelis 1987; Maeda et al. 1998). For example, *P. persimilis* is attracted by the volatiles emitted by plants that are being attacked by spider mites (Dicke et al. 1990). Moreover, *P. persimilis* is able to discriminate between volatiles released by plants infested with *T. urticae* versus other, non-prey herbivores (De Boer et al. 2004c). Apparently, methyl salicylate plays an important role in the detection of spider mite patches (de Boer and Dicke 2004a; De Boer and Dicke 2004b). Manjunatha et al. (1998) reported that the plant volatile germacrene-D plays an important role in the response of *N. cucumeris* to chrysanthemum (*Denranthemct morifolium*) plants infested with *F. occidentalis*. In *A. swirskii*, experience was necessary for the mites to recognize the volatiles emitted by plants infested with whiteflies (*B. tabaci*), but this acquired response diminished after a few hours (Nomikou et al. 2005). The searching capacity of predatory mites depends on predator and prey species, density of the prey, type and developmental stage of the host plant and several abiotic factors (e.g. light and temperature) (Maeda et al. 2000; Dicke and Baldwin 2010).

3. Specialist versus generalist feeding

Although earlier it was assumed that the most optimal biocontrol agents were specialists (Rosen and Huffaker 1983; DeBach and Rosen 1991; Hoy 1994), there has been quite some debate on whether this conventional idea holds true (Symondson et al. 2002; Janssen and Sabelis 2015). The main concerns for not using generalist predators were their possible feeding on non-pest species, unsynchronized dynamics with the pest, and commonly lower population increase (Murdoch et al. 1985; van Lenteren et al. 2003; Janssen and Sabelis

2015). As a result, the specialist spider mite predator *P. persimilis* was, for several decades, the only phytoseiid to be successfully commercialized (Cock et al. 2010). Today, within acarine biocontrol agents both specialist (type I) and generalist (Type II, III and IV) predators have proven to be successful biocontrol agents (McMurtry and Croft 1997a). On one hand, specialists only forage for and kill their target prey without interfering with alternative prey. On the other hand, such predators will go extinct once the prey has been eradicated. Therefore, repeated introductions will be necessary for successful control. It has been suggested that an increased application of generalist predators may contribute to a more sustainable pest management with less introductions, as they are able to survive and persist by feeding on several prey and non-prey foods (Symondson et al. 2002; Messelink et al. 2014). Indeed, the high level of polyphagy was one of the properties mentioned by Calvo et al. (2015) making *A. swirskii* such a successful biocontrol agent. This change in perception was further demonstrated with the recent launching of the type IV pollen feeder *E. gallicus* for the control of thrips (Biobest 2014; Pijnakker et al. 2014).

1.7. Release techniques for predatory mites

An efficient release technique for natural enemies is a prerequisite to optimize their applicability. Mites can be introduced in the crop using several release techniques. The release strategy is linked to the need for preventive or curative use. When pest numbers are high, predatory mites are being released in high numbers, whereas at a lower prey density or in a preventive strategy, lower numbers of predators are needed.

Phytoseiids are usually distributed in the crop using bran or vermiculite as a carrier material, with or without one or more species of prey mites (Biobest 2015, Koppert 2015, Syngenta Bioline 2015). This mixture can be distributed on the crop plants by manual application using cardboard or plastic dispensers. The bran mixture can also be distributed using automatic blower devices (Opit et al. 2005; van Schelt et al. 2008), without compromising the viability of the phytoseiids (Pezzi et al. 2015). The mites (with carrier material and, if present, prey mites) are dispersed in the air and the mixture is subsequently accelerated by ventilation or a stronger air stream (van Schelt et al. 2008) (Figure 1.12). A critical issue is to achieve an even distribution in the crop and to obtain a high coverage of the crop leaves.

Figure 1.12: Mini-airbug and Aerobug marketed by Koppert B.V. to distribute predatory mites in the crop (left: Koppert, 2015; right: Photo author)

Figure 1.13: Biobolo® predatory mite dispenser marketed by Biobest N.V. (Biobest 2015)

An automatic predatory mite dispenser that can be fixed on a spray boom or other transport system in the greenhouse was developed by Biobest N.V. (Biobolo®, Figure 1.13). By using regular rotary motions, predatory mites are distributed homogeneously in the crop. By adjusting the rotation speed, the acquired number of predatory mites needed in the crop can be adjusted (Biobest 2015).

Another method consists of distributing slow-release sachets in the crop containing a carrier material such as wheat bran with prey mites (Figure 1.14) (Jacobson et al. 2001). These prey mites can be *C. lactis*, *S. medanensis* or *Thyreophagus entomophagus* (Laboulbène and

Robin) for *A. swirskii* (Bolckmans and van Houten 2006; Midthassel et al. 2013; Calvo et al. 2015) or *T. putrescentiae* for *N. cucumeris* (Buitenhuis et al. 2014a). For several weeks, predatory mites can feed and reproduce in the sachet. The mites can leave the sachet through a small hole punctured in the sachet, and disperse into the crop until the resources in the sachet are depleted (Midthassel et al. 2014). The greenhouse conditions are a crucial factor to maximize dispersal rates from the sachets. Buitenhuis et al. (2014a) found that by shading the sachet with plant canopy, temperature peaks were mitigated and humidity was higher than in exposed sachets, resulting in better dispersal of *N. cucumeris*.

Figure 1.14: Sachet for the release of *N. cucumeris* (Syngenta 2015)

1.8. Conservation of predatory mites

In conservation biological control, the environment of natural enemies may need to be manipulated to increase the efficacy of generalist predators (Landis et al. 2000; Eilenberg et al. 2001; Jonsson et al. 2008). This can be obtained by mitigating detrimental conditions or ameliorating favorable conditions (Barbosa 1998). As an example of reducing the more harmful conditions, selective pesticides can be used instead of broad spectrum pesticides (Ruberson et al. 1998). Habitat management to improve the persistence of beneficials is based on the provisioning of refuges, hibernation or aestivation sites, alternative host plants or prey as well as plant resources, such as pollen or (extra)floral nectar (Landis et al. 2000). Females of *N. fallacis* and *N. californicus* have been reported to overwinter in ground cover and debris beneath apple trees (Johnson and Croft 1981; Nyrop et al. 1994; Raworth et al. 1994; Stanyard et al. 1997). Kawashima and Jung (2011) used artificial microstructures as an overwintering site for *Neoseiulus makuwa* (Ehara) and *N. womersleyi* in apple orchards.

Protected crops are characterized by a closed environment with a low level of plant diversity. Pests in greenhouses usually cause more damage inside the greenhouse than outside. This can be attributed to the warm and moist environment and the lack of natural enemies or pathogens (Pilkington et al. 2010). Additionally, excessive pesticide use has led to the development of pesticide resistance (Bielza 2008). As a result, the adoption of biocontrol-based integrated pest management strategies has become more and more important in this cultivation system. In greenhouse crops, mass-reared natural enemies are released periodically, which is called augmentation biological control (van Lenteren 2012). Messelink et al. (2014) compiled a list of practices that could improve establishment of natural enemies in order to reduce the often high costs associated with frequent (e.g. weekly) releases. We will briefly discuss the methods listed by these authors with an emphasis on their relevance for phytoseiid predatory mites.

1.8.1. Pest-in-first

In this strategy, growers allow small infestations of the pest, giving the predator the chance to establish and disperse in order to prevent pest outbreaks (Markkula and Tiittanen 1976; Havelka and Kindlmann 1984; Waite 2001). For *P. persimilis*, this strategy has been successful and economically viable to establish the predator in strawberry and pepper crops, thereby preventing outbreaks of spider mites (Jarošík and Plíva 1990; Waite 2001). However, it is obvious that growers are skeptical about intentionally introducing pests in their crop and often consider this strategy too risky (Starý 1993; Huang et al. 2011).

1.8.2. Banker plant system

The earliest banker plant systems were actually an attempt to fine-tune the pest-in-first technique (Parr and Stacey 1975), as the banker plant and crop were the same plant species. Banker plants (or open-rearing systems) are usually non-crop plants that have been infested with a non-pest herbivore or provide alternative foods (such as pollen) for the released parasitoid or predator (Bennison and Corless 1993; Ramakers and Voet 1995; Osborne et al.

2005; Frank 2010; Huang et al. 2011). In this strategy, advantages of both augmentation and conservation biological control are combined, aiming at a preventive and long-term suppression of the pest (Frank 2010; Xiao et al. 2012).

A widely used method consists of monocotyledonous plants infested with cereal aphids. These aphids serve as an alternative host for parasitoids or predators of aphids attacking the crop. As these cereal aphids are specific for monocotyledons, they constitute no threat to the dicotyledonous crop (Huang et al. 2011; Messelink et al. 2014). For the thrips predator *I. degenerans*, *R. communis* plants were demonstrated to be an excellent host plant with the provisioning of both pollen and extra-floral nectar resulting in a 100-fold increase of predators after 4 months (Ramakers and Voet 1995). In spite of these high numerical increases, dispersal of *I. degenerans* into a sweet pepper crop was relatively slow (Ramakers and Voet 1996). A similar result was obtained with *A. swirskii*, the highest density being close to the *R. communis* banker plant and a decreasing numbers of predator deeper into the crop (tested on sweet pepper, eggplant and cucumber) (Messelink et al. 2005) (Figure 1.15). A disadvantage of using *R. communis* is its attractiveness for pollen-feeding thrips such as *F. occidentalis*. In a recent study performed by Kumar et al. (2015) higher numbers of *A. swirskii* were counted on a pepper crop when using ornamental pepper (*Capsicum annuum* cv. Explosive Ember) as a banker plant compared to a direct inoculation of predators on the crop plants. In another study, Xiao et al. (2012) tested three varieties of ornamental pepper to serve as banker plants for *A. swirskii*. Dispersal from these ornamental pepper plants resulted in a significant suppression of *B. tabaci*, *F. occidentalis* and the chilli thrips, *Scirtothrips dorsalis* Hood on sweet pepper crops. According to Pratt and Croft (2000), banker plants should be mobile to enhance the dispersal rate of the predators. Moreover, mobile banker plants can be replaced when pesticides or fertilizers need to be applied. The successful adoption of a banker plant system by growers depends on the labor needed to cultivate, handle and maintain the banker plant, as well as on its susceptibility to plant diseases and pests. Moreover, the banker plant should be well adapted to the greenhouse environment (e.g. tolerance to high temperatures) (Huang et al. 2011).

Figure 1.15: Young *Ricinus communis* banker plants in a sweet pepper crop (Messelink et al. 2005)

1.8.3. Importance of the host plant

A key issue in habitat management as a part of conservation biological control is checking whether a plant is a suitable host plant for the released parasitoids and predators (Landis et al. 2000; Gurr et al. 2005; Wäckers et al. 2005; Jonsson et al. 2008). Tomato plants are a good example of crop plants upon which predatory mites have difficulties to establish, mainly due to the presence of sticky glandular trichomes (Vanharen et al. 1987; Cedola et al. 2001; van Houten et al. 2013). Establishment of predacious phytoseiids is more successful in sweet pepper (*C. annuum*) (Ramakers 1988; van Houten and van Stratum 1995; Bolckmans et al. 2005; Arthurs et al. 2009). This is not surprising, as sweet pepper plants not only provide pollen but also possess domatia, which can function as both shelter and oviposition sites for predatory mites (Ferreira et al. 2008b) (see also section 1.3.1.1.). Buitenhuis et al. (2014b) reported that trichome density affected walking speed and predation rate of *A. swirskii*, which may explain the differences in efficacy of this species on the tested host plants. Recently, Kumar et al. (2015) tested 29 pepper cultivars, of which four were able to sustain *A. swirskii* during the experimental period when an initial application of pollen (*Typha* sp.) was done.

Besides functional plant structures, plant foods aid in the establishment of natural enemies. Selecting plant species that possess these food rewards may improve the establishment of biocontrol agents (Albajes and Alomar 1999; Gurr et al. 2005; Koptur 2005; Wäckers 2005).

1.8.4. Herbivore-induced plant volatiles and semiochemicals

Herbivore-induced plant volatiles (HIPVs) released by plants upon attack, guide natural enemies towards their prey and/or hosts (Paré and Tumlinson 1999). Many cases of active interaction between herbivore-attacked plants and their parasitoids and predators have been recorded in various agro-ecosystems. Phytoseiid predators lack eyes and therefore rely entirely on their chemosensory apparatus for prey searching (Hoy 1985). The spider mite predator, *P. persimilis*, is attracted by volatiles emitted by lima bean plants (*Phaseolus lunatus*) after infestation of the plants with the two-spotted spider mite, *T. urticae* (Takabayashi and Dicke 1996). Moreover, *P. persimilis* is able to discriminate between volatiles emitted by its natural prey (*T. urticae*) and non-prey herbivores (*Spodoptera exigua* (Hubner)) (De Boer et al. 2004). Predators can also learn from previous experiences with HIPV. If *P. persimilis* was reared on *T. urticae* on lima beans, the predators later preferred the odors emitted by *T. urticae*-infested lima bean plants over *T. urticae*-infested cucumber plants and vice versa. This learning behavior was found to be flexible as, when the *P. persimilis* was switched from lima bean to cucumber, the preference changed gradually towards a preference for *T. urticae*-infested cucumber plants (De Boer and Dicke 2006). Several studies found that methyl salicylate is a crucial compound in the interaction between plants and phytoseiid predators (De Boer and Dicke 2004b; Ament et al. 2010).

1.8.5. Food supplementation

When plants do not provide rewarding food themselves, food can be introduced in the crop deliberately (Wade et al. 2008; Lundgren 2009). Such foods can be either sprayed or dusted over the crop or can be provided using banker plants (Wade et al. 2008; Huang et al. 2011). For phytoseiid predators, studies on food supplementation on greenhouse crops are listed in Table 1.1. Although a great deal of studies have shown the high nutritional quality of pollen for phytoseiids (e.g. McMurtry and Scriven 1966b; van Rijn and Tanigoshi 1999b; Broufas and Koveos 2000; Goleva and Zebitz 2013) and one of earliest studies on the increased establishment of phytoseiids in a crop as a result of pollen sprays was published more than 35 years ago (Kennett et al. 1979), it is only very recently that a pollen product became commercially available for the support of phytoseiid predatory mites. Possibly, this is because harvesting pollen by hand is labor-intensive thus making it an expensive product (Linskens and Jorde 1997; Messelink et al. 2014). One pollen species that is relatively easy to harvest in larger quantities is common cattail, *T. latifolia* (Park et al. 2011). This pollen species has been tested on various crops for the support of phytoseiids (see also Table 1.1). The recently commercialized pollen product Nutrimite™ consists of pollen of another cattail species, namely narrow-leaved cattail, *Typha angustifolia* (Biobest 2014; Messelink et al. 2014). Besides directly dusting pollen on the leaves of the crop, Adar et al. (2014) developed a "pollen on-twine" technique which consists of a piece of twine provided with pollen for the support of *A. swirskii* and *E. scutalis*. In this way, both an oviposition substrate (twine) and food (pollen) are supplied to the phytoseiids. Besides pollen, Messelink et al. (2009) tested the dried fruit mite *C. lactis*, an artificial diet containing yeast, glucose and soya powder, and Aminofeed® (commercial product consisting proteins and sugars) as supporting food sources for *A. swirskii* on chrysanthemum plant. When the artificial diet was combined with *C. lactis*, equally high oviposition rates as on *T. latifolia* were obtained in initial oviposition tests. The Aminofeed® resulted in a poor establishment in the crop, whereas the artificial diet mix was able to support populations of *A. swirskii*. In the study of Leman and Messelink (2015), *E. kuehniella* eggs and *A. franciscana* cysts were compared with *T. latifolia* and *Z. mays* pollen for *A. swirskii* and *A. limonicus*. For either phytoseiids, *A. franciscana* proved to be an unsuitable food source. In a greenhouse test on chrysanthemum, the other food sources resulted in significantly fewer thrips when supplemented to *A. swirskii* in the crop than the treatment without thrips or the *A. swirskii*-only treatment.

When applying pollen in a greenhouse for the support of phytoseiids one should avoid that pollen-feeding pests such as *F. occidentalis* profit from these food resources (Hulshof et al. 2003; Leman and Messelink 2015). Although pollen have been demonstrated to enhance development and reproduction of *F. occidentalis*, a good control of the thrips pest can be obtained through a higher numerical response of the phytoseiid (van Rijn et al. 2002; Leman and Messelink 2015).

Table 1.1: Overview of studies that have tested food supplementation for phytoseiid predatory mites

Phytoseiid species[a]	Pest species[a]	Crop plant	Type of food supplementation	Effect on predator	Effect on prey	Reference
E. hibisci	*P. citri*	Citrus	*T. latifolia* pollen	Significantly higher population densities	Reduction in 50% of the cases, although no differences with untreated plots	Kennett and Hamai 1980
I. degenerans	No pest (preventive)	Cucumber	Suspension of bee-collected pollen	Increased establishment	/	Ramakers 1995
A. limonicus	*F. occidentalis*	Cucumber	*T. latifolia* pollen	Faster population increase op to ca. 250 predators/plant	Lower pest densities in pollen-treatment	van Rijn et al. 1999
I. degenerans	*F. occidentalis*	Cucumber	*T. latifolia* pollen	Faster population increase than no-pollen treatment	Lower pest densities in pollen-treatment	van Rijn et al. 1999
N. cucumeris	*F. occidentalis*	Cucumber	Prey mites in sachet	Long-term release from sachets (at least 9 weeks)	Almost complete suppression of pest	Jacobson et al. 2001
I. degenerans	*F. occidentalis*	Cucumber	*T. latifolia* pollen	Good establishment	Good control of pest	van Rijn et al. 2002
A. swirskii	No pest (preventive)	Chrysanthemum	Decapsulated *Artemia* sp. cysts	Low establishment on greenhouse crop	/	Hoogerbrugge et al. 2008
			Grinded honey bee pollen	Low establishment on greenhouse crop	/	Hoogerbrugge et al. 2008
			C. lactis	Intermediate establishment on greenhouse crop	/	Hoogerbrugge et al. 2008
			C. lactis + AD1 (unknown formulation)	High establishment on greenhouse crop	/	Hoogerbrugge et al. 2008
			AD2[b]	Low establishment on greenhouse crop	/	Hoogerbrugge et al. 2008
			AD2 + grinded honey bee pollen [b]	Low establishment on greenhouse crop	/	Hoogerbrugge et al. 2008
			E. kuehniella eggs + *Z. mays* pollen	Intermediate establishment on greenhouse crop	/	Hoogerbrugge et al. 2008
A. swirskii	No pest (preventive)	Chrysanthemum	*T. latifolia* pollen	High oviposition rate + high densities in greenhouse crops	/	Messelink et al. 2009
			Grinded bee-collected pollen (Cistaceae)	Intermediate to low ovipoisition rate + intermediate to high densities in greenhouse crop	/	Messelink et al. 2009
			Mixture of yeast, glucose and soya powder	Low oviposition rate + intermediate densities on greenhouse crop	/	Messelink et al. 2009
			C. lactis	Intermediate oviposition rate + intermediate densities on greenhouse crop	/	Messelink et al. 2009
			C. lactis + Mixture of yeast, glucose and soya powder	High oviposition rate + high densities in greenhouse crops	/	Messelink et al. 2009
			Aminofeed	Very low to no oviposition rate + Very low densities on greenhouse crop	/	Messelink et al. 2009

E. finlandicus	*P. pallidus*	Strawberry	*T. latifolia* pollen	Successful establishment	Higher numbers than other treatments	Tuovinen & Lindqvist 2010
N. cucumeris	*P. pallidus*	Strawberry	*T. latifolia* pollen	Successful establishment	Good control of pest	Tuovinen & Lindqvist 2010
A. rhenanus	*P. pallidus*	Strawberry	*T. latifolia* pollen	Successful establishment	Good control of pest	Tuovinen & Lindqvist 2010
N.barkeri	*P. pallidus*	Strawberry	*T. latifolia* pollen	Failed to reproduce on pollen	Good control of pest	Tuovinen & Lindqvist 2010
A. swirskii	*B. tabaci*	Cucumber	*T. latifolia* pollen	Higher densities on greenhouse crop	Lower densities on greenhouse crops	Nomikou et al. 2010
A. swirskii	*F. occidentalis*	Sweet pepper	*P. brutia* pollen	Increased establishment	Lower number of F. occidentalis than no-pollen treatment	Kutuk & Yigit 2011
A. swirskii	No pest (preventive)	Sweet pepper	*Z. mays* pollen	Increased establishment (twofold)	/	Adar et al. 2014
			Q. ithaburensis pollen	Increased establishment (twofold)	/	Adar et al. 2014
E. scutalis	No pest (preventive)	Sweet pepper	*Z. mays* pollen	Increased establishment (tenfold)	/	Adar et al. 2014
			Q ithaburensis pollen	Increased establishment (tenfold)	/	Adar et al. 2014
A. swirskii	*F. occidentalis*	Chrysanthemum	Apple (cv. Red delicious) pollen	Increased establishment on greenhouse crops	Less crop damage and better pest control	Delisle et al. 2014
A. limonicus	*F. occidentalis*	Chrysanthemum	*Z. mays* pollen	Intermediate oviposition rate (± 1 eggs/female/day)	Increased oviposition rates, but significant reduction thrips density in field test	Leman & Messelink 2014
			T. latifolia pollen	High oviposition rates (± 1.7 eggs/female/day)	Increased oviposition rates, but significant reduction thrips density in field test	Leman & Messelink 2014
			E. kuehniella eggs	Very low oviposition rate(<0.5 eggs/female/day)	Increased oviposition rates, but significant reduction thrips density in field test	Leman & Messelink 2014
			hydrated decapsultated *A. franciscana* cysts	Very low oviposition rate (<0.5 eggs/female/day±)	No effect on predation	Leman & Messelink 2014
A. swirskii	*F. occidentalis*	Chrysanthemum	*Z. mays* pollen	High oviposition rates (± 1.5 eggs/female/day)	Increased oviposition rates, but significant reduction thrips density in field test	Leman & Messelink 2014
			T. latifolia pollen	High oviposition rates (± 1.8 eggs/female/day)	Increased oviposition rates, but significant reduction thrips density in field test	Leman & Messelink 2014
			E. kuehniella eggs	High oviposition rate(± 1.7 eggs/female/day)	Increased oviposition rates, but significant reduction thrips density in field test	Leman & Messelink 2014
			hydrated decapsultated *A. franciscana* cysts	Very low oviposition rate (<0.5 eggs/female/day±)	No effect on predation	Leman & Messelink 2014
A. swirskii	No pest (preventive)	Pepper	*T. latifolia* pollen	Increased establishment	/	Kumar et al. 2015

[a] genus names: for phytoseiid and pest species= A: *Amblyseius* (*swirskii*), A: *Amblydromalus* (*limonicus*) or A: *Anthoseius* (*rhenanus*); E: *Euseius*; I: *Iphiseius*; N: *Neoseiulus*; C: Carpoglypgus; P: *Panonychus* (*P. citri*) or P: *Phytonemus* (*P. pallidus*); F: *Frankliniella*; B: *Bemisia*; for the food supplements= T: *Typha*; C: *Carpoglyphus*; E: *Ephestia*; Z: *Zea*; P: *Pinus*; Q: *Quercus*;

[b] AD= artificial diet

1.9. Adaptation to the greenhouse climate

Like crop plants, insects and mites inside the greenhouse are influenced by several abiotic parameters, such as temperature, humidity, light quality, light intensity, and day length (Seemann 1979; van Lenteren and Woets 1988; Baille 2000; van Lenteren 2000; Hewitt et al. 2013).

As a result of year-round production, growers are increasingly using supplementary lighting, which may affect both pests and natural enemies, either indirectly by light mediated changes in crop plants (Vänninen et al. 2010), or directly by affecting arthropod ecology and biology (Auger et al. 1999; Johansen et al. 2011). For example, extended photoperiods may prevent diapause induction of pests and beneficials that normally go into reproductive diapause under short-day conditions (Morewood and Gilkeson 1991; Zilahi-Balogh et al. 2007). For predatory mites, such issues can be overcome by selecting strains that are non-diapausing (van Houten et al. 1995a). Walzer et al. (2007) were able to select strains of *N. californicus* that were adapted to higher temperatures and lower humidity.

More than ever, greenhouse growers are facing increasing energy costs for crop production. Especially in temperate climates greenhouses are heated with a central boiler and cooled by means of ventilation (Körner and Challa 2003). Because of the set point for heating or ventilation only having a narrow bandwidth (usually only 1 to 2°C) in combination with daily temperature variations, there is a constant alternation of heating and cooling (Tap et al. 1994). As a result, considerable amounts of fossil energy are being consumed. Innovative energy saving systems are being implemented to limit these increasing energy costs (Bot 2001). In the temperature integration approach, growers allow temperatures to vary within certain boundaries of the crop’s thermal window for non-destructive growth (Pollet et al. 2009), although an average temperature is premised over a certain period. This approach relies on the ability of plants to react to the mean temperature over a period exceeding one day or within a 24h period (Buwalda et al. 2000; Körner and Challa 2004). As a result, deviations of the aimed average temperature can be offset with temperature deviations in the opposite direction. Temperature variations within 24h are often referred to as “DIF” (i.e. difference between average daytime and average nighttime temperature) or “DTR” (diurnal temperature range). When introducing this temperature integration strategy, greenhouse growers can save up to

20% of their energy expenses depending on the crop and the allowed DTR (Erwin et al. 1989; Tantau 1998; Rijsdijk and Vogelezang 2000; Dieleman et al. 2005).

Being ectothermic organisms, the thermal biology of both pests and beneficial arthropods present in a greenhouse system will be affected by such temperature variations, as was also discussed in section 1.6.1.2.

Information on how ectotherms are being influenced by diurnal temperature variations is scanty and information on the biology and predatory capacity of phytoseiids under such conditions is practically lacking.

2

Diurnal temperature variations affect development and population growth of *Tetranychus urticae* and it phytoseiid predators *Phytoseiulus persimilis* and *Neoseiulus californicus*

This chapter has been redrafted from:

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Vangansbeke D., Audenaert, J., Nguyen, D.T., Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. (2015) Diurnal Temperature Variations Affect Development of a Herbivorous Arthropod Pest and its Predators. PloS ONE 10 (4): e0124898

2.1. Introduction

Temperature is a key abiotic factor driving population dynamics of arthropods, which has resulted in a plethora of studies on the relationship between arthropod developmental biology and temperature (e.g.Wagner et al. 1984; Ratte 1985; Honek 1999; Logan et al. 2006). To predict developmental rates of poikilothermic arthropods, both linear and nonlinear models have been developed (Campbell et al. 1974; Kontodimas et al. 2004). Linear models allow the estimation of the lower developmental threshold (i.e. the temperature at which the development rate approaches zero) and the thermal constant for development (expressed in degree-days) (Honek and Kocourek 1990; Honek 1999), but fail to predict developmental rates at low and high extreme temperatures (Campbell et al. 1974; Davis et al. 2006). Nonlinear models more accurately describe the usually curvilinear relationship between arthropod developmental rate and temperature over the whole temperature range (Stinner et al. 1974; Sharpe and Demichele 1977; Brière et al. 1999; Luypaert et al. 2014). Hitherto, these models were mainly based on data from constant temperatures, which is surprising as in most environments varying temperature regimes are the rule, rather than the exception (Howe 1967; Hagstrum and Hagstrum 1970; Beck 1983; Worner 1992; Liu et al. 1995; Fantinou et al. 2003). Diurnal temperature ranges (hereafter referred to as DTR) have been shown to severely impact developmental rates of poikilothermic arthropods (Matteson and Decker 1965; Hagstrum and Hagstrum 1970; Hagstrum and Milliken 1991; Liu et al. 1995; Garcia-Ruiz et al. 2011). Usually, at varying temperature regimes, poikilotherm developmental rate tends to be higher at low temperatures and lower in the higher temperature range, as compared to the corresponding mean constant temperature (Ratte 1985; Paaijmans et al. 2010). At intermediate temperatures, little to no difference in developmental rates has been observed (Tanigoshi et al. 1976; Ratte 1985; Bryant et al. 1999). This effect has been attributed to the typically nonlinear relationship between poikilothermic developmental rates and temperature (Sharpe and Demichele 1977; Lactin et al. 1995), and has been referred to as the rate summation effect or Kaufmann effect (Worner 1992). Generally, this phenomenon is a consequence of Jensen’s inequality (Ruel and Ayres 1999), which states that the average value of a nonlinear function of a variable not necessarily equals the value of the nonlinear function evaluated at the average variable (Smallwood 1996) **(see also section 1.8.6)**. This mathematical property may, at least partly, explain the variation in arthropod developmental rates between constant and varying temperature regimes (Liu et al. 1995; Smallwood 1996; Estay et al. 2014). Other possible explanations for the observed differences in developmental rates between constant and varying temperature regimes refer to (yet unknown) physiological responses that act in addition to the rate summation effect (Ratte 1985; Worner 1992; Liu et al. 1995), or have been attributed to the presence or lack of a diurnal rhythm, as it would occur in the organism's natural environment (Behrens et al. 1983).

With rising energy costs, glasshouse growers are under pressure to reduce their energy consumption (Adams et al. 2009). Besides lowering the overall mean glasshouse temperature and growing more cold-tolerant cultivars of both vegetable and ornamental crops (van der Ploeg and Heuvelink 2005; van der Ploeg et al. 2009), variable temperatures can be implemented as an energy saving strategy (Pollet et al. 2009). This approach relies on the ability of plants to react to the mean temperature over a period exceeding one day (temperature integration) (Buwalda et al. 2000; Körner et al. 2004) or within 24 h (DIF: difference between average daytime and average nighttime temperature or DTR: diurnal temperature range) rather than to the exact daily temperature course. By allowing higher daytime and lower nighttime temperatures in the greenhouse, an annual energy saving of up to 20% can be achieved (Erwin et al. 1989; Tantau 1998; Dieleman et al. 2005), depending on the crop and the amplitude of the fluctuations (Rijsdijk and Vogelezang 2000; Körner and Challa 2003; Bakker 2009). However, little attention has been given to the impact of energy saving strategies in protected cultivation on the performance of both pests and their natural enemies used in augmentative biological control programs.

In pest management strategies, knowledge about the basic thermal biology of both pests and natural enemies is crucial to predict and manage pest outbreaks (Wagner et al. 1991; Pedigo 1998; Schowalter 2006). Temperature-driven models are an essential tool for predicting and managing agricultural and horticultural pests (Ayres 1993; Moerkens et al. 2011). Evidently, as temperature regimes affect developmental rates and other life history parameters, diurnal temperature variations should best be included in such models (Blanford et al. 2013; Paaijmans et al. 2013).

In this study, we focused on the predatory mites *P. persimilis* and *N. californicus*, two economically important and commercially available predators of the two-spotted spider mite, *T. urticae*, both in protected and field crops (van Lenteren 2012). The two-spotted spider mite is an extremely polyphagous agricultural pest with an unmatched level of pesticide resistance (Van Leeuwen et al. 2010; Grbić et al. 2011). In protected crops, introduction of these phytoseiid predators of *T. urticae* has shown to be a successful alternative for chemical control (e.g. Schausberger and Walzer 2001; Greco et al. 2005; Rhodes et al. 2006; Weintraub and Palevsky 2008).

This chapter investigated the relationship between developmental rate of *P. persimilis*, *N. californicus* and *T. urticae* and temperature, under both constant and alternating temperature regimes at four amplitudes (i.e., DTR of 0, +5, +10 and +15, resulting in a difference of 0, 5, 10 and 15°C between day and night temperatures). We evaluated linear and nonlinear models to predict developmental rates and assessed whether we could use data derived from constant temperatures to predict the effects of alternating temperatures, thereby assessing whether Jensen’s inequality is the main factor explaining the observed differences. Additionally, we explored the impact of the mites' responses to these temperature variations on their performance in biological control programs. In a second experiment, we assessed how the population growth of the three mite species respond to constant and alternating temperatures in the lower temperature range (≤ 20°C), which are commonly used in the production of ornamental plants like pot azalea, roses or English ivy (Pemberton and Wilkins 1985; Khayat et al. 1988; Pollet et al. 2009; Meijón et al. 2011). The reproduction and life table parameters of the prey and its key predators were assessed at two constant (15°C and 20°C) versus one alternating temperature regime (20°C/5°C). The results should allow growers to better understand the impact of using an energy saving strategy with variable temperatures on one of the major pests in protected cultivation and its biological control agents.

2.2. Materials and Methods

2.2.1. Mite rearing

2.2.1.1. Spider mites

Two-spotted spider mites, *T. urticae,* were collected from *Ricinus communis* L. (Euphorbiaceae) grown at the grounds of the Faculty of Bioscience Engineering (Ghent, Belgium) and a laboratory colony was set up using kidney bean plants (*Phaseolus vulgaris* L.). The infested plants were kept in Plexiglas containers (60 x 60 x 60 cm) in a climatized room at 25 ± 1°C, 70 ± 5 % RH and a 16:8 (L:D) h photoperiod.

2.2.1.2. Predatory mites

Colonies of *P. persimilis* and *N. californicus* were initiated with mites acquired from Biobest N.V. (Westerlo, Belgium) and were reared on excised kidney bean leaves heavily infested with two-spotted spider mites. Each leaf was placed upside down on water soaked cotton in a glass petri dish (ø 133 mm), with an extra cotton layer on the leaf edges, providing free water and preventing the mites from escaping. The petri dishes were kept inside Plexiglas containers at the climatic conditions mentioned above.

2.2.2. Experiment 1: Developmental rates

2.2.2.1. Experimental set-up

The development of *T. urticae* and its predators *P. persimilis* and *N. californicus* was studied at a 16:8h (L:D) photoperiod and at different constant and alternating temperature regimes between 12.5 and 40°C with 4 different amplitudes (constant: 0°C and alternating: 5, 10 and 15°C) were tested (see Table 2.1).

For *P. persimilis* and *N. californicus,* 40 eggs (<10h) were collected from the stock colony and were transferred individually to square bean leaf arenas (25 x 25mm) using a fine needle. The leaf arenas were placed upside down on a water-soaked polyurethane sponge (10 x 50 x 50 mm) in polystyrene insect breeding dishes (ø 100 mm, H 40 mm) (SPL Life Sciences, Korea). Ventilation was provided with a mesh covered hole (ø 40 mm) in the lid. To prevent the mites from escaping and to provide free water, moist tissue paper was used to cover the edges of the bean leaf arenas. Leaf arenas were infested with *T. urticae* 5 days before the introduction of a predatory mite egg by transferring 3 gravid female spider mites to the arena. Hence, a sufficient amount of both eggs and motile stages of *T. urticae* was supplied as a food source for the phytoseiid immatures.

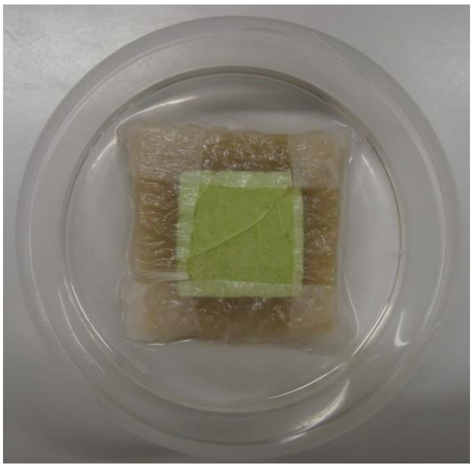

Figure 2.1: Experimental bean leaf arena

For the experiments with *T. urticae*, 3 gravid female spider mites from the stock colony were introduced to each leaf arena as described above, 4h prior to the onset of the test. Thereafter, the females were removed and the amount of spider mites eggs was reduced to a single egg per arena by piercing the excess of eggs.

The development of the mites was monitored twice a day (at 8 am and 6 pm) when the average daily temperature was equal or higher than 25°C. When the average temperature was lower than 25°C, development was checked daily.

When the developmental period of both phytoseiids and *T. urticae* exceeded 10 days, mites were transferred to fresh leaf arenas.

Relative humidity is an additional factor determining the developmental success of *P. persimilis*, *N. californicus* and *T. urticae* (Mori and Chant 1966; Walzer et al. 2007). Inside the insect breeding dishes, relative humidity was measured using HOBO H8 RH/Temp Loggers (Onset Computer, Bourne, MA, USA) and always exceeded 90%. Therefore, relative humidity during the experiments was assumed not to be a limiting factor for development of the mites.

2.2.2.2. Statistical analysis

Mean developmental times were compared using non-parametric Kruskal-Wallis ANOVAs as data were found not to be normally distributed. Means were separated using Mann-Whitney tests. The level of significance was set at 0.05.

2.2.2.3. Modelling

For further analysis, developmental rates (D_r, in day^{-1}) were derived by calculating the reciprocal of the developmental times (D) obtained from the experiments. Developmental rates were subjected to both linear and nonlinear regression. To describe the nonlinear relationship between developmental rate and temperature, a variety of functions have been constructed with different levels of complexity, numbers of parameters, different assumptions about high and low temperature limits and inclusion of biologically relevant parameters, such as optimal temperature (T_{opt}) and upper and lower developmental threshold (T_L and T_0, respectively) (Wagner et al. 1984; Roy et al. 2002; Kontodimas et al. 2004). We selected two nonlinear equations with a low level of complexity, which predict biologically relevant parameters and have the ability to intersect with the x-axis, thereby allowing an estimation of the lower developmental threshold, namely the Brière-2 and Lactin-2 model (Brière et al. 1999; Roy et al. 2002) using SigmaPlot version 12 (SYSTAT Software Inc.).

2.2.2.3.1. Linear regression

Data that deviated from the straight line were omitted for calculation of the linear regression model (Campbell et al. 1974; De Clercq and Degheele 1992).

$$D_r = a + b * T \qquad (1)$$

with

D_r= developmental rate (day^{-1})

T= temperature (°C)

a= developmental rate when T is 0°C

b= slope of the regression line

The lower developmental threshold (T_0) was estimated from the linear model as the intercept of the developmental rate-temperature curve with the temperature axis. The standard error (SE) of T_0 can be calculated using the formula described by Campbell et al. (1974):

$$SE_{T_0} = \frac{r}{b}\sqrt{\frac{s^2}{N*r^2} + (\frac{SE_b}{b})^2} \qquad (2)$$

where s^2 is the residual mean square of D_r , r is the sample mean and N is the sample size.

The thermal constant (K) indicates the amount of thermal units (in degree-days) that are needed to complete development and can be derived from the linear model as the reciprocal of the slope b (K=1/b). The SE of K can be estimated as follows (Campbell et al. 1974; Kontodimas et al. 2004):

$$SE_K = \frac{SE_b}{b^2} \qquad (3)$$

2.2.2.3.2. Nonlinear regression

Brière-2

$$D_r = a * T * (T - T_0) * (T_L - T)^{\frac{1}{d}} \qquad (4)$$

with

D_r= developmental rate (day^{-1})

T= temperature (°C)

a, d= empirical constants

T_0= low temperature developmental threshold (°C)

T_L =lethal temperature threshold (°C)

Lactin-2

$$\mathrm{D}_r = e^{(\rho * T)} - e^{\left(\rho T_L - \left(\frac{T_L - T}{\Delta T}\right)\right)} + \lambda \qquad (5)$$

with

D_r= developmental rate (day^{-1})

T= temperature (°C)

ρ= constant defining developmental rate at optimal temperature

λ= constant forcing the curve to intercept with the x-axis, thereby allowing an estimation of the lower developmental threshold T_0

ΔT= temperature range between T_{opt} and T_L

T_L =lethal maximum temperature

The optimal temperature (T_{opt}) is the temperature at which the developmental rate reaches its highest value and was calculated as the first derivative of the abovementioned nonlinear functions (value of T when $d(T)/d(D_r)=0$).

2.2.2.4. Model evaluation

The quality of the tested models was evaluated by means of the adjusted R² (R^2_{adj}) and Akaike's information criterion (AIC) (Akaike 1974) in addition to R² (coefficient of determination) and RSS (residual sum of squares) by using the following formulae:

$$R^2_{adj} = 1 - \left(\frac{n-1}{n-p}\right) * (1 - R^2) \qquad (6)$$

and

$$AIC = n * \ln\left(\frac{RSS}{n}\right) + 2 * p \qquad (7)$$

where n is the number of observations, p equals the number of model parameters and RSS is the residual sum of squares. The higher R^2_{adj} and the lower the value of AIC, the more accurately the model fits the observed developmental rates.

When accepting that the observed differences between varying and constant temperatures are exclusively due to the rate summation effect or Jensen's inequality based on the curvilinear relationship between temperature and developmental rate, it should be possible to calculate the amount of development by accumulating the proportion of development per time-unit using the following formula (Worner 1992; Liu et al. 1995):

$$D_{r,exp} = \sum_{t=a}^{b} D_{r,obs}\,[T(t)]dt \qquad (8)$$

where developmental rate $D_{r,\,exp}$ (developmental rate as expected by the rate summation) is a function of temperature (T), which in turn is a function of time (t), r is the corresponding developmental rate (r= 1/D), and a and b are the start and end of the developmental period under a given temperature regime (here a=0h and b=24h). $D_{r,obs}$ are the observed developmental rates as calculated by the reciprocal of developmental time D. For example, the expected developmental rate at 25°C/15°C (i.e. DTR+10) can be calculated as follows:

$$Dr,exp\ (25°C/15°C) = \ 16/24 * Dr,obs, 25°C \ + \ 8/24 * \ Dr,obs, 15°C \qquad (9)$$

where $D_{r,exp}$ (25°C/15°C) is the expected developmental rate when accepting the rate summation effect and $D_{r,\ obs,\ 25°C}$ and $D_{r,\ obs,\ 15°C}$ are the observed developmental rates at a constant 25°C and 15°C, respectively, and a 16:8 (L:D) photoperiod.

Next, we compared the obtained expected developmental rates with the observed developmental rates at a given temperature regime as follows (Liu et al. 1995):

$$d = \left(\frac{D_{r,exp}}{D_{r,obs}} - 1\right) * 100 \qquad (10)$$

where d equals the percentage deviation. A negative value of d indicates that the rate summation effect underestimates the actual developmental rate, whereas a positive value indicates that the rate summation effect predicts higher developmental rates than what is observed. The higher the deviation, the more we can assume that the observed difference is not solely due to the rate summation effect, but that an additional physiological response is present and that the developmental rate at a specific temperature is not independent of the present temperature regime (Worner 1992; Liu et al. 1995).

2.2.2.5. Potential impact on biological control

To assess the potential impact of the investigated temperature variations on the dynamics between *P. persimilis*, *N. californicus* and *T. urticae*, we calculated the ratio of the developmental rates at DTR+5, +10 and +15 and that at constant temperature (ΔD_r = [D_r (alternating T)/ D_r (constant T)]) as predicted by the Brière-2 nonlinear model. A species will be positively or negatively affected by the alternating temperature regime if ΔD_r is higher or lower than 100%, respectively. For fast developing species, a reduction in developmental period contributes more to population growth than a similar increase in reproduction (Lewontin 1965). Therefore, differences in developmental rate will benefit one species over another and can thus affect the outcome of a biological control program. Here, we compared the developmental rate of the predatory mites with their prey, *T. urticae*, at alternating versus constant temperature regime.

Per amplitude, we plotted the value of ΔD_r (using the Brière-2 nonlinear model) in a temperature range between 10 and 36°C using an interval of 1°C.

2.2.3. Experiment 2: Population growth

2.2.3.1. Experimental set-up

Experiments to assess the population growth of both spider mites and predatory mites were conducted at two constant temperature regimes (referred to as 15°C/15°C and 20°C/20°C) and one alternating regime (DTR+15: 20°C/5°C). The alternating regime had a corresponding mean temperature of 15°C, given the 16:8 (L:D) h photoperiod (i.e. 16h at 20°C and 8h at 5°C). The three temperature regimes were tested in climatic chambers set at 65 ± 5% RH.

Experiments were performed on similar arenas as described in section 2.1.2.1.

2.2.3.2. Reproduction

Newly molted females of both phytoseiids, derived from experiment 1, were paired with a male with the same age and that had developed to adulthood at the same temperature regime, and were placed on a leaf disc infested with mixed stages of *T. urticae*. The male was only removed after three days to allow multiple matings (Gotoh and Tsuchiya 2008; personal observation). The number of eggs laid was counted daily and eggs deposited by females of the same age were placed together on leaf discs in order to determine the sex ratio of the offspring.

Freshly molted females of *T. urticae* were provided with a male that was isolated 24h before introduction on the arena to ensure a complete insemination. As a single mating is sufficient for optimal egg production in *T. urticae* (Helle 1967; Potter and Wrensch 1978) males were kept for 24h together with females. Deposited eggs were removed from the arena on a daily basis and transferred to new leaf discs in order to determine the sex ratio of the offspring. Female spider mites were transferred to fresh bean leaf discs once a week.

2.2.3.3. Life table parameters

The intrinsic rate of increase (r_m) was calculated for *T. urticae* and both phytoseiids according to the formula of Birch (1948):

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

where x equals the female age (days), l_x is the age specific survival of the females at age x and m_x is the number of daughters produced per female at age x. The latter parameter is obtained by multiplying the mean number of eggs laid per female by the proportion of female offspring produced at age x. The Jackknife procedure was used according to Meyer et al. (1986) and Hulting et al. (1990) to calculate the standard error of r_m. Other parameters calculated were (Maia et al. 2000):

-the net reproductive rate, R_0, i.e. the mean number of female offspring produced per female (females/female)

$$R_0 = \sum l_x m_x \qquad (11)$$

- the mean doubling time, DT, i.e. mean time span necessary to double the initial population (days)

$$DT = \frac{\ln 2}{r_m} \qquad (12)$$

2.2.4. Statistical analysis

Two-way analysis of variance (ANOVA) (IBM, SPSS Statistics 20) was conducted to evaluate the effects of temperature regime on preoviposition periods, fecundity and oviposition rate of *P. persimilis* and *N. californicus*. When an interaction was detected between the main factors (temperature regime and species), means were compared within species and a pairwise multiple comparison procedure was used (Kutner et al. 2005). A Kolmogorov-Smirnov test was conducted to evaluate normality of the data. In case of normality, the effect of temperature regime was tested by means of a one-way-ANOVA. A Tukey (homoscedasticity) or Tamhane test (heteroscedasticity) was performed to separate the means, depending on the outcome of a Levene-test. When data were not normally distributed, a non-parametric Kruskal-Wallis H test was conducted and means were separated using Mann-Whitney U tests. A one-way-ANOVA or Kruskal-Wallis H test was conducted to evaluate effects of temperature on developmental and reproductive parameters of *T. urticae.*

A logistic regression was conducted to compare offspring sex ratios. In this regression a generalized linear model is constructed using a probit (log odds) link and a binomial error function. For each test, a regression coefficient is calculated and tested for being significantly different from zero, for which P-values are presented (McCullagh and Nelder 1989).

Life table parameters of *P. persimilis*, *N. californicus* and *T. urticae* at the tested temperature regimes were also analyzed with two-way ANOVAs. In all cases, P-values are considered to be significant when smaller than or equal to 0.05.

2.3. Results

2.3.1. Experiment 1: Developmental rates

Total developmental times (egg-adult) of *P. persimilis*, *N. californicus* and their prey *T. urticae* are shown in Table 2.1. Temperature affected the developmental times of all mite species (Kruskal-Wallis: *P. persimilis*: χ^2=737.956; df= 29; p<0.001, *N. californicus*: χ^2=728.697; df= 33; p<0.001; *T. urticae*: χ^2=827.341; df= 33; p<0.001). Immature *P. persimilis* were not able to reach adulthood at constant temperatures at or above 35°C, whereas *N. californicus* and *T. urticae* succeeded in completing development at a daytime temperature of 37.5°C as long as a colder nighttime temperature was maintained.

Table 2.1: Effect of different temperature regimes on the total developmental time (means ± SE) of *Phytoseiulus persimilis*, *Neoseiulus californicus* and *Tetranychus urticae*

	Temperature (°C)		DTR (°C)[a]	Average daily temperature (°C)	Developmental time (D) (days)[b]					
	Day (°C)	Night (°C)			n[a]	*P. persimilis*	n[a]	*N. californicus*	n[a]	*T. urticae*
1	12.5	12.5	0	12.5	27	24.41 ± 0.43 p	18	28.78 ± 0.82 y	21	42.83 ± 0.83 x
2	15	5	10	11.7	29	17.62 ± 0.25 o	28	21.82 ± 0.33 x	26	38.58 ± 0.21 w
3	15	10	5	13.3	28	18.29 ± 0.26 o	22	18.61 ± 0.24 w	22	34.32 ± 0.40 v
4	15	15	0	15	22	14.43 ± 0.27 n	19	15.11 ± 0.37 u	40	27.44 ± 0.20 t
5	17.5	2.5	15	12.5	32	13.88 ± 0.11 m	27	17.19 ± 0.29 v	21	30.40 ± 0.32 u
6	17.5	17.5	0	17.5	29	8.97 ± 0.12 j	28	11.11 ± 0.16 t	17	21.50 ± 0.15 s
7	20	5	15	15	20	10.70 ± 0.15 l	27	10.67 ± 0.12 s	34	20.38 ± 0.16 r
8	20	10	10	16.7	26	9.38 ± 0,15 k	19	10.08 ± 0.16 r	23	20.07 ± 0.19 r
9	20	15	5	18.3	27	7.59 ± 0.10 i	28	9.39 ± 0.14 q	23	17.67 ± 0.12 q
10	20	20	0	20	25	6.40 ± 0.10 h	23	7.15 ± 0.20 p	37	14.42 ± 0.12 p
11	22.5	22.5	0	22.5	34	5.12 ± 0.04 g	21	5.81 ± 0.11 n	18	11.61 ± 0.16 n
12	25	10	15	20	22	6.41 ± 0.08 h	22	6.73 ± 0.12 p	34	12.50 ± 0.08 o
13	25	15	10	21.7	26	5.23 ± 0.08 g	30	6.10 ± 0.09 o	31	11.69 ± 0.07 n
14	25	20	5	23.3	29	5.05 ± 0.05 g	26	5.48 ± 0.08 m	30	9.87 ± 0.08 l
15	25	25	0	25	27	3.87 ± 0.05 d	26	4.65 ± 0.07 ij	25	9.28 ± 0.07 j
16	27.5	12.5	15	22.5	26	5.00 ± 0.06 g	28	5.21 ± 0.05 l	25	10.76 ± 0.08 m
17	27.5	17.5	10	24.2	30	4.28 ± 0.06 f	18	5.00 ± 0.06 k	20	9.55 ± 0.07 k
18	27.5	22.5	5	25.8	22	4.02 ± 0.05 e	14	4.46 ± 0.06 i	21	8.55 ± 0.04 h
19	27.5	27.5	0	27.5	30	3.52 ± 0.04 b	15	4.17 ± 0.08 fg	24	7.63 ± 0.06 f
20	30	15	15	25	29	4.21 ± 0.08 ef	21	4.83 ± 0.09 jk	27	8.91 ± 0.07 i
21	30	20	10	26.7	24	3.83 ± 0.06 cd	24	4.17 ± 0.06 fg	26	7.52 ± 0.02 f
22	30	25	5	28.3	28	3.30 ± 0.06 a	28	3.82 ± 0.06 de	21	7.10 ± 0.04 e
23	30	30	0	30	34	3.34 ± 0.05 a	22	3.86 ± 0.05 e	30	6.28 ± 0.06 b
24	32.5	17.5	15	27.5	24	3.81 ± 0.08 cd	26	4.19 ± 0.07 fgh	29	8.03 ± 0.07 g
25	32.5	22.5	10	29.2	22	3.66 ± 0.07 c	22	3.73 ± 0.08 cd	21	6.88 ± 0.07 d
26	32.5	27.5	5	30.8	29	3.38 ± 0.06 ab	24	3.33 ± 0.07 a	27	5.98 ± 0.02 a
27	32.5	32.5	0	32.5	26	4.25 ± 0.11 ef	25	3.41 ± 0.06 ab	26	6.20 ± 0.10 ab
28	35	20	15	30	15	6.50 ± 0.19 h	22	4.25 ± 0.06 gh	24	7.75 ± 0.13 fg
29	35	25	10	31.7	22	4.05 ± 0.09 de	35	4.20 ± 0.06 fgh	27	6.63 ± 0.11 cd
30	35	30	5	33.3	17	4.00 ± 0.09 de	26	3.56 ± 0.05 bc	24	6.04 ± 0.07 a
31	35	35	0	35	/	/	17	4.03 ± 0.10 ef	9	7.50 ± 0.14 f
32	37.5	22.5	15	32.5	/	/	14	5.04 ± 0.11 kl	23	7.54 ± 0.11 f
33	37.5	27.5	10	34.2	/	/	21	4.67 ± 0.13 i	23	6.83 ± 0.13 d
34	37.5	32.5	5	35.8	/	/	6	4.75 ± 0.36 hi	16	6.31 ± 0.17 abc
35	37.5	37.5	0	37.5	/	/	/	/	/	/
36	40	25	15	35			/	/	/	/
37	40	30	10	36.7			/	/	/	/
38	40	35	5	38.3			/	/	/	/

[a] n= number of females that successfully developed to adult

[b] Means (± SE) within a column followed by the same letter are not significantly different (P>0.05, Kruskal-Wallis, means

were separated using Mann-Whitney tests)

2.3.1.1. Linear regression

When developmental rates at the highest temperatures were omitted from the regression analysis, the linear model showed a good fit to the data (Figure 2.1), as demonstrated by high values of R^2 and R^2_{adj} (all >0.98) and low values of RSS (Table 2.2). Diurnal temperature range had an effect on the lower developmental thresholds for egg-adult development of all mite species, with lower T_0-values with increasing DTR (Figure 2.3). T_0-values were about 3°C lower at a DTR+15 temperature regime as compared to the constant temperature regime for all species. When developmental zeros decreased, the thermal constants increased (Table 2.2).

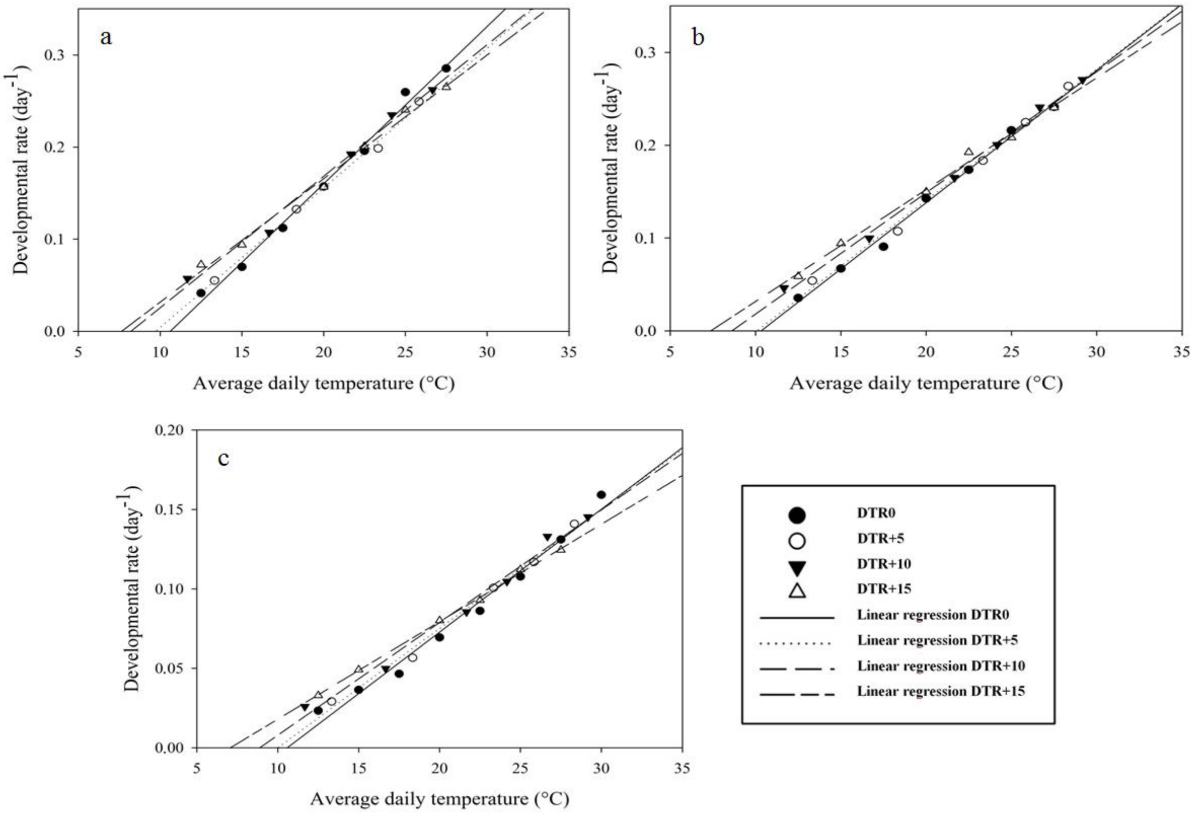

Figure 2.1: Linear regression of egg-adult developmental rate versus temperature for *Phytoseiulus persimilis* (a), *Neoseiulus californicus* (b) and *Tetranychus urticae* (c) exposed to different constant and alternating temperature regimes

Table 2.2: Fitted parameters of linear regression (D_r= a+b*T) of developmental rates, developmental threshold (T_0) and thermal constant (K) for total immature development of *Phytoseiulus persimilis*, *Neoseiulus californicus* and *Tetranychus urticae* at 4 thermoperiods (DTR)

Species	DTR (°C)	a	b	R^2	R^2_{adj}	RSS	T_0 (°C)	K (DD)
P. persimilis	0	-1.814 ± 0.0140	0.0171 ± 0.0007	0.9921	0.9906	0.0004	10.61 ± 0.43	58.48 ± 2.39
	5	-0.1475 ± 0.0147	0.0152 ± 0.0007	0.9957	0.9935	0.00009	9.70 ± 0.53	65.79 ± 3.03
	10	-0.1174 ± 0.0172	0.0143 ± 0.0008	0.9901	0.9869	0.0003	8.21 ± 0.74	69.93 ± 3.91
	15	-0.1026 ± 0.0121	0.0134 ± 0.0006	0.9928	0.991	0.0002	7.66 ± 0.62	74.63 ± 3.34
N. californicus	0	-0.1474 ± 0.0107	0.0143 ± 0.0005	0.9934	0.9921	0.0002	10.31 ± 0.38	69.93 ± 2.44
	5	-0.1427 ± 0.0154	0.0142 ± 0.0007	0.9931	0.9907	0.0002	10.05 ± 0.63	70.42 ± 3.47
	10	-0.1126 ± 0.0091	0.0131 ± 0.0004	0.9962	0.9952	0.0001	8.60 ± 0.44	76.34 ± 3.42
	15	-0.089 ± 0.0098	0.0121 ± 0.0005	0.9941	0.9926	0.0001	7.36 ± 0.57	82.64 ± 3.42
T. urticae	0	-0.0819 ± 0.0091	0.0077 ± 0.0004	0.9831	0.9803	0.0003	10.64 ± 0.64	129.87 ± 6.75
	5	-0.0752 ± 0.0089	0.0075 ± 0.0004	0.9918	0.9891	0.00007	10.03 ± 0.69	133.33 ± 7.11
	10	-0.0631 ± 0.0062	0.0071 ± 0.0004	0.9856	0.982	0.0002	8.89 ± 0.80	140.85 ± 7.93
	15	-0.0366 ± 0.0061	0.0058 ± 0.0003	0.989	0.9868	0.00009	6.31 ± 0.84	172.41 ± 8.92

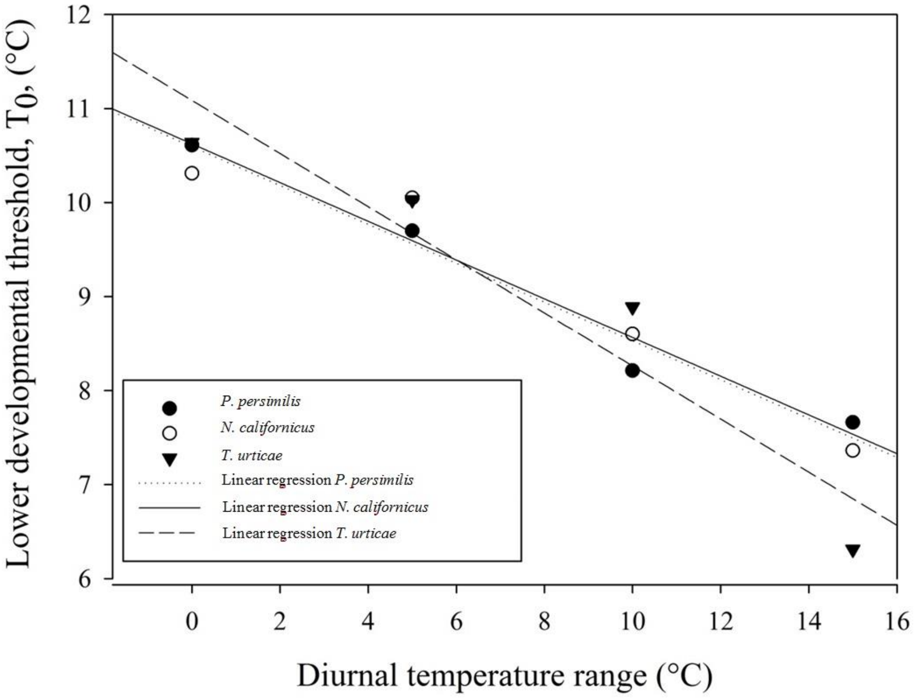

Figure 2.2: Linear relationship between lower developmental threshold (T_0) and thermoperiod for *Phytoseiulus persimilis*, *Neoseiulus californicus* and *Tetranychus urticae* exposed to different constant and alternating temperature regimes

2.3.1.2. Nonlinear models

2.3.1.2.1. Brière-2

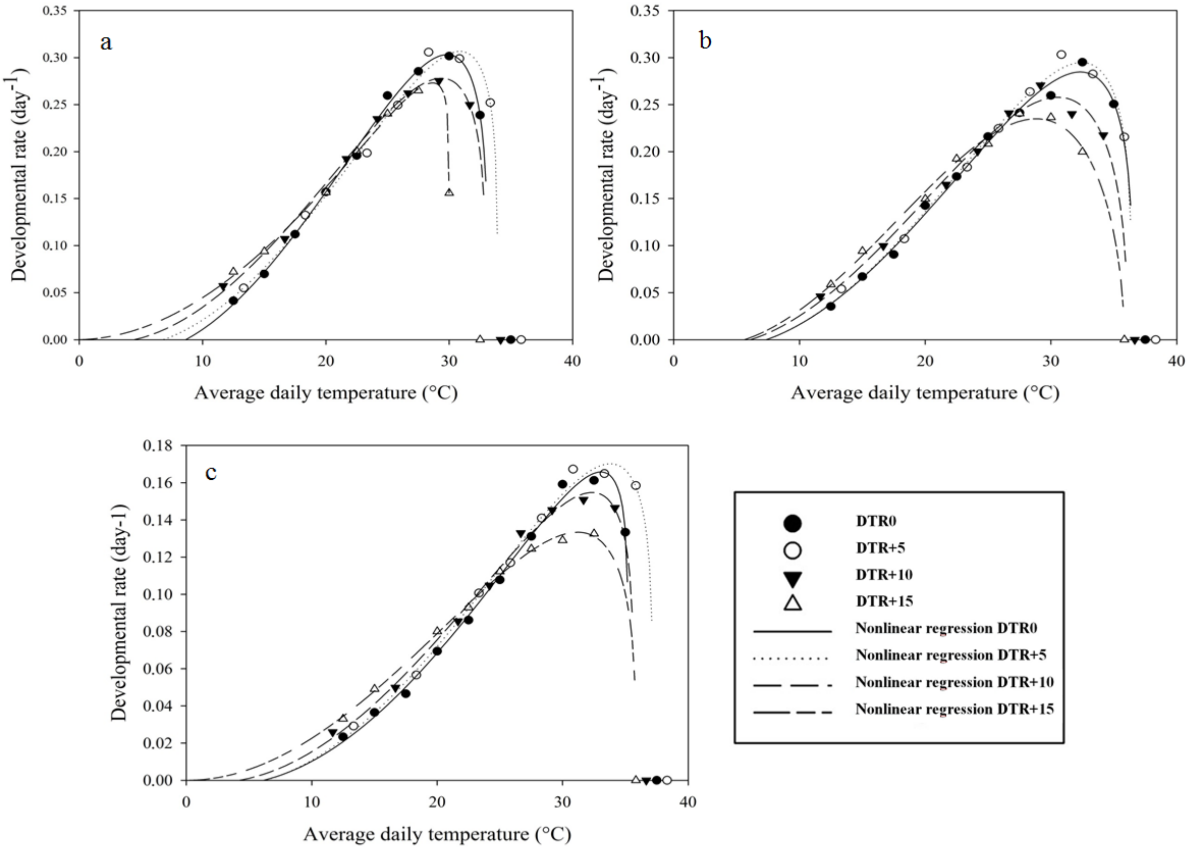

Figure 2.3: Nonlinear regression (Brière-2) of egg-adult developmental rate versus temperature for *Phytoseiulus persimilis* (a), *Neoseiulus californicus* (b) and *Tetranychus urticae* (c) exposed to different constant and alternating temperature regimes

2.3.1.2.2. Lactin-2

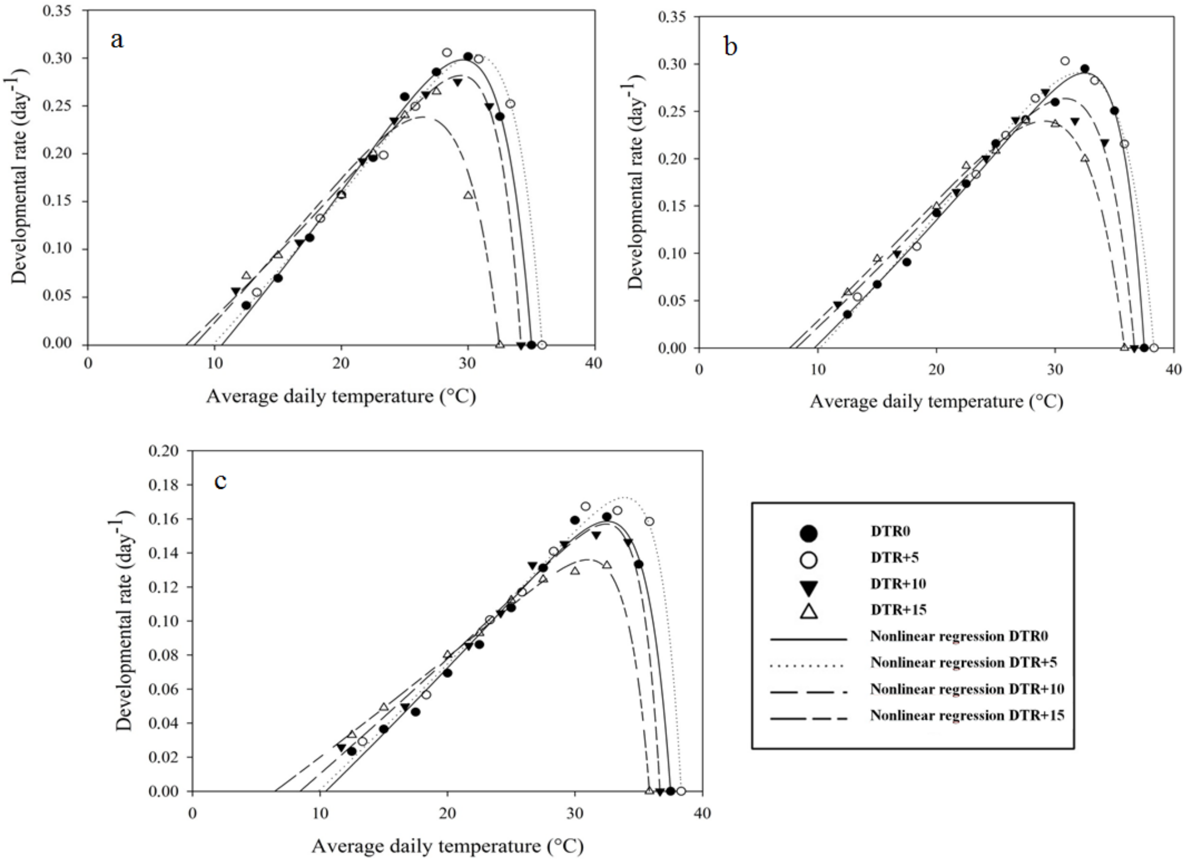

Figure 2.4: Nonlinear regression (Lactin-2) of egg-adult developmental rate versus temperature for *Phytoseiulus persimilis* (a), *Neoseiulus californicus* (b) and *Tetranychus urticae* (c) exposed to different constant and alternating temperature regimes

Table 2.3: Estimated parameters (means ± SE) of the Brière-2 and Lactin-2 model and corresponding evaluation criteria for total development of *Phytoseiulus persimilis*, *Neoseiulus californicus* and *Tetranychus urticae* at 4 thermoperiods

	P. persimilis				*N. californicus*				*T. urticae*			
	DTR0	DTR+5	DTR+10	DTR+15	DTR0	DTR+5	DTR+10	DTR+15	DTR0	DTR+5	DTR+10	DTR+15
Brière-2												
a	0.0003 ± 0.0000219	0.0003 ± 0.0000401	0.0003 ± 0.0000332	0.0003 ± 0.000017	0.0002 ± 0.000039	0.0002 ± 0.000022	0.0002 ± 0.00001108	0.0001 ± 0.000007	0.0002 ± 0.000008	0.0001 ± 0.000021	0.0001 ± 0.000021	0.00009 ± 0.000003
T_0	8.6323 ± 0.7035	6.8228 ± 2.4626	4.4634 ± 1.6586	-0.7012 ± 1.4968	7.3540 ± 1.4526	7.3687 ± 1.7074	6.6235 ± 1.7799	5.6214 ± 1.2741	6.1661 ± 1.4257	6.2958 ± 2.2196	4.2534 ± 2.3079	0.3548 ± 1.6866
T_L	33.1147 ± 0.2432	33.9198 ± 0.5946	32.9537 ± 0.7228	30.0013 ± 0.0007	36.4915 ± 0.9132	36.4035 ± 0.3302	36.6661 ± 0.0017	35.8244 ± 0.0035	35.1955 ± 0.1790	37.1671 ± 1.1029	35.6531 ± 1.2038	35.8330 ± 0.1377
d	3.7773 ± 0.5929	4.4301 ± 1.8358	3.7546 ± 1.0791	10.6917 ± 0.7996	3.3894 ± 1.0194	3.5695 ± 0.7829	2.1471 ± 0.2321	1.8359 ± 0.1422	6.9259 ± 1.9145	4.5173 ± 1.8670	4.4539 ± 1.9186	3.3140 ± 0.3769
R^2	0.9974	0.99	0.997	0.9976	0.9936	0.9928	0.9854	0.9924	0.9954	0.9941	0.9952	0.9977
R^2_{adj}	0.9961	0.9826	0.9948	0.9959	0.9908	0.9885	0.9767	0.9878	0.9934	0.9906	0.9923	0.9963
RSS	0.0002	0.0006	0.0001	0.00008	0.0005	0.0004	0.0007	0.0003	0.0001	0.0001	0.000082249	0.000025064
AIC	-100.1977828	-67.98417956	-82.31825531	-84.10340372	-101.9867751	-82.19143529	-77.1548932	-84.78057395	-119.6905921	-94.66808454	-96.42685513	-107.1217232
T_{opt}	29.83	30.87	29.36	28.65	32.33	32.44	30.44	28.82	33.05	33.81	32.28	31.16
$D_{r.opt}$	0.303	0.307	0.278	0.273	0.285	0.295	0.260	0.239	0.166	0.170	0.156	0.133
Lactin-2												
ρ	0.0139 ± 0.0005	0.0128 ± 0.0007	0.0116 ± 0.0005	0.0129 ± 0.0024	0.0110 ± 0.0004	0.0120 ± 0.0006	0.0108 ± 0.0008	0.0110 ± 0.0005	0.0068 ± 0.0003	0.0067 ± 0.0003	0.0062 ± 0.0003	0.0054 ± 0.0002
T_L	38.1573 ± 0.2250	38.6998 ± 0.3126	36.6978 ± 0.2271	36.5505 ± 1.0625	40.00 ± 0.2115	41.9620 ± 0.3421	40.1049 ± 0.4409	40.2740 ± 0.2769	40.9589 ± 0.3896	41.1241 ± 0.3136	39.3645 ± 0.3331	39.1898 ± 0.3004
λ	-1.1575 ± 0.0123	-1.1343 ± 0.0202	-1.0936 ± 0.0130	-1.1140 ± 0.0498	-1.1104 ± 0.0102	-1.1302 ± 0.0179	-1.0919 ± 0.0196	-1.0872 ± 0.0101	-1.0741 ± 0.0084	-1.0682 ± 0.0090	-1.0534 ± 0.0080	-1.0356 ± 0.0048
ΔT	2.4500 ± 0.1839	2.2095 ± 0.2518	1.8573 ± 0.1731	2.9801 ± 0.8846	1.8463 ± 0.1593	2.8176 ± 0.2796	2.5073 ± 0.3368	3.2379 ± 0.2262	1.9220 ± 0.2250	1.5743 ± 0.1827	1.4573 ± 0.1859	1.7333 ± 0.1629
R^2	0.9962	0.9939	0.9963	0.962	0.9949	0.9936	0.9885	0.9974	0.9905	0.994	0.9935	0.9972
R^2_{adj}	0.9943	0.9893	0.9935	0.9336	0.9927	0.9898	0.9817	0.9958	0.9864	0.9904	0.9897	0.9955
RSS	0.0004	0.0006	0.0003	0.0021	0.0005	0.0006	0.0008	0.0002	0.0003	0.0002	0.0002	0.000050435
AIC	-93.26631104	-67.98417956	-73.529357	-57.96207581	-101.9867751	-78.54224932	-75.95311067	-88.42975992	-107.6058569	-88.42975992	-88.42975992	-100.8284478
T_0	10.517	9.8417	7.7256	8.3798	9.5213	10.203	8.1509	7.6072	10.516	9.8483	8.3969	6.484
T_{opt}	29.5849	30.592444	29.41323	26.451389	32.65642352	32,08493723	30,80119532	29,07701184	32,5118906	33,88175206	32,443	31,01648
$D_{r,opt}$	0.300	0.303	0.283	0.239	0.293	0.290	0.265	0.239	0.157	0.173	0.158	0.136

Both nonlinear models fitted the data well, as reflected by the high R^2 and R^2_{adj} and low RSS and AIC values (Table 3.3). A similar trend as for the linear models was observed regarding the effect of DTR on the low temperature developmental threshold, with decreasing T_0-values as the difference between day and night temperatures increased. In general, lethal temperatures decreased with an increasing DTR. Optimal temperatures, calculated by the first derivative of the model equation, were higher at DTR+5 than at a constant temperature (DTR0). For *P. persimilis* and *T. urticae*, optimal temperatures at DTR+5 were about 1°C higher than at DTR0, whereas for *N. californicus* the relationship between optimal temperatures at constant and alternating temperatures was less clear.

2.3.1.3. Contribution of the rate summation effect to observed differences in developmental rates at constant and alternating temperatures

The percent deviation values shown in Table 2.4, 2.5 and 2.6 indicate that it is not possible to use developmental rates obtained at constant temperatures to accurately predict the rates at alternating temperatures over the whole temperature range. The deviation is more pronounced at lower and higher average temperatures, with values of over 50% in the higher temperature range. At intermediate average temperatures, the percent deviation was overall low (< 10%). Thus, a physiological response that acts in addition to the rate summation effect can be expected in the lower and higher temperature range.

Table 2.4: Percent deviation (d) of expected (according to the rate summation effect) from observed developmental rates at a DTR+5 temperature regime and a 16L:8D h photoperiod for *Phytoseiulus persimilis, Neoseiulus californicus* and *Tetranychus urticae*

Temperature (°C)			d (%)		
Day	Night	Average	*P. persimilis*	*N. californicus*	*T. urticae*
15	10	13.3	-14.9	-16.9	-16.6
20	15	18.3	-2.8	10.2	3.2
25	20	23.3	13.8	4.9	-6.3
27.5	22.5	25.8	2.7	-2.5	-0.7
30	25	28.3	-5.1	-3.1	0.8
32.5	27.5	30.8	-14.0	-7.7	-9.6
35	30	33.3	-59.8	-9.7	-14.3
37.5	32.5	35.8	/[a]	-53.3	-66.1

[a] Immatures were not able to develop to adult

Table 2.5: Percent deviation (d) of expected (according to the rate summation effect) from observed developmental rates at a DTR+10 temperature regime and a 16L:8D h photoperiod for *Phytoseiulus persimilis, Neoseiulus californicus* and *Tetranychus urticae*

Temperature (°C)			d (%)		
day	night	Average	*P. persimilis*	*N. californicus*	*T. urticae*
15	5	11.7	-18.0	-2.5	-6.3
20	10	16.7	-1.8	-4.2	-7.2
25	15	21.7	2.7	1.5	-1.8
27.5	17.5	24.2	8.4	-4.5	-1.7
30	20	26.7	-12.8	-8.0	-2.8
32.5	22.5	29.2	-17.8	-5.1	-6.3
35	25	31.7	-65.0	0.4	-17.3
37.5	27.5	34.2	/[a]	-62.4	-70.1

[a] Immatures were not able to develop to adult

Table 2.6: Percent deviation (d) of expected (according to the rate summation effect) from observed developmental rates at a DTR+15 temperature regime and a 16L:8D h photoperiod for *Phytoseiulus persimilis, Neoseiulus californicus* and *Tetranychus urticae*

Temperature (°C)			d (%)		
day	night	Average	*P. persimilis*	*N. californicus*	*T. urticae*
17.5	2.5	12.5	3.7	3.7	-5.7
20	5	15	12.1	1.4	-5.8
25	10	20	10.9	-3.1	-10.2
27.5	12.5	22.5	2.0	-10.1	2.5
30	15	25	-5.6	-5.6	5.4
32.5	17.5	27.5	-25.1	-5.0	-1.2
35	20	30	-66.0	-8.8	-13.2
37.5	22.5	32.5	/ [a]	-70.9	-78.4

[a] Immatures were not able to develop to adult

2.3.1.4. Potential impact on biological control

As shown in Figure 2.6, 2.7 and 2.8, temperature variations have a different impact on the different mite species. For example, a DTR+5 temperature regime resulted in a faster development of the phytoseiid predator *N. californicus* and of its prey, the two-spotted spider mite *T. urticae,* than at the corresponding mean constant temperature in a range between 20°C and 30°C. For the other predatory mite *P. persimilis,* however, development in the latter temperature range was always faster at the constant temperature regime. When mean temperatures dropped below 15°C, *P. persimilis* benefited more from temperature variations than *N. californicus* and *T. urticae* at each tested thermoperiod.

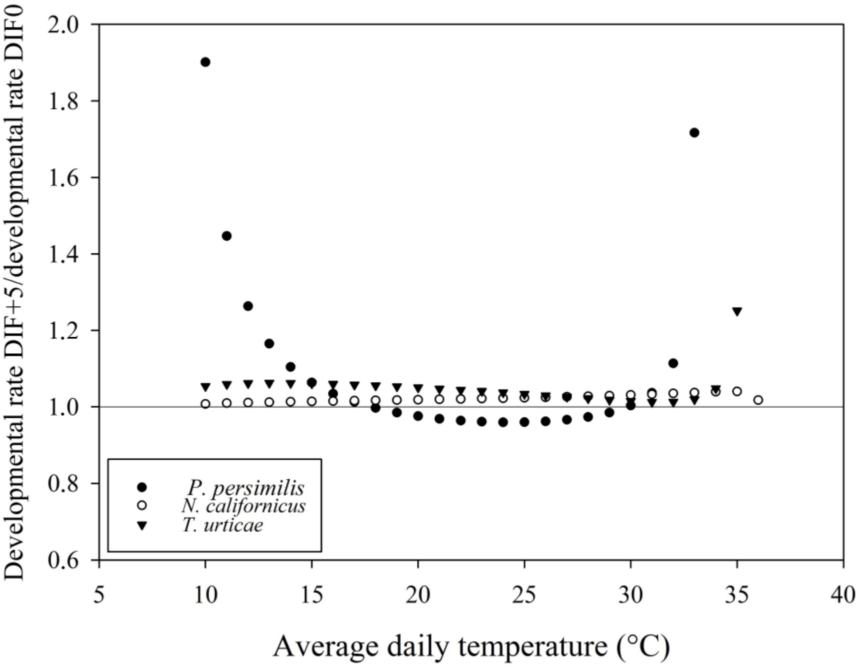

Figure 2.6: Ratio of developmental rate between DTR0 and DTR+5 for *Phytoseiulus persimilis*, *Neoseiulus californicus* and *Tetranychus urticae*

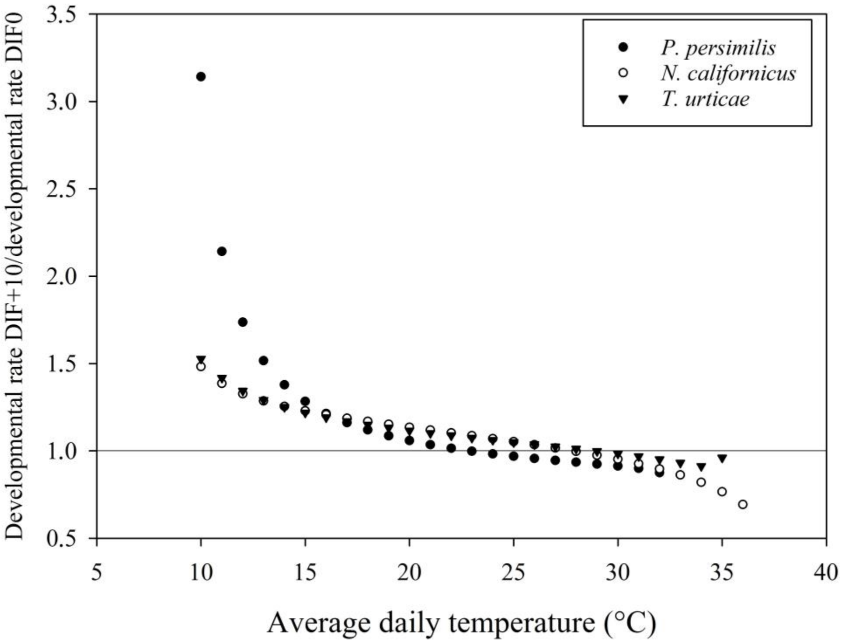

Figure 2.7: Ratio in developmental rate between DTR0 and DTR+10 for *Phytoseiulus persimilis*, *Neoseiulus californicus* and *Tetranychus urticae*

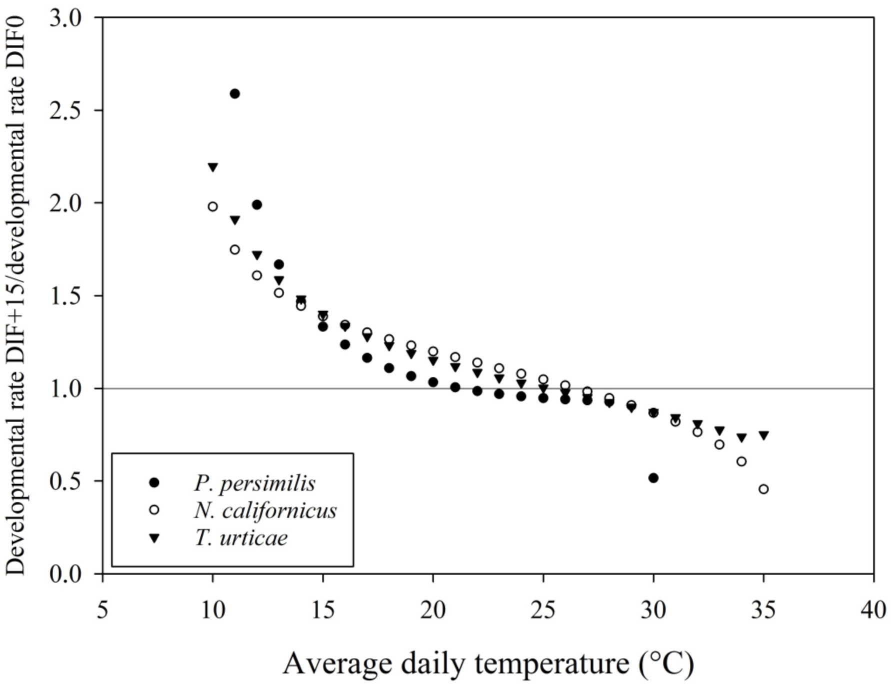

Figure 2.8: Ratio of developmental rate between DTR0 and DTR+15 for *Phytoseiulus persimilis*, *Neoseiulus californicus* and *Tetranychus urticae*

2.3.2. Experiment 2: Reproduction and population growth

Temperature had a significant effect on the reproductive parameters of both phytoseiids (Tables 2.8). The interaction between temperature regime and species was significant for all parameters except for oviposition period (Table 2.7). Preoviposition period was longer (*P. persimilis*: χ^2= 50.26; df= 2; P<0.001 and *N. californicus*: χ^2= 46.39; df= 2; P<0.001) and daily fecundity was greater (*P. persimilis*: F= 281.41; df= 2, 59; P<0.001 and *N. californicus*: F= 432.94; df= 2, 60; P<0.001) at 20°C/5°C than at the corresponding mean temperature (15°C/15°C). A significantly lower lifetime fecundity was found for *P. persimilis* at 15°C/15°C than at 20°C/5°C and 20°C/20°C (F= 11.23; df= 2, 59; P<0.001). Alternating the temperature (20°C/5°C) did not affect the fecundity of *N. californicus* compared to the corresponding mean constant temperature (15°C/15°C). However, lowering the night temperature from 20°C to 5°C resulted in a lower number of deposited eggs (F= 11.71; df= 2, 60; P<0.001) for *N. californicus*. Whereas the proportion of female offspring in *P. persimilis* was similar for the three temperature regimes (Wald Chi-square= 4.29; df= 2; P=0.117), a significantly lower percentage of female offspring was obtained at a constant temperature of 15°C than at the alternating temperature (Wald Chi-square= 42.76; df= 2; $P<0.001$) for *N. californicus.*

All reproductive parameters of *T. urticae* were affected by temperature (Table 2.8). Rearing at the alternating temperature regime resulted in significantly different preoviposition periods (Kruskal-Wallis; χ^2= 34.85; df= 2; $P<0.001$), fecundities (F= 15.27; df=2, 78; $P<0.001$) and oviposition rates (F= 146.08; df= 2, 78; $P<0.001$) compared to rearing at the corresponding mean constant temperature regime. Oviposition periods (F= 7.41; df= 2, 78; $P<0.001$) were similar at both constant regimes, but a low night temperature of 5°C resulted in a 40% longer oviposition period than a night temperature of 20°C. An average increase of approximately 50 eggs per female was achieved at the alternating temperature compared to a constant 15°C. Raising the night temperature from 5°C to 20°C increased oviposition rate by about 65 %. Temperature regime had no effect on the sex ratio of the spider mite offspring with ca. 70% females in all cases.

Two-way ANOVA indicated no interaction between temperature regime and species (*P. persimilis, N. californicus* and *T. urticae*) for the net reproductive rate, R_0 (Table 2.9), with a significant effect of both main factors. For all other parameters the interaction between temperature and species was found to be significant.

Table 2.7: Results of a logistic regression or a two-way ANOVA indicating the effect of temperature regime (15°C/15°C, 20°C/5°C or 20°C/20°C) and predator species on oviposition parameters, sex ratio and life table parameters of *P. persimilis* and *N. californicus* fed on *T. urticae*

Parameter	Temperature			Species			Temperature * Species			Error Term
	F	df	p	F	df	p	F	df	P	df
Preoviposition period[a]	156.8	2	<0.001	83.84	1	<0.001	8.173	2	<0.001	119
Fecundity[a]	18.68	2	<0.001	126.6	1	<0.001	4.533	2	0.013	119
Oviposition period[a]	34.71	2	<0.001	12.14	1	<0.001	1.192	2	0.307	119
Oviposition rate[a]	624.0	2	<0.001	285.8	1	<0.001	13.23	2	<0.001	119
Sex ratio[b]	-	-	0.002	-	-	<0.001	-	-	<0.001	-
r_m[a]	4323.5	2	<0.001	355.45	2	<0.001	39.273	4	<0.001	197
R_0[a]	15.57	2	<0.001	96.4	2	<0.001	2.084	4	0.084	197
DT[a]	4281.3	2	<0.001	453.070	2	<0.001	125.6	4	<0.001	197

Two-way ANOVAs of life table parameters r_m, R_0 and DT include *T. urticae* as a species; other parameters only include *P. persimilis* and *N. californicus* in the factor species

[a] Two-way ANOVA

[b] Probit

Table 2.8: Reproductive parameters of *P. persimilis*, *N. californicus* and *T. urticae* reared at three temperature regimes

Species	Temperature regime	n[a]	Preoviposition period (days)	Oviposition period (days)	Fecundity (eggs/female)	Oviposition rate (eggs/female/day)	n[b]	Offspring sex ratio (%♀♀)
P. persimilis	15°C/15°C	19	4.0 ± 0.1 c	48.6 ± 4.4 c	61.3 ± 5.4 b	1.27 ± 0.04 c	988	77.23 ± 1.34 a
	20°C/5°C	19	2.8 ± 0.1 b	43.3 ± 2.2 b	86.6 ± 4.1 a	2.01 ± 0.05 b	1207	74.15 ± 1.26 a
	20°C/20°C	24	1.9 ± 0.1 a	29.6 ± 0.9 a	82.7 ± 2.4 a	2.81 ± 0.05 a	1609	73.77 ± 1.10 a
N. californicus	15°C/15°C	19	5.6 ± 0.3 c	43.1 ± 1.9 c	41.2 ± 1.6 b	0.96 ± 0.02 c	673	54.23 ± 1.92 b
	20°C/5°C	23	4.1 ± 0.2 b	33.4 ± 1.5 b	47.5 ± 1.8 b	1.43 ± 0.03 b	890	69.44 ± 1.55 a
	20°C/20°C	21	2.3 ± 0.1 a	26.2 ± 1.2 a	55.5 ± 2.5 a	2.12 ± 0.03 a	937	67.24 ± 1.53 a
T. urticae	15°C/15°C	28	3.0 ± 0.1 c	29.3 ± 1.7 ab	107.6 ± 6.4 b	3.67 ± 0.07 c	786	70.74 ± 1.62 a
	20°C/5°C	29	2.5 ± 0.1 b	35.2 ± 2.3 b	156.6 ± 10.9 a	4.46 ± 0.15 b	1451	70.23 ± 1.20 a
	20°C/20°C	24	1.7 ± 0.1 a	24.7 ± 1.5 a	180.3 ± 10.2 a	7.39 ± 0.23 a	1491	69.15 ± 1.20 a

Means ± SE within a column and a species followed by the same letter are not significantly different (P>0.05; Mann-Whitney U (preoviposition period), Tukey (*P. persimilis*: oviposition rate; *N. californicus*: fecundity, oviposition period; *T. urticae*: fecundity, oviposition peiod), Tamhane (*P. persimilis*: fecundity, oviposition period; *N. californicus*: oviposition rate; *T. urticae*: oviposition rate) or probit test (offspring sex ratio)

[a] number of tested females

[b] number of observed offsprin

Table 2.9: Life table parameters of *P. persimilis*, *N. californicus* and *T. urticae* reared at three temperature regimes

Species	Temperature regime	n[a]	r_m (females/female/day)	R_0 (females/female)	DT (days)
P. persimilis	15°C/15°C	19	0.118 ± 0.001 f	42.3 ± 4.7 d	5.89 ± 0.04 f
	20°C/5°C	19	0.156 ± 0.002 d	59.0 ± 3.7 c	4.44 ± 0.02 d
	20°C/20°C	24	0.235 ± 0.001 a	58.0 ± 2.4 c	2.95 ± 0.02 a
N. californicus	15°C/15°C	19	0.084 ± 0.001 h	22.7 ± 1.1 f	8.31 ± 0.05 h
	20°C/5°C	23	0.132 ± 0.001 e	33.9 ± 1.5 e	5.27 ± 0.05 e
	20°C/20°C	21	0.204 ± 0.001 b	37.5 ± 1.6 e	3.40 ± 0.02 b
T. urticae	15°C/15°C	28	0.102 ± 0.001 g	67.0 ± 4.9 b	6.82 ± 0.06 g
	20°C/5°C	29	0.136 ± 0.002 e	99.0 ± 8.1 a	5.11 ± 0.06 e
	20°C/20°C	24	0.192 ± 0.003 c	107.7 ± 9.1 a	3.63 ± 0.06 c

Means ± SE within a column followed by the same letter are not significantly different ($P>0.05$; Tamhane (r_m and DT) or Tukey test (R_0))

[a] number of tested females

The intrinsic rates of increase (r_m) (F= 1217.40; df= 8, 197; $P<0.001$) were superior for *P. persimilis* at all temperature regimes (Table 2.9). *Neoseiulus californicus* had a lower, similar and higher r_m-value than *T. urticae* at 15°C/15°C, 20°C/5°C and 20°C/20°C, respectively. For all three mite species, a lower net reproductive rate (R_0) was found at 15°C/15°C compared to the two other temperature regimes. The alternating temperature regime resulted in a shorter doubling time (DT) (F= 1243.34; df=8, 197; $P<0.001$) than the corresponding constant temperature for all three mite species.

2.4. Discussion

Temperature alternations had a substantial impact on the egg-adult developmental rates (experiment 1) and the population growth (experiment 2) of the phytoseiid predators *P. persimilis* and *N. californicus* and their prey *T. urticae* as compared to the rates at the corresponding mean constant temperatures. In line with earlier studies on thermal responses of arthropods (e.g., Champlain and Butler 1967; Ratte 1985; Worner 1992; Bryant et al. 1999; Liu et al. 1995; Fantinou et al. 2003) developmental rates were higher at varying temperatures in the lower temperature range, whereas lower developmental rates were observed at higher temperatures compared to the corresponding constant temperature regimes. However, not all deviations could be explained by the rate summation effect (see Tables 2.4, 2.5 and 2.6).

Intriguingly, the highest developmental rates were observed at a DTR+5 and not at an optimal constant temperature. A direct consequence of the rate summation effect -and therefore also Jensen's inequality- is that a weighted average (16h light and 8h dark) of developmental rates at constant temperatures used to predict rates at alternating temperatures can never exceed the maximum rate at optimal constant temperature. However, for *P. persimilis*, *N. californicus* and *T. urticae,* alternating temperatures with an amplitude of 5°C (29.2°C/24.2°C, 30.8°C/25.8°C and 32.1/27.1°C, respectively) resulted in a faster development than the optimal rate at the optimal constant temperature. This is, at least for the species tested in this study, an indication that rate summation might be insufficient to explain the observed differences between developmental rates obtained at constant and alternating temperature regimes.

The paradoxical idea that a temperature lower than T_{opt} is the temperature at which fitness is maximized was discussed by Martin and Huey (2008). As the asymmetric temperature-rate curve of ectothermic organisms rapidly declines when temperatures exceed the optimal temperature, a slight increase in temperature above T_{opt} has a tremendous detrimental effect on the development rate, whereas a similar slight decrease below T_{opt} has relatively little impact. Therefore, ectotherms might experience an increased fitness when experiencing a temperature somewhat lower than T_{opt} to avoid temperatures higher than T_{opt} and the corresponding drop in developmental rate ("suboptimal is optimal", Martin and Huey 2008).

Possible additional physiological mechanisms in addition to the rate summation effect have been reported, albeit explained vaguely (Liu et al. 1995; Xu 1996; Worner 1992). Some authors (Huffaker 1944; Ratte 1985) have attributed these responses to a disorganized metabolism, an inadequate supply of nutrients and oxygen, or desiccation; the latter reason does not apply to our study as our experiments were conducted at high humidity (>90% RH). Behrens et al. (1983) pointed out that ectothermic organisms have evolved in an environment with fluctuating temperature and are therefore adapted to diurnally changing temperatures. Therefore, the lack of a diurnal rhythm (i.e. diurnal periodicity of activity and rest) per se might affect certain metabolic reactions. Additionally, the energy demand during the day is likely to be higher than during the night, as the optimal temperature for metabolic reactions during the night is lower than that during the light and probably more active part of the day (Ratte 1985). For *T. urticae*, a higher activity (feeding and oviposition) was observed during the day (light) than during the night (dark) (Maeda et al. 2000). In the same study, the predatory mite *Amblyseius womersleyi* Schicha (Acari: Phytoseiidae) showed a similar activity pattern, with reduced dispersal and predation rates during the night. Accordingly, we found a lower hourly predation rate of *P. persimilis* and *N. californicus* on *T. urticae* eggs during the night than during the day at a constant temperature of 20°C (Vangansbeke et al., unpublished data). As a result, if the food requirements are satisfied during the day, energy demands (such as respiration) during periods of rest should be lower under alternating temperatures than under corresponding constant temperatures (Ratte 1985).

Other possible mechanisms, that might act in addition to the rate summation effect, may be found in the production of cryoprotectants when the organism is exposed to low night temperatures (Bale 2002) or heat shock proteins when exposed to high daytime temperatures (Otsuka et al. 1997; Rinehart et al. 2007). Revealing such mechanisms warrants further analysis at the molecular level. Recently, the differentially expressed genes of diapausing versus non-diapausing *T. urticae* females were documented (Bryon et al. 2013). Similarly, such expression analysis could reveal which genes are differentially expressed when temperatures are allowed to vary instead of being kept constant. Possibly, the expression of certain genes is triggered by a diurnal rhythm (Tomanek 2010). Additionally, we cannot exclude indirect effects of the DTR on the development of *T. urticae* via nutritional value of the leaf discs. For example, temperature variations have been reported to alter the levels of gibberellin in different plant species (Myster and Moe 1995; Stavang et al. 2005), which may affect the performance of *T. urticae* feeding on those plants (Eichmeier and Guyer 1960). Possibly, also the levels of other components are affected by DTR, which in turn could influence the performance of herbivores.

The resulting lower developmental thresholds (T_0) were affected by the amplitude of the temperature alternation, with a decreasing T_0 as the amplitude of the temperature variation increased. The average temperature at which total immature development approximates zero is 3 to 4 °C lower at DTR+15 compared to the constant temperature regime. This trend was confirmed by both nonlinear models. Degree-day modelling is a widely used tool to predict the timing of a wide range of biological processes and has been successfully adopted in the management of arthropod pests (Pedigo 1998; Morrison et al. 2013). The amount of degree-days necessary to complete an event is calculated as the number of heat units above T_0 (Campbell et al. 1974). Evidently, the number of degree-days required will increase as T_0 decreases, as the organism will start to develop from a lower temperature onwards (Honek and Kocourek 1990). Our results highlight the impact of diurnal temperature variations, emphasizing the need to integrate temperature variations in predictive degree-day models. As such, population build-up of pests early in the season can be expected to happen earlier than when using constant temperature models.

Temperature alternations also had a significant influence on all reproductive parameters tested in the second experiment, but the effect differed among the examined species. For instance, lifetime fecundity was not affected by alternating temperature in *N. californicus*, whereas more progeny was produced by *P. persimilis* and *T. urticae* at 20°C/5°C compared to 15°C/15°C. Most authors who examined the fecundity of arthropods under both constant and variable temperature regimes generally found a higher number of produced eggs under variable conditions (Messenger 1964; Davis et al. 2006), although some studies reported no effect (Hagstrum and Leach 1973). In the present study, the oviposition rate was significantly higher at the alternating temperature regime than at the constant mean temperature for all three mite species. Furthermore, lowering the night temperature from 20°C to 5°C with a 20°C day temperature during the photophase resulted in a lower oviposition rate and longer oviposition period for all examined mite species, indicating the role of scotophase temperature for reproduction.

The sex ratio of the progeny was not affected by temperature regime in *P. persimilis* and *T. urticae*. In contrast, *N. californicus* produced a higher proportion of female offspring at the alternating temperature regime compared to the corresponding constant 15°C. The latter species has been shown to lower its proportion of female offspring under unfavorable conditions (Helle and Sabelis 1985; Castagnoli and Simoni 1999). Consequently, if sex ratio is considered to be a factor determining the response of phytoseiids to their environment, the alternating temperature regime of 20°C/5°C should be more favorable for *N. californicus* than a constant 15°C. Variations in the proportion of female offspring under unfavorable conditions have been reported for *P. persimilis* and *T. urticae* as well (Margolies and Wrensch 1996; Toyoshima and Amano 1998), but no such effect was observed in the present study.

The intrinsic rate of increase assembles several life table parameters such as age-specific survivorship, developmental time, daily fecundity and sex ratio of the progeny into one value. Lewontin (1965) calculated that a 10% increase of development rate roughly has the same effect on the growth rate as a 100% increase in fertility, indicating the relative importance of developmental time in the calculations of population growth. As mentioned above, developmental rate was strongly influenced by alternation of the temperature, and therefore a significant effect on the intrinsic rate of increase should be expected. Alternating the temperature indeed enhanced the population growth rate for all three species. At the tested temperature regimes, *P. persimilis* showed higher intrinsic rates of increase than *N. californicus* and its target prey. Whereas at 15°C/15°C *N. californicus* had a lower r_m-value than *T. urticae*, the predator had a higher growth rate than the pest at 20°C/20°C and a similar growth rate at the alternating temperature regime. If r_m-values are an indication of the control potential of a predator (Helle and Sabelis 1985; Nomikou et al. 2001; Stavrinides and Mills 2011), *N. californicus* should be able to control *T. urticae* at 20°C/5°C, whereas this may not be the case at the corresponding mean constant temperature of 15°C. Thus, allowing alternating temperatures in a crop may benefit the biological control potential of the latter phytoseiid against spider mite pests.

Moreover, in protected crops there is an increasing tendency to allow temperature variations within certain boundaries, as an energy-saving strategy (Körner and Challa 2003; Messelink et al. 2014). This so-called temperature integration approach allows greenhouse growers to save up to 20% of their energy costs (Tantau 1998; Dieleman et al. 2005). Both the pests and their natural enemies introduced in the crop for their management are arguably affected by these temperature variations, which may have its implications for the success of the biological control programs. Our results demonstrate a substantial impact of temperature variations on the immature development of the studied mite species. For example, in a temperature range between 15°C and 25°C, the effect of a DTR+15 is more pronounced for *T. urticae*, resulting in a relatively faster development of the pest than its predator *P. persimilis* as compared to a constant temperature regime (Figure 2.8). Differences were not only visible between pest and predator, but also among the studied phytoseiid predators (*P. persimilis* versus *N. californicus*). Between average daily temperatures of 15 and 25°C, *N. californicus* experienced a more positive effect on developmental rates at alternating temperatures than *P. persimilis*. Below an average daily temperature of 15°C, *P. persimilis* benefited more from alternating temperature regimes. Additionally, predation rates of the studied phytoseiid predators are also affected by temperature alternations (see **Chapter 3**). Therefore, we advocate that information on the impact of temperature variations should be included in models on biological control interactions as well as the selection procedure of the most suitable natural enemies.

Arguably, the findings from experiments using small arenas should be interpreted with caution and field trials are needed to confirm the outcome of the present laboratory study. For instance, the humidity inside the insect breeding dishes fluctuated between 85-100% RH, which is considerably higher than the 65% RH setting of the climatic chambers. This may have influenced the outcome of our experiments, since egg hatchability (van Houten et al. 1995; de Vis et al. 2006), development (Stenseth 1979), reproduction (Kumari and Sadana 1991) and predation (Shipp et al. 1996) of phytoseiids are affected by the level of humidity. The high relative humidity may have favored the phytoseiids, while it may have disadvantaged the spider mites as Boudreaux (1958) found that survival, oviposition and egg hatchability of tetranychids decreased at high humidity conditions. Consequently, our laboratory study may have underestimated the developmental and reproductive performance of *T. urticae.*

In summary, our results indicate that the rate summation effect alone does not suffice to explain the observed and predicted differences in developmental rates between constant and varying temperatures, especially in the lower and higher temperature ranges. Developmental rates and possibly other life history parameters at a specific temperature cannot be evaluated independently from the prevailing temperature regime. Further research should investigate possible physiological mechanisms that act in addition to the rate summation effect to fully appreciate the impact of diurnal cycling temperatures on life history traits of ectotherms. Diurnal temperature variations should be incorporated in predictive models on ectotherm ecology to generate more accurate predictions on the phenology of agricultural pests and their natural enemies. Finally, our findings may aid in further understanding the effects of climate change, as not only mean temperatures will increase, but also diurnal temperature ranges will be altered (Easterling et al. 1997). This is particularly relevant for ectotherms as they are more sensitive to temperature variation and are thus expected to be more vulnerable to the consequences of climate change (Paaijmans et al. 2013; Aguilar-Fenollosa and Jacas 2014).

3

Prey consumption by phytoseiid spider mite predators as affected by diurnal temperature variations

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3.1. Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most devastating agricultural pests worldwide (Jeppson et al. 1975; Helle and Sabelis 1985; Gerson and Weintraub 2012). Its host range comprises over 1100 plant species (Grbić et al. 2011), many of which are economically important crops. In addition, the pest has the ability to rapidly develop resistance against chemical pesticides (Van Leeuwen et al. 2014). To overcome this issue, along with a growing public demand for residue-free foods (van Lenteren 2000), alternative control strategies have become increasingly important. Increasing sustainability in agricultural production by way of integrated pest management (IPM) strategies has become a primary objective in policy. For instance, the European Directive 2009/128/EC aims at a substantial reduction of pesticide use by obliging professional users of pesticides in Europe to implement the principles of IPM. An important component of IPM is the use of biological control practices, such as the augmentative release of natural enemies. Phytoseiid predatory mites, especially *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* McGregor (Acari: Phytoseiidae), have demonstrated effective control of *T. urticae* outbreaks, both in protected and open field crops (Oatman et al. 1968; Schausberger and Walzer 2001; Rhodes et al. 2006; Weintraub and Palevsky 2008). The success of a biological control program depends on a variety of biotic and abiotic factors. One of the most influential abiotic factors affecting pest/predator interactions is temperature (Sabelis 1981; Logan et al. 2006; Öhlund et al. 2015). For predatory mites, a large body of information is available on the effects of temperature on life history traits and predation rates (e.g., Hardman and Rogers 1991; Shipp et al. 1996; Gotoh et al. 2004; Kim et al. 2009). However, whereas previous research has predominantly focused on the effects of constant temperatures, natural temperature regimes usually undergo diurnal cycles (Hagstrum and Hagstrum 1970; Beck 1983; Brakefield and Mazzotta 1995) which may affect performance of the predatory mites. Additionally, there is an increasing tendency in protected crops to allow higher daily temperature variations as an energy-saving strategy (Tantau 1998; Pollet et al. 2009), which may affect biological control (Messelink et al. 2014).

Both theoretical and empirical studies have been conducted to understand the effects of temperature variations on the biology of ectotherms (e.g., Siddiqui et al. 1973; Worner 1992; Liu et al. 1995; Paaijmans et al. 2010; Estay et al. 2014). These studies have demonstrated an impact on developmental rate (Garcia-Ruiz et al. 2011; Gotoh et al. 2014), reproduction (Messenger 1964; Behrens et al. 1983; Mironidis and Savopoulou-Soultani 2008), sex ratio

(**Chapter 2**), vector capacity (Paaijmans et al. 2010) and stress resistance (Terblanche et al. 2010) in various arthropods. These observed and predicted differences between ectothermic performance at constant and varying temperature regimes have been attributed to the typically nonlinear relationship between the ectotherm's performance and temperature (Worner 1992). As a consequence of Jensen's inequality (Ruel and Ayres 1999), temperature variations over a concave part of a thermal fitness curve (i.e. the cold end) tend to result in an increase of performance, whereas variations over the convex part (i.e. the warmer part of the curve) result in a net decrease of performance (Estay et al. 2014). In arthropod thermal biology and physiology, this phenomenon is known as the Kaufmann-effect (Worner 1992). Up to now, little is known about the effects of temperature variations on the predation rate of arthropod predators (Audenaert et al. 2014). If prey consumption tends to have a similar nonlinear relationship with temperature like other life history traits (e.g. developmental rate), a similar response is to be expected, where higher predation rates would occur under diurnal temperature variations in the low temperature range, whereas the opposite would be expected in the higher temperature range, as compared to the corresponding mean constant temperatures.

In the present study, we assessed the effect of temperature variations on the body size, predation capacity and oviposition rate of *P. persimilis* and *N. californicus* presented with eggs of their target prey *T. urticae.* We tested 5 temperatures both under a constant (DTR0) and an alternating temperature regime with a diurnal amplitude of 15°C (DTR+15).

3.2. Material and methods

3.2.1. Mite rearing

Mite colonies were reared as described in section 2.2.1.

3.2.2. Experimental set-up

For each temperature regime, about 50 eggs (less than 8h old) of either *P. persimilis* or *N. californicus* were collected from the colony and were placed in groups of 10 eggs on circular bean leaf arenas (25 x 25 mm) placed upside down on a wet polyurethane sponge in an insect breeding dish (ø 100 mm, H 40 mm) (SPL Life Sciences, Korea). The edges of the leaf arena were covered with moist tissue paper to provide free water and prevent the mites from escaping. Three days before the introduction of the phytoseiid eggs, 5 female *T. urticae* were introduced to each leaf arena, thus ensuring the presence of both eggs and mobile stages on the leaf disc during the feeding stages of the predators. After the introduction of the predator eggs, the dishes were placed in an environmental test chamber (Sanyo Electric Co., Ltd., Japan) at the temperature regime to be tested. The test temperatures were 15, 20, 25, 27.5 and 30°C both under a constant (DTR0) and alternating regime with a difference of 15°C between day and night temperatures (DTR+15). Thus, the corresponding alternating temperatures were 20°C/5°C, 25°C/10°C, 30°C/15°C, 32.5°C/17.5°C and 35°C/20°C (day temperature/night temperature, at a 16:8 h L:D photoperiod). Once the predatory mites reached adulthood, females were placed together with a male on an individual leaf arena infested with a mixture of all stages of *T. urticae*. Next, 2 to 5 days after the first egg was laid (i.e. the time at which oviposition rate peaks, Janssen and Sabelis 1992; Abad-Moyano et al. 2009), depending on the temperature regime, females were individually transferred to a bean leaf arena on which 40 *T. urticae* eggs were presented. For this purpose, 10 female *T. urticae* had been allowed to oviposit on the leaf arena for 5h prior to the experiment, after which the females were removed from the arena and the number of eggs was reduced to 40 by destroying excess eggs with a fine needle. Twenty-four hours after their introduction to the test arenas, predator females were removed from the arena and their consumption rate was determined by counting the number of remaining *T. urticae* eggs. Additionally, the number of phytoseiid eggs deposited during the 24-h experimental period was counted. The oviposition rate of phytoseiid predatory mites at peak oviposition is strongly correlated with the intrinsic rate of increase (Janssen and Sabelis 1992), and is thus believed to be a good indicator for population growth. Twenty-five replicates were done for each temperature regime.

To assess the effect of temperature regime on body size of both phytoseiids, females used in the predation experiment were mounted on glass slides in a Marc-André II medium (Upton 1993) which were then kept for one week at 50°C. The length of the dorsal shield was measured under a microscope at a 200X magnification (Leica Microsystems, Leica Application Suite version 3.8.8, Wetzlar, Germany). In Phytoseiidae, the length of the dorsal shield has been noted to be a reliable measure of body size (Toyoshima and Amano 1998; Croft et al. 1999).

3.2.3. Statistical analysis

Data were analyzed using SPSS version 20 (IBM SPSS statistics). Predation rates, oviposition rates and body size measures were tested for normality using a Kolmogorov-Smirnov test before being subjected to two-way analysis of variance (ANOVA) with average daily temperature and temperature variation (constant versus alternating) as main factors. The level of significance was set at 0.05.

3.3. Results

The predatory mites were able to complete their development at all temperature regimes with an immature survival of 100%, except for *P. persimilis* at 35°C/20°C. Under the latter regime, only 3 *P. persimilis* females reached the adult stage. Two of these drowned while trying to escape before the start of the predation experiment, whereas the third died during the experiment. Hence, no data were obtained for *P. persimilis* at 35°C/20°C.

For both *P. persimilis* and *N. californicus*, two-way ANOVA indicated a significant effect of average daily temperature, temperature variation and their interaction on the number of prey killed (Table 3.1). A significant interaction indicates that temperature variation influenced the effect of average daily temperature on the predation rate of the phytoseiids. Figure 3.1 and 3.2 show similar trends in predation rate as a function of temperature regime for *P. persimilis* and *N. californicus*. Higher predation rates were observed at alternating temperature regimes with average daily temperatures of 15 and 20°C as compared with the corresponding constant temperature regimes. However, when the average daily temperature exceeded 25°C, less prey eggs were killed at alternating temperatures than at constant ones.

Oviposition rate of *P. persimilis* was affected by the average daily temperature but not by temperature variation (Table 3.2). The interaction among the main factors, however, was found to be significant. For *N. californicus*, oviposition rate was affected by average temperature, temperature variation and the interaction thereof. In general, oviposition rates followed the same trends as predation rates (Figure 3.3 and 3.4). However, whereas at a constant 30°C predation rates were lower than at 27.5°C, an opposite trend was observed for oviposition rates.

Table 3.1: Results of two-way ANOVA assessing the impact of average daily temperature and temperature alternation on prey consumption, oviposition rate and body size of *Phytoseiulus persimilis* and *Neoseiulus californicus* presented with *Tetranychus urticae* eggs

		P. persimilis			*N. californicus*		
Parameter	Source	F	df	p	F	df	p
Prey consumption							
	Average temperature	215.823	3	<0.001	343.797	4	<0.001
	Temperature variation	11.043	1	0.001	122.573	1	<0.001
	Average temperature x temperature variation	62.789	3	<0.001	274.965	4	<0.001
	Error		202			262	
Oviposition rate							
	Average temperature	218.806	3	<0.001	272.984	4	<0.001
	Temperature variation	0.161	1	0.689	99.948	1	<0.001
	Average temperature x temperature variation	63.431	3	<0.001	286.813	4	<0.001
	Error		202			262	
Body size							
	Average temperature	29.506	3	<0.001	97.608	4	<0.001
	Temperature variation	0.077	1	0.781	210.847	1	<0.001
	Average temperature x temperature variation	0.718	3	0.543	5.595	4	<0.001
	Error		174			280	

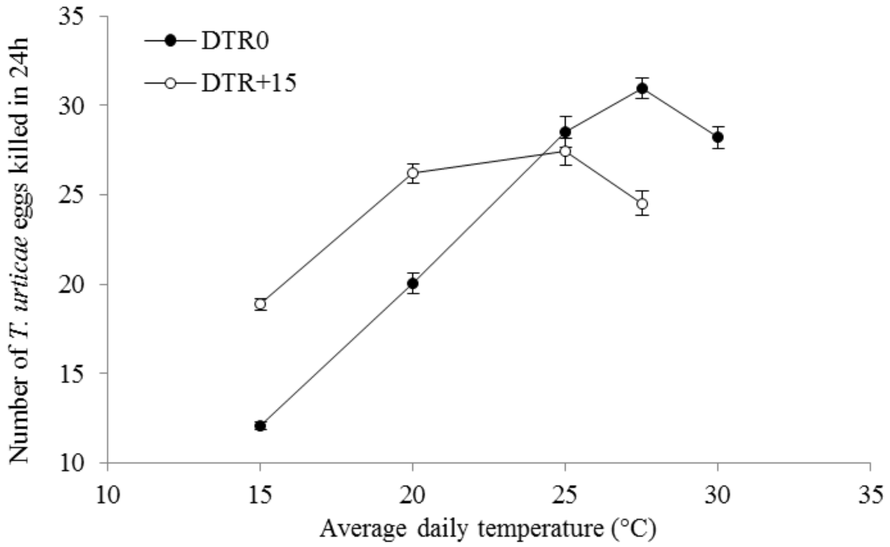

Figure 3.1: Effect of average daily temperature and temperature alternation (DTR0 versus DTR+15) on the consumption of *Tetranychus urticae* eggs by *Phytoseiulus persimilis*

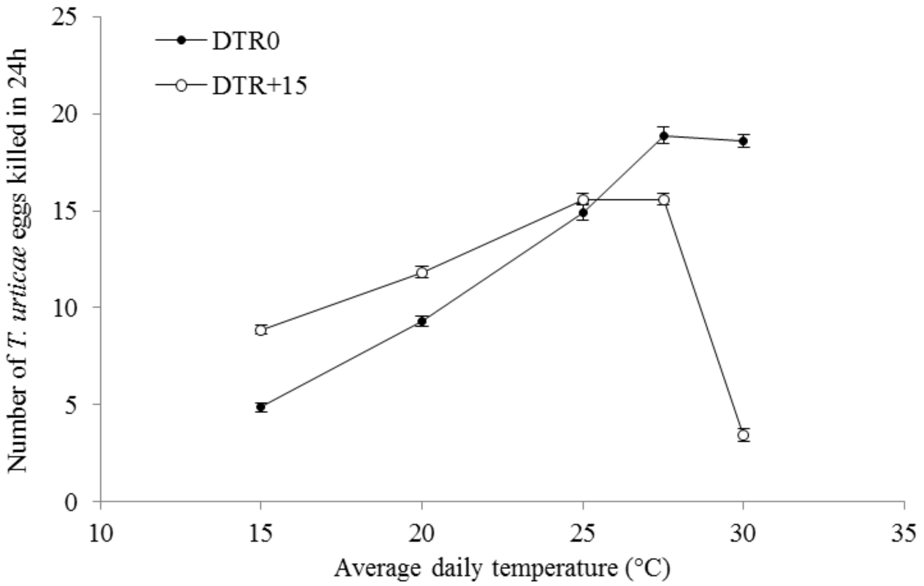

Figure 3.2: Effect of average daily temperature and temperature alternation (DTR0 versus DTR+15) on the consumption of *Tetranychus urticae* eggs by *Neoseiulus californicus*

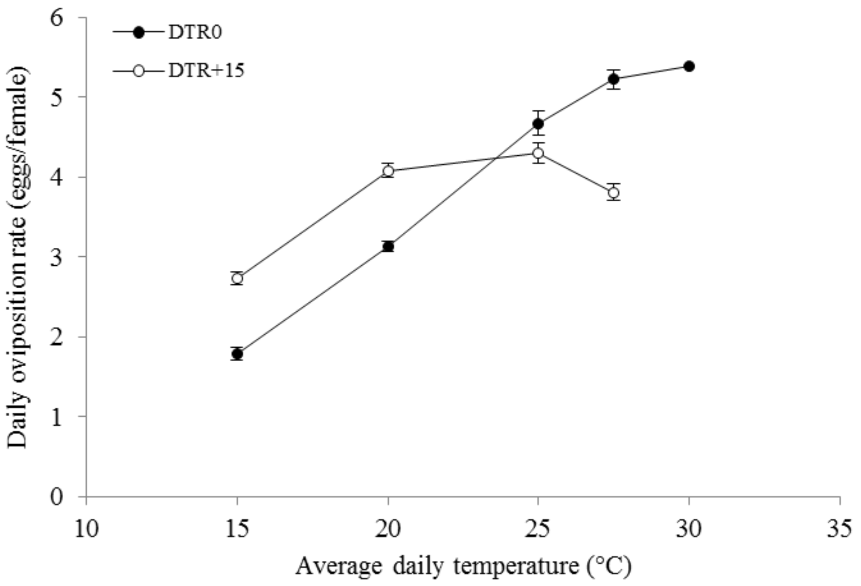

Figure 3.3: Effect of average daily temperature and temperature alternation (DTR0 versus DTR+15) on oviposition rates of *Phytoseiulus persimilis* feeding on *Tetranychus urticae* eggs

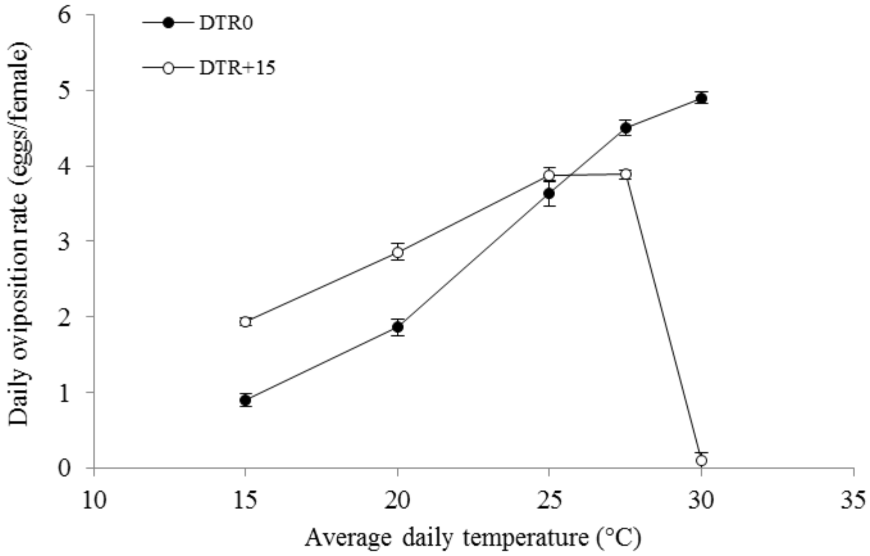

Figure 3.4: Effect of average daily temperature and temperature alternation (DTR0 versus DTR+15) on oviposition rates of *Neoseiulus californicus* feeding on *Tetranychus urticae* eggs.

The body size of *P. persimilis* was affected by average daily temperature, but not by temperature variation (Table 3.1). For *P. persimilis*, an average daily temperature of 15°C, both under constant and alternating temperature regimes, resulted in larger body size than at the other temperatures regimes. However, body sizes of *P. persimilis* females were similar at average daily temperatures above 15°C (Figure 3.5). For *N. californicus*, body size was affected by average temperature, temperature variation and their interaction (Table 3.1) and there was a negative relationship between temperature and body size with larger females appearing at lower temperatures (Figure 3.6).

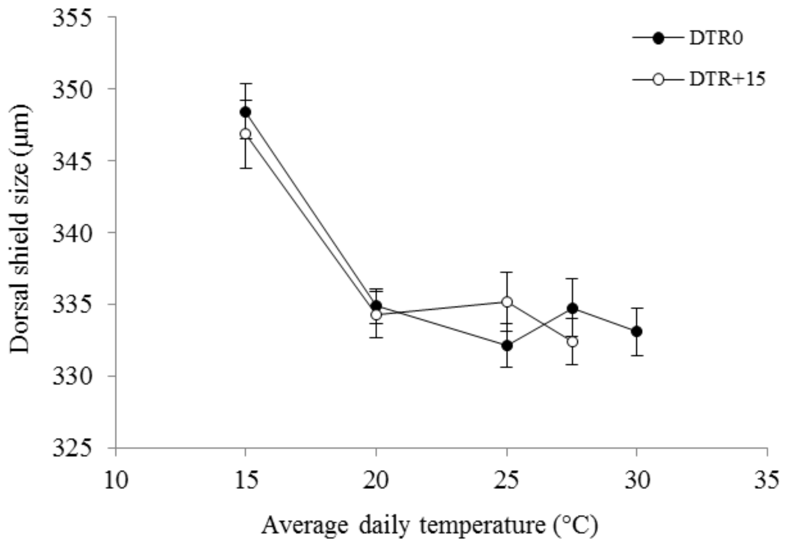

Figure 3.5: Effect of average daily temperature and temperature alternation (DTR0 versus DTR+15) on the body size of *Phytoseiulus persimilis* as measured by the length of the dorsal shield (µm)

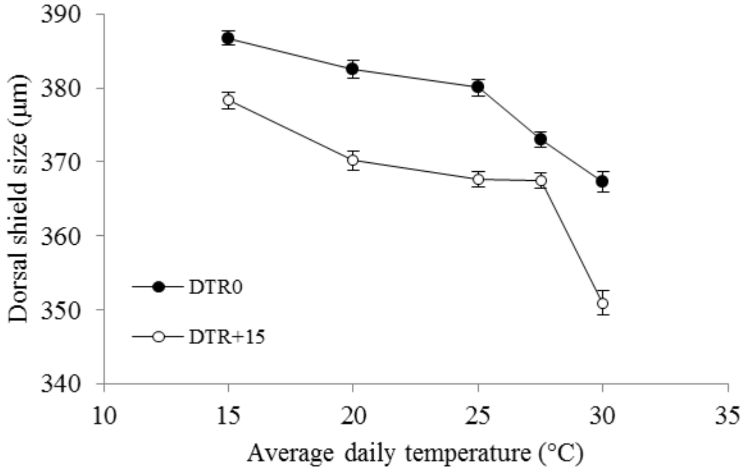

Figure 3.6: Effect of average daily temperature and temperature alternation (DTR0 versus DTR+15) on the body size of *Neoseiulus californicus* as measured by the length of the dorsal shield (µm)

3.4. Discussion

Our results demonstrate the impact of temperature and its diurnal alternations on body size, prey consumption and oviposition rate of the phytoseiid predators *P. persimilis* and *N. californicus* when feeding on *T. urticae* eggs. In general, our results are in line with previous studies assessing the effect of diurnal temperature variations on the performance of ectotherms (e.g. Messenger and Flitters 1958; Worner 1992; Liu et al. 1995; Bryant et al. 1999; Fantinou et al. 2003; Paaijmans et al. 2013): both predators showed increased predation and oviposition rates at alternating temperatures in the lower temperatures range (<25°C) and lower rates in the higher temperature range (>25°C), as compared with the corresponding average daily temperature. The observed relationship between predation rate of *P. persimilis* and *N. californicus* and temperature was nonlinear (Figure 3.1 and 3.2), reflecting the curvilinear relationship between their developmental rate and temperature, similar to the curves describing the relationship between development rate and temperature in **Chapter 2**. However, it may be warranted to test more temperatures to ascertain the shape of the curves over the complete temperature range. The high juvenile mortality rates at 35°C/20°C did not allow investigation of the predation and oviposition rates of *P. persimilis*, confirming earlier studies that temperatures above 30°C are detrimental for this species (Skirvin and Fenlon 2003; **Chapter 2**). For *N. californicus*, higher temperatures should be tested to reveal at which temperature prey consumption and oviposition rate reach their peak value.

As a result of the increasing energy prices, greenhouse growers in temperate areas tend to allow higher temperature variations in order to reduce their energy cost (Pollet et al. 2009; Messelink et al. 2014). In this so-called temperature integration approach (Körner and Challa 2003), growers allow temperatures to vary within certain boundaries. Hence, less heating during the night and less ventilation during the day can save growers up to 20% of their energy expenses (Dieleman et al. 2005; Pollet et al. 2009). The results from our laboratory experiments suggest that this approach may impact the predation capacity of natural enemies and may as such affect the outcome of a biological control program. For example, when during summer average daily temperatures of 30°C are allowed to vary with an amplitude of 15°C, *P. persimilis* and *N. californicus* will have difficulty controlling *T. urticae* outbreaks based on the low consumption rates, as these higher temperatures still support development and reproduction of *T. urticae* (Skirvin and Fenlon 2003, **Chapter 2**). On the other hand, higher kill rates were observed at a DTR+15 regime with average daily temperatures of 15 and 20°C: predation rates were 56.3 and 82.5% higher at DTR+15 than at DTR0 at an average daily temperature of 15°C for *P. persimilis* and *N. californicus*, respectively. In a previous study, we found that developmental rate increased by 35 to 41.6 % both for the predators and the prey at an average daily temperature of 15°C (**Chapter 2**). At an average daily temperature of 20°C, *P. persimilis* showed a similar developmental rate at DTR0 and DTR+15 (6.4 days, see **Chapter 2**), but the predation rate of female adults was about 30.8 % higher at DTR+15 than at DTR0 (this Chapter). The opposite scenario was observed in the higher temperature range. Thus, temperature alternations differentially impact predation rates and developmental rates.

Temperature has demonstrated to affect the body size of ectotherms (Angilletta et al. 2004). According to the “temperature-size rule”, ectotherms develop faster at higher temperatures at the expense of a smaller body size at maturity, whereas growing at colder temperatures results in larger body sizes (Atkinson 1994). Accordingly, *N. californicus* females matured at smaller sizes with increasing temperature. Furthermore, rearing at the alternating temperature regime resulted in smaller body sizes as compared with constant temperatures. This effect was less pronounced for *P. persimilis*, in which temperature variation did not affect female body size, but larger individuals emerged at 15°C than at higher average daily temperatures. Body size has been demonstrated to influence various life history traits in phytoseiids. For example, Walzer and Schausberger (2013) found that smaller body sizes of *P. persimilis*, as induced by food limitation during the juvenile stages, negatively affected survival, reproduction and attractiveness as a potential mating partner. Additionally, in the latter study smaller females deposited smaller eggs, from which smaller daughters developed, demonstrating a transgenerational effect of a reduced body size. Further, body size may influence interactions in a multispecies predator-prey system, including intraguild predation. If one species is more affected by temperature variations than another, such as *N. californicus* versus *P. persimilis* in the present study, the outcome of intraguild predation may differ at varying temperature regimes. *Neoseiulus californicus* is usually a superior intraguild predator of *P. persimilis* (Walzer and Schausberger 1999; Blumel and Walzer 2002; Barber et al. 2003) and a combined release of these two predators has been suggested as a long-term biocontrol strategy against spider mites (Schausberger and Walzer 2001). However, at a high alternating temperature regime, female *N. californicus* are smaller than at the corresponding mean constant temperature and may therefore be more vulnerable to be preyed upon by (larger) intraguild predators (Polis et al. 1989; Schausberger 1999; Walzer and Schausberger 2011). This is not the case for *P. persimilis*, with overall similar body sizes at constant and alternating temperature regimes in the intermediate and high range. All of the above suggests that *N. californicus* is more likely to be negatively affected by high temperature variations than *P. persimilis* in terms of body size.

In conclusion, diurnally changing temperatures have a substantial impact on the predation and oviposition rates of *P. persimilis* and *N. californicus,* two key biological control agents of the two-spotted spider mite, *T. urticae*. Results derived from this chapter, along with those from **Chapter 2** emphasize the need to incorporate temperature variations in predictive models of developmental, reproductive and predatory performance of arthropod predators in biological control programs. Our results need to be validated on whole plants in the field to determine the effect of temperature alternations on development, reproduction and prey consumption of predatory mites and their efficiency as biological control agents in field conditions.

4

Food supplementation affects interactions between a phytoseiid predator and its omnivorous prey

This chapter has been redrafted from:

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Vangansbeke D., Nguyen, D.T, Audenaert, J., Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. (2014) Food supplementation affects interactions between a phytoseiid predator and its omnivorous prey. Biological Control 76: 95-100.

4.1. Introduction

Plants use direct (e.g. leaf hairiness or thickened leaves) and indirect strategies (e.g. attracting predators) to enhance protection against herbivores (Turlings and Wäckers 2004; Wäckers et al. 2005; Howe and Jander 2008). Indirect protection can be obtained by providing plant volatiles and alternative food sources (e.g. nectar) or shelter (e.g. domatia) to attract and/or reward the natural enemies of herbivores (Pullin and Gilbert 1989; Dicke et al. 1990; English-Loeb et al. 2002; Koller et al. 2007; Howe and Jander 2008). Natural enemies have been shown to benefit from the presence of (extrafloral) nectar and pollen, resulting in enhanced pest suppression (Bakker and Klein 1992; van Rijn et al. 2002; Wäckers and van Rijn 2005). When the plants themselves do not provide such food sources, they can be introduced deliberately in the crop or the wider habitat to increase predator numbers (Gurr et al. 2005; Nomikou et al. 2010) or to conserve populations of natural enemies in times when prey are scarce (Nomikou et al. 2003a). Several laboratory and greenhouse experiments have demonstrated a beneficial effect of providing alternative foods to various arthropod generalist predators (Castañé et al. 2006; Hoogerbrugge et al. 2008; Nomikou et al. 2010; Wong and Frank 2012). However, when introducing such foods in the crop, one should consider that not all pests are exclusively herbivorous. Omnivorous pests may also profit from additional food sources (Agrawal and Klein 2000; Coll and Guershon 2002). Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is such an omnivorous pest that can feed on both animal prey, like tetranychid eggs, and plant materials, like pollen (Agrawal et al. 1999; Hulshof and Vänninen 2002; van Maanen et al. 2012). Furthermore, this cosmopolitan pest has been shown to display antipredator behavior by killing eggs of phytoseiid mites (Janssen et al. 2002; 2003), which are key predators of thrips (van Lenteren 2012). In another study, we have demonstrated a significant reduction in population growth of *Amblydromalus limonicus* Garman & McGregor (Acari: Phytoseiidae) due to counterattacking thrips larvae destroying the predator eggs (Vangansbeke et al. 2014).

Previous studies indicated the benefits of supplementing pollen from different plants to generalist phytoseiid mites both in the laboratory and in the field (van Rijn et al. 1999; van Rijn and Tanigoshi 1999; Hoogerbrugge et al. 2011a; Nguyen et al. 2013; Janssen and Sabelis 2015). Factitious prey such as the eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and cysts of the brine shrimp, *Artemia franciscana* Kellogg (Branchiopoda: Artemiidae) are commercially available and are adequate food sources for various arthropod predators, including *Macrolophus* spp. (Castañé et al. 2006;

Vandekerkhove et al. 2009) and *Orius* spp. (Cocuzza et al. 1997; Arijs and De Clercq 2001; Bonte and De Clercq 2008; Bonte et al. 2012). Hoogerbrugge et al. (2011a) showed that the population growth of *A. limonicus* accelerated when *T. latifolia* pollen was offered as an alternative food in strawberries, thereby improving the control of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae). Populations of *Iphiseius degenerans* Berlese and *A. limonicus* developed faster when *T. latifolia* pollen was supplemented on cucumber plants, resulting in a suppression of the thrips population (van Rijn et al. 1999). Eggs of *E. kuehniella* and cysts of *Artemia* sp. were tested as supplementary foods for another generalist phytoseiid, *Amblyseius swirskii* Athias-Henriot, in chrysanthemum. Eggs of *E. kuehniella* supplemented with corn pollen resulted in an increase of the *A. swirskii* population, whereas *Artemia* cysts alone did not appear to support population growth of the predator (Hoogerbrugge et al. 2008). In a laboratory study, Vantornhout et al. (2004) showed that *I. degenerans* successfully developed to adulthood on both *E. kuehniella* eggs and decapsulated *A. franciscana* cysts. Cysts of the brine shrimp *A. franciscana* and eggs of the Mediterranean flour moth *E. kuehniella* were found to support the population increase of both *A. swirskii* more than *T. latifolia* pollen did (Nguyen et al. 2013; 2014).

In the present chapter, we focus on *A. limonicus* which is a commercially available generalist predatory mite, used for the control of key arthropod pests in protected crops, including thrips and whiteflies (Messelink et al. 2006; Hoogerbrugge et al. 2011b). It also feeds on plant-derived food sources such as pollen and extra-floral nectar as well as on spores of fungi (McMurtry and Scriven 1965; Bakker and Klein 1992; McMurtry and Croft 1997).

We investigated the implications of food supplementation to a tritrophic system (see Figure 4.1) in the laboratory consisting of kidney bean (*Phaseolus vulgaris* L.), the omnivorous pest *F. occidentalis* and the predator *A. limonicus* (Figure 4.1). We tested 1) the population growth of *A. limonicus* on potential food supplements; 2) whether applying supplemental foods affects development of *F. occidentalis* immatures; 3) whether the predation rate of *A. limonicus* on thrips larvae is affected by the presence of additional food; 4) the influence of food supplementation on antipredator behavior of thrips larvae on predator eggs; 5) whether supplemental foods reduce leaf damage caused by thrips.

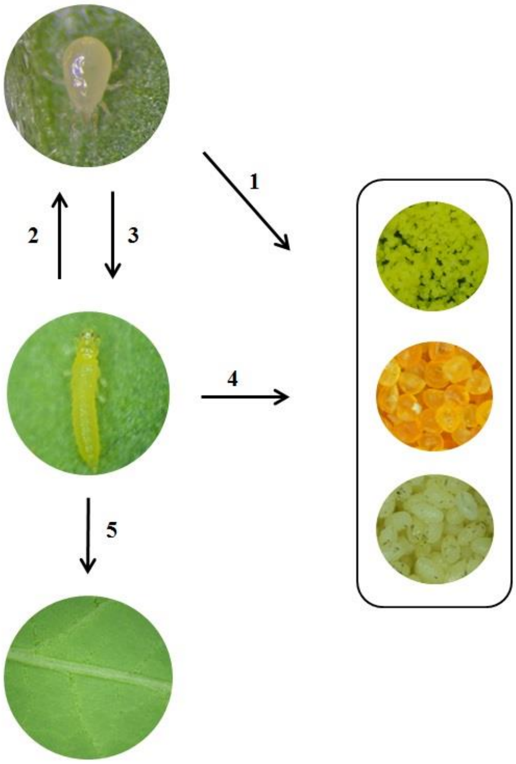

Figure 4.1: Effects of supplementing food on the interactions between a phytoseiid predator (*Amblydromalus limonicus*), an omnivorous pest (*Frankliniella occidentalis*) and a kidney bean plant (*Phaseolus vulgaris*). 1: Effect of food supplements on population growth of *Amblydromalus limonicus*; 2: Antipredator behavior of *Frankliniella occidentalis* killing *Amblydromalus limonicus* eggs; 3: Predation by *Amblydromalus limonicus* on *Frankliniella occidentalis* larvae; 4: Feeding by thrips on supplemental foods; 5: Feeding by thrips on bean leaves

4.2. Materials and Methods

4.2.1. Insect and mite colonies

A laboratory colony of *A. limonicus* was initiated with mites acquired from Koppert B. V. (Berkel en Rodenrijs, The Netherlands) and was maintained on reversed leaf discs of kidney bean (*Phaseolus vulgaris* L.). The leaves were placed upside down on a layer of water-soaked cotton in a petri dish (ø 14cm), with a surrounding layer of cotton along the edges of the leaf to provide moisture to the mites and prevent them from escaping. Fresh cattail pollen (*Typha latifolia* L.) was supplied by Biobest N.V. (Westerlo, Belgium) and was dusted twice a week over the leaf discs. Threads of cotton were used as an egg-laying substrate and were transferred to new leaf discs twice a week. The petri dishes were kept in Plexiglas containers (60 x 60 x 60 cm) in a climatic cabinet. Western flower thrips were collected from rose plants

(*Rosa* cv. 'Red Naomi') and cultured in plastic boxes on green bean pods (*P. vulgaris*) placed on a layer of vermiculite. The colonies were maintained in a controlled environment of 25 ± 1°C, 65 ± 5% RH and a photoperiod of 16:8 h (L:D).

4.2.2. Experiment 1: Performance of *A. limonicus* on food supplements

Eggs (<8h old) from the stock colony were transferred individually to a bean leaf arena (2.5 x 2.5 cm) placed upside down on a water-soaked polyurethane sponge (1 x 5 x 5 cm), of which the edges were covered with moist pieces of tissue paper, in a polystyrene insect breeding dish (10 x 4 cm) with a mesh hole (ø 40 mm) in the lid (SPL Life Sciences Co. Ltd., Korea). From the larval stage on, the mites were supplemented ad libitum with fresh *T. latifolia* pollen, dry decapsulated cysts of *A. franciscana* or frozen eggs of *E. kuehniella*. The *A. franciscana* cysts were provided by the *Artemia* Reference Center (ARC) at Ghent University (Ghent, Belgium) and originated from Great Salt Lake (Utah, USA). Frozen eggs of *E. kuehniella* were supplied by Koppert B.V. (Berkel en Rodenrijs, The Netherlands). The foods were replenished every other day. Survival and development were monitored daily. Fifty replicates were used for each diet-substrate combination. The arenas were kept in a climatic cabinet at 23 ± 1°C, 65 ± 5% RH and a photoperiod of 16:8 (L:D) h.

Adult females obtained from the development test were paired with a male of the same age and with the same feeding history. Since there is no information on the number of matings necessary to obtain full reproduction for *A. limonicus*, dead males were replaced by a new one to ensure the continuous presence of a mating partner during the experiment. Observations were made on a daily basis to determine the preoviposition and oviposition period, fecundity and adult longevity. Eggs deposited by females of the same age were collected, transferred to bean leaf discs and fed with the same diet as their parents. The sex of the progeny was checked when reaching the adult stage in order to determine the age-specific sex ratio. The intrinsic rate of increase, r_m, was calculated according to Birch (1948) (see also section 2.2.3.3). The standard error of the r_m-value was calculated using the Jackknife procedure (Meyer et al. 1986; Hulting et al. 1990).

4.2.3. Experiment 2: Development of *F. occidentalis* on food supplements

First instars of *F. occidentalis* (< 6h old) were transferred from the colony to individual insect breeding cages (50 x 15 mm) (SPL Life Sciences Co. Ltd., Korea) furnished with a circular bean (*P. vulgaris*) leaf disc (ø 50 mm) placed on a 7 mm layer of agar (1.5% w/w). Leaf discs were either not supplied with extra food (control) or supplemented once (at the start of the experiment) with *ad libitum* amounts of *T. latifolia* pollen, *A. franciscana* cysts or *E. kuehniella* eggs (corresponding with about 50, 350 and 250 µg per dish, respectively). Each treatment was replicated 25 times and thrips development was monitored daily.

4.2.4. Experiment 3: Influence of food supplements on predation by *A. limonicus*

Gravid four-day-old females of *A. limonicus* fed on either *T. latifolia*, *A. franciscana* or *E. kuehniella* (in the second generation on the respective diets) were starved individually for 24 h without water in glass tubes (ø=7 mm, L=45 mm) sealed with a cotton plug. For each diet, two groups of 20 starved females (with or without supplemental food) were individually transferred to a reversed square bean leaf disc (25 x 25 mm) placed on a polyurethane sponge soaked in tap water and supplied with 15 *F. occidentalis* first instars. The first group of females received only thrips, whereas the second group received thrips supplemented with the same food source the mites were previously reared on. The number of killed thrips larvae was counted after 24 h.

4.2.5. Experiment 4: Influence of food supplements on counterattack behavior by *F. occidentalis*

Ten predatory mite eggs (<14h old) were collected from the colony and were placed in a circle on the edges of a square bean leaf disc prepared as described above. A single second instar of *F. occidentalis* was transferred from the colony to the center of the circle of *A. limonicus* eggs. The larvae were allowed to feed on the bean leaf disc and mite eggs. After 24h the number of damaged *A. limonicus* eggs was counted. Four treatments were replicated 20 times. The first treatment consisted of a bean leaf disc without supplemental food, whereas in the other three either *T. latifolia*, *A. franciscana* or *E. kuehniella* was provided.

4.2.6. Experiment 5: Influence of food supplements on plant damage caused by *F. occidentalis* females

Five adult females of *F. occidentalis* (5-10 days old) were introduced to similar arenas as those used in experiment 1 and were allowed to feed on the bean leaf tissue for 24h. In a first treatment, females received no additional food, whereas in the remaining treatments leaf discs were supplied with either *T. latifolia, A. franciscana* or *E. kuehniella*. After 24h thrips and foods were carefully removed from the leaf discs, after which the discs were scanned and the damaged leaf area was quantified using the software CompuEye (Bakr 2005). For each treatment 30 replicates were set up.

All experiments were carried out in climate chambers set at 23 ± 1°C, 65 ± 5% RH and a 16:8 h (L:D) photoperiod.

4.2.7. Data analysis

Data were analyzed using SPSS Statistics 20 (IBM). A two-way ANOVA was conducted to assess the impact of sex and diet on the developmental times of *A. limonicus*. As interactions occurred between the factors sex and diet for the total immature development and mean developmental times demonstrated a non-normal distribution (checked using a Kolmogorov-Smirnov test), means were also compared using Kruskal-Wallis H followed by Mann-Whitney U tests to separate the means. Developmental times of *F. occidentalis* were analyzed similarly.

When a Kolmogorov-Smirnov test indicated that the data of the reproductive parameters were normally distributed, a one-way ANOVA was conducted. Equality of variances was checked using a Levene-test. When the variances were shown to be equal (homoscedasticity), means were separated using Tukey's test (experiment 3). A Tamhane test was used to separate the means in case of heteroscedasticity (experiment 5). When the means were not normally distributed, a non-parametric Kruskal-Wallis H test was conducted followed by Mann-Whitney U tests to separate the means.

Likewise, reproductive parameters and intrinsic rates of increase were analyzed using two-way-ANOVAs. When no interaction was observed between the main factors, means were compared using a Tukey test. In case of interaction, the same procedures as described above were used.

Survival rates and sex ratios had a binomial distribution and were analyzed using a logistic regression. This generalized linear model makes use of a probit (log odds) link and a binomial error function (McCullagh and Nelder 1989). For all tests, the level of significance was set at $P=0.05$.

A two-way ANOVA was conducted to evaluate the impact of previous diet and food supplementation (i.e. with or without food supplement) on prey consumption (experiment 3). As no interactions between the main factors were found, mean predation rates were separated by way of a Tukey test.

The level of significance was set at 0.05 for all tests.

4.3. Results

4.3.1. Experiment 1: Performance of *A. limonicus* on food supplements

When no food was added to the leaf arenas, none of the *A. limonicus* reached the adult stage. When predators were supplied with the tested foods, all diets resulted in high survival rates (>98%). A two-way ANOVA analysis revealed no interaction between the factors sex and diet for the developmental periods of the different development stages, except for the duration of the total immature development (Table 4.1). Egg and protonymphal stage were not affected by sex, whereas during the larval and deutonymphal stages males tended to develop faster than female juveniles. Diet affected the developmental period for all stages, except the (non-feeding) egg stage.

Females fed on *T. latifolia* pollen deposited significantly fewer eggs than those fed on *A. franciscana* or *E. kuehniella* (Table 4.3). Oviposition rates were lowest when females were fed on *T. latifolia* pollen. The oviposition rate of *A. limonicus* females fed on *E. kuehniella* eggs (ca. 3 eggs per female per day) exceeded that of the other diets. Longevity, oviposition period and the proportion of female progeny (ca. 66%) were not affected by diet. The highest growth rate was found when the mites were offered *E. kuehniella*, followed by *A. franciscana* cysts, while the lowest growth rates were observed when fed on *T. latifolia*.

Table 4.1: Two-way ANOVA analysis of the developmental period of *A. limonicus* as affected by the factors sex and diet (*Typha latifolia*, *Artemia franciscana* and *Ephestia kuehniella*)

Source		Developmental stage				
		Egg	Larva	Protonymph	Deutonymph	Total immature
Sex	F	1.479	5.367	3.338	11.658	12.323
	df	1	1	1	1	1
	P	0.226	0.022	0.07	0.001	0.001
Diet	F	1.129	19.752	87.227	15.751	47.583
	df	2	2	2	2	2
	P	0.326	<0.001	<0.001	<0.001	<0.001
Sex x Diet	F	0.580	1.724	0.110	2.467	3.898
	df	2	2	2	2	2
	P	0.561	0.182	0.896	0.088	0.022
Error	df	144	144	144	144	144

Table 4.2: Developmental times of *A. limonicus* males and females fed on three food sources (*T. latifolia*, *A. franciscana* or *E. kuehniella*)

Sex	Diet		Developmental time (days)[b]				
		n[a]	Egg	Larva	Protonymph	Deutonymph	Total immature
Male	*T. latifolia*	20	2.33 ± 0.09 a	1.48 ± 0.10 b	1.75 ± 0.10 d	1.43 ± 0.10 ab	6.98 ± 0.21 c
	A. franciscana	15	2.43 ± 0.08 a	1.13 ± 0.08 a	1.30 ± 0.08 b	1.60 ± 0.17 b	6.47 ± 0.24 bc
	E. kuehniella	20	2.50 ± 0.04 a	1.23 ± 0.07 ab	0.95 ± 0.05 a	1.20 ± 0.06 a	5.88 ± 0.10 a
Female	*T. latifolia*	29	2.33 ± 0.06 a	1.76 ± 0.09 c	1.84 ± 0.07 d	1.95 ± 0.09 d	7.88 ± 0.16 d
	A. franciscana	35	2.37 ± 0.05 a	1.30 ± 0.06 ab	1.43 ± 0.05 b	1.83 ± 0.10 cd	6.93 ± 0.13 bc
	E. kuehniella	29	2.36 ± 0.06 a	1.22 ± 0.05 a	1.02 ± 0.04 a	1.29 ± 0.06 a	5.90 ± 0.11 a
	χ^2		6.550	31.741	86.775	46.288	72.303
	df		5	5	5	5	5
	P		0.256	<0.001	<0.001	<0.001	<0.001

[a] Number of tested individuals

[b] Means ± SE within a column followed by the same letter are not significantly different (P>0.05; Kruskal-Wallis ANOVA and Mann-Whitney U)

Table 4.3: Reproductive parameters and intrinsic rates of increase of *A. limonicus* females fed on three foods (*T. latifolia*, *A. franciscana* or *E. kuehniella*)

Diet	n[a]	Preoviposition period (days)[b]	Oviposition period (days)[b]	Fecundity (eggs/female)[b]	Oviposition rate (eggs/female/day)[b]	Female longevity (days)[b]	Offspring sex ratio (%♀♀)[b]	r_m (females/female/day)[b]
T. latifolia	26	2.81 ± 0.18 c	16.0 ± 1.1 a	27.0 ± 1.2 c	1.80 ± 0.10 c	27.5 ± 1.4 a	64.8 ± 2.6 a	0.166 ± 0.004 c
A. franciscana	31	2.35 ± 0.10 b	14.5 ± 0.7 a	36.4 ± 2.1 b	2.52 ± 0.06 b	28.5 ± 2.1 a	68.7 ± 1.8 a	0.215 ± 0.002 b
E. kuehniella	29	1.93 ± 0.12 a	15.4 ± 0.6 a	45.9 ± 2.0 a	3.00 ± 0.08 a	25.5 ± 1.4 a	66.9 ± 1.3 a	0.256 ± 0.003 a
	χ^{2*}/F^{**}	18.587*	0.918**	25.085**	53.666**	0.857**	1.801*	260.528**
	df	2	2, 83	2, 83	2, 83	2, 83	2	2, 83
	p	<0.001	0.403	<0.001	<0.001	0.428	0.406	<0.001

[a] Number of tested females

[b] Means ± SE within a column followed by the same letter are not significantly different (P>0.05; Kruskal-Wallis (*) followed by Mann-Whitney U tests, one-way-ANOVA (**) Tukey or Tamhane or probit test (offspring sex ratio))

4.3.2. Experiment 2: Development of *F. occidentalis* on food supplements

Thrips larvae were observed to feed on all food sources provided. Diet did not affect the survival rates of thrips immatures, as no mortality was observed in all treatments. Offering *T. latifolia* pollen and *A. franciscana* cysts in addition to bean leaves accelerated the development of immature *F. occidentalis* (Table 4.4). Adding *E. kuehniella* eggs to the leaf disc did, however, not result in faster development. Accelerated development of the larval stages was not associated with faster (pre-)pupal development of the thrips.

Table 4.4: Developmental time (days) of *Frankliniella occidentalis* on bean leaf discs in the presence or absence of additional food sources (*Typha latifolia* pollen, *Artemia franciscana* cysts or *Ephestia kuehniella* eggs)

Diet	First + second instar	Prepupa	Pupa	Total
P. vulgaris	6.25 ± 0.16 c	1.20 ± 0.08 a	2.96 ± 0.07 a	10.41 ± 0.16 c
P. vulgaris + *T. latifolia*	5.22 ± 0.12 a	1.12 ± 0.07 a	2.88 ± 0.11 a	9.22 ± 0.14 a
P. vulgaris + *A. franciscana*	5.78 ± 0.14 b	1.16 ± 0.07 a	2.80 ± 0.08 a	9.74 ± 0.14 b
P. vulgaris + *E. kuehniella*	5.82 ± 0.21 bc	1.18 ± 0.09 a	2.94 ± 0.11 a	9.94 ± 0.21 bc
χ^2	18.379	0.53	1.993	22.107
df	3	3	3	3
P	<0.001	0.912	0.574	<0.001

Means (± standard error) within a column followed by the same letter are not significantly different (p>0.05; Mann-Whitney U tests; χ^2-, df- and p-values refer to Kruskal-Wallis ANOVAs)

4.3.3. Experiment 3: Influence of food supplements on predation by *A. limonicus*

A two-way ANOVA revealed no interaction between the factors previous diet and food supplementation ($F_{2,120}$= 0.792; p= 0.455) on the number of *F. occidentalis* first instars killed by *A. limonicus* females after 24 h. Both main factors had a significant effect on the number of thrips killed ($F_{2,120}$= 10.153; p<0.001 and $F_{1,120}$= 91.77; p<0.001, previous diet and food supplementation, respectively). Additionally, a one-way ANOVA was performed for pairwise comparison of the treatments (one-way ANOVA, F= 22.652, df= 5, 114; p<0.001; Tamhane's test). When fed on cattail pollen for 2 generations, the phytoseiid killed fewer thrips larvae than when fed on moth eggs or brine shrimp cysts (Figure 4.2). When *A. limonicus* females were offered thrips larvae together with a food supplement, their predation rates on the thrips decreased by about 30% on all three supplemental food sources as compared to being presented with thrips alone.

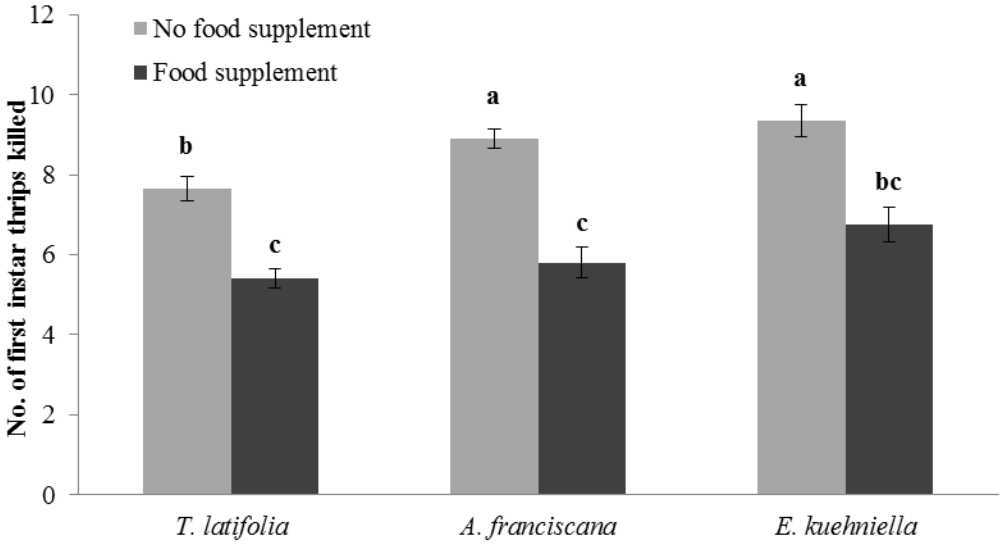

Figure 4.2: Influence of previous diet and food supplementation (*Typha latifolia* pollen, *Artemia franciscana* cysts or *Ephestia kuehniella* eggs) on 24-h predation rate (means ± SE) of *Amblydromalus limonicus* females on first instars of *Frankliniella occidentalis*. Bars with the same letter are not significantly different (p>0.05; Tukey test)

4.3.4. Experiment 4: Influence of food supplements on counterattack behavior by *F. occidentalis*

The presence of alternative food on the leaf disc affected antipredator behavior of *F. occidentalis* larvae (Kruskal-Wallis, χ^2=44.622; df=3; p<0.001) (Figure 4.3). Second instar *F. occidentalis* consumed an average of 2.5 *A. limonicus* eggs per day in the absence of food supplements. When brine shrimp cysts or moth eggs were added to the experimental arena, only about 1.1 and 0.4 predator eggs were killed, respectively. No antipredator behavior was observed in the presence of *T. latifolia* pollen.

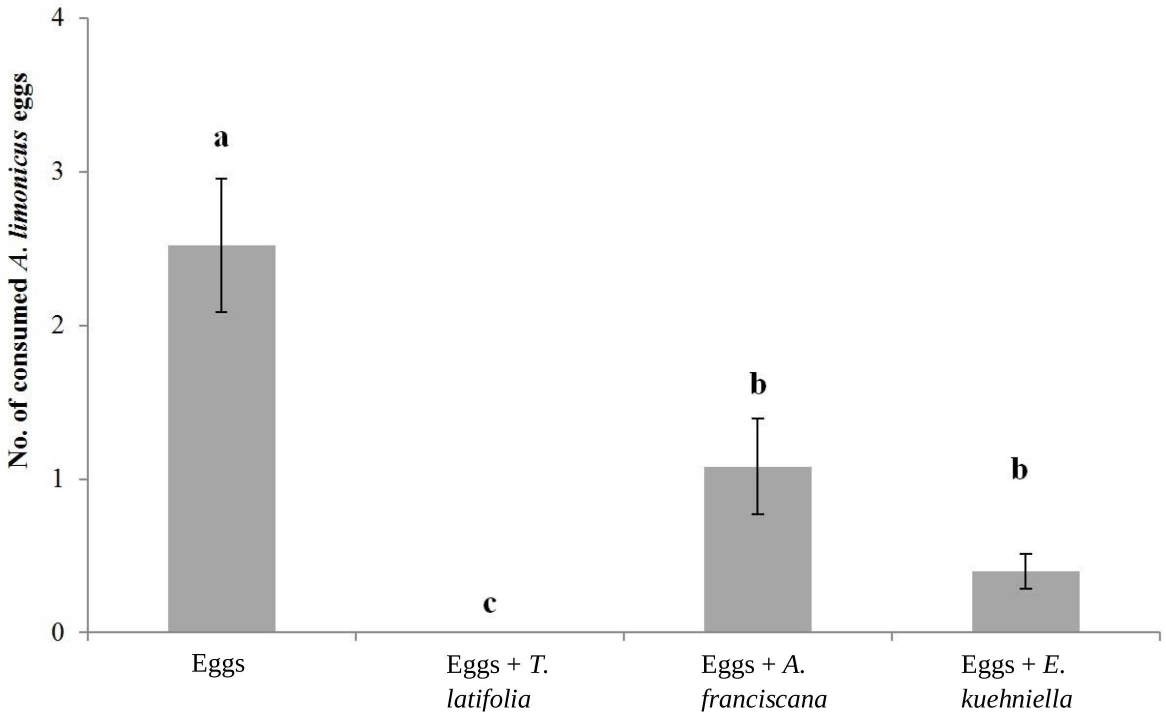

Figure 4.3: Mean (± SE) number of *Amblydromalus limonicus* eggs killed by antipredator behavior of *Frankliniella occidentalis* second instars in the absence (control) or presence of food supplements (*Typha latifolia* pollen, *Artemia franciscana* cysts or *Ephestia kuehniella* eggs). Bars with the same letter are not significantly different (p > 0.05; Mann-Whitney U tests)

4.3.5. Experiment 5: Influence of food supplements on plant damage caused by *F. occidentalis* females

Diet significantly affected the bean leaf area damaged by adult thrips (one-way ANOVA, F=87.388; df=3,113; p<0.001). In the presence of *T. latifolia* pollen, the damaged leaf area was only one tenth of that in the control without additional food (Figure 4.4). Supplementing bean leaf discs with *E. kuehniella* eggs did not reduce leaf injury levels, whereas a supplement of *A. franciscana* cysts did.

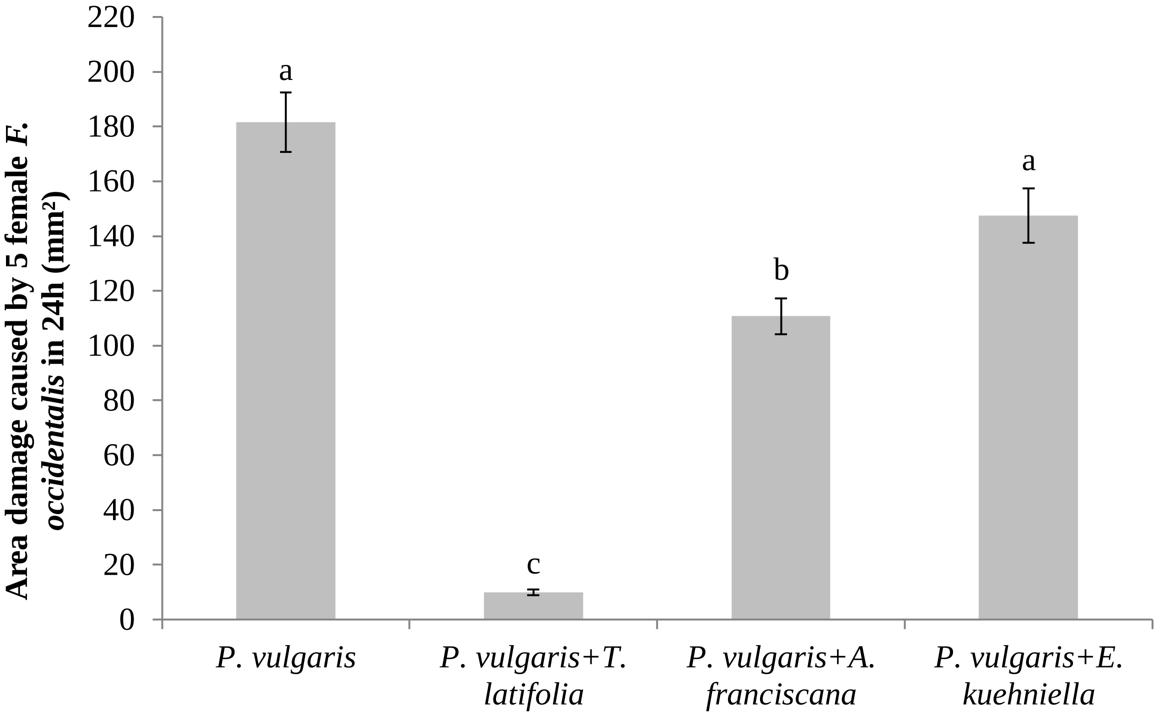

Figure 4.4: Damaged leaf area (means ± SE) due to feeding by *Frankliniella occidentalis* adults in the absence (control) or presence of food supplements (*Typha latifolia* pollen, *Artemia franciscana* cysts or *Ephestia kuehniella* eggs). Bars with the same letter are not significantly different ($p > 0.05$; Tamhane tests)

4.4. Discussion

In biological control programs, food supplements can be introduced in the crop to support natural enemy populations and as such enhance top-down cascading effects to minimize plant damage. However, the impact of food supplementation on pest suppression may not be straightforward when foods can be utilized by the target pest as well. In line with other studies (Hulshof et al. 2003; Zhi et al. 2005; Leman and Messelink 2015), our results show that *F. occidentalis* larvae developed faster on bean leaves when pollen was present. Moreover, thrips larvae were also observed to feed on *A. franciscana* cysts and *E. kuehniella* eggs offered as supplemental foods. On brine shrimp cysts, this even resulted in a faster development of the pest. Despite the ability of thrips to use pollen as a food source, van Rijn et al. (1999, 2002) observed an increased level of thrips suppression when *T. latifolia* pollen was supplemented to different phytoseiid predators. The authors suggested that the patch-wise application of pollen resulted in a different vertical distribution of pest and predator.

Predatory mites tended to aggregate thus monopolizing pollen-treated leaves, whereas thrips were more abundant on the top of the plant. This hypothesis could not be tested in the present laboratory study using leaf disc arenas.

To enhance the efficacy of a biological control program against thrips in protected cultivation, a supplemental food source should be selected which favors population increase of thrips predators more than that of thrips (van Rijn and Tanigoshi 1999; Hulshof et al. 2003). In the first experiment, we found that both *A. franciscana* and *E. kuehniella* were superior food sources to *T. latifolia* pollen in terms of their potential to support the population increase of the generalist predator *A. limonicus*. Results from the second experiment showed that *A. franciscana* cysts and *E. kuehniella* eggs have some nutritional benefit for thrips but not to the same extent as *T. latifolia* pollen. Therefore, the above factitious food sources may be candidates for use as supplemental food sources to enhance thrips control by *A. limonicus* in protected cultivation. In the present study we have only focused on the effect of food supplements on immature development of *F. occidentalis*. Although generally the intrinsic rate of increase (r_m) is more affected by changes in developmental parameters than in reproduction (Lewontin 1965) life table analysis is warranted to fully appreciate the impact of these foods on the population growth of *F. occidentalis*.

Diet affected the predation rate of *A. limonicus* females on first instars of *F. occidentalis*, with less prey killed when the predator was previously fed on *T. latifolia*. When supplemental foods were present on the leaf discs less prey were consumed (about 30%) than in the absence of additional food. Similar observations were obtained for other predatory mites offered pollen supplements (McMurtry and Scriven 1966b; van Rijn and Sabelis 1993; Fouly 1997; Pappas et al. 2013; Leman and Messelink 2015). Food supplementation may lead to a decrease in the per capita predation rate, but considering the expected resulting numerical increases of the predator population, the overall predation level may be higher and thus yield better pest suppression.

In another laboratory experiment, we reported a reduced population growth of *A. limonicus* due to antipredator responses of *F. occidentalis* larvae destroying predator eggs (Vangansbeke et al. 2014). Thrips are believed to pierce the eggs of their phytoseiid predators rather as a mechanism to reduce the future risk of predation (Janssen et al. 2002; Walzer and Schausberger 2009) than for nutritionally optimizing their diet (Walzer and Schausberger 2009). In the present study, individual second instars of *F. occidentalis* killed no less than 2.5

predatory mite eggs per 24h on bean leaf discs, a rate similar to that reported by Janssen et al. (2003) for second instar *F. occidentalis* killing eggs of *I. degenerans*. In the presence of cattail pollen, these counterattacks were substantially reduced in the latter study. Likewise, no egg-killing was observed in the present study when cattail pollen was added to the leaf disc. Brine shrimp cysts and moth eggs also reduced antipredator behavior, but not to the same extent as pollen. This indicates that not only plant quality (Janssen et al. 2003) but also the quality of food supplements may alter counterattack behavior of thrips. Further, it has previously been suggested that thrips kill more eggs of predatory mites that are considered to be more harmful (Janssen et al. 2002). High numbers of *A. limonicus* eggs killed by *F. occidentalis* larvae in the present study therefore suggest that the phytoseiid was identified by the thrips to be a harmful predator with an average of 2.5 eggs killed per larvae per day. Interestingly, however, no antipredator behavior was observed in the presence of pollen. It may thus be more beneficial for western flower thrips to invest time in population growth (by consuming a high quality food like pollen) rather than to spend time destroying potentially harmful predators. As an alternative hypothesis, the risk of being attacked by a phytoseiid predator in the presence of pollen might be reduced to such a level that it is not worthwhile for thrips to invest energy in antipredator behavior. Finally, we should note that extrapolation of the findings from our laboratory study using simple leaf disc arenas to field level should be done carefully. Being thigmotactic organisms (Lewis 1997), thrips need to abandon their hiding places to feed on food supplements in the crop. To what extent food supplements will lead to behavioral changes in *F. occidentalis* is subject for future research.

The supplementation of pollen resulted in diet switch of *F. occidentalis* females yielding a tenfold reduction of leaf damage. Adding *A. franciscana* to the arena reduced leaf damage compared to the control (no food supplementation) but not to the same level as pollen supplementation, whereas supplying *E. kuehniella* eggs did not reduce leaf damage. Western flower thrips are a key pest of a wide range of vegetable crops, but also cause serious economic damage to ornamental crops. In ornamental plant production, there is often a zero tolerance towards pest presence for aesthetic reasons (Skirvin et al. 2002; Schumacher et al. 2006). If supplementing pollen in a pest-predator system reduces plant damage by thrips to a minimum, this approach may be more suitable than using *A. franciscana* cysts or *E. kuehniella* eggs for food supplementation to support populations of phytoseiids or other arthropod predators in ornamentals. Obviously, the results from our small scale laboratory trials would need to be confirmed in longer-term experiments on plants.

In the mass production of generalist phytoseiids, including *A. limonicus*, astigmatid mites are the main food source (Zhang 2003; Bolckmans and van Houten 2006; Midthassel et al. 2013). However, the use of astigmatid prey mites may lead to health problems for workers in mass production facilities caused by allergens originating from these mites (Fernandez-Caldas et al. 2007). Good performance of *A. limonicus* on *Ephestia* eggs and *Artemia* cysts in our study suggests that these factitious foods may provide an alternative to astigmatid prey mites and contribute to optimizing the rearing process for this phytoseiid. However, in the present study the predator was reared only for a single generation on these factitious foods. As nutrient imbalances may be expressed only after several generations of rearing on an unnatural diet (De Clercq et al. 2005), the developmental and reproductive performance of *A. limonicus* on the studied foods needs to be tested for multiple generations.

Although a diet of *E. kuehniella* eggs resulted in the shortest developmental and preoviposition periods and the highest reproduction rates of *A. limonicus*, this factitious food may be less suitable to sustain laboratory or field populations of the mite in terms of its cost-effectiveness. Whereas the market price of *E. kuehniella* remains above 500 US $/kg, the price of the brine shrimp cysts varies, but can be as low as one tenth of that amount (G. Van Stappen, ARC, personal communication). Therefore, the high price of the lepidopteran eggs can not offset its sligthly better performance, making the *Artemia* cysts a more cost-effective food source to support the population growth of *A. limonicus*.

Food supplementation in agricultural crops is usually done with the intention to induce density-mediated direct interactions (i.e. higher number of predators kill more prey) resulting in density-mediated indirect interactions (i.e. more predators ultimately diminish plant damage) (Abrams 1995). However, the results from our laboratory study suggest that the unintended nutritional benefits of supplementing pollen to an omnivorous pest like *F. occidentalis* might be leveled off by both top-down (reduced leaf damage) and bottom-up (reduced antipredator behavior) cascade effects. We expect that the density-mediated direct interactions related to the supplementing of *A. franciscana* cysts and *E. kuehniella* eggs will be greater as compared with pollen supplementation, as the former animal foods have been shown to yield a higher population increase of the predator. However, based on our results, direct reduction of leaf damage and antipredatory responses by the pest may be less pronounced when brine shrimp cysts and moth eggs are supplied than when cattail pollen is applied. To what level food supplementation using the food sources tested in this study will result in better pest suppression needs to be further investigated in the field.

5

Supplemental food for *Amblyseius swirskii* in the control of thrips: feeding friend or foe?

This chapter has been redrafted from:

Vangansbeke D., Nguyen, D.T, Audenaert, J., Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. (2015) Supplemental food for *Amblyseius swirskii* in the control of thrips: feeding friend or foe? Pest Management Science doi: 10.1002/ps.4000

5.1. Introduction

The Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a major destructive pest in greenhouse crops worldwide (Lewis 1997; Kirk and Terry 2003) and remains especially problematic in ornamental crops with a zero tolerance for crop damage or for crops destined for export (Parrella 1995; Shipp 1995; Reitz 2009). This virus-vectoring thrips species is primarily herbivorous, feeding on leaf tissue, but also on other plant materials, such as pollen (Hulshof et al. 2003) and extrafloral nectar (Yokoyama 1978). To a lesser extent, *F. occidentalis* is also known to feed on animal foods, such as tetranychid and phytoseiid mite eggs (Trichilo and Leigh 1986; Janssen et al. 2002), and whitefly crawlers (van Maanen et al. 2012). Due to the pest's cryptic lifestyle and rapid development of resistance to insecticides, growers increasingly use biological control strategies against this pest (Immaraju et al. 1992; Jensen 2000; Bielza 2008; van Lenteren 2012).

The generalist predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) is well-known for its efficacy to control WFT infestations (Messelink et al. 2006; Calvo et al. 2011) and is being used worldwide as a biological control agent in a wide range of crops (van Lenteren 2012; Buitenhuis et al. 2015; Calvo et al. 2015). To cope with poor establishment of the predator after release and/or its persistence in periods of low prey levels in ornamentals, growers may have to re-introduce predatory mites repeatedly ("insurance policy", Cock et al. 2010; Messelink et al. 2014), which adds a considerable cost for pest control. Messelink et al. 2014 Messelink et al. (2014) and Buitenhuis et al. (2015) discussed an array of practices that can be implemented for the population enhancement of natural enemies in greenhouse crops. One of the suggested approaches to support populations of augmentatively released natural enemies is to procure supplemental foods. These foods are meant to provide the natural enemies with necessary resources to overcome periods with little availability of prey or plant foods. Provision of food supplements has been shown to enhance the biological control function of a wide range of omnivorous beneficial insects and mite (Wäckers 2005; Aguilar-Fenollosa et al. 2011; Wong and Frank 2012;Leman and Messelink 2015). The use of pollen has repeatedly been suggested for supporting populations of generalist predatory mites (McMurtry and Scriven 1966;van Rijn and Sabelis 1993; van Rijn et al. 1999; Nomikou et al. 2010; Maoz et al. 2011; Adar et al. 2014; Messelink et al. 2014). Recently, a commercial product consisting of pollen of narrow-leaved cattail, *Typha angustifolia* L. (Nutrimite^{TM}, Biobest N.V., Belgium), was launched to enhance the population growth of different generalist predatory mites. The application of pollen products in greenhouse crops has to be done with caution, especially when (one of) the target prey is a pollen-feeder as well (Kirk 1984). Another cattail species, *Typha latifolia* L., was found to promote the development and reproduction of WFT (Hulshof and Vänninen 2002; Leman and Messelink 2015). Pollen supplementation may result in a twofold potential benefit for WFT: an increase in population growth and a reduced predator pressure. Although such benefits to the pest have been demonstrated in a number of short-term experiments (Skirvin 2007; Leman and Messelink 2015), a higher numerical response of the predator as a result of pollen supplementation has been reported to yield improved control of thrips (van Rijn et al. 1999;Leman and Messelink 2015). Dry decapsulated brine shrimp cysts, *Artemia* sp. (Branchiopoda: Artemiidae) constitute another potential food source that has been shown to support generalist phytoseiids. The cysts are the diapausing eggs of the brine shrimps, which are harvested from salt lakes worldwide and are routinely being used in aquaculture as a food source for fish and shellfish (Lavens and Sorgeloos 2000). Cysts of *Artemia franciscana* Kellogg have shown to be a suitable food source for several predatory mites including *Iphiseius degenerans* (Berlese) (Vantornhout et al. 2004), *Amblydromalus limonicus* Garman and McGregor (see **Chapter 4**) and *A. swirskii* (Nguyen et al. 2014). However, *A. franciscana* cysts were found to be unsuitable for *F. occidentalis* by Leman and Messelink (2015) and less supportive for the thrips development than *T. latifolia* pollen (**Chapter 4**) .

In the present study, we compared the nutritional value of two types of brine shrimp cysts with that of *T. angustifolia* pollen for both WFT and its predator *A. swirskii*. Further, we also investigated the effect of providing a combined diet of supplemental food (pollen, both types of brine shrimp cysts) and thrips to *A. swirskii*. Finally, we assessed the impact of the presence of the three food sources on the predation efficacy of *A. swirskii* on WFT larvae.

5.2. Material and methods

5.2.1. Predatory mite and thrips rearing

A stock colony of *A. swirskii* was established with mites obtained from Biobest N.V. (Westerlo, Belgium) and was maintained on plastic arenas, the edges of which were covered with moist tissue paper to provide free water and prevent the mites from escaping. Twice a week, pollen of *T. latifolia* was dusted over the arenas as a food for the mites. The laboratory colony of *F. occidentalis* was maintained as described in section 4.2.1.

5.2.2. Food sources

Pollen of *T. angustifolia* (Nutrimite^{TM}) was supplied by Biobest N.V. and was stored at -18 °C. Weekly, a small amount of pollen was taken out of the freezer and kept in a refrigerator at 5 °C before being used in the experiments. Two types of decapsulated *Artemia* cysts were tested: the first material was a commercial product consisting of cysts of a non-specified *Artemia* species (Artefeed, Koppert B.V., The Netherlands) whereas the second material was from an *A. franciscana* strain originating from the Great Salt Lake (Utah, USA) and kept at a research institute (Artemia Reference Center, Ghent University, Belgium). Both *Artemia* products were stored at 5 °C.

5.2.3. Population growth of WFT

For each experiment, 4 groups of 50 first instars (<6 h old) of *F. occidentalis* were collected from the stock colony and were transferred individually to bean leaf discs (ø 5 cm) placed upside down on a 7 mm layer of agar (1% w/w) in a closed insect breeding dish (ø 5 and H 1.5 cm). The first group of 50 larvae received no food supplements, whereas for the other three groups, bean leaf discs were supplemented with either *T. angustifolia* pollen (ca. 50 µg), *A. franciscana* cysts or Artefeed cysts (ca. 300 µg). Foods were replenished once a week. Development and survival were monitored twice a day up to adulthood. Next, females were mated with a male that was reared on the same diet as the female and reproduction was followed daily during the first 5 days by transferring pairs of thrips to fresh leaf discs with or without food supplements; thereafter thrips reproduction was monitored every other day. Reproductive performance of thrips was assessed by counting the number of hatched larvae. Egg hatch was assessed by counting the number of non-hatched eggs 6 days after the first larvae had hatched. To determine egg incubation period, 10 gravid female WFT were placed in individual cages with a bean leaf disc for 8h, after which the females were removed. Next, egg hatching was monitored every 12 h. All experiments were conducted in climate chambers set at 25±1 °C, 65±5 % RH and a 16L:8D h photoperiod.

5.2.4. Population growth of *A. swirskii*

For each diet, 50 predatory mite eggs (<8 h old) were collected from the stock colony and were placed individually on square (2.5 x 2.5 cm) bean leaf arenas (*P. vulgaris*). The leaf arenas were placed upside down on a water-soaked sponge and the edges were covered with moist tissue paper to provide free water and prevent the mites from escaping. Development and survival were monitored twice a day. Once the adult stage was reached, females were paired with a male and oviposition was monitored daily. Eggs from females of the same age were transferred to new leaf discs and were allowed to develop to adulthood to assess the sex ratio of the progeny. The different diet combinations tested were: thrips larvae, *T. angustifolia* pollen, *T. angustifolia* + thrips larvae, *A. franciscana*, *A. franciscana* + thrips larvae, Artefeed, and Artefeed + thrips larvae. For tests including WFT, about 20 first instars of the pest were added to the leaf arena and this number was kept constant by daily adding newly hatched larvae. Whenever second instars developed to the prepupal stage, they were removed from the leaf arena and replaced by fresh first instars. Experiments were conducted in climate chambers set at 25±1 °C, 65±5 % RH and a 16L:8D h photoperiod.

5.2.5. Influence of supplemental foods on predation capacity of *A. swirskii*

Gravid four-day-old females of *A. swirskii*, previously fed on either *T. angustifolia* pollen + thrips, *A. franciscana* + thrips or Artefeed + thrips, were used to assess their predation capacity on first instars of *F. occidentalis*, either in the presence or absence of the aforementioned supplemental foods on which they had been reared during both their immature and adult stages. For each diet treatment, 2 groups of 30 females were transferred to individual square bean leaf discs (2.5 x 2.5 cm) placed upside down on a polyurethane sponge soaked in tap water. Each female was supplied with 15 WFT first instars with or without supplemental food. The first group of 30 females received only thrips, whereas the second group received thrips supplemented with the same food the mites were previously reared on. The number of killed thrips larvae was counted after 24 h.

5.2.6. Statistical analysis

Data were analyzed using SPSS Statistics (Version 20, IBM). Intrinsic rates of increase (r_m) were calculated using the methods described by Birch (1948) and Maia et al. (2000). We used one-way analysis of variance (ANOVA) to compare the jackknife values of r_m (Meyer et al. 1986) and means were separated using Tukey's (in case of homoscedasticity) or Tamhane's test (in case of heteroscedasticity). *Frankliniella occidentalis* is known to kill phytoseiid eggs (Janssen et al. 2002), which can substantially affect the predators' intrinsic rate of increase (Vangansbeke et al. 2014). Therefore, r_m-values were calculated both including and excluding eggs killed by the antipredator behavior of thrips larvae. To account for antipredator behavior, the number of damaged eggs was subtracted from the total amount of eggs counted per day. Differences in developmental and reproductive parameters were assessed by one-way ANOVA, when a Kolmogorov-Smirnov test indicated data were normally distributed. When data were not normally distributed they were analyzed by Kruskal-Wallis ANOVA and Mann-Whitney tests were used to separate the means. Binomially distributed sex ratios were analyzed using logistic regression, consisting of a generalized linear model using a probit link function and a binomial error function (McCullagh and Nelder 1989). Differences in predation rates were analyzed using two-way ANOVA analysis with previous diet and presence versus absence of food supplements as main factors. The level of significance was set at 0.05.

5.3. Results

5.3.1. Population growth of WFT

Egg incubation time and egg hatch of WFT averaged 3.18 ± 0.02 days (mean ± SE) and 95.9%, respectively (n=395). Supplementing food on bean leaf discs affected immature developmental time for both male and female *F. occidentalis* (Table 5.1). When provided with *T. angustifolia* pollen on bean leaf discs, WFT reached adulthood faster than on the other diets, except for males supplemented with Artefeed. Supplementing either types of *Artemia* cysts did, however, not result in a faster development of female thrips. Differences in developmental time were mainly observed in both larval stages, whereas diet did not affect the duration of the (pre-)pupal stages.

Table 5.1: Developmental time (days) of *F. occidentalis* immatures fed on several diet combinations at 25 °C

			Developmental time (days)[b]				
Sex	Diet	n[a]	L1[c]	L2[c]	PP[c]	P[c]	Total immature
♂	*P. vulgaris*	21	1.90 ± 0.06 b	2.48 ± 0.12 a	1.12 ± 0.05 a	2.45 ± 0.08 a	7.95 ± 0.17 b
	P. vulgaris + *T. angustifolia*	23	1.50 ± 0.04 a	2.39 ± 0.07 a	0.98 ± 0.04 a	2.65 ± 0.10 a	7.52 ± 0.11 a
	P. vulgaris + *A. franciscana*	9	2.00 ± 0.12 b	2.61 ± 0.18 a	1.17 ± 0.08 a	2.39 ± 0.11 a	8.17 ± 0.12 b
	P. vulgaris + Artefeed	16	2.00 ± 0.10 b	2.40 ± 0.13 a	1.06 ± 0.04 a	2.38 ± 0.06 a	7.84 ± 0.12 ab
	χ^2		27.592	1.370	7.364	4.836	10.731
	df		3	3	3	3	3
	p		<0.001	0.713	0.061	0.184	0.013
♀	*P. vulgaris*	24	1.83 ± 0.07 b	2.81 ± 0.08 b	1.06 ± 0.03 a	2.65 ± 0.08 a	8.35 ± 0.12 b
	P. vulgaris + *T. angustifolia*	25	1.54 ± 0.06 a	2.18 ± 0.06 a	1.04 ± 0.03 a	2.42 ± 0.07 a	7.18 ± 0.10 a
	P. vulgaris + *A. franciscana*	36	2.06 ± 0.06 c	2.86 ± 0.06 b	1.04 ± 0.02 a	2.40 ± 0.05 a	8.36 ± 0.08 b
	P. vulgaris + Artefeed	32	1.98 ± 0.04 bc	2.88 ± 0.08 b	1.09 ± 0.05 a	2.42 ± 0.05 a	8.38 ± 0.11 b
	χ^2		34.213	39.753	1.226	6.765	45.163
	df		3	3	3	3	3
	p		<0.001	<0.001	0.184	0.080	<0.001

[a] number of tested individuals

[b] Means (± standard error) within a column and sex followed by the same letter are not significantly different (p>0.05; Kruskal-Wallis ANOVA, followed by Mann-Whitney U tests)

[c] L1= first instar, L2= second instar, PP= prepupa, P= pupa

Pollen substantially enhanced the fecundity of *F. occidentalis* in terms of the number of hatched larvae (Figure 5.1). During the peak oviposition period (after 5 days), the number of produced larvae was about three times higher in the presence of pollen than in its absence. Brine shrimp cysts had no beneficial effect on the pest's fecundity, as indicated by the lower lifetime fecundity and oviposition rate compared to pollen subsidy (Table 5.2). Sex ratio was not affected by food supplementation with about 2 females for each male. Pollen provisioning reduced female longevity as compared with the other food combinations. The intrinsic rate of increase of thrips supplemented with pollen was about 38% higher than that on bean leaves only. Supplementing either tested materials of *Artemia* cysts resulted in a less promoting effect on population growth as compared with pollen (Table 5.2 and 5.3). Thrips population growth increased with about 5% and 9% for *A. franciscana* and Artefeed supplements compared to only bean leaves, respectively.

Table 5.2: Reproductive parameters of *F. occidentalis* fed on *P. vulgaris* with or without food supplements

Diet	n[b]	Preoviposition period (days)[a]	Fecundity (larvae/female)[a]	Oviposition period (days)[a]	Oviposition rate (larvae/female/day)[a]	Longevity (days)[a]	Sex ratio (% female offspring)[a]	r_m (females/female/day)[a]
P. vulgaris	17	2.18 ± 0.13 ab	106.9 ± 10.2 b	23.0 ± 2.1 ab	4.7 ± 0.3 c	28.8 ± 2.1 a	64.0 ± 2.5 a	0.204 ± 0.003 c
P. vulgaris + *T. angustifolia*	20	2.10 ± 0.10 a	236.7 ± 21.9 a	17.1 ± 1.5 a	14.1 ± 0.7 a	20.3 ± 1.5 b	64.7 ± 2.3 a	0.281 ± 0.004 a
P. vulgaris + *A. franciscana*	25	2.36 ± 0.10 b	123.5 ± 6.2 b	23.2 ± 1.3 b	5.4 ± 0.2 bc	28.8 ± 1.4 a	67.2 ± 2.3 a	0.214 ± 0.003 bc
P. vulgaris + Artefeed	24	2.29 ± 0.06 b	134.9 ± 10.7 b	20.9 ± 1.6 ab	6.6 ± 0.4 b	26.3 ± 1.6 ab	65.9 ± 2.7 a	0.223 ± 0.004 b
χ^2/F		9.43	6.758	3.045	2.403	5.556	1.101	78.160
Df		3	3,82	3,82	3,82	3,82	3	3,82
P		0.024	<0.001	0.033	<0.001	0.002	0.777	<0.001

[a] Means (± standard error) within a column followed by the same letter are not significantly different (P>0.05; Kruskal-Wallis ANOVA, followed by Mann-Whitney tests (preoviposition period); one-way ANOVA, followed by Tukey test (fecundity, oviposition period, oviposition rate, longevity and r_m) or probit test (sex ratio))

[b] Number of individuals that successfully reached adulthood

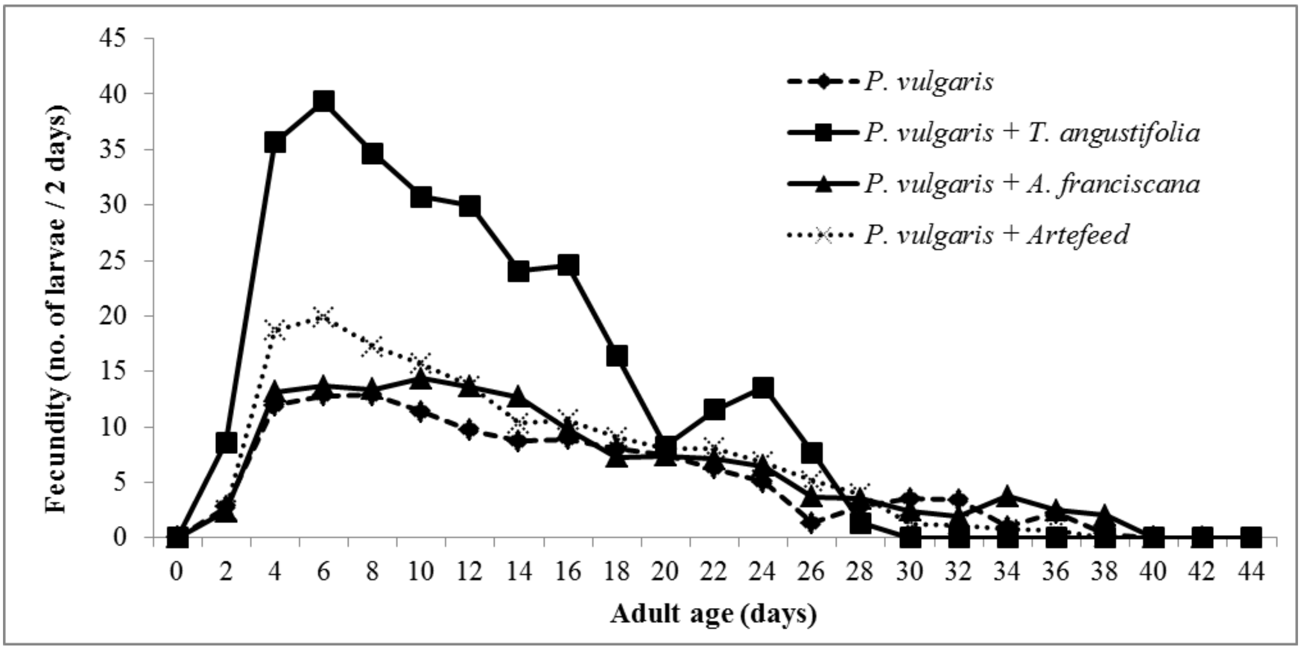

Figure 5.1: Fecundity (expressed as the number of hatched larvae/2 days) of *F. occidentalis* when fed on different diets at 25°C

5.3.2. Population growth of *A. swirskii*

With a 100% survival and developmental times of less than 7 days, all food supplements, whether or not in combination with thrips larvae, supported the predator's development. For both sexes, the slowest development was observed on a diet solely consisting of thrips larvae, followed by a diet of only Artefeed (Table 5.3). Pollen of *T. angustifolia* resulted in the fastest development, irrespective of the presence of thrips larvae. When fed either types of *Artemia* cysts, mites developed faster when thrips larvae were added.

Table 5.3: Immature developmental time (days) of *Amblyseius swirskii* fed on different diets at 25°C

Sex	Diet	n[a]	Larva	Protonymph	Deutonymph	Total immature
♂	*F. occidentalis*	10	0.95 ± 0.05 d	1.75 ± 0.13 d	1.95 ± 0.28 c	6.65 ± 0.32 b
	T. angustifolia	12	0.71 ± 0.07 ab	1.21 ± 0.07 ab	1.08 ± 0.06 a	5.04 ± 0.07 a
	T. angustifolia + *F. occidentalis*	21	0.83 ± 0.05 bcd	1.14 ± 0.05 a	1.14 ± 0.09 ab	5.12 ± 0.10 a
	A. franciscana	14	0.61 ± 0.06 a	1.36 ± 0.08 bc	1.36 ± 0.17 abc	5.32 ± 0.19 a
	A. franciscana + *F. occidentalis*	14	0.57 ± 0.05 a	1.32 ± 0.08 abc	1.29 ± 0.10 ab	5.25 ± 0.10 a
	Artefeed	12	0.92 ± 0.06 cd	1.50 ± 0.11 cd	1.46 ± 0.18 bc	5.92 ± 0.21 b
	Artefeed + *F. occidentalis*	11	0.73 ± 0.08 abc	1.18 ± 0.08 ab	1.18 ± 0.14 ab	5.18 ± 0.12 a
		χ^2	25.896	22.807	13.215	25.216
		df	6	6	6	6
		p	<0.001	0.001	0.040	<0.001
♀	*F. occidentalis*	40	0.90 ± 0.03 e	1.58 ± 0.06 de	2.31 ± 0.13 d	6.81 ± 0.15 d
	T. angustifolia	38	0.61 ± 0.04 ab	1.29 ± 0.04 b	1.38 ± 0.12 a	5.14 ± 0.08 a
	T. angustifolia + *F. occidentalis*	29	0.70 ± 0.05 bc	1.15 ± 0.04 a	1.33 ± 0.08 ab	5.10 ± 0.10 a
	A. franciscana	36	0.59 ± 0.03 ab	1.49 ± 0.06 cd	1.56 ± 0.07 c	5.64 ± 0.11 b
	A. franciscana + *F. occidentalis*	36	0.56 ± 0.03 a	1.35 ± 0.06 bc	1.36 ± 0.05 ab	5.22 ± 0.08 a
	Artefeed	38	0.86 ± 0.04 de	1.71 ± 0.07 e	1.66 ± 0.08 c	6.21 ± 0.09 c
	Artefeed + *F. occidentalis*	39	0.77 ± 0.04 cd	1.33 ± 0.05 bc	1.56 ± 0.08 bc	5.67 ± 0.10 b
		χ^2	63.653	50.230	55.072	11.676
		df	6	6	6	6
		p	<0.001	<0.001	<0.001	<0.001

[a] number of tested individuals

[b] Means (± standard error) within a column and sex followed by the same letter are not significantly different (p>0.05; Kruskal-Wallis ANOVA, followed by Mann-Whitney U tests)

The diets affected all reproductive parameters of *A. swirskii*, except progeny sex ratio (Table 9.4). Preoviposition period was longest when the predator was fed on *A. franciscana* cysts (with or without thrips larvae) or Artefeed. Lifetime fecundity was highest on *A. franciscana* cysts or *T. angustifolia* pollen, both with or without thrips larvae. The lowest number of eggs was observed on Artefeed. However, when combined with thrips larvae, the number of eggs produced increased to the level of *A. franciscana* and pollen. Female mites lived longer when solely fed on either types of *Artemia* cysts than on the other diets. When thrips larvae were combined with any of the food supplements, longevity decreased. Highest intrinsic rates of increase were obtained when *A. swirskii* was fed on pollen, pollen + WFT and *A. franciscana* + WFT. Intrinsic rates of increase presented in Table 5.4 do not include the predator eggs that were killed by thrips larvae. When considering this egg killing, the r_m-value of *A. swirskii* fed on *F. occidentalis* decreased by 17% (0.175 versus 0.210 females/female/day). With food supplementation, r_m-values decreased by only 8.9% for pollen, and by 10% for either *Artemia* products.

Table 5.4: Reproductive parameters of *Amblyseius swirskii* fed on different diets

Diet	n[b]	Preoviposition period (days)[a]	Fecundity (eggs/female)[a]	Oviposition period (days)[a]	Oviposition rate (eggs/female/day)[a]	Female longevity (days)[a]	Sex ratio (%♀)[a]	r_m (females/female/day)[a]
F. occidentalis	35	2.29 ± 0.13 ab	29.7 ± 1.7 b	15.2 ± 0.8 c	1.96 ± 0.05 bc	20.5 ± 1.2 cd	78.3 ± 1.7 a	0.210 ± 0.002 c
T. angustifolia	35	2.20 ± 0.09 ab	36.3 ± 1.5 ab	17.9 ± 0.9 abc	2.07 ± 0.04 ab	24.9 ± 1.3 bcd	80.4 ± 1.3 a	0.254 ± 0.002 a
F. occidentalis + *T. angustifolia*	23	2.13 ± 0.10 ab	34.8 ± 1.9 ab	15.7 ± 0.9 bc	2.24 ± 0.05 a	20.0 ± 1.1 d	78.6 ± 1.8 a	0.258 ± 0.003 a
A. franciscana	35	2.34 ± 0.09 bc	40.1 ± 2.2 a	22.5 ± 1.4 a	1.85 ± 0.05 c	37.4 ± 2.8 a	78.9 ± 1.9 a	0.237 ± 0.002 b
F. occidentalis + *A. franciscana*	34	2.34 ± 0.10 bc	37.3 ± 1.5 a	18.2 ± 0.8 abc	2.08 ± 0.04 ab	26.6 ± 1.6 bc	78.9 ± 1.4 a	0.251 ± 0.002 a
Artefeed	36	2.72 ± 0.15 c	17.7 ± 1.2 c	21.0 ± 1.4 ab	0.92 ± 0.06 d	31.1 ± 1.9 ab	76.5 ± 2.6 a	0.167 ± 0.004 d
F. occidentalis + Artefeed	34	2.09 ± 0.05 a	34.4 ± 1.3 ab	17.0 ± 0.6 b	2.02 ± 0.03 bc	22.0 ± 1.1 cd	78.9 ± 1.6 a	0.242 ± 0.002 b
χ^2/F		18.637	21.564	6.613	85.582	13.542	2.390	189.223
Df		6	6,225	6,225	6,225	6,225	6	6,225
P		0.005	<0.001	<0.001	<0.001	<0.001	0.881	<0.001

[a] Means (± standard error) within a column followed by the same letter are not significantly different (P>0.05; Kruskal-Wallis ANOVA, followed by Mann-Whitney tests (preoviposition period); one-way ANOVA, followed by Tamhane test (fecundity, oviposition period, oviposition rate, longevity and r_m) or probit test (sex ratio))

[b] Number of females tested

5.3.3. Influence of supplemental foods on predation capacity of *A. swirskii*

Predation rates were affected by previous diet, presence of food supplements and also their combined effect (F= 7.145; df=2; p=0.001, F= 94.807; df=1; p<0.001 and F=10.328; df=2; p<0.001, respectively) (Figure 5.2). More prey was killed when *A. swirskii* was previously fed on pollen and thrips larvae than on either *Artemia* products and thrips larvae. The predation rate of *A. swirskii* females was lower in the presence of food supplements. When *T. angustifolia* pollen was the food supplement, prey consumption dropped by 45.2%, as compared with 34.0% and 20.4% for *A. franciscana* and Artefeed, respectively.

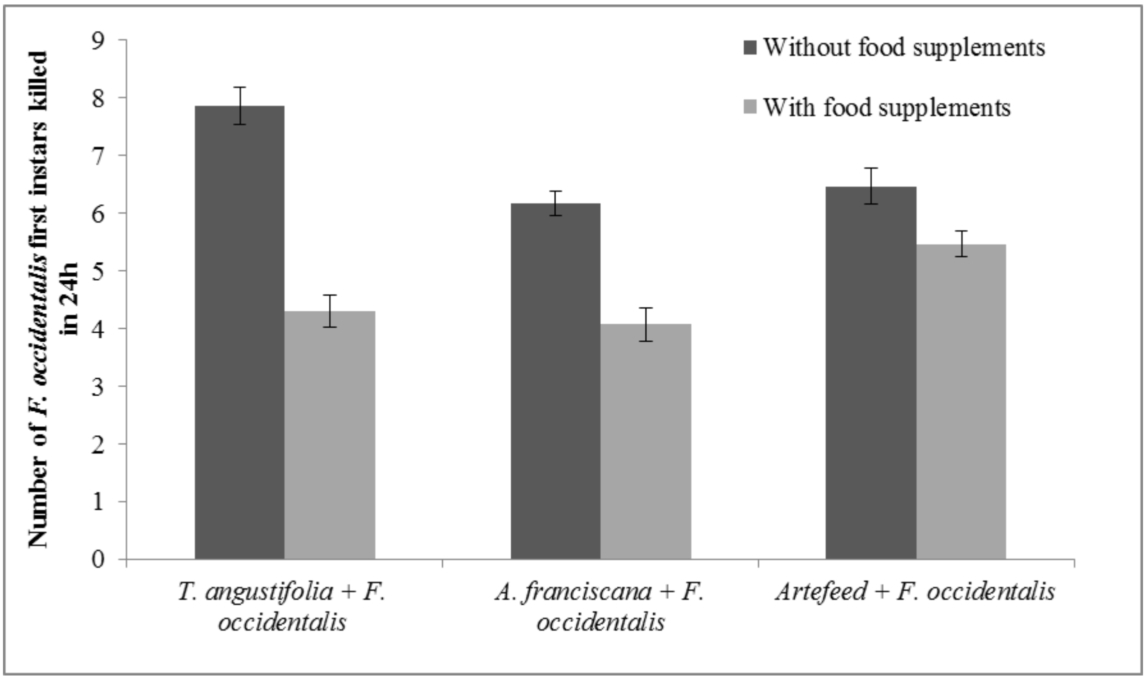

Figure 5.2: Effect of previous diet (*T. angustifolia, A. franciscana* cysts and Artefeed) and presence of food supplements on the 24-h predation rate (means ± SE) of *A. swirskii* on first instars of *F. occidentalis*.

5.4. Discussion

Providing alternative food to predators has been shown to improve pest control in various crops (van Rijn et al. 2002;Wade et al. 2008; Messelink et al. 2014). Even when the pest itself can feed on the supplemented resources and benefit in terms of its population growth, a higher population increase of the predator can outweigh the population increase of the pest, as was observed when supplementing pollen to the generalist phytoseiid mites *I. degenerans* and *A.*

swirskii for WFT control (van Rijn et al. 2002; Leman and Messelink 2015). Nonetheless, it is warranted to search for supplemental foods that do not support pest populations in the crop (Wäckers et al. 2005). Results obtained in the present laboratory study indicate that brine shrimp cysts have potential to serve as a supporting food source for *A. swirskii* in the management of *F. occidentalis*. First, both materials of *Artemia* cysts tested here had only a limited promoting effect on the development and reproduction of the thrips, whereas supplementing pollen yielded a faster development and a doubling of the pest's reproductive output. Likewise, Hulshof et al. (2003) reported that providing *T. latifolia* pollen doubled the reproduction of *F. occidentalis* on cucumber leaf discs, compared to a cucumber-leaf-only treatment. A similar result was reported on chrysanthemum (Leman and Messelink 2015). Although *Typha* pollen did increase the reproduction of *F. occidentalis*, this increase was relatively low as compared to supplementation of other species, such as pine pollen on cucumber leaves which quadruplicated the fecundity of the thrips as compared to a cucumber-only treatment.

The intrinsic rate of increase of *A. swirskii* was highest when pollen of *T. angustifolia* was supplied and this plant food even proved to be a better food source than the target prey, *F. occidentalis*. A similar result was obtained when females were fed on a combination of both pollen and thrips larvae. The reproductive performance of *A. swirskii* presented with the non-commercial material consisting of decapsulated *A. franciscana* cysts combined with *F. occidentalis* larvae equaled that of the mites fed on *T. angustifolia* pollen. The commercial brine shrimp cyst product Artefeed was found to be a less adequate food source for the phytoseiid resulting in the lowest reproductive output. Moreover, it was not possible to maintain a colony of *A. swirskii* solely on Artefeed from the second generation onwards. However, when combining Artefeed cysts with *F. occidentalis* larvae, fecundity of the predator was higher than when providing the two food sources separately. This confirms earlier observations that generalist predators usually have an increased reproductive performance on a mixed diet compared to single-diet treatments (Evans et al. 1999; Messelink et al. 2008; Messelink et al. 2010; Calvo et al. 2011; Leman and Messelink 2014; Muñoz-Cárdenas et al. 2014; Calvo et al. 2015). However, this was not observed for combining either *A. franciscana* cysts or *T. angustifolia* pollen with thrips larvae. Our results also suggest that lower quality food sources such as Artefeed (yielding the lowest r_m-value for *A. swirskii*) can still be useful when thrips (or other pests present at low numbers) are present in the crop, as the combination of both foods resulted in a higher population growth of the phytoseiid than on the separate food sources.

The presence of food supplements lowered the prey consumption of individual *A. swirskii* females. The number of thrips killed when pollen was supplemented on the leaf disc arena, was only half of that without pollen, confirming recent observations made by Leman and Messelink (2014). In comparison, the phytoseiid's predation rate was reduced by 34% and 20% in the presence of *A. franciscana* cysts and Artefeed, respectively. Thus, the per capita predation capacity of *A. swirskii* is expected to be higher when *Artemia* cysts (of various quality) are supplemented in the crop as compared with pollen subsidies. Our study also indicates that previous diet affects the predation capacity of *A. swirskii* females. Mites previously fed on a combination of *T. angustifolia* pollen and *F. occidentalis* larvae killed about 25% more thrips larvae than those reared on a combination of either type of *Artemia* cysts and thrips. Possibly, this is due to different satiation levels as a result of previous diet (Sabelis 1990) and may have resulted in more vigorous females.

Food supplements not only provide food when prey is scarce or absent, but also enable small larvae and protonymphs of *A. swirskii* to consume food during those more vulnerable developmental stages when predator:prey size ratios are low. In the presence of supplemental foods, predatory mites reached adulthood faster than on a diet solely consisting of WFT first instars. Larvae of *A. swirskii* are facultative feeders: they do not require food to reach the protonymphal stage, but they will eat when suitable food is available (Chittenden and Saito 2001). In our study, larvae of *A. swirskii* were never observed to kill and feed on thrips larvae. However, some larvae were observed to feed on pollen or *Artemia* cysts. *Amblyseius swirskii* protonymphs have difficulty capturing larger *F. occidentalis* larvae (personal observations). Additionally, thrips are known to fight off predators by swinging with their abdomen (Bakker and Sabelis 1989; De Bruijn et al. 2006). In our experiments, young mites may have concentrated on the supplemental food in the early stage of their development to avoid encounters with counterattacking prey, thus speeding up their development. However, it is unclear whether lower developmental rates of *A. swirskii* on *F. occidentalis* in our experiments are due to the lower nutritional quality of thrips larvae, to antipredator behaviours of the prey, or to a combination of both.

Artefeed is a cheaper product than Nutrimite^{TM}, with market prices of about 150€ and 300€ per kg, respectively. The current market price of the highest quality cysts of *A. franciscana* used for aquaculture (i.e. yielding the highest number of nauplii hatching per gram of cysts) is approximately 200€ (G. Van Stappen, Ghent University, personal communication), excluding the costs for decapsulation. Whether the use of lower quality -and thus cheaper- cysts will be allow to maintain a predatory mite colony in the crop is subject for future research.

A possible drawback of using materials that are harvested in nature, like cattail pollen or *Artemia* cysts, is that quality and harvestable quantity may vary, which may impede a continuous supply of materials with a given quality and thus influence their market price. In contrast to our results, *A. franciscana* cysts proved to be unsuitable for *A. swirskii* in the study of Leman and Messelink (2014). This might be due to the variable intrinsic quality of the *Artemia* strains used in the experiments and thus their nutritional value for the predators or to differences in the decapsulation process (Leman and Messelink 2014). Also, the cysts in our study were not fully hydrated by immersing them in water as was done in the study of Leman and Messelink (2014), but were only partially hydrated by absorbing water from the humid environment (over 90% RH) in our experimental cages (Vandekerkhove et al. 2009). The hydration process of the cysts might have affected the mites' access to the content of the cysts.

In practice, commercial pollen products, such as Nutrimite™, are being applied onto the crop using blower devices, targeting an even distribution of the pollen (Adar et al. 2014; Pijnakker et al. 2014). However, according to van Rijn et al. (2002), pollen should best be applied patch-wise, as predatory mites tend to colonize these patches, impeding the access of thrips to the pollen resources. Uniformly distributed pollen may enable thrips to feed more from the pollen subsidies. This issue might be less important when *Artemia* cysts are used as food supplements, as thrips do not feed on the brine shrimp cysts to the same extent as they do on pollen.

Pollen and *Artemia* cysts could also be used together or alternately, as is presently done for supporting the omnivorous predatory bug *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) in various greenhouse crops. In this strategy, field populations of *M. pygmaeus* are initially supported using relatively expensive *Ephestia kuehniella* (Zeller) eggs (Lepidoptera: Pyralidae) (Lenfant et al. 2000; De Clercq et al. 2014; Messelink et al. 2014) , whereas later in the season, cheaper products consisting of *Artemia* cysts are distributed in the crop. For predatory mites, a similar strategy could be applied. When monitoring indicates an increased level of thrips infestation, growers might switch to brine shrimp cysts as a supplemental food to reduce the beneficial effect of previous pollen subsidies on the population growth of thrips.

Other food supplements than those tested in the present study may be useful to support populations of predatory mites. For instance, astigmatid mites have been shown to support the population growth of *A. swirskii* (Calvo et al. 2015). It is warranted to investigate the supportive value of these alternative food sources for omnivorous pests like *F. occidentalis* in order to develop an optimal food supplementation strategy. Results may also differ depending on the host plant species (Leman and Messelink 2014) and accessibility of food supplements may be affected by the prevailing temperature and humidity conditions. In addition, more research is necessary to optimize the provision of food supplements in different greenhouse crops, including the timing and rate of application.

In conclusion, our study demonstrates the potential of *Artemia* cysts as a supplemental food source for the management of thrips using the predatory mite *A. swirskii,* and possibly for other generalist phytoseiid predators as well, like *A. limonicus* as was reported in **Chapter 4**. *Artemia* cysts provide little support for the population growth of the omnivorous *F. occidentalis*, but are a suitable food source for predatory mites. The benefits and drawbacks of food subsidies of pollen and *Artemia* cysts are presently being assessed in the greenhouse to test our hypotheses. The potential of artificial food sprays composed of easily available ingredients warrants further investigation. Recently, liquid and solid artificial diets have been developed for *A. swirskii* (Nguyen et al. 2014a, b). These diets can also be tested for their applicability as supplemental foods to support predatory mites in the crop and for their attractiveness to omnivorous pests like thrips. Finally, it is warranted to investigate whether artificial diets can be modified in such a way that, when applied as supplemental foods in the crop, they exclusively support populations of the predator and not those of the pest.

6

Pre-establishment of *Amblyseius swirskii* with food supplements in greenhouse crops

6.1. Introduction

In augmentative biological control programs, large numbers of mass-produced beneficial arthropods are released in crops to obtain immediate pest control (Stinner 1977; Collier and Van Steenwyk 2004). Although this type of biological control is now a widely adopted standard practice for pest control, efficacy can be low due to poor establishment of the natural enemies in the crop. Moreover, the necessity of periodic releases is not always economically viable for the producers. To increase the resilience of beneficial arthropods in crops after release, several strategies can be adopted, such as providing alternative food or hosts, oviposition sites and/or shelters (Messelink et al. 2014, see also **section 1.8**). Food supplementation to natural enemies has been shown to increase the efficiency of pest control and to enhance conservation of predators in the crop (van Rijn et al. 2002; Wäckers et al. 2005; Put et al. 2012; Messelink et al. 2014). A summary of the literature on the provisioning of foods for phytoseiid predatory mites is presented in **Table 1.4**. By providing phytoseiids with suitable food supplements, a so-called “standing army” of predators can be created which can provide a prophylactic biological control strategy before pests invade the crop.

Here, we report the results of two greenhouse experiments where food supplements for the pre-establishment of *A. swirskii* were tested. This generalist predatory mite is an efficient biocontrol agent of key greenhouse pests, such as thrips (Messelink et al. 2006, 2008; Calvo et al. 2011), whiteflies (Nomikou et al. 2002; Messelink et al. 2008; Calvo et al. 2012) and broad mites (van Maanen et al. 2010). Ever since its first commercial introduction in 2005, *A. swirskii* became one of the most successful biocontrol agents in protected cultivation, and has been used in over 50 countries (Calvo et al. 2015) We used food supplements that were previously tested in the laboratory in **Chapter 5**. In the first experiment, we compared a commercially available pollen product of *T. angustifolia* (Nutrimite^{TM}), decapsulated cysts of *Artemia* sp. (Artefeed) and an artificial powder diet that was developed by Nguyen et al. (2014b) for the pre-establishment of *A. swirskii* on potted chrysanthemum plants. In a second experiment on ivy plants we tested the establishment of *A. swirskii* using the same foods, except for *Artemia* cysts of the commercial product Artefeed, which were replaced with dry decapsulated cysts of *A. franciscana* from a non-commercial strain. The greenhouse experiment with ivy plants was performed at both a constant and an alternating temperature regime to also assess the impact of climate on the establishment.

6.2. Material and Methods

6.2.1. Greenhouse experiment 1: Chrysanthemum

The first experiment was performed in a greenhouse at the Ornamental Plant Research station (Destelbergen, Belgium) between May and August 2014 on young potted chrysanthemum plants (*Dendranthema* X *grandiflorum*) (Figure 6.1). Four young plants with 10 to 12 leaves (ca. 12 cm high) were planted in one pot (H 9.4cm, ø 12 cm). Four such pots were placed inside a mesh cage (40 x 90 x 80 cm) and per diet treatment we used 8 mesh cages. One mesh cage containing 4 pots with four plants each was considered as one replication. Pots were placed on a water-saturated irrigation matting to prevent the predatory mites from migrating. In each mesh cage, five adult females of *A. swirskii* from the stock colony (see **section 5.2.1**) were transferred to the chrysanthemum plants using a fine brush. In this experiment, we tested 3 food supplements: pollen of *T. angustifolia* (Nutrimite^{TM}, Biobest N.V., Westerlo, Belgium), dry decapsulated cysts of *Artemia* sp. (Artefeed, Koppert B.V., Berkel en Rodenrijs, The Netherlands) and a powdered artificial diet (AD) developed by Nguyen et al. (2014). This artificial diet was composed of 16.6% sucrose, 16.6% tryptone, 16.6% yeast extract, 6.7% glucose (MP Biomedicals LLC, Illkirch, France), 6.7% fructose (Sigma Aldrich Chemie GmbH, Steinheim, Germany), 16.6% egg yolk powder (Bouwhuis Enthoven BV, Raalte, The Netherlands), 0.13% vitamin mix based on the composition of bovine liver (Vandekerkhove et al. 2006) (weight percentages: 25.4 % nicotinic acid, 4.9 % riboflavin, 0.5 % thiamine, 1.5 % vitamin B6, 12.4 % Ca-pantothenate, 1 % folic acid, 0.1 % biotin and 54.2 % vitamin C) and 20% (w/w) dry decapsulated cysts of *A. franciscana* (*Artemia* Reference Center (ARC), Ghent, Belgium); all ingredients of the artificial diet were ground to powder using a pestle. All diets were applied on the plants using a dusting applicator (Nutrigun, provided by Biobest N.V.). The amount of food provided on the plants was recalculated from 500g/ha, which is the recommended dosage for Nutrimite^{TM} (Biobest, 2015). A control treatment with *A. swirskii* but without food supplements was included in the experiment. Every other week, the population growth of the predatory mites was monitored by counting the number of motile stages of *A. swirskii* on 20 leaves per cage *in situ.* After counting, the diets were distributed over the plants. Temperature was monitored every 5 minutes and during the experiment an average temperature of 23.9°C and average relative humidity of 61.8% was recorded. Although a constant temperature regime was set in the greenhouse, actual temperatures tended to fluctuate between 26°C (daytime peak temperature) and 18°C (lowest nighttime temperature) (**Appendix A.1a**). Relative humidity also fluctuated, dropping to about 50% RH along with peak daytime temperatures. During the night, lower temperatures were accompanied with high relative humidities up to 80% (**Appendix A.1b**).

6.2.2. Greenhouse experiment 2: Ivy

A similar experiment was carried out between January and March 2015 on young ivy plants (*Hedera helix* L. cv. "Green ripple"). Ten cuttings of ivy (5-10 leaves) were planted in one pot (H 9.4cm, ø 12 cm). Nine pots were placed in one mesh cage (similar set-up as those described for experiment 1) and per diet treatment 6 cages were used. Counting of the predators and distribution of the diets were done in the same way as described for experiment 1. Pollen and artificial diet were similar to those described for experiment 1. Dry decapsulated cysts of *Artemia franciscana* originated from the Great Salt Lake (Utah, USA) and were provided by the *Artemia* Reference Center (ARC, Ghent, Belgium).

This experiment was conducted at two different temperature regimes in separate greenhouse compartments. In the first greenhouse, a constant temperature of 25°C was set, whereas in the other greenhouse compartment an alternating temperature regime with a daytime temperature of 30°C and a nighttime temperature of 15°C at a 16:8 h (L:D) photoperiod (DTR+15) was chosen. However, the actual temperature and relative humidity data recorded in both compartments are displayed in **Appendix A.2**. For the constant temperature regime, average daytime and nighttime temperature were 24.0 and 22.4°C, respectively. Relative humidity averaged 57% under the constant temperature regime. In the treatment where temperatures were allowed to fluctuate, an average daytime temperature of 27.0°C was obtained with an average nighttime temperature of 18.3°C. In this regime, relative humidity during the day was on average 54.9%, whereas this was 70.7% during the night.

Figure 6.1: Experimental set-up of the greenhouse experiment using chrysanthemum (left) and ivy plants (right)

6.2.3. Statistical analysis

We used repeated measures ANOVA to analyze the population growth of *A. swirskii* as affected by different food supplements (IBM, SPSS Statistics 20). For the first experiment, diet was considered as the *between-subject* factor and time of sampling as the *within-subject* factor. Population densities at the end of the experiment (16 weeks after the initial introduction) were compared by means of a Kruskal-Wallis analysis. Mann-Whitney U tests were used to separate the means. In the second experiment on ivy plants, data was analyzed in the same way as in experiment 1 and temperature was added as a *between-subject* factor. The level of significance was set at 0.05 in all tests.

6.3. Results

6.3.1. Greenhouse experiment 1: Chrysanthemum

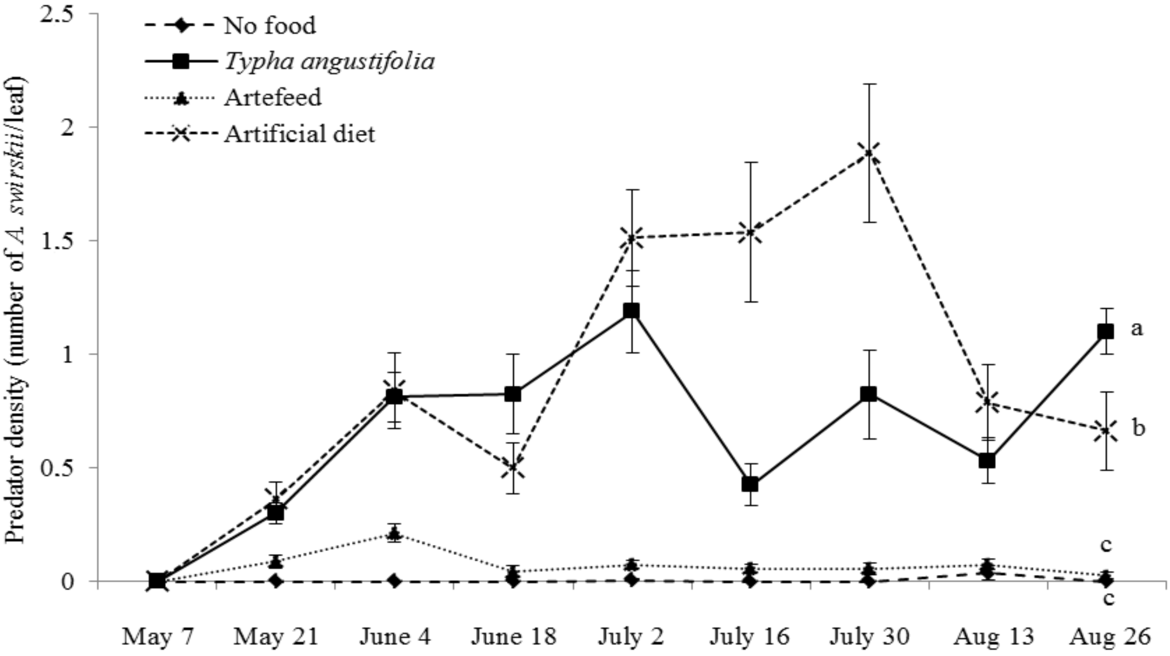

Figure 6.2: Population density of *A. swirskii* motiles (mean numbers per leaf ± SE) on caged chrysanthemum plants as affected by food supplements (*Typha angustifolia*, Artefeed and an artificial powder diet developed by Nguyen et al. (2014))

The density of *A. swirskii* on the chrysanthemum plants was significantly affected by diet, time of sampling and the interaction thereof (Table 6.1). Figure 6.2 shows that supplementation with *T. angustifolia* pollen or the artificial powder diet resulted in significantly higher predator densities than the treatment with *Artemia* sp. cysts and the treatment without food supplements. Two weeks after the introduction of *A. swirskii* (from May 7 to May 21), no predatory mites were observed on the chrysanthemum leaves in the absence of food supplements. In general, pollen and the artificial diet resulted in similar population growth numbers up to 8 weeks. Thereafter, higher numbers of *A. swirskii* were counted with the artificial diet as a food supplement until week 12 of the experiment. The last monitoring resulted in higher phytoseiid numbers in the pollen treatment than the treatment with the AD (Kruskal-Wallis: χ^2= 25.544; df=3; $p<0.001$).

Table 6.1: Results of repeated measures ANOVA on the effects of diet and sampling time (ST) on the population density of *A. swirskii* on chrysanthemum

Experiment 1 (chrysanthemum)	Source	df	F	P
within-subject factor	ST	7	8.669	<0.001
	ST x diet	21	7.048	<0.001
	Error	196		
between-subject factor	Diet	3	79.585	<0.001
	Error	28		

6.3.2. Greenhouse experiment 2: Ivy

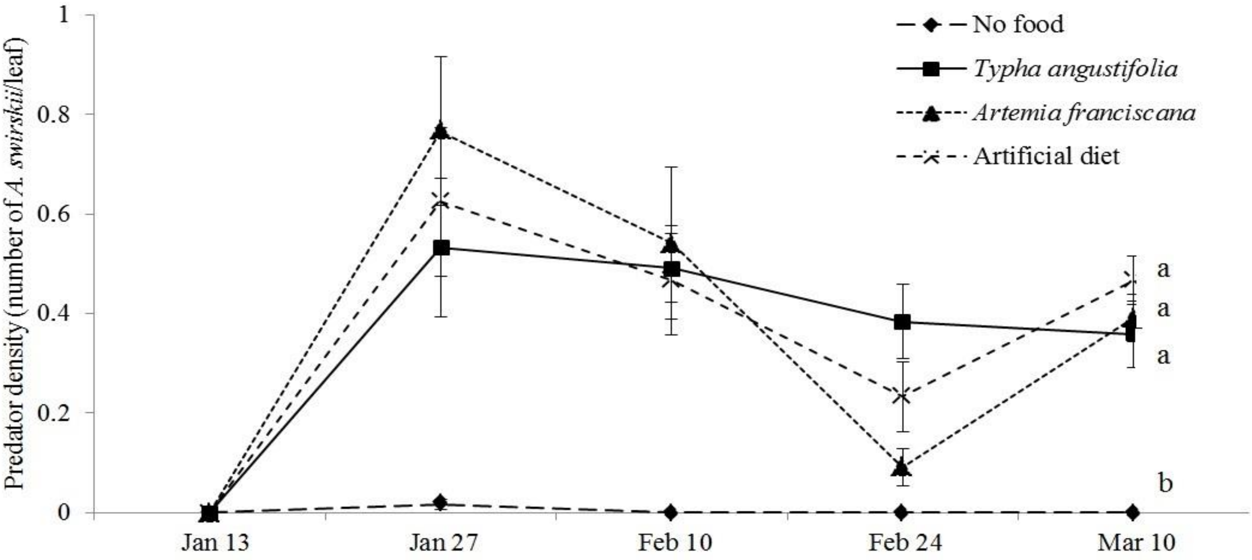

Figure 6.3: Population density of *A.* swirskii (mean numbers per leaf ± SE) on caged ivy plants as affected by food supplements (*Typha angustifolia*, *Artemia franciscana* and an artificial powder diet developed by Nguyen et al. (2014)) at a constant temperature regime of 25°C

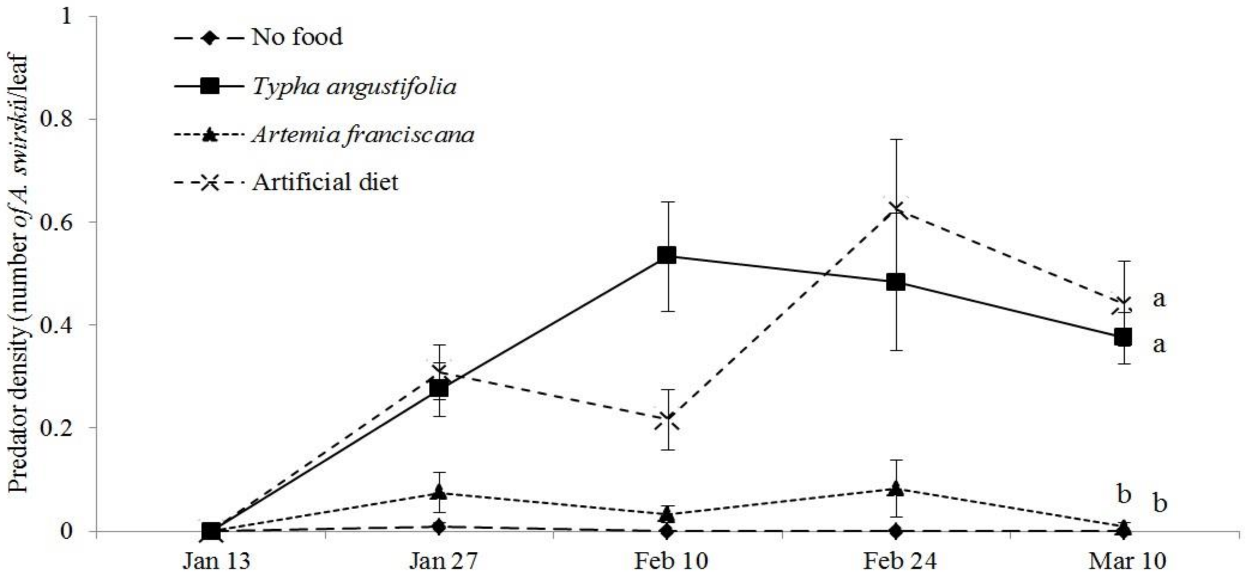

Figure 6.4: Population density of *A. swirskii* (mean numbers per leaf ± SE) on caged ivy plants as affected by food supplements (*Typha angustifolia*, *Artemia franciscana* and an artificial powder diet developed by Nguyen et al. (2014)) at an alternating temperature regime of 30°C/15°C.

All tested factors and their interactions had a significant effect on the population density of *A. swirskii* on the ivy plants (Table 6.2). The only factor that did not significantly affect the establishment of *A. swirskii* was sampling time. The effect of temperature regime on the population density of the predator was most pronounced when *A. swirskii* was provided with *A. franciscana* cysts (Figures 6.3 and 6.4). Establishment of *A. swirskii* when supplied with *A. franciscana* cysts was similar to that in the treatments with *T. angustifolia* pollen or artificial diet at the set temperature of 25°C. However, predator densities were substantially lower in the *Artemia* treatment at the alternating temperature regime. A significant interaction was observed between diet and temperature (Table 6.2). When no foods were provided on the ivy plants, no population of *A. swirskii* could be established. The last monitoring in the treatment at 25°C demonstrated similar population densities of *A. swirskii* when foods were supplemented (Kruskal-Wallis: χ^2= 19.342; df=3; p<0.001). In the treatment at the alternating temperature regime, pollen and the artificial diet resulted in similar densities, but a significantly lower population density when *Artemia* cysts or no food were supplemented (Kruskal-Wallis: χ^2= 13.928; df=3; p=0.003).

Table 6.2: Results of repeated measures ANOVA analyzing the effects of diet, temperature and sampling time (ST) on the population density of *A. swirskii* on ivy plants

Experiment 2 (ivy)	Source	df	F	P
within-subject factor	ST	3	2.269	0.084
	ST x diet	9	2.626	0.008
	ST x temperature	3	12.771	<0.001
	ST x diet x treatment	9	2.387	0.016
	Error	120		
between-subject factor	Diet	3	36.673	<0.001
	Temperature	1	12.903	0.001
	Diet x temperature	3	8.011	<0.001
	Error	40		

6.4. Discussion

There is a growing interest in conserving populations of released natural enemies in crops by providing food supplements. For generalist phytoseiids, pollen has been repeatedly suggested as a suitable food source for supporting the population build-up of the predators and thus improving pest control (van Rijn and Tanigoshi 1999; van Rijn et al. 2002; Delisle et al. 2015; Leman and Messelink 2015). Results from the present greenhouse experiments confirm that pollen of *T. angustifolia* (Nutrimite^{TM}) are a suitable food source for the prophylactic release of *A. swirskii* in chrysanthemum and ivy crops. An average of about one motile *A. swirskii* was found per chrysanthemum leaf up to 16 weeks after the initial release of only 5 female *A. swirskii* per 4 plants. The outcome of this cage study confirms the results obtained in the laboratory study reported in **Chapter 5** for *A. swirskii* fed on *T. angustifolia* pollen. A population of predatory mites could not establish when provided with cysts of a commercial strain of *Artemia* sp. (Artefeed), which was in line with the laboratory findings in **Chapter 5** that *A. swirskii* could not be maintained for more than two generations on this commercial product of *Artemia* cysts. In the second experiment on ivy plants, dry decapsulated *A. franciscana* cysts from a non-commercial strain did support the establishment of *A. swirskii*. However, there was a marked difference between the two tested temperature regimes in terms of population increase. Whereas a similar amount of predatory mites was found in the treatment with pollen at the two tested temperature regimes, a substantially lower number of *A. swirskii* motiles was detected on the ivy plants in the greenhouse at the alternating temperature regime than at the constant one. Possibly, humidity effects may have played an important role in the lower establishment in the alternating temperature regime. The diurnal cycle of high and low temperatures is accompanied by large diurnal variations in humidity (**Appendix A2**). As was also emphasized in **Chapters 4** and **5,** humidity might play a crucial role for phytoseiids in their ability to take up the content of *Artemia* cysts. Greenhouse climate data presented in the figures in **Appendix A2** show that relative humidity at the alternating temperature regime was about 20% higher than at the constant temperature regime during the night, whereas similar humidity patterns were observed during the day. These large fluctuations in humidity might have caused cyclic hydration and dehydration of the *Artemia* cysts, preventing predators from piercing them. Moreover, similar relative humidities during the day, but higher daytime temperatures resulted in higher “vapour pressure deficit” values at the alternating temperature regime, thereby causing greater dehydration of the cysts as compared to the constant temperature regime (**Appendix A.3**). This was confirmed by personal observations during the monitoring. In the first experiment, where a constant temperature regime was programmed, **Appendix A.1** shows a similar diurnal pattern as for the alternating temperature regime in experiment 2. The poor performance of *A. swirskii* on *Artemia* cysts (Artefeed) in the first experiment may thus also have (partly) resulted from these diurnally varying relative humidity levels. Alternatively, the cycling hydration/dehydration levels of the *Artemia* cysts may also have resulted in a decreased energy content, loss in vitamins and reduced fatty acid concentrations in the *Artemia* cysts as was demonstrated by El-Magsodi et al. (2014). Similarly, Lavens and Sorgeloos (1987) reported a decreased hatching rate of cysts after several hydration/dehydration cycles. As hatching rate is correlated with the nutritional content of the cysts, it is likely that this had an effect on the population growth of the predatory mites as well.

Higher *A. swirskii* densities were observed in the first greenhouse experiment on chrysanthemum than in the second experiment on ivy. This may be due to several reasons, such as the longer experimental time in the first experiment than in the second experiment. Secondly, the plot with ivy plants had a higher number of leaves than the plots with chrysanthemum plants (ca. 200 versus 50 leaves/plots at the end of the experiment, respectively). Chrysanthemum leaves have trichomes which may influence the performance of predatory mites (predation rate and walking speed, Buitenhuis et al. 2014), including *A. swirskii*. On the other hand, trichomes could serve as oviposition substrates or refuge for predatory mites (Faraji et al. 2002; Loughner et al. 2008). Ivy plants have glabrous leaves and thus lack such oviposition substrates, which may have influenced oviposition rate and population growth.

The powdered artificial diet developed by Nguyen et al. (2014b) for *A. swirskii* proved to support the population increase of this predator on both chrysanthemum and ivy plants. The population build-up in the treatment with the artificial diet was similar to that on *T. angustifolia* pollen. As opposed to *A. franciscana* cysts, the consumption of the artificial diet was not negatively affected by large temperature variations (and associated relative humidity variations). A previous attempt by Hoogerbrugge et al. (2008) to establish *A. swirskii* on chrysanthemum plants using an artificial diet (with unknown composition) failed. In the study by Messelink et al. (2009) a mixture of yeast, glucose and soya powder allowed to maintain a population of *A. swirskii* on chrysanthemum plants, albeit not to the same degree as cattail pollen (*T. latifolia*). Supplementing an artificial diet with components from insects or other invertebrates (such as *Artemia*) may improve the nutritional quality of the diet for the phytoseiid (Nguyen et al. 2013, 2014a, 2015). Indeed, the addition of insect components to artificial diets has been shown to increase their nutritional quality and appreciation by arthropod predators (Grenier and De Clercq 2003). Although solid diets have some practical advantages, including non-stickiness, longer shelf life and lower levels of plant surface soiling, (Nguyen et al. 2014b), liquid diets (whether or not encapsulated) should also be tested for their value to support phytoseiid predators.

During the course of the experiment, fungal contamination of the artificial diet was frequently observed when the diet was not sprinkled finely enough and as a result the diet particles clumped together. Additionally, in the first experiment on chrysanthemum we frequently found Collembola species and oribatid mites, usually soil-dwelling species, migrating to the leaves, to feed on the artificial diet. Likewise, albeit to a much lesser extent, these scavengers were also found feeding on the *Artemia* cysts. These organisms were not observed to occur on the plants in the treatments with pollen. Whether these migrating soil dwellers could be used as a food source by the predatory mites is not known.

An important criterion for selecting a food supplement to be adopted and applied by greenhouse growers is the cost. Prices of the tested foods per weight unit are presented in Table 6.3. The prices of Artefeed and Nutrimite^{TM} are based on current market prices. The market price of *A. franciscana* cysts can vary substantially, as a result of fluctuations in harvestable quantity and quality (see also Section 5.4). This holds true also for other foods that are harvested in nature, such as pollen. To tackle these issues, an artificial diet with known components that are easily available on the market may be a more reliable food source. The cost of the decapsulation process of *A. franciscana* was not quantified but will likely increase the total cost of decapsulated cysts. The calculated cost to compose the artificial diet is about half the market price of Nutrimite™. This price could be further reduced if the more expensive components of the diet (like tryptone) are to be replaced with cheaper ones (like casein).

Table 6.3: Approximate cost of food supplements for *A. swirskii*

Food	Price (€/kg)
Artefeed	150
A. franciscana [a]	200
Nutrimite™	300
AD [b]	166

[a] Price of high quality encapsulated cysts (G. Van Stappen, personal communication)

[b] AD= artificial diet (D.T. Nguyen, personal communication)

As *Artemia* cysts are relatively large compared to a pollen grain (ca. 250 µm vs. 20 µm (Vanhaecke and Sorgeloos 1980; van Rijn and Tanigoshi 1999, respectively)), less leaf area was covered by cysts as compared to pollen when a similar weight of food supplements was sprinkled over the crop. Additionally, after sprinkling, *Artemia* cysts tended to accumulate in the leaf axils, whereas a more even distribution was achieved in the application of pollen or artificial diet. Possibly, this may have affected the availability of the foods and thus the establishment of the phytoseiid. Furthermore, decapsulating *Artemia* cysts could lead to a weight reduction of approximately 50%, which will double the price per weight unit as compared to the encapsulated *Artemia* product. This is another factor that should be taken into account when comparing the prices of supplemental foods.

Western flower thrips is the most important pest in greenhouse crop production in the Netherlands (Messelink 2014). It is especially problematic in ornamental crops, seen the zero-tolerance for crop damage. The continuous presence of an effective thrips predator such as *A. swirskii* in the crop should contribute to a better efficacy in controlling this pest. However, developers of artificial food supplements should take into consideration that some nutrients may stimulate the reproduction of thrips. Hulshof et al. (2003) analyzed the value of several pollen species to support the reproduction of *F. occidentalis*, and found substantial differences in fecundity. As was mentioned in the previous chapter, *Typha* pollen did increase the reproduction of *F. occidentalis* in the study of Hulshof et al. (2003), but other pollen (e.g. pine pollen) were found to have a much higher positive impact on the thrips' fecundity. The nutritional composition of the pollen may explain its potential nutritional value for thrips. For example, the number of *F. occidentalis* females detected in flower heads was found to be positively correlated with the percentage of phenylalanine in the total amino acid profile (Brodbeck et al. 2001). In **Chapter 5**, we reported on the lower performance of the omnivorous *F. occidentalis* on *Artemia* cysts as compared with *T. angustifolia* pollen. However, we did not investigate whether the artificial diet could benefit the development and/or reproduction of *F. occidentalis*. The effects of artificial diets on thrips populations remain to be addressed in future studies.

In summary, *T. angustifolia* pollen and the powdered artificial diet were found to greatly enhance the population build-up of *A. swirskii* on caged chrysanthemum and ivy plants in the greenhouse. In accordance with our findings in **Chapter 5**, the selected commercial cysts of *Artemia* sp. (Artefeed) were not suitable to support a population of *A. swirskii*. In contrast, cysts from a non-commercial *A. franciscana* strain did support population growth of the phytoseiid, but only when diurnal temperature variations (and associated variations in relative humidity) were limited. When temperature and humidity levels strongly fluctuate in the crop environment, we hypothesize that *A. franciscana* cysts are not easily accessible for the predators.

7

General discussion, conclusion and future perspectives

Biologically-based pest management strategies are becoming increasingly important, especially in protected crops. In response to the increasing costs for energy expenditures, greenhouse growers are forced to adopt strategies to reduce these costs. By allowing temperatures to vary within a certain range, up to 20% of the energy cost can be saved. However, the ectothermic fauna present in the greenhouse will also be affected by these temperature variations. Nowadays, over 230 arthropod species are commercially available worldwide for biological pest suppression in greenhouse crops. With about 30 species marketed, predatory mites represent the second largest group of natural enemies released in protected crops (van Lenteren et al. 2012; Messelink 2014). In the first part of this study, we focused on the phytoseiid spider mite predators *Phytoseiulus persimilis* and *Neoseiulus californicus* and their natural prey *Tetranychus urticae* as model organisms to evaluate and predict the effects of diurnal temperature variations on the outcome of biological control.

In **Chapter 2**, we demonstrated that the theoretical population growth of *P. persimilis* and *N. californicus* as well as of their spider mite prey *T. urticae* was substantially affected by temperature alternations when compared to their growth rates at constant temperatures. The intrinsic rate of increase (r_m) was 32, 57 and 33% higher at an alternating temperature regime of 20°C/5°C (at a 16:8h L:D photoperiod) than at a constant 15°C for *P. persimilis*, *N. californicus* and *T. urticae*, respectively. This emphasizes on the one hand the impact of diurnal temperature ranges (DTR) on the population growth of the three mite species, but also highlights the interspecific differences. Whereas a practically similar impact was observed on population growth of *P. persimilis* and *T. urticae*, population growth of *N. californicus* was over 20% more positively influenced as compared with the two other mite species when developing and reproducing at the alternating temperature. This highlights the need to implement data on varying temperature regimes in predictive models for biological control. When allowing temperatures to vary, rather than keeping them constant, greenhouse growers could introduce natural enemies earlier in the season as they will be able to establish a population. Furthermore, we examined the developmental rate of *P. persimilis*, *N. californicus* and *T. urticae* over a temperature range with an average daily temperature of 11.7 to 38.3°C, with night temperatures as low as 2.5 °C and day temperatures as high as 40°C. We compared the development at four diurnal temperature ranges, with a difference of 0, 5, 10 and 15°C between day temperature and night temperature. For fast developing species, such as predatory and spider mites, development has a much higher proportionate impact on the population growth than reproduction does (Lewontin 1965). Hence, it was assumed that results of our developmental data have predictive value for population growth rates. By using both linear and nonlinear models, we were able to assess the impact of the different DTRs on the development of the mite species. We found that the developmental threshold was markedly affected by DTR. This shows that the investigated mite species will resume their development from lower average temperatures onwards when confronted with variable temperatures. The higher the DTR, the lower the developmental threshold, as was confirmed by both linear and nonlinear regression. This opens perspectives to introduce predatory mites earlier in the season and to allow them to establish before pests arrive in the crop. Likewise, however, pest outbreaks could occur earlier in the season when growers use varying temperature regimes in their greenhouses. Fine-tuning predictions of pest outbreaks, by taking variable temperatures in consideration, could assist in reducing the need for chemical control measures. Whereas our result focused on mites, possibly similar mechanisms may be in play for other ectothermic organisms.

A direct impact of a diurnal temperature range with a high amplitude of 15°C on biological control efficacy of predatory mites was demonstrated in **Chapter 3**, where predation rates of *P. persimilis* and *N. californicus* were compared. At an average of 15 and 20°C, predation rates were significantly higher at a DTR of 15°C as compared with those at constant temperatures regimes. If pest and predator are equally affected by the varying temperatures in terms of population growth (as was demonstrated for the impact on the developmental rates of *P. persimilis* and *T. urticae* in **Chapter 2**), then the increased predation rates at the alternating temperature regimes should result in better pest control than that at constant temperatures. If so, allowing temperature to vary in the colder temperature range (for example early in the season) should be beneficial for biocontrol. The opposite scenario is then expected to occur in the higher temperature range (>25°C). However, it deserves emphasis that our experiments were carried out at alternating temperatures regimes. Here, the higher daytime temperatures are being maintained during the entire photophase (16h). Fluctuating temperatures more realistically resemble temperature regimes occurring in a greenhouse or natural environment. In that case, exposure of the organism to the highest temperatures in the range will be much shorter. Hence, more research in this area should focus on the critical exposure time at which ectotherms can survive these high temperatures. Similarly, time spent at near-zero temperatures during the night when higher daytime temperatures occur should be investigated. Besides laboratory experiments, effects of varying temperatures regimes should be tested on plants in a (semi-)field situation.

The relevance of our findings may not be restricted to pest and predator interactions in a greenhouse environment.

Over the past 120 years, over 2600 species of exotic natural enemies have been introduced for the control of plant and arthropod pest species (Cock et al. 2010). Although the majority of introductions did not result in negative effects on the environment (van Lenteren et al. 2003), there is a growing concern about adverse non-target effects of exotics (De Clercq et al. 2011). Many exotic biocontrol agents have been introduced in greenhouses and establishment outside these protected environments may provoke undesired effects on native species (Howarth 1991; Jolly 2000). Temperature is a crucial factor for the establishment of non-native biological control agents. Several proxies have been proposed to determine the risk of establishment of an exotic biocontrol agent, among which the developmental threshold and thermal budget (Hatherly et al. 2005). In most cases, these parameters are derived from experiments using constant temperatures. However, these parameters were found to be substantially affected by diurnal temperature ranges in the present study (**Chapter 2**). In the light of our results, it is advisable to include DTRs in such risk indices (van Lenteren et al. 2003) to fine-tune risk the environmental risk assessment of exotic biological control agents. Risk assessments solely based on constant temperature effects may substantially underestimate the establishment potential of exotics.

Attention to the effects of temperature variations is also warranted in studies on the impact of global change. Climate change will not only alter mean temperatures, but also DTRs (Easterling et al. 1997). However, there is no consensus whether diurnal temperature variations will increase (Schwartz et al. 2006; Makowski et al. 2008; Folguera et al. 2009) or decrease (Walther et al. 2002) due to climate change. Nevertheless, it is recommendable to incorporate temperature variations in predictive models, especially since ectotherms are more sensitive to temperature variation and thus more vulnerable to climate change (Paaijmans et al. 2013). For example, Paaijmans et al. (2010) showed the necessity of including temperature fluctuations in models predicting malaria transmission as affected by climate change. It is widely accepted that organisms react individualistically to climate changes (Bale et al. 2002), resulting in differential species responses, which in turn will alter species interactions (Davis et al. 1998; Voigt et al. 2003; Aguilar-Fenollosa and Jacas 2014). The results of our study indicate that not only the impact of temperature is species-specific, but also that temperature variations affect different species differentially. If certain changes in temperature (variations) benefit the pests more than their natural enemies, this may lead to an intensification of pesticide use, which could compromise a sustainable agriculture (Aguilar-Fenollosa and Jacas 2014).

In **Chapter 2** we demonstrated that developmental rates derived at constant temperature regimes do not enable reliable prediction of developmental rates at varying temperature regimes in the low and high temperature regime. Possibly, other physiological mechanisms are involved in these deviations. The response to thermal stress (either high daytime temperatures or low nighttime temperatures in this study) may be associated with the production of protective agents, such as heat shock proteins and/or cryoprotectants (Sørensen et al. 2003; Rinehart et al. 2007). Exposing ectotherms to diurnal temperature regimes has been shown to increase cold hardiness which involves accumulation of sugars and glycerol and modifications of cell membrane lipids. A rapid cold hardening can be induced by only a short exposure to moderately low temperatures (Lee et al. 1987), providing organisms with protection against otherwise detrimental low temperatures. Diurnal cycles are characterized by rapid temperature cooling and may therefore contribute to increasing cold hardiness in ectotherms. This may have implications for the practice of biological control. In biocontrol, efficient storage methods for commercially available natural enemies are a crucial factor for transport and allow more flexibility for the end-users in terms of release time in the crop (Leopold 1998; Ghazy et al. 2012). Rearing natural enemies at varying temperatures may not only reduce rearing costs, but could allow them to better survive cold-storage during transport and cope with temperature extremes in the greenhouse.

Obtaining more insight in the ways in which diurnal temperature regimes affect physiological processes in ectotherms is of paramount importance to predict ecological outcomes, including predictive systems of pest outbreaks, the establishment of alien species in new environments and responses to climate change (Bale and Walters 2001; Bale et al. 2002). This research area is woefully understudied and deserves more attention.

Food supplementation for generalist beneficials has been demonstrated to be a major asset for increasing their efficacy (Wäckers 2005; Lundgren 2009). This emerging strategy allows establishment of natural enemies in crops that do not provide such food sources themselves or provide food sources of low quality. Rather than inundative releases of massive numbers of natural enemies on a periodical basis, a more permanent population of natural enemies is desired in greenhouse crops. Conservation biological control is well-developed in outdoor crops with habitat management techniques, such as providing flower strips and pollinating cover crops (Barbosa 1998; Wäckers and van Rijn 2012). Similar strategies could be developed for use in greenhouse crops (Messelink et al. 2014). Although quite some research reported promising results using pollen as a food supplement for generalist phytoseiid predators over the past decades (van Rijn and Tanigoshi 1999; van Rijn et al. 2002; Leman and Messelink 2015), it is only very recently that a commercial pollen-based product was marketed for the support of generalist phytoseiid predatory mites released in the crop (*Typha* sp., Nutrimite^{TM}, Biobest, Westerlo, Belgium). However, one should be cautious when providing pollen in a greenhouse ecosystem, as omnivorous pollen-feeding pests, such as the western flower thrips *Frankliniella occidentalis*, may also benefit from these foods. Besides an increased fecundity, prey may also temporarily escape predation when generalist predators get satiated from feeding on other foods present in the crop (Cottrell and Yeargan 1998; Skirvin et al. 2007; Pappas et al. 2013; Leman and Messelink 2014), with the risk of increasing levels of crop damage (van Maanen et al. 2012). In the long run, however, an increased reproduction is expected to result in a positive numerical response of the predator, which in turn promotes biocontrol (van Rijn et al. 2002; Leman and Messelink 2014). However, to eliminate short-term satiation and lower predation rates of the predators, foods should to be selected that have a minimal beneficial impact on the herbivore, but do result in a population increase of the predator.

In the present work, we assessed the potential of several alternative food sources besides *Typha* pollen for the support of two generalist phytoseiid predators, *Amblyseius swirskii* and *Amblydromalus limonicus*. We found that a non-commercial batch of dry decapsulated cysts of the brine shrimp *Artemia franciscana* (derived from the Artemia Reference Center (ARC), Ghent, Belgium) was a suitable food source for both *A. limonicus* (**Chapter 4**) and *A. swirskii* (**Chapter 5**) in laboratory trials. However, a commercial product of brine shrimp cysts (Artefeed), which is marketed to support predatory bugs such as *Macrolophus* sp. in addition to *E. kuehniella* eggs (Lenfant et al. 2000; De Clercq et al. 2014), proved less adequate and did not allow to maintain a colony of *A. swirskii* for more than two generations in the laboratory. This outcome was confirmed in the first greenhouse experiment reported in **Chapter 6**, where it was not possible to establish a greenhouse colony of *A. swirskii* on chrysanthemum plants using the Artefeed product. A similar result was obtained by Leman and Messelink (2015) who reported a low oviposition rate for *A. swirskii* and *A. limonicus* on leaf discs in the laboratory with another strain of decapsulated *Artemia* cysts. Hoogerbrugge et al. (2008) observed no population growth of *A. swirskii* on chrysanthemum plants. In contrast, the cysts from the non-commercial strain population of *A. franciscana* supplied by ARC, Ghent University, were found to be suitable food allowing to maintain a colony of both *A. limonicus* and *A. swirskii* for over 10 generations. In greenhouse trials on chrysanthemum and ivy plants, *A. franciscana* cysts also showed to be an appropriate food source for *A. swirskii*. However, this was not the case in the greenhouse test with ivy plants where the temperature was allowed to vary between 30°C (during the light phase) and 15°C (during the dark phase). We hypothesized that the large diurnal humidity variations accompanied with the diurnal temperature alternations caused the content of the cysts to become inaccessible for *A. swirskii*. This confirms previous reports on the importance of water content and environmental humidity for the ability of different arthropod predators handle the cysts and take up their content (Arijs and De Clercq 2001; De Clercq et al. 2005; Vandekerkhove et al. 2008). The appreciation of *Artemia* cysts by generalist phytoseiids thus appears to be a combination of:

1. The intrinsic nutritional quality of the cysts: in optimal high humidity conditions in the laboratory, a substantial difference was observed in the performance for *A. swirskii* between the commercial and non-commercial *Artemia* strain.
2. The level of decapsulation: Vandekerkhove et al. (2009) reported that a longer decapsulation time increased the accessibility of brine shrimp cysts to nymphs of the mirid bug *M. caliginosus* increasing their survival. Although these bugs use a different feeding mechanism (piercing-sucking feeding mode), similar results may be obtained for predatory mites.
3. Water content of the cysts: fully hydrated *Artemia* cysts allowed the anthocorid bug *Orius laevigatus* to pierce the cysts and imbibe the content in experiments performed by Arijs and De Clercq (2001). In *Macrolophus* bugs, however, partial hydration of the cysts in a humid environment sufficed to allow successful exploitation of the cysts (Vandekerkhove et al. 2009).

4. Ambient humidity and temperature regime: continuously fluctuating humidity levels may lead to desiccation of the cysts disabling the mites from feeding on them as was shown in the second greenhouse experiment on ivy in **Chapter 6.** Fluctuating levels of hydration/dehydration as such may have affected the nutritional quality of the cysts (El-Magsodi et al. 2014).

Variations in nutritional content among batches of *Artemia* cysts may also be responsible for the observed differences in developmental rates of thrips in **Chapters 4** and **5**. In **Chapter 4**, *A. franciscana* cysts provided *F. occidentalis* with a developmental benefit, whereas in **Chapter 5** the cysts were not advantageous for the thrips' development. These cysts were derived from two different batches obtained from the ARC, both originating from Great Salt Lake (Utah, USA). Another explanation may be found in the different temperatures during the experiments (23 and 25°C, in **Chapters 4** and **5**, respectively). A higher ambient temperature may have forced the thrips larvae to increase the leaf consumption to compensate for water loss.

Further biochemical analysis could be performed to assess nutritional differences among the investigated food sources. For example, De Clercq et al. (2005) found similar protein amounts when comparing *E. kuehniella* eggs and *A. franciscana* cysts, but also reported that the flour moth eggs were three times richer in fatty acids than the brine shrimp cysts. Both food sources were rich in oleic acid (18:1) which comprised over 30% of the total fatty acid content. Delisle et al. (2014) found that soluble proteins are not a key determinant in the nutritional quality of pollen for some phytoseiids (including *A. swirskii*). It is not obvious on which components to focus as the nutritional content of a food source may not be indicative for the bioavailability of these nutrients.

Although we found that *Typha* pollen boosts the reproduction of *F. occidentalis*, we also observed a reduced level of leaf damage when these pollen were present. The increased number of produced thrips offspring may thus not be that devastating for the crop if the thrips are less interested in the leaf tissue itself, but primarily feed on the pollen. In addition to developing predictive models on population growth of thrips and their predators, as was done by van Rijn et al. (2002), models could be developed to estimate the level of crop damage associated with the population increase or decline. If the pollen result in more thrips larvae, a larger number of prey individuals present in the crop will also be beneficial for the population growth of the predator, when the pollen starts to decay (i.e. at the end of the two-week application interval). Moreover, when the thrips are not damaging the crop, higher levels of thrips may to tolerated.

Further research could also focus on the spatial distribution of the food supplements and the according changes in the (vertical) distribution of the (omnivorous) pest and its predators on crop plants. Van Rijn et al. (2002) showed that leaves containing pollen harbored the vast majority of the predator population (>90% in the first weeks after application). Thrips usually tend to be most abundant in the top layer of the crop. Therefore, it could be useful to investigate whether supplying food supplements in the middle of the plant could reduce promoting effects on thrips.

Besides the factitious foods tested in the present study, several artificial diets have been tested for generalist phytoseiid predators (Ogawa and Osakabe 2008; Nguyen et al. 2013, 2014a ,b , c). In the field experiment in this study, a powdered artificial diet developed by Nguyen et al. (2014) demonstrated to possess great potential for supporting *A. swirskii* and possibly also other generalist phytoseiids. Although applied in a dry, solid formulation, the hygroscopic diet quickly became semi-liquid, which may have enhanced its accessibility for the mites. However, if phytoseiids excrete saliva for pre-digesting their foods (Evans and Till 1965; Flechtmann and McMurtry 1992), this partial liquefaction may not be essential for optimal food uptake. Solid diets remain more stable and thus do not easily liquefy and have a better “shelf life” in the crop. Further optimizing liquid artificial diets may include (micro)encapsulation. However, basic information on capsule size and shell thickness allowing the predatory mites to access the content of a diet capsule is lacking. Further study is also warranted to develop formulation types that allow predatory mites but not thrips to feed from the capsule contents.

Alternatively, a food source could be developed with a nutritional composition that exclusively supports natural enemies. Pollen can differ greatly in their nutritional value for *F. occidentalis* (Hulshof et al. 2003; Roulston and Cane 2000). In-depth analysis of nutrient requirements of thrips may aid in selecting pollen species as food supplement or in the development of artificial diets that have only a limited beneficial effect on thrips.

With the use of foods supplements to support populations of natural enemies in the crop, biological control in protected crops tends to shift from augmentative biocontrol to a conjunction of augmentative releases and conservation biological control methods in the crop. A wider commercial availability of food supplements could be a real game-changer in integrated pest management and contribute to a better adoption of biological control, thus increasing sustainability in crop production.

Summary

Nowadays, phytoseiid predatory mites are a crucial factor in integrated pest management strategies, especially in covered crops. To further improve sustainability and cost-effectiveness of these production systems, it is necessary to understand how phytoseiids and their target pests behave under realistic greenhouse conditions. For cold-blooded organisms, such as predatory mites, temperature is a major factor affecting survival, development and reproduction. Similar to the temperature regimes experienced in a natural environment, greenhouse conditions are subjected to daily temperature variations. Although a great deal of work has been conducted on the effects of constant temperatures, the impact of these diurnal temperature ranges (DTRs) is less clear and largely understudied. A first objective of this study was to investigate how DTRs affect the developmental and reproductive performance of spider mites and their phytoseiid predators. In a second part of this study, we focused on food supplementation for generalist phytoseiid predators, which is an emerging strategy to increase their establishment and persistence in the crop. We tested several potential food supplements and investigated the impact on tritrophic interactions when the omnivorous thrips, *Frankliniella occidentalis*, is the target pest.

Results presented in the first experimental chapter (**Chapter 2**) demonstrate the impact of DTRs on the development of the two-spotted spider mite, *Tetranychus urticae* and its phytoseiid predators *Phytoseiulus persimilis* and *Neoseiulus californicus*. In the lower temperature range (<20°C), developmental rates increased with an increasing DTR for both pest and predators, whereas at the higher temperature range (>25°C) the opposite scenario was observed. In the moderate temperature range, little effect of DTRs on developmental rates were observed. As a result, the mites will resume their development from a lower average temperature onwards and be able to establish earlier in the season when DTRs are taken into account. The differences in developmental rates at constant and alternating temperatures could not be explained mathematically due to the nonlinearity of the relationship between developmental rate and temperature (i.e. Jensen’s inequality or Kaufmann-effect), suggesting other factors are involved, such as cryoprotectants and heat shock proteins. The average developmental threshold temperature decreased with increasing DTR, leading to earlier development when taking DTR into account. The interspecific different effects of DTRs on the development of the tested mite species suggest a potential impact on biological control. If the development and population growth of pest and predator are differentially affected, this may impact their interactions.

A direct effect of temperature on biological control performance of predatory phytoseiids was reported in **Chapter 3**. We evaluated the impact of temperature alternations on body size, predation capacity and oviposition rate of the predatory mites *Phytoseiulus persimilis* and *Neoseiulus californicus* when presented with eggs of their natural prey, the two-spotted spider mite *Tetranychus urticae*. For both predators, mean daily temperature as well as temperature alternation had a substantial impact on the number of prey consumed. At lower average temperatures, more eggs were killed under an alternating temperature regime (20 °C/5 °C and 25 °C/10 °C) than at the corresponding mean constant temperatures (15 and 20 °C). At higher average temperatures (>25 °C), however, the opposite was observed with higher numbers of prey killed at constant temperatures than at alternating temperatures. At 25 °C, temperature variation had no effect on the predation capacity. A similar trend as for the predation rates was observed for the oviposition rates of the phytoseiids. Body size of *N. californicus* was affected both by average daily temperature and temperature variation, with smaller adult females emerging at alternating temperatures than at constant temperatures, whereas for *P. persimilis*, temperature variation had no impact on its body size. Results in this chapter demonstrate that temperature variations are likely to affect biological control of *T. urticae* by the studied phytoseiid predators.

In **Chapter 4**, we investigated the effect of food supplements (pollen of common cattail *Typha latifolia*, decapsulated cysts of the brine shrimp *Artemia franciscana* and frozen eggs of the Mediterranean flour moth *Ephestia kuehniella*) on the tritrophic interactions between the phytoseiid predator *Amblydromalus limonicus*, the omnivorous thrips pest *F. occidentalis* and bean leaves (*Phaseolus vulgaris*) as a host plant substrate. For *A. limonicus*, the fastest development was observed when offered *E. kuehniella* eggs, followed by *A. franciscana* cysts, whereas the slowest development was achieved on *T. latifolia* pollen. Fecundity and oviposition rate were higher on *E. kuehniella* and *A. franciscana* than on *T. latifolia*. The intrinsic rate of increase (r_m) was highest when *E. kuehniella* eggs were offered (0.256 females/female/day). The intrinsic rate of increase on *A. franciscana* cysts averaged 0.22 females/female/day. Larvae of *F. occidentalis* were observed to feed on all three food sources when applied to bean leaves. The immature development time of *F. occidentalis* was significantly shorter when *T. latifolia* pollen was provided compared to bean leaves only and bean leaves supplemented with *A. franciscana* or *E. kuehniella*. The predation rate of *A. limonicus* females on first instars of *F. occidentalis* decreased with about 30% irrespective of food type supplemented to the leaf discs. The presence of additional foods reduced antipredator behavior of *F. occidentalis* larvae killing predator eggs. Thrips larvae did not attack eggs of *A. limonicus* when cattail pollen was added to the leaf discs, whereas 2.5 predator eggs per day were consumed without food supplementation. Leaf damage decreased substantially when *Typha* pollen was present on the leaf discs. The findings in this chapter indicate that food supplementation shifts tritrophic interactions both top-down and bottom-up and may affect the outcome of an augmentative biological control program.

In **Chapter 5**, we compared a commercial pollen product consisting of narrow-leaved cattail *Typha angustifolia* (Nutrimite^{TM}, Biobest N.V., Westerlo, Belgium) with a commercial (Artefeed, Koppert B.V., Berkel en Rodenrijs, The Netherlands) and non-commercial strain (ARC, Ghent, Belgium) of decapsulated cysts of *Artemia* sp. as potential supplemental foods for *A. swirskii* and its thrips prey. Pollen was highly supportive for both *F. occidentalis* and *A. swirskii*, whereas *Artemia* cysts supported thrips populations to a lesser extent than those of the predator. Furthermore, a less pronounced reduction in thrips consumption by *A. swirskii* was observed in the presence of *Artemia* cysts as compared with *T. angustifolia* pollen. *Artemia* might be a valuable alternative to pollen for supporting populations of *A. swirskii* in order to improve thrips management, as they are less beneficial for the pest but do support population growth of *A. swirskii*.

In the final research chapter (**Chapter 6**), we studied the pre-establishment of *A. swirskii* on two greenhouse crops, chrysanthemum and ivy, using different food supplements. In the first greenhouse experiment, we tested establishment of *A. swirskii* on pollen of *T. angustifolia* (Nutrimite^{TM}, Biobest N.V., Westerlo, Belgium), decapsulated cysts of *Artemia* sp. (Artefeed, Koppert B.V., Berkel en Rodenrijs, The Netherlands) and a powdered artificial diet which consisted of 20% (w/w) crushed *Artemia* cysts. A biweekly supplementation of pollen or the powdered artificial diet resulted in a good establishment of *A. swirskii* on the chrysanthemum plants, whereas the cysts of the commercial *Artemia* strain did not allow establishment of the predator confirming results obtained in **Chapter 5**. In the second greenhouse trial, we tested the establishment of *A. swirskii* on an ivy crop in two greenhouses set at two different temperature regimes with an average of 25°C. In the first greenhouse, the temperature was set at a constant 25°C (DTR0), whereas in the other greenhouse the temperature was allowed to alternate with a DTR of 15°C (DTR+15). We again tested the establishment of the predatory mite on *T. angustifolia* pollen and the powdered artificial diet, but replaced the commercial strain of *Artemia* sp. with a non-commercial strain of decapsulated *A. franciscana* cysts (ARC, Ghent University) which was found to be an adequate food source for *A. limonicus* and

A. swirskii in **Chapters 4** and **5**, respectively. All three food sources provided good support for *A. swirskii* on the ivy plants located in the greenhouse with a constant temperature regime. When temperatures were allowed to vary, the cysts of *A. franciscana* did not allow the establishment of a population of *A. swirskii*. We hypothesize that the large fluctuations in relative humidity associated with the DTRs were responsible for dehydration of the cysts impeding the predators from feeding on the cysts' content. In the treatment with pollen and the artificial diet, temperature regime had no significant effect on the establishment of *A. swirskii*. Altogether, *T. angustifolia* pollen and the artificial diet showed great potential to support populations of *A. swirskii* in the crop.

In **Chapter 7**, a general discussion and future prospects are provided. The first research topic demonstrated a substantial impact of DTRs on the development, reproduction, population growth and predation rates of the phytoseiid predators and spider mites. As the effect of DTR on development differed amongst the tested species, the outcome of predictive models using constant temperatures will deviate from that of models including temperature variations. In the second research part, we found that food supplements can have a different impact on tritrophic interactions between the phytoseiid, omnivorous thrips and bean plants. The suitability of some of the food supplements tested in the laboratory was further demonstrated in two greenhouse trials on chrysanthemum and ivy plants indicating their potential to support establishment of generalist phytoseiids in the crop. Further research is warranted to develop foods that support generalist arthropod predators, but have no beneficial effect on pests.

Samenvatting

Heden ten dage vormen phytoseiid roofmijten een cruciale factor in geïntegreerde gewasbeschermingsstrategieën, vooral in beschermde teelten. Om de duurzaamheid en kost-effectiviteit van deze productiesystemen verder te optimaliseren, is het noodzakelijk te begrijpen hoe roofmijten en hun plaagorganismen zich gedragen in realistische kascondities. Temperatuur is voor koudbloedige organismen, zoals roofmijten, een belangrijke factor die hun overleving, ontwikkeling en voorplanting beïnvloedt. Net zoals de temperatuurregimes in een natuurlijke omgeving, is het klimaat in een kasomgeving onderhevig aan dagelijkse temperatuurvariaties (DTVs). Hoewel reeds een groot aantal studies gepubliceerd zijn rond de effecten van constante temperatuurregimes, blijft de impact van variabele temperaturen op de biologie van koudbloedige organismen onduidelijk en onderbestudeerd. Een eerste doelstelling van deze studie bestond uit het onderzoeken van hoe deze DTVs de ontwikkeling en reproductie van spintmijten en hun phytoseiid predatoren beinvloeden. In een tweede luik werd gefocust op het bijvoeren van generalistische phytoseiid predatoren met behulp van voedingssupplementen, wat een opkomende strategie is om hun vestiging en persistentie in het gewas te stimuleren. Verschillende voedingssupplementen werden getest en de impact op de tritrofische interacties, wanneer de omnivore trips *Frankliniella occidentalis* de te bestrijden plaag was, werd onderzocht.

Resultaten die in het eerste experimentele hoofdstuk (**Hoofdstuk 2**) gepresenteerd worden, demonstreren de impact van dagelijkse temperatuurvariaties op de ontwikkeling van de bonenspintmijt *Tetranychus urticae* en zijn phytoseiid predatoren *Phytoseiulus persimilis* en *Neoseiulus californicus*. De ontwikkelingssnelheid steeg met toenemende DTV voor zowel de plaag als de predatoren in de lagere temperatuurrange (<20°C), terwijl het omgekeerde scenario werd waargenomen in de hogere temperatuurrange (>25°C). Dagelijkse temperatuurvariaties hadden weinig tot geen impact bij de gematigde temperaturen. Bijgevolg zullen mijten hun ontwikkeling hernemen vanaf een lagere gemiddelde temperatuur en zullen zich vroeger in het seizoen kunnen vestigen wanneer rekening gehouden wordt met DTVs. De verschillen in ontwikkelingssnelheden tussen constante en alternerende temperaturen konden niet mathematisch verklaard worden als gevolg van het niet-lineair verband tussen ontwikkelingssnelheid en temperatuur (i.e. Jensen’s ongelijkheid of het Kaufmann-effect), wat suggereert dat andere factoren, zoals de aanmaak van cryprotectanten en “*heat shock proteins*”, betrokken waren. De gemiddelde drempeltemperatuur voor de ontwikkeling daalde wanneer de DTVs stegen, wat de mijten toeliet om hun ontwikkeling bij een lagere gemiddelde temperatuur te hervatten. De interspecifieke verschillende effecten van de DTVs op de ontwikkeling van de onderzochte mijtensoorten wijzen op een mogelijk effect op biologische plaagbesheersing. Als de impact van temperatuurvariaties op ontwikkeling en populatiegroei verschillend is voor de plaag en de predator, kan dit hun interacties beïnvloeden.

Een directe invloed van temperatuur op de prestaties als biologische bestrijders van roofmijten werd beschreven in **Hoofdstuk 3**. We evalueerden de impact van temperatuuralternaties op de lichaamsgrootte, predatiecapaciteit en ovipositiegraad van de roofmijten *P. persimilis* en *N. californicus* wanneer eitjes van hun natuurlijke prooi, de bonenspintmijt *T. urticae*, werden aangeboden. Voor beide predatoren hadden zowel gemiddelde dagelijkse temperatuur als temperatuuralternaties een significant effect op het aantal prooi-eitjes dat geconsumeerd werd. Bij lage gemiddelde temperaturen, werden meer eitjes gedood bij alternerende temperatuurregimes (20 °C/5 °C en 25 °C/10 °C) dan bij de corresponderende gemiddelde constante temperaturen (15 en 20°C). Bij hogere gemiddelde temperaturen werd het omgekeerde scenario waargenomen met een grotere hoeveelheid geconsumeerde eitjes bij de constante temperatuurregimes dan bij de alternerende. Bij 25°C had temperatuurvariatie geen effect op de predatiecapaciteit. Een analoge trend als voor de predatiecapaciteiten werd waargenomen voor de ovipositiegraden van de roofmijten. De lichaamsgrootte van *N. californicus* wijfjes werd beïnvloed door zowel gemiddelde dagelijkse temperatuur als temperatuurvariatie, met kleinere mijten bij de alternerende dan bij de constante temperaturen. Temperatuurvariatie had geen invloed op de lichaamsgrootte van *P. persimilis*. De resultaten die in dit hoofdstuk werden bekomen tonen duidelijk aan dat temperatuurvariaties de biologische bestrijding van bonenspintmijt met phytoseiid predatoren kan beïnvloeden.

In **Hoofdstuk 4** onderzochten we het effect van voedingssupplementen (stuifmeel van de grote lisdodde *Typha latifolia*, gedecapsuleerde cysten van het pekelkreeftje *Artemia franciscana* en diepgevroren eitjes van de meelmot *Ephestia kuehniella*) op de tritrofische interacties tussen de phytoseiid predator *Amblydromalus limonicus*, de omnivore tripsplaag, *F. occidentalis*, en bonenbladeren (*Phaseolus vulgaris*) als waardplantsubstraat. Voor *A. limonicus* werd de snelste ontwikkeling waargenomen wanneer *E. kuehniella* eitjes werden aangeboden, gevolgd door *A. franciscana* cysten, terwijl de traagste ontwikkeling werd waargenomen op een dieet van *T. latifolia* stuifmeel. De intrinsieke groeisnelheid (r_m) was het hoogst wanneer de roofmijt werd gevoed op *E. kuehniella* eitjes (0.256 wijfjes/wijfje/dag). De intrinsieke groeisnelheid op *A. franciscana* cysten bedroeg 0.22 wijfjes/wijfje/dag. De larven van *F. occidentalis* werden waargenomen zich te voeden op de drie voedingsbronnen. De ontwikkelingsduur van *F. occidentalis* was significant korter wanneer *T. latifolia* stuifmeel werd aangeboden op bonenbladeren in vergelijking met bonenbladeren voorzien van *A. franciscana* cysten of *E. kuehniella* eitjes. De predatie van *A. limonicus* wijfjes op eerste larve stadia van *F. occidentalis* daalde met 30% ongeacht het type voedingssupplement dat werd aangeboden. De aanwezigheid van voedingssupplementen reduceerde het antipredator gedrag van *F. occidentalis* larven die roofmijteitjes doodden. Tripslarven vernietigden geen eitjes van *A. limonicus* wanneer stuifmeel van lisdodde werd toegevoegd aan de bladarena’s, terwijl gemiddeld 2.5 predatoreitjes werden opgegeten op bladarena’s zonder bijvoeder. Bladschade veroorzaakt door trips wijfjes daalde significant wanneer stuifmeel werd toegediend op de bladarena’s. De bevindingen in dit hoofdstuk tonen aan dat voedingssupplementen tritrofische interacties zowel *top-down* als *bottom-up* kunnen doen verschuiven, wat het resultaat van biologische bestrijdingsprogramma’s kan beïnvloeden.

In **Hoofdstuk 5** vergeleken we een commercieel pollen product bestaande uit stuifmeel van de kleine lisdodde *Typha angustifolia* (Nutrimite^{TM}, Biobest N.V., Westerlo, België) met een commerciële (Artefeed, Koppert B.V., Berkel en Rodenrijs, Nederland) en niet-commerciële stam (ARC, Gent, België) van gedecapsuleerde cysten van *Artemia* sp. als potentiële voedingssupplementen voor *A. swirskii* en zijn trips prooi. Stuifmeel werd als ondersteunend bevonden voor zowel *F. occidentalis* als *A. swirskii*, terwijl *Artemia* cysten de tripspopulatie in mindere mate ondersteunden dan de populatie roofmijten. Verder werd een minder uitgesproken reductie in de predatiecapaciteit van *A. swirskii* op tripslarven waargenomen in de aanwezigheid van *Artemia* cysten in vergelijking met *T. angustifolia* stuifmeel. *Artemia* kan een alternatief bieden voor stuifmeel om populaties van *A. swirskii* te ondersteunen om zo de beheersing van trips te verbeteren, daar cysten minder ondersteunend zijn voor de plaag, maar een populatie *A. swirskii* kunnen ondersteunen.

In het laatste onderzoekshoofdstuk (**Hoofdstuk 6**) onderzochten we de preventieve introductie van *A. swirskii* in twee sierteeltgewassen in de kas, namelijk chrysant en klimop, door gebruik te maken van verschillende voedingssupplementen. In de eerste serreproef, onderzochten we de vestiging van *A. swirskii* met stuifmeel van *T. angustifolia*, gedecapsuleerde cysten van *Artemia* sp. en een artificieel poederdieet bestaande uit 20% (w/w) vermalen *Artemia* cysten. Een tweewekelijkse toediening van stuifmeel of het artificieel dieet resulteerde in een goede vestiging van *A. swirskii* op de chrysanten, terwijl cysten van de commerciële *Artemia* stam geen vestiging van de predator toelieten, wat de resultaten verkregen in **Hoofdstuk 5** bevestigde. De vestiging van *A. swirskii* in een klimopgewas werd nagegaan in de tweede serreproef, waar in twee kassen twee verschillende temperatuurregimes werden ingesteld met een gemiddelde temperatuur van 25°C. In de eerste kas werd een constante temperatuur van 25°C ingesteld, terwijl in de andere kas de temperatuur kon alterneren met een DTV van 15°C (DTV+15). Opnieuw werd de vestiging van de roofmijt *A. swirskii* getest op *T. angustifolia* stuifmeel en het artificieel poederdieet, maar nu werd de commerciële *Artemia* sp. stam vervangen door een niet-commerciële stam van gedecapsuleerde *A. franciscana* cysten (ARC, Universiteit Gent) wat een geschikte voedselbron werd bevonden voor *A. limonicus* en *A. swirskii* in **Hoofdstukken 4** en **5**, respectievelijk. Al de voedingssupplementen zorgden voor een goede ondersteuning van *A. swirskii* op het klimopgewas in de kas waar een constante temperatuurregime was ingesteld. Wanneer de temperatuur kon variëren, werd geen vestiging van een *A. swirskii* populatie op cysten van *A. franciscana* waargenomen. We veronderstellen dat de grote fluctuaties in relatieve vochtigheid die geassocieerd zijn met de temperatuurvariaties verantwoordelijk waren voor de dehydratie van de cysten waardoor de predatoren niet langer konden voeden van de inhoud van de cysten. In de behandeling met stuifmeel en het artificieel dieet had temperatuurregime geen effect op de vestiginig van *A. swirskii*. Samengevat vertoonden stuifmeel van *T. angustifolia* en het artificieel poederdieet groot potentieel om populaties *A. swirskii* te ondersteunen in het gewas.

In **Hoofdstuk 7** werd een algemene discussie en toekomstperspectieven gepresenteerd. Het eerst onderzoeksluik toonde een wezenlijke impact van DTV op de ontwikkeling, reproductie, populatiegroei en predatiecapaciteit van de phytoseiid roofmijten en spintmijten. Aangezien het effect van DTVs op de ontwikkeling afhankelijk was de onderzochte species, zal de uitkomst van voorspellingsmodellen die constante temperaturen gebruiken verschillen van de uitkomst van modellen die DTVs incorporeren. In het tweede onderzoeksluik toonden we aan dat voedingssupplementen een verschillende impact hebben op de tritrofische interacties tussen phytoseeid, omnivore trips en bonenplanten. De geschiktheid van sommige voedingssupplementen getest in het laboratorium werd verder aangetoond en bevestigd in serreproeven op chrysant en klimop gewassen, wat hun potentieel aantoont voor de ondersteuning van generalistische phytoseiids in het gewas. Verder onderzoek is vereist om voedingssupplementen te ontwikkelen die generalistische geleedpotige predatoren ondersteunen, maar geen gunstig effect hebben op plaagorganismen.

References

Abad-Moyano, R., Pina, T., Ferragut, F. and Urbaneja, A. (2009) Comparative life-history traits of three phytoseiid mites associated with *Tetranychus urticae* (Acari: Tetranychidae) colonies in clementine orchards in eastern Spain: implications for biological control. Experimental and Applied Acarology 47: 121-132.

Abou-Awad, B., Reda, A. and Elsawi, S. (1992) Effects of artificial and natural diets on the development and reproduction of two phytoseiid mites *Amblyseius gossipi* and *Amblyseius swirskii* (Acari: Phytoseiidae). International Journal of Tropical Insect Science 13: 441-445.

Abrams, P.A. (1995) Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. American Naturalist 114: 112-134.

Adams, S., Valdes, V. and Fuller, D. (2009) The effects of day and night temperature on *Chrysanthemum morifolium*: investigating the safe limits for temperature integration. Journal of Horticultural Science and Biotechnology 84: 604-608.

Adar, E., Inbar, M., Gal, S., Doron, N., Zhang, Z.-Q. and Palevsky, E. (2012) Plant-feeding and non-plant feeding phytoseiids: differences in behavior and cheliceral morphology. Experimental and Applied Acarology 58: 341-357.

Adar, E., Inbar, M., Gal, S., Gan-Mor, S. and Palevsky, E. (2014) Pollen on-twine for food provisioning and oviposition of predatory mites in protected crops. BioControl 59: 307-317.

Adar, E., Inbar, M., Gal, S., Issman, L. and Palevsky, E. (2015) Plant cell piercing by a predatory mite: evidence and implications. Experimental and Applied Acarology 65: 181-193.

Agrawal, A. (1997) Do leaf domatia mediate a plant–mite mutualism? An experimental test of the effects on predators and herbivores. Ecological Entomology 22: 371-376.

Agrawal, A.A. and Karban, R. (1997) Domatia mediate plant-arthropod mutualism. Nature 387: 562-563.

Agrawal, A.A., Kobayashi, C. and Thaler, J.S. (1999) Influence of prey availability and induced host-plant resistance on omnivory by western flower thrips. Ecology 80: 518-523.

Agrawal, A.A. and Klein, C.N. (2000) What omnivores eat: direct effects of induced plant resistance on herbivores and indirect consequences for diet selection by omnivores. Journal of Animal Ecology 69: 525-535.

Aguilar-Fenollosa, E., Ibanez-Gual, M.V., Pascual-Ruiz, S., Hurtado, M. and Jacas, J.A. (2011) Effect of ground-cover management on spider mites and their phytoseiid natural enemies in clementine mandarin orchards (II): Top-down regulation mechanisms. Biological Control 59: 171-179.

Aguilar-Fenollosa, E. and Jacas, J.A. (2014) Can we forecast the effects of climate change on entomophagous biological control agents? Pest Management Science 70: 853-859.

Akaike, H. (1974) A new look at the statistical model identification. Automatic Control, IEEE Transactions on 19: 716-723.

Alatawi, F., Nechols, J.R. and Margolies, D.C. (2011) Spatial distribution of predators and prey affect biological control of twospotted spider mites by *Phytoseiulus persimilis* in greenhouses. Biological Control 56: 36-42.

Albajes, R. and Alomar, Ò. (1999) Current and potential use of polyphagous predators. Integrated pest and disease management in greenhouse crops. Springer, pp. 265-275.

Ali, F.S. (1998) Life tables of *Phytoseiulus macropilis* (Banks) (Gamasida: Phytoseiidae) at different temperatures. Experimental and Applied Acarology 22: 335-342.

Allen, W.R. and Broadbent, A. (1986) Transmission of tomato spotted wilt virus in Ontario greenhouses by *Frankliniella occidentalis*. Canadian Journal of Plant Pathology 8: 33-38.

Amano, H. and Chant, D.A. (1978) Some factors affecting reproduction and sex ratios in 2 species of predacious mites, *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius andersoni* (Chant) (Acarina Phytoseiidae). Canadian Journal of Zoology-Revue Canadienne De Zoologie 56: 1593-1607.

Ament, K., Krasikov, V., Allmann, S., Rep, M., Takken, F.L. and Schuurink, R.C. (2010) Methyl salicylate production in tomato affects biotic interactions. Plant Journal 62: 124-134.

Angilletta, M.J., Steury, T.D. and Sears, M.W. (2004) Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. Integrative and Comparative Biology 44: 498-509.

Aratchige, N.S., Sabelis, M.W. and Lesna, I. (2007) Plant structural changes due to herbivory: Do changes in Aceria-infested coconut fruits allow predatory mites to move under the perianth? Experimental and Applied Acarology 43: 97-107.

Arijs, Y. and De Clercq, P. (2001) Rearing *Orius laevigatus* on cysts of the brine shrimp *Artemia franciscana*. Biological Control 21: 79-83.

Arlian, L.G. (1989) Biology, host relations, and epidemiology of *Sarcoptes scabiei*. Annual Review of Entomology 34: 139-159.

Arnold, C.Y. (1959) The determination and significance of the base temperature in a linear heat unit system. Proceedings of theAmerican Society for Horticultural Science 74: 430-445.

Arthurs, S., McKenzie, C.L., Chen, J., Dogramaci, M., Brennan, M., Houben, K. and Osborne, L. (2009) Evaluation of *Neoseiulus cucumeris* and *Amblyseius swirskii* (Acari: Phytoseiidae) as biological control agents of chilli thrips, *Scirtothrips dorsalis* (Thysanoptera: Thripidae) on pepper. Biological Control 49: 91-96.

Atkinson, D. (1994) Temperature and organism size—a biological law for ectotherms? Advances in ecological research 25: 1-58.

Audenaert, J., Vangansbeke, D., Verhoeven, R., De Clercq, P., Tirry, L. and Gobin, B. (2014) Predation efficiency of predatory mites from different climatic origin under variable climates in Belgian greenhouses. IOBC/wprs Bulletin 102: 7-13.

Audenaert, J., Vissers, M., Haleydt, B., Verhoeven, R., Goossens, F. and Gobin, B. (2009) Acaricides and predatory mites against the begonia mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae), on *Hedera helix*. Communications in agricultural and applied biological sciences 74: 217-224.

Auger, P., Tixier, M.S., Kreiter, S. and Fauvel, G. (1999) Factors affecting ambulatory dispersal in the predaceous mite *Neoseiulus californicus* (Acari : Phytoseiidae). Experimental and Applied Acarology 23: 235-250.

Ayres, M.P. (1993) Plant defense, herbivory, and climate change. In: Biotic interactions and global change (Kareiva, P.M., Kingsolver, J.G., Huey, R.B., eds.). pp. 75-94.

Baille, A., 2000. Trends in greenhouse technology for improved climate control in mild winter climates. V International Symposium on Protected Cultivation in Mild Winter Climates: Current Trends for Suistainable Technologies 559, pp. 161-168.

Bakker, F.M. and Klein, M.E. (1992) Transtrophic interactions in cassava. Experimental and Applied Acarology 14: 293-311.

Bakker, J. (2009) Energy saving greenhouses. Chronica Horticulturae 49: 19-23.

Bakr, E. (2005) A new software for measuring leaf area, and area damaged by Tetranychus urticae Koch. Journal of Applied Entomology 129: 173-175.

Bale, J. (2002) Insects and low temperatures: from molecular biology to distributions and abundance. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 357: 849-862.

Bale, J., Van Lenteren, J. and Bigler, F. (2008) Biological control and sustainable food production. Philosophical Transactions of the Royal Society B: Biological Sciences 363: 761-776.

Barber, A., Campbell, C., Crane, H., Lilley, R. and Tregidga, E. (2003) Biocontrol of two-spotted spider mite Tetranychus urticae on dwarf hops by the phytoseiid mites *Phytoseiulus persimilis* and *Neoseiulus californicus*. Biocontrol Science and Technology 13: 275-284.

Barbosa, P.A. (1998) Conservation biological control. Academic Press.

Beck, S. (1983) Thermal and thermoperiodic effects on larval development and diapause in the European corn borer, *Ostrinia nubilalis*. Journal of Insect Physiology 29: 107-112.

Behrens, W., Hoffmann, K., Kempa, S., Gäßler, S. and Merkel-Wallner, G. (1983) Effects of diurnal thermoperiods and quickly oscillating temperatures on the development and reproduction of crickets, *Gryllus bimaculatus*. Oecologia 59: 279-287.

Bennison, J. and Corless, S. (1993) Biological control of aphids on cucumbers: Further development of open rearing units or" Banker plants" to aid establishment of aphid natural enemies. IOBC/wprs Bulletin 16: 5-8.

Berlinger, M.J., LokvanDijk, B., Dahan, R., LebiushMordechai, S. and Taylor, R.A.J. (1996) Indicator plants for monitoring pest population growth. Annals of the Entomological Society of America 89: 611-622.

Bielza, P. (2008) Insecticide resistance management strategies against the western flower thrips, *Frankliniella occidentalis*. Pest Management Science 64: 1131-1138.

Biobest (2015) www.biobest.be

Birch, L. (1948) The intrinsic rate of natural increase of an insect population. The Journal of Animal Ecology 17: 15-26.

Blanford, J.I., Blanford, S., Crane, R.G., Mann, M.E., Paaijmans, K.P., Schreiber, K.V. and Thomas, M.B. (2013) Implications of temperature variation for malaria parasite development across Africa. Scientific Reports 3.

Blumel, S. and Walzer, A. (2002) Efficacy of different release strategies of *Neoseiulus californicus* McGregor and *Phytoseiulus persimilis* Athias Henriot (Acari: Phytoseiidae) for the control of two-spotted spider mite (*Tetranychus urticae* Koch) on greenhouse cut roses. Systematic and Applied Acarology 7: 35-48.

Bolckmans, K., van Houten, Y. and Hoogerbrugge, H., (2005) Biological control of whiteflies and western flower thrips in greenhouse sweet peppers with the phytoseiid predatory mite *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae). Second International Symposium on Biological Control of Arthropods, pp. 555-565.

Bolckmans, K.J.F. and van Houten, Y., (2006) Mite composition, use thereof, method for rearing the phytoseiid predatory mite *Amblyseius swirskii*, rearing system for rearing said phytoseiid mite and methods for biological pest control on a crop. WO Patent 2,006,057,552.

Bolla, R., Bruschi, R., Davoli, F. and Cucchietti, F. (2011) Energy efficiency in the future internet: a survey of existing approaches and trends in energy-aware fixed network infrastructures. Communications Surveys & Tutorials, IEEE 13: 223-244.

Bonte, J., Vangansbeke, D., Maes, S., Bonte, M., Conlong, D. and De Clercq, P. (2012) Moisture source and diet affect development and reproduction of *Orius thripoborus* and *Orius naivashae*, two predatory anthocorids from southern Africa. Journal of Insect Science 12: 1-16.

Bonte, M. and De Clercq, P. (2008) Developmental and reproductive fitness of *Orius laevigatus* (Hemiptera: Anthocoridae) reared on factitious and artificial diets. Journal of Economic Entomology 101: 1127-1133.

Bot, G.P. (2001) Developments in indoor sustainable plant production with emphasis on energy saving. Computers and Electronics in Agriculture 30: 151-165.

Boudreaux, H. (1958) The effect of relative humidity on egg-laying, hatching, and survival in various spider mites. Journal of Insect Physiology 2: 65-72.

Brakefield, P.M. and Mazzotta, V. (1995) Matching field and laboratory environments: effects of neglecting daily temperature variation on insect reaction norms. Journal of Evolutionary Biology 8: 559-573.

Brière, J.-F., Pracros, P., Le Roux, A.-Y. and Pierre, J.-S. (1999) A novel rate model of temperature-dependent development for arthropods. Environmental Entomology 28: 22-29.

Brodbeck, B.V., Stavisky, J., Funderburk, J.E., Andersen, P.C. and Olson, S.M. (2001) Flower nitrogen status and populations of *Frankliniella occidentalis* feeding on *Lycopersicon esculentum*. Entomologia Experimentalis Et Applicata 99: 165-172.

Brodsgaard, H. and Hansen, L.S. (1992) Effect of *Amblyseius cucumeris* and *Amblyseius barkeri* as biological control agents of *Thrips tabaci* on glasshouse cucumbers. Biocontrol Science and Technology 2: 215-223.

Broufas, G. and Koveos, D. (2000) Effect of different pollens on development, survivorship and reproduction of *Euseius finlandicus* (Acari: Phytoseiidae). Environmental Entomology 29: 743-749.

Broufas, G.D. and Koveos, D.S. (2001) Development, survival and reproduction of *Euseius finlandicus* (Acari: Phytoseiidae) at different constant temperatures. Experimental and Applied Acarology 25: 441-460.

Broufas, G., Pappas, M. and Koveos, D. (2007) Development, survival, and reproduction of the predatory mite Kampimodromus aberrans (Acari: Phytoseiidae) at different constant temperatures. Environmental Entomology 36: 657-665.

Bryant, S., Bale, J. and Thomas, C. (1999) Comparison of development and growth of nettle-feeding larvae of Nymphalidae (Lepidoptera) under constant and alternating temperature regimes. European Journal of Entomology 96: 143-148.

Bryon, A., Wybouw, N., Dermauw, W., Tirry, L. and Van Leeuwen, T. (2013) Genome wide gene-expression analysis of facultative reproductive diapause in the two-spotted spider mite *Tetranychus urticae*. BMC genomics 14: 815.

Buitenhuis, R., Shipp, L. and Scott-Dupree, C. (2010) Intra-guild vs extra-guild prey: effect on predator fitness and preference of *Amblyseius swirskii* (Athias-Henriot) and *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae). Bulletin of Entomological Research 100: 167-173.

Buitenhuis, R., Glemser, E. and Brommit, A. (2014a) Practical placement improves the performance of slow release sachets of *Neoseiulus cucumeris*. Biocontrol Science and Technology 24: 1153-1166.

Buitenhuis, R., Shipp, L., Scott-Dupree, C., Brommit, A., and Lee, W. (2014b) Host plant effects on the behaviour and performance of *Amblyseius swirskii* (Acari: Phytoseiidae). Experimental and Applied Acarology 62: 171-180.

Buitenhuis, R., Murphy, G., Shipp, L. and Scott-Dupree, C. (2015) *Amblyseius swirskii* in greenhouse production systems: a floricultural perspective. Experimental and Applied Acarology 65: 451-464.

Buryn, R. and Brandl, R. (1992) Are the morphometrics of chelicerae correlated with diet in mesostigmatid mites (Acari)? Experimental and Applied Acarology 14: 67-82.

Buwalda, F., Eveleens, B. and Wertwijn, R. (2000) Ornamental crops tolerate large temperature fluctuations: a potential for more efficient greenhouse heating strategies. Acta Horticulturae 515: 141-149.

Byrne, D.N. and Miller, W.B. (1990) Carbohydrate and amino acid composition of phloem sap and honeydew produced by *Bemisia tabaci*. Journal of Insect Physiology 36: 433-439.

Byrne, D.N. and Bellows Jr, T.S. (1991) Whitefly biology. Annual Review of Entomology 36: 431-457.

Calvo, F., Bolckmans, K. and Belda, J. (2011) Control of *Bemisia tabaci* and *Frankliniella occidentalis* in cucumber by *Amblyseius swirskii*. BioControl 56: 185-192.

Calvo, F.J., Bolckmans, K. and Belda, J.E. (2012) Biological control-based IPM in sweet pepper greenhouses using *Amblyseius swirskii* (Acari: Phytoseiidae). Biocontrol Science and Technology 22: 1398-1416.

Calvo, F.J., Knapp, M., van Houten, Y.M., Hoogerbrugge, H. and Belda, J.E. (2015) *Amblyseius swirskii*: What made this predatory mite such a successful biocontrol agent? Experimental and Applied Acarology 65: 419-433.

Camin, J., Moss, W., Oliver Jr, J. and Singer, G. (1967) Cloacaridae, a new family of Cheyletoid mites from the cloaca of aquatic turtles (Acari: Acariformes: Eleutherengona). Journal of Medical Entomology 4: 261.

Campbell, A., Frazer, B., Gilbert, N., Gutierrez, A. and Mackauer, M. (1974) Temperature requirements of some aphids and their parasites. Journal of Applied Ecology 11: 431-438.

Canlas, L.J., Amano, H., Ochiai, N. and Takeda, M. (2006) Biology and predation of the Japanese strain of Neoseiulus californicus (McGregor) (Acari: Phytoseiidae) Systematic and Applied Acarology 11: 141-157.

Castagnoli, M. (1989) Biologia e prospettive di allevamento massale di *Amblyseius cucumeris* (Oud.) (Acarina: Phytoseiidae) usando *Dermatophagoides farinae* Hughes (Acarina: Pyroglyphidae) come preda. Redia 72: 389-402.

Castagnoli, M. and Simoni, S. (1991) Influenza della temperature sull'incremento delle popolazioni di *Amblyseius californicus* (McGregor) (Acari: Phytoseiidae). Redia LXXIV: 621–640. (In Italian with English summary).

Castagnoli, M., Liguori, M., Simoni, S., Pintucci, M., Guidi, S. and Falchini, L. (1996) Observations on diapause induction in three phytoseiid (Phytoseiidae) species. Acarology IX 1: 9-12.

Castagnoli, M. and Simoni, S. (1999) Effect of long-term feeding history on functional and numerical response of *Neoseiulus californicus* (Acari: Phytoseiidae). Experimental and Applied Acarology 23: 217-234.

Castagnoli, M., Simoni, S. and Biliotti, N. (1999) Mass-rearing of *Amblyseius californicus* (Acari: Phytoseiidae) on two alternative food sources. Ecology and Evolution of the Acari. Springer, pp. 425-431.

Castagnoli, M., Simoni, S. and Liguori, M. (2003) Evaluation of *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) as a candidate for the control of *Aculops lycopersici* (Tyron)(Acari Eriophyoidea): a preliminary study. Redia 86: 97-100.

Castagnoli, M., Nannelli, R., Tarchi, F. and Simoni, S. (2006) Screening of astigmatid mites for mass-rearing *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae). Redia 89: 55-58.

Castañé, C., Quero, R. and Riudavets, J. (2006) The brine shrimp *Artemia* sp. as alternative prey for rearing the predatory bug *Macrolophus caliginosus*. Biological Control 38: 405-412.

Cedola, C.V., Sanchez, N.E. and Liljesthrom, G.G. (2001) Effect of tomato leaf hairiness on functional and numerical response of *Neoseiulus californicus* (Acari : Phytoseiidae). Experimental and Applied Acarology 25: 819-831.

Champlain, R.A. and Butler, G.D. (1967) Temperature Effects on Development of Egg and Nymphal Stages of *Lygus Hesperus* (Hemiptera - Miridae). Annals of the Entomological Society of America 60: 519-521.

Chant, D.A. (1959) Phytoseiid mites (Acarina: Phytoseiidae). Memoirs of the Entomological Society of Canada 91: 5-166.

Chant, D. and Fleschner, C. (1960) Some observations on the ecology of phytoseiid mites (Acarina: Phytoseiidae) in California. Entomophaga 5: 131-139.

Chant, D. (1961) An experiment in biological control of *Tetranychus telarius* (L.) (Acarina: Tetranychidae) in a greenhouse using the predacious mite *Phytoseiulus persimilis* Athias-Henriot (Phytoseiidae). The Canadian Entomologist 93: 437-443.

Chant, D.A. (1985) External Anatomy. In: Helle, W., Sabelis, M.W., (Eds.), Spider Mites. Their Biology, natural enemies and ontrol. Elsevier, pp. 11-16.

Chant, D. (1993) Discontinuous variation in the suppression of idiosomal setae in the family Phytoseiidae (Acari: Gamasina). Experimental and Applied Acarology 17: 187-195.

Chant, D. and McMurtry, J. (1994) A review of the subfamilies Phytoseiinae and Typhlodrominae (Acari: Phytoseiidae). International Journal of Acarology 20: 223-310.

Chauve, C. (1998) The poultry red mite *Dermanyssus gallinae* (De Geer, 1778): current situation and future prospects for control. Veterinary Parasitology 79: 239-245.

Chittenden, A.R. and Saito, Y. (2001) Why are there feeding and nonfeeding larvae in phytoseiid mites (Acari, Phytoseiidae)? Journal of Ethology 19: 55-62.

Choh, Y. and Takabayashi, J. (2006) Herbivore-induced extrafloral nectar production in lima bean plants enhanced by previous exposure to volatiles from infested conspecifics. Journal of Chemical Ecology 32: 2073-2077.

Choh, Y., Kugimiya, S. and Takabayashi, J. (2006) Induced production of extrafloral nectar in intact lima bean plants in response to volatiles from spider mite-infested conspecific plants as a possible indirect defense against spider mites. Oecologia 147: 455-460.

Cock, M.J., van Lenteren, J.C., Brodeur, J., Barratt, B.I., Bigler, F., Bolckmans, K., Cônsoli, F.L., Haas, F., Mason, P.G. and Parra, J.R.P. (2010) Do new Access and Benefit Sharing procedures under the Convention on Biological Diversity threaten the future of biological control? BioControl 55: 199-218.

Cocuzza, G., De Clercq, P., Van De Veire, M., De Cock, A., Degheele, D. and Vacante, V. (1997) Reproduction of *Orius laevigatus* and *Orius albidipennis* on pollen and *Ephestia kuehniella* eggs. Entomologia Experimentalis Et Applicata 82: 101-104.

Cohen, A.C., 2003. Insect diets: science and technology. CRC.

Colin, L.J. and Jones, C.E. (1980) Pollen energetics and pollination modes. American Journal of Botany 67: 210-215.

Coll, M. and Guershon, M. (2002) Omnivory in terrestrial arthropods: mixing plant and prey diets. Annual Review of Entomology 47: 267-297.

Collier, T. and Van Steenwyk, R. (2004) A critical evaluation of augmentative biological control. Biological Control 31: 245-256.

Coop, L. and Croft, B. (1995) *Neoseiulus fallacis*: dispersal and biological control of *Tetranychus urticae* following minimal inoculations into a strawberry field. Experimental and Applied Acarology 19: 31-43.

Croft, B. and Croft, M. (1993) Larval survival and feeding by immature *Metaseiulus occidentalis*, *Neoseiulus fallacis, Amblyseius andersoni* and *Typhlodromus pyri* on life stage groups of *Tetranychus urticae* Koch and phytoseiid larvae. Experimental and Applied Acarology 17: 685-693.

Croft, B., Messing, R., Dunley, J. and Strong, W. (1993) Effects of humidity on eggs and immatures of *Neoseiulus fallacis*, *Amblysieus andersoni*, *Metaseiulus occidentalis* and *Typhlodromus pyri* (Phytoseiidae): implications for biological control on apple, caneberry, strawberry and hop. Experimental and Applied Acarology 17: 451-459.

Croft, B., Pratt, P., Koskela, G. and Kaufman, D. (1998) Predation, reproduction, and impact of phytoseiid mites (Acari: Phytoseiidae) on cyclamen mite (Acari: Tarsonemidae) on strawberry. Journal of Economic Entomology 91: 1307-1314.

Croft, B.A., Luh, H.K. and Schausberger, P. (1999) Larval size relative to larval feeding, cannibalism of larvae, egg or adult female size and larval-adult setal patterns among 13 phytoseiid mite species. Experimental and Applied Acarology 23: 599-610.

Dabbert, S., Haring, A.M. and Zanoli, R. (2004) Organic farming: policies and prospects. Zed Books.

Danks, H.V. (1987) Insect dormancy: an ecological perspective. Biological Survey of Canada (Terrestrial Artropods).

Davies, J. T., Ireson, J. E., and Allen, G. R. (2009) Pre-adult development of *Phytoseiulus persimilis* on diets of *Tetranychus urticae* and *Tetranychus lintearius*: implications for the biological control of *Ulex europaeus*. Experimental and Applied Acarology 47: 133-145.

Davis, J., Radcliffe, E. and Ragsdale, D. (2006) Effects of high and fluctuating temperatures on *Myzus persicae* (Hemiptera: Aphididae). Environmental Entomology 35: 1461-1468.

De Bach, P. (1964) Biological control of insect pests and weeds. Biological control of insect pests and weeds.

De Bach, P. and Rosen, D. (1991) Biological control by natural enemies. CUP Archive.

de Boer, J.G. and Dicke, M. (2004a) Experience with methyl salicylate affects behavioural responses of a predatory mite to blends of herbivore-induced plant volatiles. Entomologia Experimentalis Et Applicata 110: 181-189.

De Boer, J.G. and Dicke, M. (2004b) The role of methyl salicylate in prey searching behavior of the predatory mite *Phytoseiulus persimilis*. Journal of Chemical Ecology 30: 255-271.

De Boer, J.G., Posthumus, M.A. and Dicke, M. (2004c) Identification of volatiles that are used in discrimination between plants infested with prey or nonprey herbivores by a predatory mite. Journal of Chemical Ecology 30: 2215-2230.

De Boer, J.G. and Dicke, M. (2006) Olfactory learning by predatory arthropods. Animal Biology 56: 143-155.

De Clercq, P. and Degheele, D. (1992) Development and survival of *Podisus maculiventris* (Say) and *Podisus sagitta* (Fab.) (Heteroptera: Pentatomidae) at various constant temperatures. The Canadian Entomologist 124: 125-133.

De Clercq, P., Arijs, Y., Van Meir, T., Van Stappen, G., Sorgeloos, P., Dewettinck, K., Rey, M., Grenier, S. and Febvay, G. (2005) Nutritional value of brine shrimp cysts as a factitious food for *Orius laevigatus* (Heteroptera: Anthocoridae). Biocontrol Science and Technology 15: 467-479.

De Courcy Williams, M.E., Kravar-garde, L., Fenlon, J.S. and Sunderland, K.D. (2004) Phytoseiid mites in protected crops: the effect of humidity and food availability on egg hatch and adult life span of *Iphiseius degenerans*, *Neoseiulus cucumeris, N. californicus* and *Phytoseiulus persimilis* (Acari: Phytoseiidae). Experimental and Applied Acarology 32: 1-13.

De Klerk, M. and Ramakers, P. (1986) Monitoring population densities of the phytoseiid predator *Amblyseius cucumeris* and its prey after large scale introductions to control *Thrips tabaci* on sweet pepper. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent 51: 1045-1048.

de Vis, R.M.J., de Moraes, G.J. and Bellini, M.R. (2006) Effect of air humidity on the egg viability of predatory mites (Acari: Phytoseiidae, Stigmaeidae) common on rubber trees in Brazil. Experimental and Applied Acarology 38: 25-32.

Delisle, J., Brodeur, J. and Shipp, L. (2014) Evaluation of various types of supplemental food for two species of predatory mites, *Amblyseius swirskii* and *Neoseiulus cucumeris* (Acari: Phytoseiidae). Experimental and Applied Acarology 65: 483-494.

Delisle, J.F., Shipp, L. and Brodeur, J. (2015) Apple pollen as a supplemental food source for the control of western flower thrips by two predatory mites, *Amblyseius swirskii* and *Neoseiulus cucumeris* (Acari: Phytoseiidae), on potted chrysanthemum. Experimental and Applied Acarology 65: 495-509.

Demite, P.R., McMurtry, J.A. and De Moraes, G.J. (2014) Phytoseiidae Database: a website for taxonomic and distributional information on phytoseiid mites (Acari). Zootaxa 3795: 571-577.

Denmark, H.A. and Evans, G.A. (2011) Phytoseiidae of North America and Hawaii (Acari: Mesostigmata). Indira Publishing House.

Dicke, M. and Sabelis, M.W. (1987) How plants obtain predatory mites as bodyguards. Netherlands Journal of Zoology 38: 148-165.

Dicke, M., Sabelis, M.W., Takabayashi, J., Bruin, J. and Posthumus, M.A. (1990) Plant strategies of manipulating predatorprey interactions through allelochemicals: prospects for application in pest control. Journal of Chemical Ecology 16: 3091-3118.

Dicke, M., Van Beek, T., Posthumus, M., Dom, N.B., Van Bokhoven, H. and De Groot, A. (1990) Isolation and identification of volatile kairomone that affects acarine predatorprey interactions Involvement of host plant in its production. Journal of Chemical Ecology 16: 381-396.

Dicke, M. and Baldwin, I.T. (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. Trends in Plant Science 15: 167-175.

Dieleman, J., Meinen, E. and Dueck, T.A. (2005) Effects of temperature integration on growth and development of roses. Acta Horticulturae 691: 51-58.

Domingos, C.A., Da S. Melo, J.W., Gondim Jr., M. G. C., De Moraes, G.J., Hanna, R., Lawson-Balagbo, L. M. and Schausberger, P. (2010) Diet-dependent lifehistory, feeding preference and thermal requirements of the predatory mite *Neoseiulus baraki* (Acari: Phytoseiidae). Experimental and Applied Acarology 50: 201-215.

Easterbrook, M. (1996) Damage and control of eriophyoid mites in apple and pear. World crop pests 6: 527-541.

Easterbrook, M.A., Fitz Gerald, J.D. and Solomon, M.G. (2001) Biological control of strawberry tarsonemid mite *Phytonemus pallidus* and two-spotted spider mite *Tetranychus urticae* on strawberry in the UK using species of *Neoseiulus* (*Amblyseius*) (Acari: Phytoseiidae). Experimental and Applied Acarology 25: 25-36.

Easterling, D.R., Horton, B., Jones, P.D., Peterson, T.C., Karl, T.R., Parker, D.E., Salinger, M.J., Razuvayev, V., Plummer, N. and Jamason, P. (1997) Maximum and minimum temperature trends for the globe. Science 277: 364-367.

Eilenberg, J., Hajek, A. and Lomer, C. (2001) Suggestions for unifying the terminology in biological control. Biocontrol 46: 387-400.

El-Badry, E.A. (1967) Three new species of phytoseiid mites preying on the cotton whitefly, *Bemisia tabaci* in the Sudan (Acarina, Phytoseiidae). The Entomologist 100: 106-111.

Elbadry, E.A. (1968) Biological studies on *Amblyseius aleyrodis* a predator of the cotton whitefly [Acarina, Phytoseiidae]. Entomophaga 13: 323-329.

Elgar, M.A. and Crespi, B.J., 1992. Cannibalism: ecology and evolution among diverse taxa. Oxford University Press, USA.

El-Laithy, A. and Fouly, A. (1992) Life table parameters of the two phytoseiid predators *Amblyseius scutalis* (Athias-Henriot) and *A. swirskii* A.-H.(Acari, Phytoseiidae) in Egypt. Journal of Applied Entomology 113: 8-12.

El-Magsodi, M.O., Bossier, P., Sorgeloos, P. And Van Stappen, G. (2014) Hatching and nutritional quality of Artemia cysts progressively deteriorates as a fucntion of increased exposure to hydration/dehydration cycles. Aquaculture International 22: 1515-1532.

El-Taj, H.F. and Jung, C. (2012) Effect of temperature on the life-history traits of *Neoseiulus californicus* (Acari: Phytoseiidae) fed on *Panonychus ulmi.*Experimental and Applied Acarology 56: 247-260.

English-Loeb, G., Norton, A.P. and Walker, M.A. (2002) Behavioral and population consequences of acarodomatia in grapes on phytoseiid mites (Mesostigmata) and implications for plant breeding. Entomologia Experimentalis Et Applicata 104: 307-319.

Erwin, J.E., Heins, R.D. and Karlsson, M.G. (1989) Thermomorphogenesis in *Lilium longiflorum*. American Journal of Botany 76: 47-52.

Escudero, L.A. and Ferragut, F. (2005) Life-history of predatory mites *Neoseiulus californicus* and *Phytoseiulus persimilis* (Acari : Phytoseiidae) on four spider mite species as prey, with special reference to *Tetranychus evansi* (Acari : Tetranychidae). Biological Control 32: 378-384.

Escudero-Colomar, L.-A. and Chorąży, A. (2012) First record of *Amblydromalus limonicus* (Acari: Phytoseiidae) from Spain. International Journal of Acarology 38: 545-546.

Estay, S.A., Lima, M. and Bozinovic, F. (2014) The role of temperature variability on insect performance and population dynamics in a warming world. Oikos 123: 131-140.

Evans, G. and Till, W. (1965) Studies on the British Dermanyssidae (Acari: Mesostigamata). Part I: External morphology. Bulletin of the British Museum (Natural History). Zoology 13: 250-294.

Fan, Y. and Petitt, F. (1994) Biological control of broad mite, *Polyphagotarsonemus latus* (Banks), by *Neoseiulus barkeri* Hughes on pepper. Biological Control 4: 390-395.

Fantinou, A.A., Perdikis, D.C. and Chatzoglou, C.S. (2003) Development of immature stages of *Sesamia nonagrioides* (Lepidoptera: Noctuidae) under alternating and constant temperatures. Environmental Entomology 32: 1337-1342.

Faraji, F., Janssen, A., Van Rijn, P.C. and Sabelis, M.W. (2000) Kin recognition by the predatory mite *Iphiseius degenerans*: discrimination among own, conspecific, and heterospecific eggs. Ecological Entomology 25: 147-155.

Faraji, F., Janssen, A. and Sabelis, M.W. (2002) Oviposition patterns in a predatory mite reduce the risk of egg predation caused by prey. Ecological Entomology 27: 660-664.

Fernandez-Caldas, E., Iraola, V. and Carnes, J. (2007) Molecular and biochemical properties of storage mites (except Blomia species). Protein and Peptide Letters 14: 954-959.

Ferreira, J.A.M., Eshuis, B., Janssen, A. and Sabelis, M.W. (2008a) Domatia reduce larval cannibalism in predatory mites. Ecological Entomology 33: 374-379.

Ferreira, J.A.M., Zanuncio, J.C., Torres, J.B. and Molina-Rugama, A.J. (2008b) Predatory behaviour of *Podisus nigrispinus* (Heteroptera : Pentatomidae) on different densities of *Anticarsia gemmatalis* (Lepidoptera : Noctuidae) larvae. Biocontrol Science and Technology 18: 711-719.

Ferrero, M., Gigot, C., Tixier, M.S., van Houten, Y.M. and Kreiter, S. (2010) Egg hatching response to a range of air humidities for six species of predatory mites. Entomologia Experimentalis Et Applicata 135: 237-244.

Ferro, D. and Chapman, R. (1979) Effects of different constant humidities and temperatures on twospotted spider mite egg hatch. Environmental Entomology 8: 701-705.

Flechtmann, C.H.W. and McMurtry, J.A. (1992) Studies of cheliceral and deutosternal morphology of some Phytoseiidae (Acari: Mesostigmata) by scanning electron microscopy. International Journal of Acarology 18: 163-169.

Fouly, A. (1997) Effects of prey mites and pollen on the biology and life tables of *Proprioseiopsis asetus* (Chant) (Acari, Phytoseiidae). Journal of Applied Entomology 121: 435-439.

Frank, S.D. (2010) Biological control of arthropod pests using banker plant systems: Past progress and future directions. Biological Control 52: 8-16.

French, N., Parr, W.J., Gould, H.J., Williams, J.J. and Simmonds, S.P. (1976) Development of biological methods for control of *Tetranychus urticae* on tomatoes using *Phytoseiulus persimilis*. Annals of Applied Biology 83: 177-189.

Friese, D. and Gilstrap, F. (1982) Influence of prey availability on reproduction and prey consumption of *Phytoseiulus persimilis*, *Amblyseius californicus* and *Metaseiulus occidentalis* (Acarina: Phytoseiidae). International Journal of Acarology 8: 85-89.

Gadino, A.N. and Walton, V.M. (2012) Temperature-related development and population aprameters for *Typhlodromus pyri* (Acari: Phytoseiidae) found in Oregon vineyards. Experimental and Applied Acarology 58: 1-10.

Galazzi, D. and Nicoli, G. (1996) Comaprative study of strains of Phytoseiulus persimilis Athias-Henriot (Acarina: Phytoseiidae). I. Development and Adult life. Boll. Ist. Ent. "G. Grandi" Univ. Bologna 50: 215-231.

Ganjisaffar, F. Fathipour, Y., and Kamali, K. (2011) effect of temperature on prey consumption of *Typhlodromus bagdasarjani* (Acari: Phytoseiidae) on *Tetranychus urticae* (Acari: Phytoseiidae). International Journal of Acarology 37: 556-560.

Garcia-Ruiz, E., Marco, V. and Perez-Moreno, I. (2011) Effects of variable and constant temperatures on the embryonic development and survival of a new grape pest, *Xylotrechus arvicola* (Coleoptera: Cerambycidae). Environmental Entomology 40: 939-947.

Genini, M., Klay, A., Baumgärtner, J., Delucchi, V., and Baillod, M. (1991) Comparative studies on the influence of temperature and food on the development of *Amblyseius andersoni*, *Neoseiulus fallacis*, *Galendromus longipilus* and *Typhlodromus pyri* (Acari: Phytoseiidae). Entomophaga 36: 139-154.

Gerling, D. (1990) Whiteflies: their bionomics, pest status and management. Intercept Limited.

Gerson, U. (1992) Biology and control of the broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). Experimental and Applied Acarology 13: 163-178.

Gerson, U. and Weintraub, P.G. (2007) Mites for the control of pests in protected cultivation. Pest Management Science 63: 658-676.

Gerson, U., Smiley, R.L. and Ochoa, R. (2008) Mites (Acari) for pest control. John Wiley & Sons.

Gerson, U. and Weintraub, P.G. (2012) Mites (Acari) as a factor in greenhouse management. Annual Review of Entomology 57: 229-247.

Gerson, U. (2014) Pest control by mites (Acari): Present and Future. Acarologia 54: 371-394.

Gillespie, D. (1989) Biological control of thrips [Thysanoptera: Thripidae] on greenhouse cucumber by *Amblyseius cucumeris*. Entomophaga 34: 185-192.

Gillespie, D.R. and Quiring, D. (1994) Reproduction and longevity of the predatory mite, *Phytoseiulus persimilis* (Acari: Phytoseiidae) and its prey, *Tetranychus urticae* (Acari: Tetranychidae) on different host plants. Journal of the Entomological Society of British Columbia 91: 3-8.

Goleva, I. and Zebitz, C.P. (2013) Suitability of different pollen as alternative food for the predatory mite *Amblyseius swirskii* (Acari, Phytoseiidae). Experimental and Applied Acarology 61: 259-283.

Gotoh, T., Yamaguchi, K. and Mori, K. (2004) Effect of temperature on life history of the predatory mite *Amblyseius* (*Neoseiulus*) *californicus* (Acari : Phytoseiidae). Experimental and Applied Acarology 32: 15-30.

Gotoh, T., Akizawa, T., Watanabe, M., Tsuchiya, A. and Shimazaki, S. (2005) Cold hardiness of *Neoseiulus californicus* and *N. womersleyi* (Acari: Phytoseiidae). Journal of Acarological Society of Japan 14: 93-103.

Gotoh, T., Tsuchiya, A. and Kitashima, Y. (2006) Influence of prey on developmental performance, reproduction and prey consumption of *Neoseiulus californicus* (Acari : Phytoseiidae). Experimental and Applied Acarology 40: 189-204.

Gotoh, T. and Tsuchiya, A. (2008) Effect of multiple mating on reproduction and longevity of the phytoseiid mite *Neoseiulus californicus*. Experimental and Applied Acarology 44: 185-197.

Gotoh, T., Saito, M., Suzuki, A. and Nachman, G. (2014) Effects of constant and variable temperatures on development and reproduction of the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). Experimental and Applied Acarology 64: 465-478.

Gould, H. and Light, W.S.G. (1971) Biological control of *Tetranychus urticae* on stock plants of ornamental ivy. Plant Pathology 20: 18-20.

Grafton-Cardwell, E. and Ouyang, Y. (1996) Influence of citrus leaf nutrition on survivorship, sex ratio, and reproduction of *Euseius tularensis* (Acari: Phytoseiidae). Environmental Entomology 25: 1020-1025.

Grbić, M., Van Leeuwen, T., Clark, R.M., Rombauts, S., Rouzé, P., Grbić, V., Osborne, E.J., Dermauw, W., Ngoc, P.C.T. and Ortego, F. (2011) The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. Nature 479: 487-492.

Greco, N.M., Sanchez, N.E. and Liljesthrom, G.G. (2005) *Neoseiulus californicus* (Acari : Phytoseiidae) as a potential control agent of *Tetranychus urticae* (Acari : Tetranychidae): effect of pest/predator ratio on pest abundance on strawberry. Experimental and Applied Acarology 37: 57-66.

Grenier, S. and De Clercq, P. (2003) Comparison of artificially vs. naturally reared natural enemies and their potential for use in biological control. In: van Lenteren JC, editor. Quality Control and the Production of Biological Control Agents: Theory and Testing Procedures. CAB International: pp. 115-131.

Grice, E.A. and Segre, J.A. (2011) The skin microbiome. Nature Reviews Microbiology 9: 244-253.

Griffiths, D.A. (1999) Biological control of mites. Integrated Pest and Disease Management in Greenhouse Crops. Springer, pp. 217-234.

Gurr, G., Wratten, S., Tylianakis, J., Kean, J. and Keller, M. (2005) Providing plant foods for natural enemies in farming systems: balancing practicalities and theory. 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. 326-347.

Hagstrum, D.W. and Hagstrum, W.R. (1970) A simple device for producing fluctuating temperatures, with an evaluation of the ecological significance of fluctuating temperatures. Annals of the Entomological Society of America 63: 1385-1389.

Hagstrum, D.W. and Leach, C.E. (1973) Role of constant and fluctuating temperatures in determining development time and fecundity of three species of stored-products Coleoptera. Annals of the Entomological Society of America 66: 407-410.

Hagstrum, D.W. and Milliken, G.A. (1991) Modeling differences in insect developmental times between constant and fluctuating temperatures. Annals of the Entomological Society of America 84: 369-379.

Hardman, J.M. and Rogers, M.L. (1991) Effects of temperature and prey density on survival, development, and feeding rates of immature *Typhlodromus pyri* (Acari: Phytoseiidae). Environmental Entomology 20: 1089-1096.

Hart, A., Bale, J., Tullett, A., Worland, M. and Walters, K. (2002) Effects of temperature on the establishment potential of the predatory mite *Amblyseius californicus* McGregor (Acari: Phytoseiidae) in the UK. Journal of Insect Physiology 48: 593-599.

Havelka, J. and Kindlmann, P. (1984) Optimal use of the pest in first method for controlling *Tetranychus urticae* Koch (Acarina, Tetranychidae) on glasshouse cucumbers through *Phytoseiulus persimilis* A-H (Acarina, Phytoseiidae). Zeitschrift Fur Angewandte Entomologie-Journal of Applied Entomology 98: 254-263.

Heil, M. (2015) Extrafloral nectar at the plant-insect interface: A spotlight on chemical ecology, phenotypic plasticity, and food webs. Annual Review of Entomology 60: 213-232.

Helle, W. (1967) Fertilization in the two-spotted spider mite (*Tetranychus urticae*: Acari). Entomologia Experimentalis Et Applicata 10: 103-110.

Helle, W. and Sabelis, M.W. (1985) Spider mites: their biology, natural enemies and control. Elsevier Amsterdam, The Netherlands.

Hepper, P.G. (1986) Kin recognition: Functions and mechanisms a review. Biological Reviews 61: 63-93.

Hewitt, L.C., Shipp, L., Buitenhuis, R. and Scott-Dupree, C. (2013) Seasonal climatic variations influence the efficacy of predatory mites used for control of western flower thrips in greenhouse ornamental crops. Experimental and Applied Acarology: 1-16.

Hoddle, M., Van Driesche, R. and Sanderson, J. (1998) Biology and use of the whitefly parasitoid *Encarsia formosa*. Annual Review of Entomology 43: 645-669.

Hokkanen, H. (1989) Choosing an effective biocontrol agent-an evolutionary perspective. Acta Entomol. Fenn 53: 19-24.

Honek, A. (1999) Constraints on thermal requirements for insect development (Proceedings of the International Japanese-Czech New-Year Seminar of Entomology [1999]). Entomological Science 2: 615-621.

Honek, A. and Kocourek, F. (1990) Temperature and development time in insects: a general relationship between thermal constants. Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere 117: 401-439.

Hoogerbrugge, H., van Houten, Y., Van Baal, E. and Bolckmans, K. (2008) Alternative food sources to enable establishment of *Amblyseius swirskii* (Athias-Henriot) on chrysanthemum without pest presence. IOBC/wprs Bulletin 32: 79-82.

Hoogerbrugge, H., van Houten, Y.M., Knapp, M. and Bolckmans, K. (2011a) Biological control of greenhouse whitefly on roses with phytoseiid mites. IOBC/wprs Bulletin 68: 59-63.

Hoogerbrugge, H., van Houten, Y.M., Knapp, M. and Bolckmans, K. (2011b) Biological control of thrips and whitefly on strawberry with *Amblydromalus limonicus* and *Amblyseius swirskii*. IOBC/wprs Bulletin 68: 65-69.

Hoogerbrugge, H., Oude Lenferink, K., van Houten, Y. and Bolckmans, K. (2014) Screening of three phytoseiid mite species as biocontrol agents of *Echinothrips americanus*. IOBC/wprs Bulletin 102: 97-101.

Howe, R.W. (1967) Temperature effects on embryonic development in insects. Annual Review of Entomology 12: 15-42.

Howe, G.A. and Jander, G. (2008) Plant immunity to insect herbivores. Annual Review of Plant Biology 59: 41-66.

Hoy, M.A. and Smilanick, J.M. (1979) A sex pheromone produced by immature and adult females of the predatory mite, *Metaseiulus occidentalis*, Acarina: Phytoseiidae. Entomologia Experimentalis Et Applicata 26: 291-300.

Hoy, M. (1984) Genetic improvement of a biological control agent: multiple pesticide resistance and nondiapause in *Metaseiulus occidentalis* (Nesbitt) (Phytoseiidae). Acarology VI/editors, DA Griffiths and CE Bowman.

Hoy, M.A. (1985) Recent advances in genetics and genetic improvement of the Phytoseiidae. Annual Review of Entomology 30: 345-370.

Hoy, M.A. (1994) Parasitoids and predators in management of arthropod pests. Introduction to insect pest management 4: 129-198.

Hoy, M.A. (2011) Agricultural acarology: Introduction to integrated mite management. CRC Press.

Huang, N., Enkegaard, A., Osborne, L.S., Ramakers, P.M.J., Messelink, G.J., Pijnakker, J. and Murphy, G. (2011) The banker plant method in biological control. Critical Reviews in Plant Sciences 30: 259-278.

Huey, R.B. and Kingsolver, J.G. (1989) Evolution of thermal sensitivity of ectotherm performance. Trends in Ecology & Evolution 4: 131-135.

Huffaker, C.B. (1944) The temperature relations of the immature stages of the malarial mosquito, *Anopheles quadrimaculatus* Say, with a comparison of the developmental power of constant and variable temperatures in insect metabolism. Annals of the Entomological Society of America 37: 1-27.

Hulshof, J. and Vänninen, I., (2002) Western flower thrips feeding on pollen, and its implications for control. Thrips and Tospoviruses: Proc. 7th Intl. Symp. on Thysanoptera, July, pp. 2-7.

Hulshof, J., Ketoja, E. and Vänninen, I. (2003) Life history characteristics of *Frankliniella occidentalis* on cucumber leaves with and without supplemental food. Entomologia Experimentalis Et Applicata 108: 19-32.

Hulting, F.L., Orr, D.B. and Obrycki, J.J. (1990) A computer program for calculation and statistical comparison of intrinsic rates of increase and associated life table parameters. Florida Entomologist: 601-612.

Immaraju, J.A., Paine, T.D., Bethke, J.A., Robb, K.L. and Newman, J.P. (1992) Western flower thrips (Thysanoptera: Thripidae) resistance to insecticides in coastal California greenhouses. Journal of Economic Entomology 85: 9-14.

Jacobson, R., Croft, P. and Fenlon, J. (2001) Suppressing establishment of Frankliniella occidentalis Pergande (Thysanoptera: Thripidae) in cucumber crops by prophylactic release of Amblyseius cucumeris Oudemans (Acarina: Phytoseiidae). Biocontrol Science and Technology 11: 27-34.

Jafari, S., Fathipour, Y., and Faraji, F. (2012) Temperature-depedent development of *Neoseiulus barkeri* (Acari: Phytoseiidae) on *Tetranychus urticae* (Acari: Phytoseiidae) at seven constant temperatures. Insect Science 19: 220-228.

Janssen, A. and Sabelis, M. (1992) Phytoseiid life-histories, local predator-prey dynamics, and strategies for control of tetranychid mites. Experimental & applied acarology 14: 233-250.

Janssen, A., Faraji, F., Van Der Hammen, T., Magalhães, S. and Sabelis, M.W. (2002) Interspecific infanticide deters predators. Ecology Letters 5: 490-494.

Janssen, A., Willemse, E. and Van Der Hammen, T. (2003) Poor host plant quality causes omnivore to consume predator eggs. Journal of Animal Ecology 72: 478-483.

Janssen, A. and Sabelis, M.W. (2015) Alternative food and biological control by generalist predatory mites: the case of *Amblyseius swirskii*. Experimental and Applied Acarology 65: 413-418.

Jarošík, V. and Plíva, J. (1990) Efficient control of twospotted spider mite (*Tetranychus urticae* Koch) by *Phytoseiulus persimilis* A.-H. on glasshouse peppers. Journal of Applied Entomology 110: 270-274.

Jensen, S.E. (2000) Insecticide resistance in the western flower thrips, *Frankliniella occidentalis*. Integrated Pest Management Reviews 5: 131-146.

Jeppson, L.R., Keifer, H.H. and Baker, E.W. (1975) Mites injurious to economic plants. Univ of California Press.

Johansen, N., Vänninen, I., Pinto, D.M., Nissinen, A. and Shipp, L. (2011) In the light of new greenhouse technologies: 2. Direct effects of artificial lighting on arthropods and integrated pest management in greenhouse crops. Annals of Applied Biology 159: 1-27.

Johnson, D.T. and Croft, B. (1981) Dispersal of *Amblyseius fallacis* (Acarina: Phytoseiidae) in an apple ecosystem. Environmental Entomology 10: 313-319.

Jolly, R.L. (2000) The predatory mite *Neoseiulus californicus*: its potential as a biocontrol agent for the fruit tree red spider mite *Panonychus ulmi* in the UK. Bcpc Conference: Pests & Diseases 2000, Vols 1-3, Proceedings 1-3: 487-490.

Jonsson, M., Wratten, S.D., Landis, D.A. and Gurr, G.M. (2008) Recent advances in conservation biological control of arthropods by arthropods. Biological Control 45: 172-175.

Kadono, F., Shimizu, K. & Nomura, K. (1975) Effects of temperature on developmental period of *Amblyseius longispinosus*. Annual Report Kanto Plant Protection 22: 90–91. (In Japanese).

Kawashima, M. and Jung, C. (2011) Effects of sheltered ground habitats on the overwintering potential of the predacious mite *Neoseiulus californicus* (Acari: Phytoseiidae) in apple orchards on mainland Korea. Experimental and Applied Acarology 55: 375-388.

Kennett, C. and Hamai, J. (1980) Oviposition and development in predaceous mites fed with artificial and natural diets (Acari: Phytoseiidae). Entomologia Experimentalis Et Applicata 28: 116-122.

Kennett, C., Flaherty, D. and Hoffmann, R. (1979) Effect of wind-borne pollens on the population dynamics of *Amblyseius hibisci* [Acarina: Phytoseiidae]. Entomophaga 24: 83-98.

Khayat, E., Zieslin, N., Mortensen, L. and Moe, R. (1988) Effect of alternating temperature on dark respiration and ^{14}C export in rose plants. Journal of Plant Physiology 133: 199-202.

Kim, J.H., Broadbent, A.B. and Lee, S.G. (2001) Quality control of the mass-reared predatory mite, *Amblyseius cucumeris* (Acarina: Phytoseiidae). Journal of Asia-Pacific Entomology 4: 175-179.

Kim, T., Ahn, J. and Lee, J.H. (2009) Temperature-dependent developmental model of *Neoseiulus californicus* (McGregor) (Acari, Phytoseiidae). Journal of Applied Entomology 133: 284-291.

Kim, T., Ahn, J. and Lee, J.H. (2013) Age- and temperature-dependent oviposition model of *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) with *Tetranychus urticae* as prey. Journal of Applied Entomology 137: 282-288.

Kirk, W. (1984) Pollen-feeding in thrips (Insecta: Thysanoptera). Journal of Zoology 204: 107-117.

Kirk, W.D., (2002) The pest and vector from the West: *Frankliniella occidentalis*. Thrips and Tospoviruses: Proceedings of the 7th International symposium on Thysanoptera. Australian National Insect Collection Canberra, Australia, pp. 33-42.

Kirk, W.D. and Terry, L.I. (2003) The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). Agricultural and Forest Entomology 5: 301-310.

Knapp, M., van Houten, Y., Hoogerbrugge, H. and Bolckmans, K. (2013) *Amblydromalus limonicus* (Acari: Phytoseiidae) as a biocontrol agent: literature review and new findings. Acarologia 53: 191-202.

Koller, M., Knapp, M. and Schausberger, P. (2007) Direct and indirect adverse effects of tomato on the predatory mite *Neoseiulus californicus* feeding on the spider mite *Tetranychus evansi*. Entomologia Experimentalis Et Applicata 125: 297-305.

Kolokytha, P., Fantinou, A. and Papadoulis, G.T. (2011) Effect of several different pollens on the bio-ecological parameters of the predatory mite *Typhlodromus athenas* Swirski and Ragusa (Acari: Phytoseiidae). Environmental Entomology 40: 597-604.

Kontodimas, D.C., Eliopoulos, P.A., Stathas, G.J. and Economou, L.P. (2004) Comparative temperature-dependent development of *Nephus includens* (Kirsch) and *Nephus bisignatus* (Boheman)(Coleoptera: Coccinellidae) preying on *Planococcus citri* (Risso) (Homoptera: Pseudococcidae): evaluation of a linear and various nonlinear models using specific criteria. Environmental Entomology 33: 1-11.

Koppert (2015) www.koppert.nl

Koptur, S. (1992) Extrafloral nectary-mediated interactions between insects and plants. Insect Plant Interactions 4: 81-129.

Koptur, S. (2005) Nectar as fuel for plant protectors 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. 75-108.

Körner, O. and Challa, H. (2003) Design for an improved temperature integration concept in greenhouse cultivation. Computers and electronics in agriculture 39: 39-59.

Körner, O. and Challa, H. (2004) Temperature integration and process-based humidity control in chrysanthemum. Computers and Electronics in Agriculture 43: 1-21.

Körner, O., Bakker, M. and Heuvelink, E. (2004) Daily temperature integration: a simulation study to quantify energy consumption. Biosystems engineering 87: 333-343.

Kostiainen, T.S. and Hoy, M.A. (1996) The phytoseiidae as biological control agents of pest mites and Insects: A bibliography, 1960-1994. Springer.

Krantz, G.W. (1978) A manual of acarology. Texas Tech University Press.

Kreiter, S., Tixier, M., Croft, B., Auger, P. and Barret, D. (2002) Plants and leaf characteristics influencing the predaceous mite *Kampimodromus aberrans* (Acari: Phytoseiidae) in habitats surrounding vineyards. Environmental Entomology 31: 648-660.

Krips, O.E., Willems, P.E.L. and Dicke, M. (1999) Compatibility of host plant resistance and biological control of the two-spotted spider mite *Tetranychus urticae* in the ornamental crop gerbera. Biological Control 16: 155-163.

Kropczynska, D., Pilko, A., Witul, A. and Al-Mabrouk, A. (1999) Control of two-spotted spider mite with *Amblyseius californicus* (Oud.) on cotton. Bulletin OILB SROP (France) 22: 133-136.

Kumar, V., Xiao, Y., McKenzie, C.L. and Osborne, L.S. (2015) Early establishment of the phytoseiid mite *Amblyseius swirskii* (Acari: Phytoseiidae) on pepper seedlings in a predator-in-first approach. Experimental and Applied Acarology 65: 465-481.

Kumari, M. and Sadana, G. (1991) Influence of temperature and relative humidity on the development of *Amblyseius alstoniae* (Acari: Phytoseiidae). Experimental and Applied Acarology 11: 199-203.

Kutner, M., Nachtsheim, C., Neter, J. and Li, W. (2005) Applied Linear Statistical Models. McGraw-Hill. New York.

Lactin, D.J., Holliday, N.J., Johnson, D.L. and Craigen, R. (1995) Improved rate model of temperature-dependent development by arthropods. Environmental Entomology 24: 68-75.

Lamb, R. (1992) Developmental rate of *Acyrthosiphon pisum* (Homoptera: Aphididae) at low temperatures: implications for estimating rate parameters for insects. Environmental Entomology 21: 10-19.

Landis, D.A., Wratten, S.D. and Gurr, G.M. (2000) Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology 45: 175-201.

Lavens, P. and Sorgeloos, P. (1987) The cryptobiotic state of *Artemia* cysts, its diapause deactivation and hatching: a review: p. 27-63. In: Artemia research and its applications. Vol. 3. Ecology, Culturing, Use in Aquaculture. Sorgeloos, P.; Bengtson, D.A.; Decleir, W.; Jaspers, E. (Eds). Universa Press, Wetteren, Belgium, 556 p.

Lavens, P. and Sorgeloos, P. (2000) The history, present status and prospects of the availability of *Artemia* cysts for aquaculture. Aquaculture 181: 397-403.

Lee, H.S. and Gillespie, D.R. (2011) Life tables and development of *Amblyseius swirskii* (Acari: Phytoseiidae) at different temperatures. Experimental and Applied Acarology 53: 17-27.

Lee, J.H. and Ahn, J.J. (2000) Temperature effects on development, fecundity, and life table parameters of *Amblyseius womersleyi* (Acari: Phytoseiidae). Environmental Entomology 29: 265-271.

Lee, R.E., Jr., Chen, C.P. and Denlinger, D.L. (1987) A rapid cold-hardening process in insects. Science 238: 1415-1417.

Leman, A. and Messelink, G.J. (2015) Supplemental food that supports both predator and pest: A risk for biological control? Experimental and Applied Acarology 65: 511-524.

Lesna, I., da Silva, F.R., Sato, Y., Sabelis, M.W. and Lommen, S.T. (2014) *Neoseiulus paspalivorus*, a predator from coconut, as a candidate for controlling dry bulb mites infesting stored tulip bulbs. Experimental and Applied Acarology 63: 189-204.

Lesna, I., Sabelis, M. and Conijn, C. (2004) From biological control to biological insight: rust-mite induced change in bulb morphology, a new mode of indirect plant defence? Phytophaga 14: 285 - 291.

Lewis, T. (1997) Thrips as crop pests. Cab International.

Lewontin, R.C. (1965) Selection for colonizing ability, In: "The Genetics of Colonizing Species" (H.G. Baker, G. L. Stebbins et al., Eds.). Academic Press, New York.

Lindquist, R. and Short, T. (2004) Effects of greenhouse structure and function on biological control. Biocontrol in protected culture. Ball Publishing, Batavia.

Linskens, H. and Jorde, W. (1997) Pollen as food and medicine—a review. Economic Botany 51: 78-86.

Liu, S.S., Zhang, G.M. and Zhu, J. (1995) Influence of temperature variations on rate of development in insects: analysis of case studies from entomological literature. Annals of the Entomological Society of America 88: 107-119.

Logan, J.D., Wolesensky, W. and Joern, A. (2006) Temperature-dependent phenology and predation in arthropod systems. Ecological Modelling 196: 471-482.

Loughner, R., Goldman, K., Loeb, G. and Nyrop, J. (2008) Influence of leaf trichomes on predatory mite (*Typhlodromus pyri*) abundance in grape varieties. Experimental and Applied Acarology 45: 111-122.

Lundgren, J.G. (2009) Relationships of natural enemies and non-prey foods. Springer Science & Business Media.

Luypaert, G., Witters, J., Van Huylenbroeck, J., Maes, M., De Riek, J. and De Clercq, P. (2014) Temperature-dependent development of the broad mite *Polyphagotarsonemus latus* (Acari: Tarsonemidae) on *Rhododendron simsii*. Experimental and Applied Acarology 63: 389-400.

MacGill, E.I. (1939) A gamasid mite (*Typhlodromus thripsi* n. sp.), a predator of *Thrips tabaci* Lind. Annals of Applied Biology 26: 309-317.

Maeda, T., Takabayashi, J., Yano, S. and Takafuji, A. (1998) Factors affecting the resident time of the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae) in a prey patch. Applied Entomology and Zoology 33: 573-576.

Maeda, T., Takabayashi, J., Yano, S. and Takafuji, A. (2000) Effects of light on the tritrophic interaction between kidney bean plants, two-spotted spider mites and predatory mites, *Amblyseius womersleyi* (Acari: Phytoseiidae). Experimental and Applied Acarology 24: 415-425.

Magalhaes, S. and Bakker, F.M. (2002) Plant feeding by a predatory mite inhabiting cassava. Experimental and Applied Acarology 27: 27-37.

Maia, A.H.N., Luiz, A.J.B. and Campanhola, C. (2000) Statistical inference on associated fertility life parameters using jackknife technique: computational aspects. Journal of Economic Entomology 93: 511-518.

Manjunatha, M., Pickett, J., Wadhams, J. and Nazzi, F. (1998) Response of western flower thrips, *Frankliniella occidentalis* and its predator *Amblyseius cucumeris* to chrysanthemum volatiles in olfactometer and greenhouse trials. Insect Science and its Application 18: 139-144.

Maoz, Y., Gal, S., Argov, Y., Coll, M. and Palevsky, E. (2011) Biocontrol of persea mite, *Oligonychus perseae*, with an exotic spider mite predator and an indigenous pollen feeder. Biological Control 59: 147-157.

Margolies, D.C. and Wrensch, D.L. (1996) Temperature-induced changes in spider mite fitness: offsetting effects of development time, fecundity, and sex ratio. Entomologia Experimentalis Et Applicata 78: 111-118.

Markkula, M. and Tiittanen, K. (1976) " Pest in first" and" natural infestation" methods in the control of *Tetranychus urticae* Koch with *Phytoseiulus persimilis* Athias-Henriot on glasshouse cucumbers. Annales Agriculturae Fenniae 15: 81-85.

Martin, T.L. and Huey, R.B. (2008) Why “suboptimal” is optimal: Jensen’s inequality and ectotherm thermal preferences. The American Naturalist 171: 102-118.

Matteson, J.W. and Decker, G.C. (1965) Development of European corn borer at controlled constant and variable temperatures. Journal of Economic Entomology 58: 344-349.

McCullagh, P. and Nelder, J.A. (1989) Generalized linear models. Chapman & Hall, New York.

McMurtry, J. and Croft, B. (1997) Life-styles of phytoseiid mites and their roles in biological control. Annual Review of Entomology 42: 291-321.

McMurtry, J. and Scriven, G. (1964a) Biology of the predaceous mite *Typhlodromus rickeri* (Acarina: Phytoseiidae). Annals of the Entomological Society of America 57: 362-367.

McMurtry, J.A. and Scriven, G.T. (1964b) Studies on the feeding, reproduction, and development of *Amblyseius hibisci* (Acarina: Phytoseiidae) on various food substances. Annals of the Entomological Society of America 57: 649-655.

McMurtry, J.A. and Scriven, G.T. (1965) Life-history studies of *Amblyseius limonicus*, with comparative observations on *Amblyseius hibisci* (Acarina: Phytoseiidae). Annals of the Entomological Society of America 58: 106-111.

McMurtry, J. and Scriven, G. (1966a) Effects of artificial foods on reproduction and development of four species of phytoseiid mites. Annals of the Entomological Society of America 59: 267-269.

McMurtry, J. and Scriven, G. (1966b) The influence of pollen and prey density on the number of prey consumed by *Amblyseius hibisci* (Acarina: Phytoseiidae). Annals of the Entomological Society of America 59: 147-149.

McMurtry, J., Mahr, D. and Johnson, H. (1976) Geographic races in the predaceous mite, *Amblyseius potentillae* (Acari: Phytoseiidae). International Journal of Acarology 2: 23-28.

McMurtry, J.A., Oatman, E.R., Phillips, P.A. and Wood, C.W. (1978) Establishment of *Phytoseiulus persimilis* (Acari-Phytoseiidae) in Southern-California. Entomophaga 23: 175-179.

McMurtry, J., Badii, M. and Johnson, H. (1984) The broad mite, *Polyphagotarsonemus latus*, as a potential prey for phytoseiid mites in California. Entomophaga 29: 83-86.

McMurtry, J.A., De Moraes, G.J. and Sourassou, N.F. (2013) Revision of the lifestyles of phytoseiid mites (Acari: Phytoseiidae) and implications for biological control strategies. Systematic and Applied Acarology 18: 297-320.

Meijón, M., Feito, I., Valledor, L., Rodríguez, R. and Cañal, M.J. (2011) Promotion of flowering in azaleas by manipulating photoperiod and temperature induces epigenetic alterations during floral transition. Physiologia Plantarum 143: 82-92.

Melnyk, J., Smith, A., Scott-Dupree, C., Marcone, M. and Hill, A. (2010) Identification of cheese mite species inoculated on Mimolette and Milbenkase cheese through cryogenic scanning electron microscopy. Journal of dairy science 93: 3461-3468.

Messelink, G., Ramakers, P., Cortez, J. and Janssen, A. (2009) How to enhance pest control by generalist predatory mites in greenhouse crops. Proceedings of the 3rd ISBCA, Christchurch, New Zealand: 309-318.

Messelink, G., van Wensveen, W., Haaring, M.-A., Bloemhard, C. and van Slooten, M. (2005) De wonderboom *Ricinus communis*.

Messelink, G.J., Van Steenpaal, S.E.F. and Ramakers, P.M.J. (2006) Evaluation of phytoseiid predators for control of western flower thrips on greenhouse cucumber. BioControl 51: 753-768.

Messelink, G.J., Maanen, R.v., van Steenpaal, S.E. and Janssen, A. (2008) Biological control of thrips and whiteflies by a shared predator: two pests are better than one. Biological Control 44: 372-379.

Messelink, G.J., Van Maanen, R., Van Holstein-Saj, R., Sabelis, M.W. and Janssen, A. (2010) Pest species diversity enhances control of spider mites and whiteflies by a generalist phytoseiid predator. BioControl 55: 387-398.

Messelink, G.J. (2014) Persistent and emerging pests in greenhouse crops: Is there a need for new natural enemies? IOBC/wprs Bulletin 102: 143-150.

Messelink, G.J., Bennison, J., Alomar, O., Ingegno, B.L., Tavella, L., Shipp, L., Palevsky, E. and Wäckers, F.L. (2014) Approaches to conserving natural enemy populations in greenhouse crops: current methods and future prospects. BioControl 59: 377-393.

Messenger, P. and Flitters, N. (1958) Effect of constant temperature environments on the egg stage of three species of Hawaiian fruit flies. Annals of the Entomological Society of America 51: 109-119.

Messenger, P.S. (1964) Influence of rhythmically fluctuating temperatures on development + reproduction of spotted alfalfa aphid *Therioaphis maculata*. Journal of Economic Entomology 57: 71-76.

Meyer, J.S., Ingersoll, C.G., McDonald, L.L. and Boyce, M.S. (1986) Estimating uncertainty in population growth rates: jackknife vs. bootstrap techniques. Ecology 67: 1156-1166.

Meyerdirk, D.E. and Coudriet, D.L. (1985) Predation and developmental studies of *Euseius hibisci* (Chant)(Acarina: Phytoseiidae) feeding on *Bemisia tabaci* (Gennadius)(Homoptera: Aleyrodidae). Environmental Entomology 14: 24-27.

Meyerdirk, D.E. and Coudriet, D.L. (1986) Evaluation of two biotypes of *Euseius scutalis* (Acari: Phytoseiidae) as predators of *Bemisia tabaci* (Homoptera: Aleyrodidae). Journal of Economic Entomology 79: 659-663.

Midthassel, A., Leather, S.R. and Baxter, I.H. (2013) Life table parameters and capture success ratio studies of *Typhlodromips swirskii* (Acari: Phytoseiidae) to the factitious prey *Suidasia medanensis* (Acari: Suidasidae). Experimental and Applied Acarology 61: 69-78.

Midthassel, A., Leather, S.R., Wright, D.J. and Baxter, I.H. (2014) The functional and numerical response of *Typhlodromips swirskii* (Acari: Phytoseiidae) to the factitious prey *Suidasia medanensis* (Acari: Suidasidae) in the context of a breeding sachet. Biocontrol Science and Technology 24: 361-374.

Mironidis, G. and Savopoulou-Soultani, M. (2008) Development, survivorship, and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae) under constant and alternating temperatures. Environmental Entomology 37: 16-28.

Moerkens, R., Gobin, B., Peusens, G., Helsen, H., Hilton, R., Dib, H., Suckling, D.M. and Leirs, H. (2011) Optimizing biocontrol using phenological day degree models: the European earwig in pipfruit orchards. Agricultural and Forest Entomology 13: 301-312.

Momen, F. (1996) Effect of prey density on reproduction, prey consumption and sex-ratio of *Amblyseius barkeri* (Acari: Phytoseiidae). Acarologia 37: 3-6.

Momen, F.M. and El-Laithy, A.Y. (2007) Suitability of the flour moth *Ephestia kuehniella* (Lepidoptera: Pyralidae) for three predatory phytoseiid mites (Acari: Phytoseiidae) in Egypt. International Journal of Tropical Insect Science 27: 102-107.

Momen, F. and Abdel-Khalek, A. (2009) Cannibalism and intraguild predation in the phytoseiid mites *Typhlodromips swirskii*, *Euseius scutalis* and *Typhlodromus athiasae* (Acari: Phytoseiidae). Acarina 17: 223-229.

Momen, F. (2011) Life tables and feeding habits of *Proprioseiopsis cabonus*, a specific predator of tydeid mites (Acari: Phytoseiidae and Tydeidae). Acarina 19: 103-109.

Montserrat, M., Guzmán, C., Sahún, R., Belda, J. and Hormaza, J. (2013) Pollen supply promotes, but high temperatures demote, predatory mite abundance in avocado orchards. Agriculture, Ecosystems & Environment 164: 155-161.

Morewood, W. and Gilkeson, L. (1991) Diapause induction in the thrips predator *Amblyseius cucumeris* [Acarina: phytoseiidae] under greenhouse conditions. Entomophaga 36: 253-263.

Mori, H. and Chant, D.A. (1966) Influence of humidity on activity of *Phytoseiulus persimilis* Athias-Henriot and its prey *Tetranychus urticae* (C L Koch) (Acarina - Phytoseiidae Tetranychidae). Canadian Journal of Zoology 44: 863-871.

Morris, M., Berry, R. and Croft, B. (1999) Phytoseiid mites on peppermint and effectiveness of *Neoseiulus fallacis* to control *Tetranychus urticae* (Acari: Phytoseiidae, Tetranychidae) in arid growing regions. Journal of Economic Entomology 92: 1072-1078.

Morrison, W.R., Andresen, J. and Szendrei, Z. (2013) The development of the asparagus miner (*Ophiomyia simplex* Loew; Diptera: Agromyzidae) in temperate zones: a degree-day model. Pest Management Science 70: 1105-1113.

Murdoch, W.W., Chesson, J. and Chesson, P.L. (1985) Biological control in theory and practice. American Naturalist: 344-366.

Nagelkerke, C.J. and Sabelis, M.W. (1998) Precise control of sex allocation in pseudo-arrhenotokous phytoseiid mites. Journal of Evolutionary Biology 11: 649-684.

Nakagawa, T. (1984) Growth and prey quantity of *Amblyseius longispinosus* (Evans) as a predator of *Tetranychus kanzawai* Kishida. Proceedings of the Association for Plant Protection, Kyushu 30: 158–161. (In Japanese)

Nguyen, D.T., Vangansbeke, D., Lü, X. and De Clercq, P. (2013) Development and reproduction of the predatory mite *Amblyseius swirskii* on artificial diets. BioControl 58: 369-377.

Nguyen, D., Bouguet, V., Spranghers, T., Vangansbeke, D. and De Clercq, P. (2014a) Beneficial effect of supplementing an artificial diet for *Amblyseius swirskii* with *Hermetia illucens* haemolymph. Journal of Applied Entomology 139: 342-351.

Nguyen, D.T., Vangansbeke, D. and De Clercq, P. (2014b) Solid artificial diets for the phytoseiid predator *Amblyseius swirskii*. BioControl 59: 719-727.

Nguyen, D.T., Vangansbeke, D. and De Clercq, P. (2014c) Artificial and factitious foods support the development and reproduction of the predatory mite *Amblyseius swirskii*. Experimental and Applied Acarology 62: 181-194.

Nguyen, D.T., Vangansbeke, D. and De Clercq, P. (2015) Performance of four species of phytoseiid mites on artificial and natural diets. Biological Control 80: 56-62.

Nomikou, M., Janssen, A., Schraag, R. and Sabelis, M. (2001) Phytoseiid predators as potential biological control agents for *Bemisia tabaci*. Experimental and Applied Acarology 25: 271-291.

Nomikou, M., Janssen, A., Schraag, R. and Sabelis, M.W. (2002) Phytoseiid predators suppress populations of *Bemisia tabaci* on cucumber plants with alternative food. Experimental and Applied Acarology 27: 57-68.

Nomikou, M., Janssen, A. and Sabelis, M.W. (2003a) Phytoseiid predators of whiteflies feed and reproduce on non-prey food sources. Experimental and Applied Acarology 31: 15-26.

Nomikou, M., Janssen, A. and Sabelis, M.W. (2003b) Phytoseiid predator of whitefly feeds on plant tissue. Experimental and Applied Acarology 31: 27-36.

Nomikou, M., Meng, R., Schraag, R., Sabelis, M.W. and Janssen, A. (2005) How predatory mites find plants with whitefly prey. Experimental and Applied Acarology 36: 263-275.

Nomikou, M., Sabelis, M.W. and Janssen, A. (2010) Pollen subsidies promote whitefly control through the numerical response of predatory mites. BioControl 55: 253-260.

Nyrop, J.P., Minns, J.C. and Herring, C.P. (1994) Influence of ground cover on dynamics of *Amblyseius fallacis* Garman (Acarina; Phytoseiidae) in New York apple orchards. Agriculture, Ecosystems & Environment 50: 61-72.

Oatman, E.R., Mcmurtry, J.A. and Voth, V. (1968) Suppression of 2-spotted spider mite on strawberry with mass releases of *Phytoseiulus persimilis*. Journal of Economic Entomology 61: 1517-1521.

Ochieng, R., Oloo, G. and Amboga, E. (1987) An artificial diet for rearing the phytoseiid mite, *Amblyseius teke* Pritchard and Baker. Experimental and Applied Acarology 3: 169-173.

Oerke, E.C. (2006) Crop losses to pests. The Journal of Agricultural Science 144: 31-43.

Öhlund, G., Hedström, P., Norman, S., Hein, C.L. and Englund, G. (2015) Temperature-dependence of predation depends on relative performance of predators and prey. Proceedings of the Royal Society B-Biological Sciences.

Onzo, A., Houedokoho, A.F. and Hanna, R. (2012) Potential of the predatory mite, *Amblyseius swirskii* to suppress the broad mite, *Polyphagotarsonemus latus* on the gboma eggplant, *Solanum macrocarpon*. Journal of Insect Science 12: 7.

Opit, G., Peterson, B., Gillespie, D. and Costello, R. (1997) The life cycle and management of *Echinothrips americanus* (Thysanoptera: Thripidae). Journal of the Entomological Society of British Columbia 94: 3-6.

Opit, G.P., Nechols, J.R., Margolies, D.C. and Williams, K.A. (2005) Survival, horizontal distribution, and economics of releasing predatory mites (Acari : Phytoseiidae) using mechanical blowers. Biological Control 33: 344-351.

Osborne, L.S., Landa, Z., Taylor, D.J. and Tyson, R.V. (2005) Using banker plants to control insects in greenhouse vegetables. Proceedings of the Florida State Horticulture Society, pp. 127-128.

Otsuka, Y., Takano, T.S. and Yamazaki, T. (1997) Genetic variation in the expression of the six hsp genes in the presence of heat shock in *Drosophila melanogaster*. Genes & genetic systems 72: 19-24.

Paaijmans, K.P., Blanford, S., Bell, A.S., Blanford, J.I., Read, A.F. and Thomas, M.B. (2010) Influence of climate on malaria transmission depends on daily temperature variation. Proceedings of the National Academy of Sciences of the United States of America 107: 15135-15139.

Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C. and Thomas, M.B. (2013) Temperature variation makes ectotherms more sensitive to climate change. Global Change Biology 19: 2373-2380.

Pacini, E., Guarnieri, M. and Nepi, M. (2006) Pollen carbohydrates and water content during development, presentation, and dispersal: a short review. Protoplasma 228: 73-77.

Palevsky, E., Reuveny, H., Okonis, O. and Gerson, U. (1999) Comparative behavioural studies of larval and adult stages of the phytoseiids (Acari: Mesostigmata) *Typhlodromus athiasae* and *Neoseiulus californicus*. Experimental and Applied Acarology 23: 467-485.

Palevsky, E., Walzer, A., Gal, S. and Schausberger, P. (2008) Evaluation of dry-adapted strains of the predatory mite *Neoseiulus californicus* for spider mite control on cucumber, strawberry and pepper. Experimental and Applied Acarology 45: 15-27.

Pappas, M.L., Broufas, G.D. and Koveos, D.S. (2007) Effect of mating frequency on fecundity and longevity of the predatory mite *Kampimodromus aberrans* (Acari: Phytoseiidae). Experimental and Applied Acarology 43: 161-170.

Pappas, M.L., Xanthis, C., Samaras, K., Koveos, D.S. and Broufas, G.D. (2013) Potential of the predatory mite Phytoseius finitimus (Acari: Phytoseiidae) to feed and reproduce on greenhouse pests. Experimental and Applied Acarology 61: 387-401.

Paré, P.W. and Tumlinson, J.H. (1999) Plant volatiles as a defense against insect herbivores. Plant Physiology 121: 325-332.

Park, H.-H., Shipp, L. and Buitenhuis, R. (2010) Predation, development, and oviposition by the predatory mite *Amblyseius swirkii* (Acari: Phytoseiidae) on tomato russet mite (Acari: Eriophyidae). Journal of Economic Entomology 103: 563-569.

Park, H.H., Shipp, L., Buitenhuis, R. and Ahn, J.J. (2011) Life history parameters of a commercially available *Amblyseius swirskii* (Acari: Phytoseiidae) fed on cattail (*Typha latifolia*) pollen and tomato russet mite (*Aculops lycopersici*). Journal of Asia-Pacific Entomology 14: 497-501.

Parr, W. and Stacey, D. (1975) ‘Banker’-plant system of whitefly parasite release on tomatoes. Report of the Glasshouse Crops Research tnstitute 96.

Parrella, M.P. (1995) IPM—approaches and prospects. Thrips biology and management. Springer, pp. 357-363.

Pedigo, L.P. (1998) Entomology and pest management. Prentice-Hall International.

Pemberton, H. and Wilkins, H. (1985) Seasonal variation on the influence of low temperature, photoperiod, light source, and GA in floral development of the evergreen azalea. Journal of the American Society for Horticultural Science 110: 730-737.

Peña, J. and Osborne, L. (1996) Biological control of *Polyphagotarsonemus latus* (Acarina: Tarsonemidae) in greenhouses and field trials using introductions of predacious mites (Acarina: Phytoseiidae). Entomophaga 41: 279-285.

Petanidou, T. and Vokou, D. (1990) Pollination and pollen energetics in Mediterranean ecosystems. American Journal of Botany 77: 986-992.

Petrova, V., Cudare, Z. and Steinite, I. (2000) The efficiency of the predatory mite *Amblyseius cucumeris* (Acari: Phytoseiidae) as a control agent of the strawberry mite *Phytonemus pallidus* (Acari: Tarsonemidae) on field strawberry. IV International Strawberry Symposium 567, pp. 675-678.

Pezzi, F., Martelli, R., Lanzoni, A. and Maini, S. (2015) Effects of mechanical distribution on survival and reproduction of *Phytoseiulus persimilis* and *Amblyseius swirskii*. Biosystems engineering 129: 11-19.

Pfennig, D.W., Ho, S.G. and Hoffman, E.A. (1998) Pathogen transmission as a selective force against cannibalism. Animal Behaviour 55: 1255-1261.

Pijnakker, J., de Souza, A. and Wäckers, F. (2014) *Euseius gallicus*, a bodyguard for roses. IOBC/wprs Bulletin 102: 191-195.

Pilkington, L.J., Messelink, G., van Lenteren, J.C. and Le Mottee, K. (2010) "Protected Biological Control"–Biological pest management in the greenhouse industry. Biological Control 52: 216-220.

Polis, G.A., Myers, C.A. and Holt, R.D. (1989) The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics 20: 297-330.

Pollet, B., Steppe, K., Dambre, P., Van Labeke, M.-C. and Lemeur, R. (2009) Temperature integration of *Hedera helix* L.: Quality aspects and growth response. Scientia Horticulturae 120: 89-95.

Porres, M., McMurtry, J. and March, R. (1975) Investigations of leaf sap feeding by three species of phytoseiid mites by labelling with radioactive phosphoric acid (H3 32PO4). Annals of the Entomological Society of America 68: 871-872.

Potter, D.A. and Wrensch, D.L. (1978) Interrupted matings and the effectiveness of second inseminations in the twospotted spider mite. Annals of the Entomological Society of America 71: 882-885.

Pradhan, S. (1945) Insect population studies. II. Rate of insect development under variable temperature of the field. Proceedings of the National Institute of Sciences of India, pp. 74-80.

Pratt, P. and Croft, B. (2000) Banker plants: evaluation of release strategies for predatory mites. Journal of Environmental Horticulture 18: 211-217.

Pratt, P.D., Schausberger, P. and Croft, B.A. (1999) Prey-food types of *Neoseiulus fallacis* (Acari : Phytoseiidae) and literature versus experimentally derived prey-food estimates for five phytoseiid species. Experimental and Applied Acarology 23: 551-565.

Pruszynski, S. and Cone, W.W. (1972) Relationships between *Phytoseiulus persimilis* and other enemies of the twospotted spider mite on hops. Environmental Entomology 1: 431-433.

Pullin, A.S. and Gilbert, J.E. (1989) The stinging nettle, *Urtica dioica*, increases trichome density after herbivore and mechanical damage. Oikos 54: 275-280.

Put, K., Bollens, T., Wackers, F.L. and Pekas, A. (2012) Type and spatial distribution of food supplements impact population development and dispersal of the omnivore predator *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae). Biological Control 63: 172-180.

Ragusa, S. and Swirski, E. (1977) Feeding habits, post-embryonic and adult survival, mating, virility and fecundity of the predacious mite *Amblyseius swirskii* [Acarina: Phytoseiidae] on some coccids and mealybugs. Entomophaga 22: 383-392.

Rahman, V.J., Babu, A., Roobakkumar, A., and Perumalsamy, K. (2013) Life table and predation of *Neoseiulus longispinosus* (Acari: Phytoseiidae) on *Oligonychus coffeae* (Acari: Tetranychidae) infesting tea. Experimental and Applied Acarology 60: 229-240.

Ramakers, P. (1980) Biological control of *Thrips tabaci* (Thysanoptera: Thripidae) with *Amblyseius* spp.(Acari: Phytoseiidae). Bulletin SROP 3: 203-207.

Ramakers, P. (1983) Mass prodution and introduction of *Amblyseius mckenziei* and *A. cucumeris*. Bulletin SROP 6: 203-206.

Ramakers, P. (1988) Population dynamics of the thrips predators *Amblyseius mckenziei* and *Amblyseius cucumeris* (Acarina: Phytoseiidae) on sweet pepper. Netherlands Journal of Agricultural Science (Netherlands) 36: 247–252.

Ramakers, P., Dissevelt, M. and Peeters, K. (1989) Large scale introductions of phytoseiid predators to control thrips on cucumber. Mededelingen van de Faculteit Landbouwwetenschappen, Rijksuniversiteit Gent 54: 923-929.

Ramakers, P.M. (1995) Biological control using oligophagous predators. Thrips Biology and Management. Springer, pp. 225-229.

Ramakers, P. and Voet, S. (1996) Introduction of *Amblyseius degenerans* for thrips control in sweet peppers with potted castor beans as banker plants. Bulletin OILB SROP (France).

Rasmy, A.H., Abou-El-Ella, G.M. and Hussein, H.E. (2004) Cannibalism and interspecific predation of the phytoseiid mite, *Amblyseius swirskii*. Journal of Pest Science 77: 23-25.

Ratte, H. (1985) Temperature and insect development. In: Hoffmann KH (ed) Environmental physiology and biochemistry of insects. Springer, New York. Environmental physiology and biochemistry of insects: 33-66.

Raworth, D.A., Fauvel, G. and Auger, P. (1994) Location, reproduction and movement of *Neoseiulus californicus* (Acari, Phytoseiidae) during the autumn, winter and spring in orchards in the south of France. Experimental and Applied Acarology 18: 593-602.

Reitz, S.R. (2009) Biology and ecology of the western flower thrips (Thysanoptera: Thripidae): The making of a pest. Florida Entomologist 92: 7-13.

Rencken, C. and Pringle, K.L. (1998) Developmental biology of *Amblyseius californicus* (McGregor) (Acarina: Phytoseiidae), a predator of tetranychid mites, at three temperatures. African Entomology 6: 41–45.

Rhodes, E.M., Liburd, O.E., Kelts, C., Rondon, S.I. and Francis, R.R. (2006) Comparison of single and combination treatments of *Phytoseiulus persimilis*, *Neoseiulus californicus*, and Acramite (bifenazate) for control of twospotted spider mites in strawberries. Experimental and Applied Acarology 39: 213-225.

Riddick, E.W. (2009) Benefits and limitations of factitious prey and artificial diets on life parameters of predatory beetles, bugs, and lacewings: a mini-review. BioControl 54: 325-339.

Rijsdijk, A. and Vogelezang, J. (2000) Temperature integration on a 24-hour base: a more efficient climate control strategy. Acta Horticulturae 515: 141-149.

Rinehart, J.P., Li, A., Yocum, G.D., Robich, R.M., Hayward, S.A. and Denlinger, D.L. (2007) Up-regulation of heat shock proteins is essential for cold survival during insect diapause. Proceedings of the National Academy of Sciences 104: 11130-11137.

Rodríguez, H. and Ramos, M. (2004) Biology and feeding behavior of *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) on *Polyphagotarsonemus latus* (Banks)(Acari: Tarsonemidae). Revista de Protección Vegetal 19: 73-79.

Rosen, D. and Huffaker, C. (1983) An overview of desired attributes of effective biological control agents, with particular emphasis on mites. Biological control of pests by mites. Univ Calif Agric Exp Stn Special Publ 3304: 2-11.

Rott, A.S. and Ponsonby, D.J. (2000) Improving the control of *Tetranychus urticae* on edible glasshouse crops using a specialist coccinellid (*Stethorus punctillum* Weise) and a generalist mite (*Amblyseius californicus* McGregor) as biocontrol agents. Biocontrol Science and Technology 10: 487-498.

Roulston, T. and Cane, J.H. (2000) Pollen nutritional content and digestibility for animals. Plant Systematics and Evolution 222: 187-209.

Roy, M., Brodeur, J. and Cloutier, C. (2002) Relationship between temperature and developmental rate of *Stethorus punctillum* (Coleoptera: Coccinellidae) and its prey *Tetranychus mcdanieli* (Acarina: Tetranychidae). Environmental Entomology 31: 177-187.

Ruberson, J., Nemoto, H. and Hirose, Y. (1998) Pesticides and conservation of natural enemies in pest management. Conservation biological control, pp. 207-220.

Ruel, J.J. and Ayres, M.P. (1999) Jensen's inequality predicts effects of environmental variation. Trends in Ecology & Evolution 14: 361-366.

Sabelis, M. (1981) Biological control of two-spotted spider mites using phytoseiid predators. Part 1. Modelling the predator-prey interaction at the individual level. Pudoc., Wageningen, the Netherlands.

Sabelis, M. and Nagelkerke, C. (1988) Evolution of pseudo-arrhenotoky. Experimental and Applied Acarology 4: 301-318.

Sabelis, M. (1991) Life-history evolution of spider mites. The Acari. Springer, pp. 23-49.

Sabelis, M.W. and Bakker, F.M. (1992) How predatory mites cope with the web of their tetranychid prey: a functional view on dorsal chaetotaxy in the Phytoseiidae. Experimental and Applied Acarology 16: 203-225.

Sabelis, M. and Bruin, J. (1996) 1.5. 3. Evolutionary ecology: Life history patterns, food plant choice and dispersal. World crop pests 6: 329-366.

Sakimura, K. (1962) *Frankliniella occidentalis* (Thysanoptera: Thripidae), a vector of the tomato spotted wilt virus, with special reference to the color forms. Annals of the Entomological Society of America 55: 387-389.

Sammataro, D., Gerson, U. and Needham, G. (2000) Parasitic mites of honey bees: life history, implications, and impact. Annual Review of Entomology 45: 519-548.

Sandström, J. and Moran, N. (2001) Amino acid budgets in three aphid species using the same host plant. Physiological Entomology 26: 202-211.

Schausberger, P. (1998) The influence of relative humidity on egg hatch in *Euseius filandicus*, *Typhlodromus pyri* and *Kampimodromus aberrans* (Acari, Phytoseiidae). Journal of Applied Entomology 122: 497-500.

Schausberger, P. (1999) Predation preference of *Typhlodromus pyri* and *Kampimodromus aberrans* (Acari: Phytoseiidae) when offered con-and heterospecific immature life stages. Experimental and Applied Acarology 23: 389-398.

Schausberger, P. and Croft, B.A. (1999a) Activity, feeding, and development among larvae of specialist and generalist phytoseiid mite species (Acari: Phytoseiidae). Environmental Entomology 28: 322-329.

Schausberger, P. and Croft, B.A. (1999b) Predation on and discrimination between con- and heterospecific eggs among specialist and generalist phytoseiid mites (Acari: Phytoseiidae). Environmental Entomology 28: 523-528.

Schausberger, P. and Croft, B.A. (2000) Nutritional benefits of intraguild predation and cannibalism among generalist and specialist phytoseiid mites. Ecological Entomology 25: 473-480.

Schausberger, P. and Croft, B.A. (2001) Kin recognition and larval cannibalism by adult females in specialist predaceous mites. Animal Behaviour 61: 459-464.

Schausberger, P. and Walzer, A. (2001) Combined versus single species release of predaceous mites: predator–predator interactions and pest suppression. Biological Control 20: 269-278.

Schausberger, P. (2003) Cannibalism among phytoseiid mites: a review. Experimental and Applied Acarology 29: 173-191.

Schausberger, P. (2007) Kin recognition by juvenile predatory mites: prior association or phenotype matching? Behavioral Ecology and Sociobiology 62: 119-125.

Schowalter, T.D. (2006) Insect ecology: an ecosystem approach. Academic Press.

Schumacher, S.K., Marsh, T.L. and Williams, K.A. (2006) Optimal pest control in greenhouse production of ornamental crops. Agricultural Economics 34: 39-50.

Seemann, J. (1979) Greenhouse climate. Agrometeorology. Springer, pp. 165-178.

Sengonca, C., Khan, I. and Blaeser, P. (2004) The predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) causes feeding scars on leaves and fruits of apple. Experimental and Applied Acarology 33: 45-53.

Sharpe, P.J.H. and Demichele, D.W. (1977) Reaction-Kinetics of Poikilotherm Development. Journal of Theoretical Biology 64: 649-670.

Shehata, K. and Weismann, L. (1972) Rearing the predacious mite *Phytoseiulus persimilis* Athias-Henriot on artificial diet (Acarina: Phytoseiidae). Biologia Bratislava 27: 609-615.

Shipp, J. and Wang, K. (2003) Evaluation of *Amblyseius cucumeris* (Acari: Phytoseiidae) and *Orius insidiosus* (Hemiptera: Anthocoridae) for control of *Frankliniella occidentalis* (Thysanoptera: Thripidae) on greenhouse tomatoes. Biological Control 28: 271-281.

Shipp, J. and Whitfield, G. (1991) Functional response of the predatory mite, *Amblyseius cucumeris* (Acari: Phytoseiidae), on western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Environmental Entomology 20: 694-699.

Shipp, L.J. (1995) Monitoring of western flower thrips on glasshouse and vegetable crops. Thrips Biology and Management. Springer, pp. 547-555.

Shipp, J., Ward, K. and Gillespie, T. (1996) Influence of temperature and vapor pressure deficit on the rate of predation by the predatory mite, *Amblyseius cucumeris*, on *Frankliniella occidentalis*. Entomologia Experimentalis Et Applicata 78: 31-38.

Siddiqui, W., Barlow, C. and Randolph, P. (1973) Effects of some constant and alternating temperatures on population growth of the pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphididae). The Canadian Entomologist 105: 145-156.

Simoni, S., Nannelli, R., Goggioli, D., Guidi, S. and Castagnoli, M. (2006) Biological and demographic parameters of *Neoseiulus californicus* (McGregors)(Acari Phytoseiidae) reared on two astigmatid mites. Redia 89: 59-63.

Singh, P. (1977) Artificial diets for insects, mites and spiders. IFI/Plenum Data Company.

Skirvin, D.J., Williams, M.E.D., Fenlon, J.S. and Sunderland, K.D. (2002) Modelling the effects of plant species on biocontrol effectiveness in ornamental nursery crops. Journal of Applied Ecology 39: 469-480.

Skirvin, D.J. and Fenlon, J.S. (2003) The effect of temperature on the functional response of *Phytoseiulus persimilis* (Acari: Phytoseiidae). Experimental and Applied Acarology 31: 37-49.

Skirvin, D.J. (2007) Modelling plant canopies for biocontrol and biodiversity. Functional-Structural Plant Modelling in Crop Production 22: 253-264.

Smallwood, P.D. (1996) An introduction to risk sensitivity: The use of Jensen's inequality to clarify evolutionary arguments of adaptation and constraint. American Zoologist 36: 392-401.

Smith, D. and Papacek, D. (1991) Studies of the predatory mite *Amblyseius victoriensis* (Acarina: Phytoseiidae) in citrus orchards in south-east Queensland: Control of *Tegolophus australis* and *Phyllocoptruta oleivora* (Acarina: Eriophyidae), effect of pesticides, alternative host plants and augmentative release. Experimental and Applied Acarology 12: 195-217.

Smytheman, P. (2011) Mite composition. European Patent Office, WO 2008/104807.

Solberg, Y. and Remedios, G. (1980) Chemical composition of pure and bee-collected pollen. Meldinger fra Norges Landbrukshoegskole 59: 2-12.

Stanyard, M.J., Foster, R.E. and Gibb, T.J. (1997) Effects of orchard ground cover and mite management options on the population dynamics of European red mite (Acari: Tetranychidae) and *Amblyseius fallacis* (Acari: Phytoseiidae) in apple. Journal of Economic Entomology 90: 595-603.

Starý, P. (1993) Alternative host and parasitoid in first method in aphid pest management in glasshouses. Journal of Applied Entomology 116: 187-191.

Stavrinides, M.C. and Mills, N.J. (2011) Influence of temperature on the reproductive and demographic parameters of two spider mite pests of vineyards and their natural predator. Biocontrol 56: 315-325.

Stenseth, C. (1979) Effect of temperature and humidity on the development of *Phytoseiulus persimilis* and its ability to regulate populations of *Tetranychus urticae* [Acarina: Phytoseiidae. Tetranychidae]. BioControl 24: 311-317.

Stewart, G.A. (1995) Dust mite allergens. Clinical reviews in allergy and immunology 13: 135-150.

Stinner, R., Gutierrez, A. and Butler, G. (1974) An algorithm for temperature-dependent growth rate simulation. The Canadian Entomologist 106: 519-524.

Stinner, R. (1977) Efficacy of inundative releases. Annual Review of Entomology 22: 515-531.

Strapazzon, A. and Monta, L. (1988) Role and distribution of *Amblyseius andersoni* (Chant) and Zetzellia mali (Ewing) in apple orchards infested by *Aculus schlechtendali* (Nalepa). Redia 71: 39-54.

Strong, W., Croft, B. and Slone, D. (1997) Spatial aggregation and refugia of the mites *Tetranychus urticae* and *Neoseiulus fallacis* (Acari: Tetranychidae, Phytoseiidae) on hop. Environmental Entomology 26: 859-865.

Styrsky, J.D. and Eubanks, M.D. (2007) Ecological consequences of interactions between ants and honeydew-producing insects. Proceedings of the Royal Society B: Biological Sciences 274: 151-164.

Swirski, E., Amitai, S. and Dorzia, N. (1967) Laboratory studies on feeding development and reproduction of predaceous mites *Amblyseius rubini* Swirski and Amitai and *Amblyseius* *swirskii* Athias (Acarina-Phytoseiidae) on various kinds of food substances. Israel Journal of Agricultural Research 17: 101-119.

Swirski, E. and Dorzia, N. (1968) Studies on the feeding, development and oviposition of the predaceous mite *Amblyseius limonicus* Garman & McGregor (Acarina: Phytoseiidae) on various kinds of food sources. The Israel Journal of Agricultural Research 18: 71-75.

Symondson, W., Sunderland, K. and Greenstone, M. (2002) Can generalist predators be effective biocontrol agents? 1. Annual Review of Entomology 47: 561-594.

Syngenta Bioline (2015) www3.syngenta.com

Takabayashi, J. and Dicke, M. (1996) Plant—carnivore mutualism through herbivore-induced carnivore attractants. Trends in Plant Science 1: 109-113.

Takahashi, F. and Chant, D. (1994) Adaptive strategies in the genus *Phytoseiulus* Evans (Acari: Phytoseiidae). II. Survivorship and reproduction. International Journal of Acarology 20: 87-97.

Tal, C., Coll, M. and Weibtraub, P. (2007) Biological control of *Polyphagotarsonemus latus* by the predaceous mite *Amblyseius swirskii*. IOBC/wprs Bulletin 30: 111-115.

Tanigoshi, L., Browne, R., Hoyt, S. and Lagier, R. (1976) Empirical analysis of variable temperature regimes on life stage development and population growth of *Tetranychus mcdanieli* (Acarina: Tetranychidae). Annals of the Entomological Society of America 69: 712-716.

Tantau, H.J. (1998) Energy saving potential of greenhouse climate control. Mathematics and computers in simulation 48: 93-101.

Tap, R., Van Willigenburg, L. and Van Straten, G., 1994. Experimental results of receding horizon optimal control of greenhouse climate. II IFAC/ISHS Workshop: Mathematical & Control Applications in Agriculture & Horticulture 406, pp. 229-238.

Tauber, M.J., Tauber, C.A. and Masaki, S. (1986) Seasonal adaptations of insects. Oxford University Press.

Teich, Y., 1966. Mites of family Phytoseiidae as predators of tobacco whitefly *Bemisia tabaci* Gennadius. The Israel Journal of Agricultural Research 16: 141-142.

Tentcheva, D., Gauthier, L., Zappulla, N., Dainat, B., Cousserans, F., Colin, M.E. and Bergoin, M. (2004) Prevalence and seasonal variations of six bee viruses in *Apis mellifera* L. and *Varroa destructo*r mite populations in France. Applied and environmental microbiology 70: 7185-7191.

Terblanche, J.S., Nyamukondiwa, C. and Kleynhans, E. (2010) Thermal variability alters climatic stress resistance and plastic responses in a globally invasive pest, the Mediterranean fruit fly (*Ceratitis capitata*). Entomologia Experimentalis Et Applicata 137: 304-315.

Tomanek, L. (2010) Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. The Journal of experimental biology 213: 971-979.

Toyoshima, S. and Amano, H. (1998) Effect of prey density on sex ratio of two predacious mites, *Phytoseiulus persimilis* and *Amblyseius womersleyi* (Acari: Phytoseiidae). Experimental and Applied Acarology 22: 709-723.

Treat, A.E. (1975) Mites of moths and butterflies. Comstock Publishing Associates, Cornell University Press.

Trichilo, P.J. and Leigh, T.F. (1986) Predation on spider mite eggs by the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), an opportunist in a cotton agroecosystem. Environmental Entomology 15: 821-825.

Tsoukanas, V., Papadopoulos, G., Fantinou, A. and Papadoulis, G.T. (2006) Temperature-dependent development and life table of *Iphiseius degenerans* (Acari: Phytoseiidae). Environmental Entomology 35: 212-218.

Turlings, T.C. and Wäckers, F. (2004) Recruitment of predators and parasitoids by herbivore-injured plants. Advances in Insect Chemical Ecology 2: 21-75.

Upton, M.S. (1993) Aqueous gum-chloral slide mounting media: an historical review. Bulletin of Entomological Research 83: 267-267.

van der Ploeg, A. and Heuvelink, E. (2005) Influence of sub-optimal temperature on tomato growth and yield. Journal of Horticultural Science & Biotechnology 80: 652-659.

van der Ploeg, A., Carvalho, S.M.P. and Heuvelink, E. (2009) Genotypic variation in the response to suboptimal temperature at different plant densities in cut chrysanthemum. Journal of the American Society for Horticultural Science 134: 31-40.

Van Dinh, N., Janssen, A. and Sabelis, M. (1988) Reproductive success of *Amblyseius idaeus* and *A. anonymus* on a diet of two-spotted spider mites. Experimental and Applied Acarology 4: 41-51.

van Houten, Y.M., van Rijn, P.C.J., Tanigoshi, L.K., van Stratum, P. and Bruin, J. (1995a) Preselection of predatory mites to improve year-round biological control of western flower thrips in greenhouse crops. Entomologia Experimentalis Et Applicata 74: 225-234.

van Houten, Y.M., van Stratum, P., Bruin, J. and Veerman, A. (1995b) Selection for non-diapause in *Amblyseius cucumeris* and *Amblyseius barkeri* and exploration of the effectiveness of selected strains for thrips control. Entomologia Experimentalis Et Applicata 77: 289-295.

van Houten, Y.M. and van Stratum, P. (1995) Control of western flower thrips on sweet pepper in winter with *Amblyseius cucumeris* (Oudemans) and *A. degenerans* Berlese. Thrips biology and management. Springer, pp. 245-248.

van Houten, Y.M., Hoogerbrugge, H. and Bolckmans, K.J. (2007) Spider mite control by four phytoseiid species with different degrees of polyphagy. IOBC/wprs Bulletin 30: 123-127.

van Houten, Y.M., Rothe, J. and Bolckmans, K.J.F. (2008) The generalist predator *Typhlodromalus limonicus* (Acari: Phytoseiidae): a potential biological control agent of thrips and whiteflies. IOBC/wprs Bulletin 32: 237-240.

van Houten, Y., Glas, J., Hoogerbrugge, H., Rothe, J., Bolckmans, K., Simoni, S., Van Arkel, J., Alba, J., Kant, M. and Sabelis, M. (2013) Herbivory-associated degradation of tomato trichomes and its impact on biological control of Aculops lycopersici. Experimental and Applied Acarology 60: 127-138.

Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W. and Tirry, L. (2010a) Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: A review. Insect biochemistry and molecular biology 40: 563-572.

Van Leeuwen, T., Witters, J., Nauen, R., Duso, C. and Tirry, L. (2010b) The control of eriophyoid mites: state of the art and future challenges. Eriophyoid Mites: Progress and Prognoses. Springer, pp. 205-224.

Van Leeuwen, T., Tirry, L., Yamamoto, A., Nauen, R. and Dermauw, W. (2014) The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. Pesticide Biochemistry and Physiology 121: 12-21.

van Lenteren, J. and Woets, J.v. (1988) Biological and integrated pest control in greenhouses. Annual Review of Entomology 33: 239-269.

van Lenteren, J., Babendreier, D., Bigler, F., Burgio, G., Hokkanen, H., Kuske, S., Loomans, A., Menzler-Hokkanen, I., Van Rijn, P. and Thomas, M. (2003) Environmental risk assessment of exotic natural enemies used in inundative biological control. BioControl 48: 3-38.

van Lenteren, J.C. (2000) A greenhouse without pesticides: fact or fantasy? Crop Protection 19: 375-384.

van Lenteren, J.C. (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl 57: 1-20.

van Maanen, R., Vila, E., Sabelis, M.W. and Janssen, A. (2010) Biological control of broad mites (*Polyphagotarsonemus latus*) with the generalist predator *Amblyseius swirskii*. Experimental and Applied Acarology 52: 29-34.

van Maanen, R., Broufas, G., Oveja, M.F., Sabelis, M.W. and Janssen, A. (2012) Intraguild predation among plant pests: western flower thrips larvae feed on whitefly crawlers. BioControl 57: 533-539.

van Rijn, P. and Sabelis, M. (1993) Does alternative food always enhance biological control? The effect of pollen on the interaction between western flower thrips and its predators [*Amblyseius cucumeris*]. IOBC/wprs Bulletin 16: 123-125.

van Rijn, P.C. and Tanigoshi, L.K. (1999a) The contribution of extrafloral nectar to survival and reproduction of the predatory mite *Iphiseius degenerans* on *Ricinus communis*. Experimental and Applied Acarology 23: 281-296.

van Rijn, P.C.J. and Tanigoshi, L.K. (1999b) Pollen as food for the predatory mites *Iphiseius degenerans* and *Neoseiulus cucumeris* (Acari: Phytoseiidae): dietary range and life history. Experimental and Applied Acarology 23: 785-802.

van Rijn, P.C.J., van Houten, Y.M. and Sabelis, M.W. (1999) Pollen improves thrips control with predatory mites. IOBC/wprs Bulletin 22: 209-212.

van Rijn, P.C., van Houten, Y.M. and Sabelis, M.W. (2002) How plants benefit from providing food to predators even when it is also edible to herbivores. Ecology 83: 2664-2679.

van Schelt, J., Tetteroo, A., Hoogerbrugge, H., Veenman, R. and Bolckmans, K. (2008) The release of beneficials in greenhouses with an air blower, a new wind in biocontrol. IOBC/wprs Bulletin 32: 245-248.

Van Stappen, G. (1996) Introduction, biology and ecology of *Artemia*. Manual on the production and use of live food for aquaculture: pp. 79-136.

Vandekerkhove, B., Parmentier, L., Van Stappen, G., Grenier, S., Febvay, G., Rey, M. and De Clercq, P. (2009) *Artemia* cysts as an alternative food for the predatory bug *Macrolophus pygmaeus*. Journal of Applied Entomology 133: 133-142.

Vangansbeke, D., Nguyen, D.T., Audenaert, J., Verhoeven, R., Deforce, K., Gobin, B., Tirry, L. and De Clercq, P. (2014) Diet-dependent cannibalism in the omnivorous phytoseiid mite *Amblydromalus limonicus*. Biological Control 74: 30-35.

Vanhaecke, P. and Sorgeloos, P. (1980) International study on *Artemia* .14. Growth and survival of *Artemia* larvae of different geographical origin in a standard culture test. Marine Ecology Progress Series 3: 303-307.

Vanharen, R.J.F., Steenhuis, M.M., Sabelis, M.W. and Deponti, O.M.B. (1987) Tomato stem trichomes and dispersal success of *Phytoseiulus persimilis* relative to its prey *Tetranychus urticae*. Experimental & applied acarology 3: 115-121.

Vänninen, I., Pinto, D., Nissinen, A., Johansen, N. and Shipp, L. (2010) In the light of new greenhouse technologies: 1. Plant-mediated effects of artificial lighting on arthropods and tritrophic interactions. Annals of Applied Biology 157: 393-414.

Vantornhout, I., Minnaert, H., Tirry, L. and De Clercq, P. (2004) Effect of pollen, natural prey and factitious prey on the development of *Iphiseius degenerans*. Biocontrol 49: 627-644.

Veerman, A. (1992) Diapause in phytoseiid mites: a review. Experimental and Applied Acarology 14: 1-60.

Vierbergen, G., Cean, M., Hatalane Szeller, I., Jenser, G., Masten, T. and Simala, M. (2006) Spread of two thrips pests in Europe: *Echinothrips americanus* and *Microcephalothrips abdominalis* (Thysanoptera: Thripidae). Acta Phytopathologica et Entomologica Hungarica 41: 287-296.

Völkl, W., Woodring, J., Fischer, M., Lorenz, M.W. and Hoffmann, K.H. (1999) Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. Oecologia 118: 483-491.

Wäckers, F., Zuber, D., Wunderlin, R. and Keller, F. (2001) The effect of herbivory on temporal and spatial dynamics of foliar nectar production in cotton and castor. Annals of Botany 87: 365-370.

Wäckers, F. (2005) Suitability of (extra-) floral nectar, pollen, and honeydew as insect food sources. . 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, New York: pp. 17-74.

Wäckers, F. and van Rijn, P. (2005) Food for protection: an introduction. 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, New York: 1-14.

Wäckers, F.L., Rijn, P.C.J. and Bruin, J. (2005) Plant-provided food for carnivorous insects: a protective mutualism and its applications. Cambridge University Press.

Wade, M.R., Zalucki, M.P., Wratten, S.D. and Robinson, K.A. (2008) Conservation biological control of arthropods using artificial food sprays: current status and future challenges. Biological Control 45: 185-199.

Wagner, T.L., Wu, H.-I., Sharpe, P.J., Schoolfield, R.M. and Coulson, R.N. (1984) Modeling insect development rates: a literature review and application of a biophysical model. Annals of the Entomological Society of America 77: 208-225.

Wagner, T., Olson, R. and Willers, J. (1991) Modeling arthropod development time. Journal of agricultural entomology (USA).

Waite, G., 2001. Managing spider mites in field-grown strawberries using *Phytoseiulus persimilis* and the ‘pest-in-first' technique. Acarology: Proceedings of the 10th International Congress. CSIRO Publishing, pp. 381-386.

Walde, S.J., Nyrop, J.P. and Hardman, J.M. (1992) Dynamics of *Panonychus ulmi* and *Typhlodromus pyri:* factors contributing to persistence. Experimental and Applied Acarology 14: 261-291.

Waldman, B. (1988) The ecology of kin recognition. Annual Review of Ecology and Systematics: 543-571.

Walter, D.E. and O'Dowd, D.J. (1992) Leaves with domatia have more mites. Ecology 73: 1514-1518.

Walter, D.E., O'Dowd, D.J., Lowman, M. and Nadkarni, N. (1995) Life on the forest phylloplane: hairs, little houses, and myriad mites. Forest canopies, pp. 325-351.

Walter, D.E. (1996) Living on leaves: mites, tomenta, and leaf domatia. Annual Review of Entomology 41: 101-114.

Walter, D.E. and Proctor, H.C. (1999) Mites: ecology, evolution, and behaviour. Springer.

Walzer, A. and Schausberger, P. (1999a) Cannibalism and interspecific predation in the phytoseiid mites *Phytoseiulus persimilis* and *Neoseiulus californicus*: predation rates and effects on reproduction and juvenile development. BioControl 43: 457-468.

Walzer, A. and Schausberger, P. (1999b) Predation preferences and discrimination between con- and heterospecific prey by the phytoseiid mites *Phytoseiulus persimilis* and *Neoseiulus californicus*. BioControl 43: 469-478.

Walzer, A., Blumel, S. and Schausberger, P. (2001) Population dynamics of interacting predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus*, held on detached bean leaves. Experimental and Applied Acarology 25: 731-743.

Walzer, A., Paulus, H.F. and Schausberger, P. (2004) Ontogenetic shifts in intraguild predation on thrips by phytoseiid mites: the relevance of body size and diet specialization. Bulletin of Entomological Research 94: 577-584.

Walzer, A., Castagnoli, M., Simoni, S., Liguori, M., Palevsky, E. and Schausberger, P. (2007) Intraspecific variation in humidity susceptibility of the predatory mite *Neoseiulus californicus*: Survival, development and reproduction. Biological Control 41: 42-52.

Walzer, A. and Schausberger, P. (2009) Non-consumptive effects of predatory mites on thrips and its host plant. Oikos 118: 934-940.

Walzer, A. and Schausberger, P. (2011) Threat-sensitive anti-intraguild predation behaviour: maternal strategies to reduce offspring predation risk in mites. Animal behaviour 81: 177-184.

Walzer, A. and Schausberger, P. (2013) Intra-and trans-generational costs of reduced female body size caused by food limitation early in life in mites. Plos One 8: e79089.

Way, M.J. (1963) Mutualism between ants and honeydew-producing Homoptera. Annual Review of Entomology 8: 307-344.

Weintraub, P. and Palevsky, E. (2008) Evaluation of the predatory mite, *Neoseiulus californicus*, for spider mite control on greenhouse sweet pepper under hot arid field conditions. Experimental and Applied Acarology 45: 29-37.

Weintraub, P., Kleitman, S., Shapira, N., Argov, Y. and Palevsky, E. (2006) Efficacy of *Phytoseiulus persimilis* versus *Neoseiulus californicus* for controlling spider mites on greenhouse sweet pepper. IOBC/wprs Bulletin 29: 121-125.

Weintraub, P.G., Kleitman, S., Mori, R., Shapira, N. and Palevsky, E. (2003) Control of the broad mite (*Polyphagotarsonemus latus* (Banks)) on organic greenhouse sweet peppers (*Capsicum annuum* L.) with the predatory mite, *Neoseiulus cucumeris* (Oudemans). Biological Control 27: 300-309.

Wimmer, D., Hoffmann, D. and Schausberger, P. (2008) Prey suitability of western flower thrips, *Frankliniella occidentalis*, and onion thrips, *Thrips tabaci*, for the predatory mite *Amblyseius swirskii*. Biocontrol Science and Technology 18: 533-542.

Wong, S.K. and Frank, S.D. (2012) Pollen increases fitness and abundance of *Orius insidiosus* Say (Heteroptera: Anthocoridae) on banker plants. Biological Control 64: 45-50.

Worner, S.P. (1992) Performance of phenological models under variable temperature regimes: consequences of the Kaufmann or rate summation effect. Environmental Entomology 21: 689-699.

Xia, B., Zou, Z., Li, P. and Lin, P. (2012) Effect of temperature on development and reproduction of *Neoseiulus barkeri* (Acari: Phytoseiidae) fed on *Aleuroglyphus ovatus*. Experimental and Applied Acarology 56: 33-41.

Xiao, Y., Avery, P., Chen, J., McKenzie, C. and Osborne, L. (2012) Ornamental pepper as banker plants for establishment of Amblyseius swirskii (Acari: Phytoseiidae) for biological control of multiple pests in greenhouse vegetable production. Biological Control 63: 279-286.

Xu, X.M. (1996) On estimating non-linear response of fungal development under fluctuating temperatures. Plant Pathology 45: 163-171.

Xu, X.N. and Enkegaard, A. (2010) Prey preference of the predatory mite, *Amblyseius swirskii* between first instar western flower thrips *Frankliniella occidentalis* and nymphs of the twospotted spider mite *Tetranychus urticae*. Journal of Insect Science 10: 149.

Yao, D. and Chant, D. (1989) Population growth and predation interference between two species of predatory phytoseiid mites (Acarina: Phytoseiidae) in interactive systems. Oecologia 80: 443-455.

Yee, W.L., Toscano, N.C., Chu, C.-C., Henneberry, T.J. and Nichols, R.L. (1996*) Bemisia argentifolii* (Homoptera: Aleyrodidae) action thresholds and cotton photosynthesis. Environmental Entomology 25: 1267-1273.

Yokoyama, V.Y. (1978) Relation of seasonal changes in extrafloral nectar and foliar protein and arthropod populations in cotton. Environmental Entomology 7: 799-802.

Zaher, M., El-Bishlawy, S. and Ali, F. (2007) Some ecological and biological studies on *Typhlodrompis swirskii* (Athias-Henriot) (Acari: Phytoseiidae). Acarines 1: 23-27.

Zhang, Z.Q. and Croft, B.A. (1994) A comparative life history study of immature *Amblyseius fallacis*, *Amblyseius andersoni*, *Typhlodromus occidentalis* and *Typhlodromus pyri* (Acari: Phytoseiidae) with a review of larval feeding patterns in the family. Experimental and Applied Acarology 18: 631-657.

Zhang, Z.-Q. and Croft, B.A. (1995) Interspecific competition and predation between immature *Amblyseius fallacis*, *Amblyseius andersoni*, *Typhlodromus occidentalis* and *Typhlodromus pyri* (Acari: Phytoseiidae). Experimental and Applied Acarology 19: 247-257.

Zhang, Z.Q. (2003) Mites of greenhouses: identification, biology and control. CABI International.

Zhi, J., Fitch, G.K., Margolies, D.C. and Nechols, J.R. (2005) Apple pollen as a supplemental food for the western flower thrips, Frankliniella occidentalis: response of individuals and populations. Entomologia Experimentalis Et Applicata 117: 185-192.

Zhimo, Z. and McMurtry, J. (1990) Development and reproduction of three *Euseius* (Acari: Phytoseiidae) species in the presence and absence of supplementary foods. Experimental and Applied Acarology 8: 233-242.

Zilahi-Balogh, G., Shipp, J., Cloutier, C. and Brodeur, J. (2007) Predation by *Neoseiulus cucumeris* on western flower thrips, and its oviposition on greenhouse cucumber under winter vs. summer conditions in a temperate climate. Biological Control 40: 160-167.

Zoebelein, G. (1956) Der Honigtau als Nahrung der Insekten: Teil I. Zeitschrift für Angewandte Entomologie 38: 369-416.

Appendices

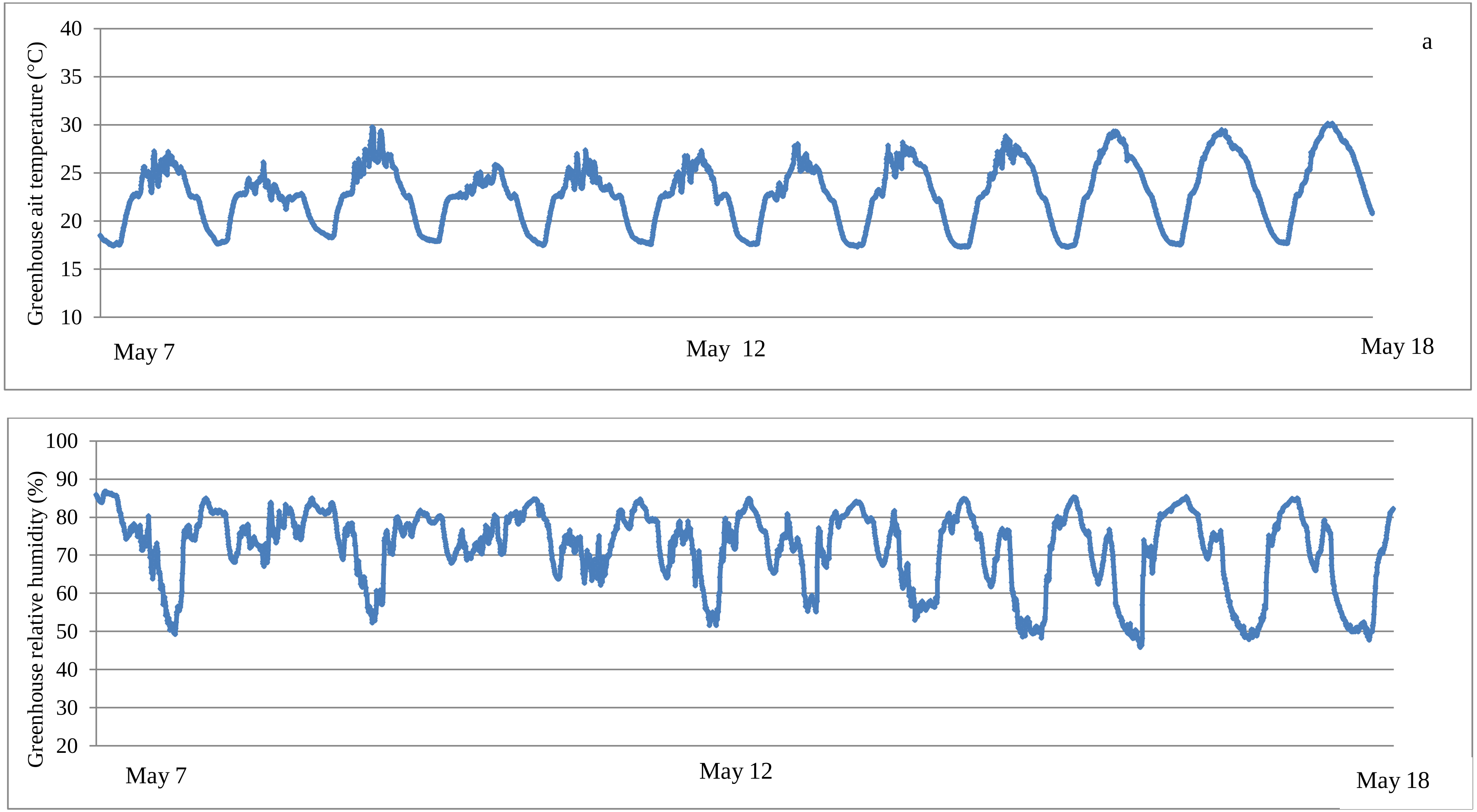

Figure A.1: Diurnal temperature (a) and relative humidity (b) variations between May 1 and May 12 for experiment 2

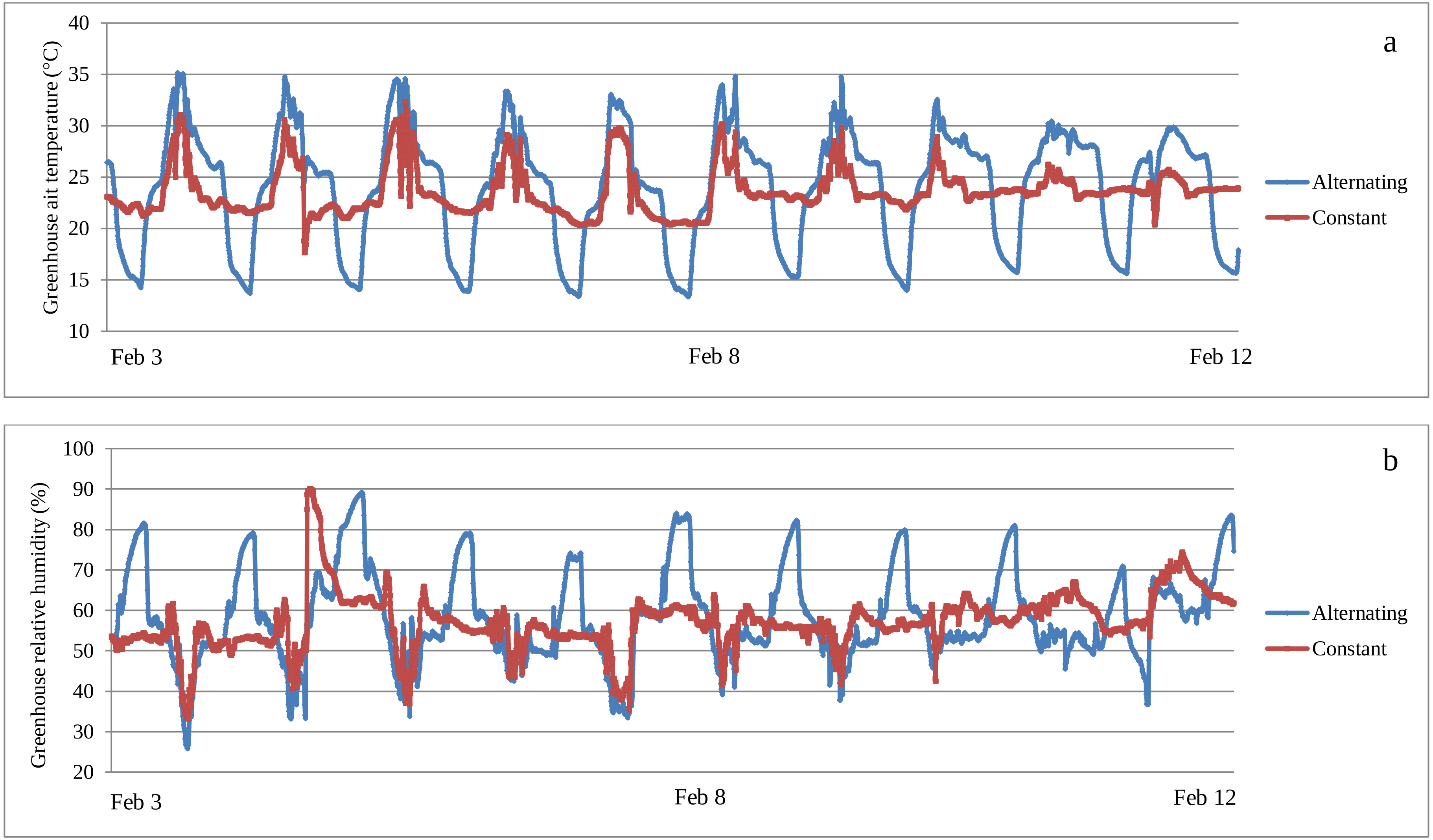

Figure A.2: Diurnal temperature (a) and relative humidity (b) variations between February 3 and February 12 for experiment 2

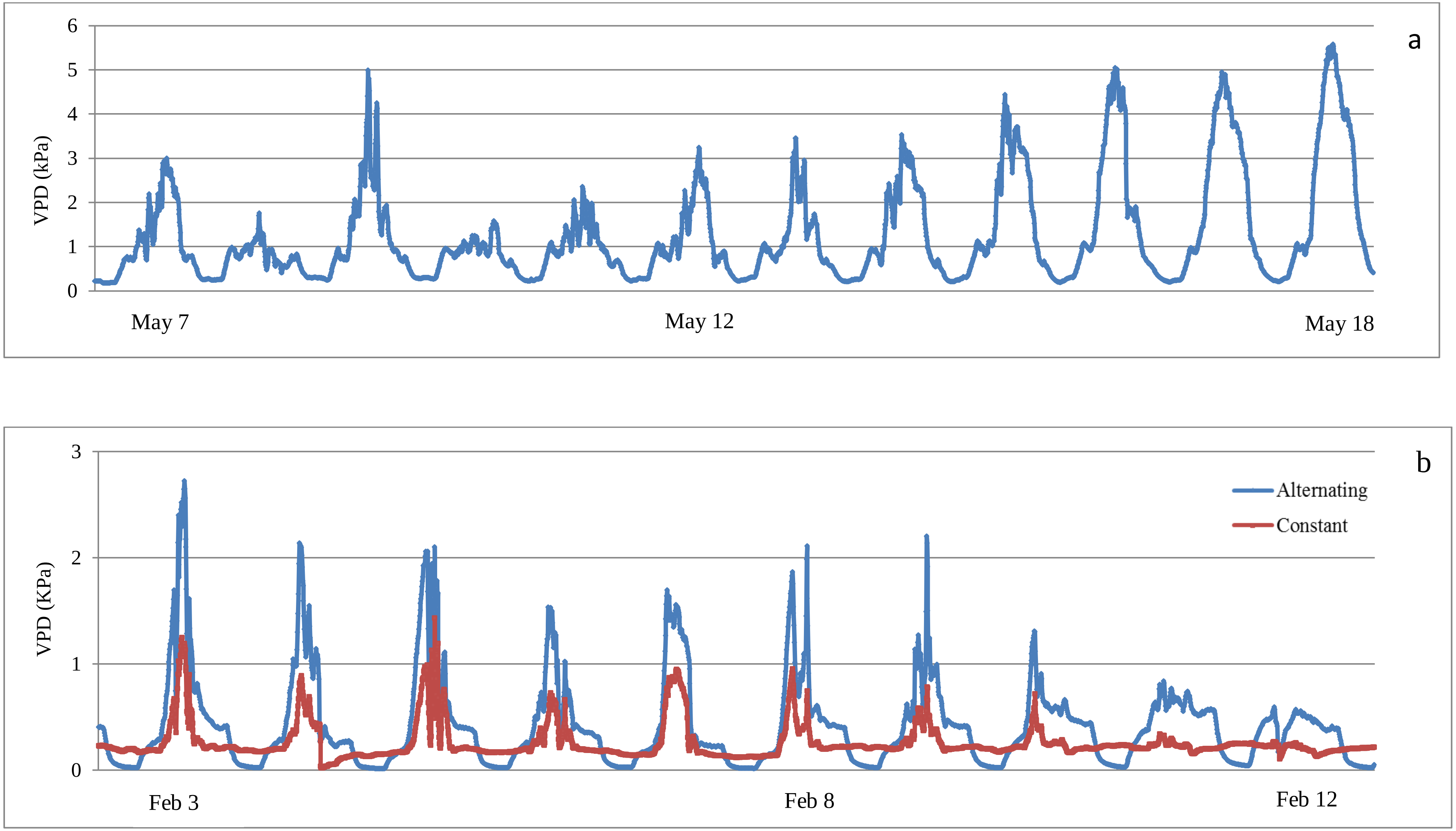

Figure A.3: Vapour pressure deficit (VPD) values (in kPa) of experiment 1 on chrysanthemum (a) and experiment 2 on ivy (b)

Curriculum vitae

1. Personalia

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2. Education

2011-2014: PhD Student at the Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Belgium

Promoters: Prof. Dr. Ir. P. De Clercq, Prof Dr. Ir. L. Tirry, Dr. B. Gobin

Thesis: Effects of temperature regime and food supplementation on the performance of phytoseiid mites as biological control agents

2008-2010: M.Sc. in Applied Biological Sciences

Agriculture (Major in Crop Protection)

Faculty of Bioscience Engineering, Ghent University, Belgium

Thesis: Alternatieve voedselbronnen voor roofwantsen van het genus *Orius* uit Zuid-Afrika

Promoter: Prof. Dr. Ir. P. De Clercq

2004-2008: B.Sc. in Applied Biological Sciences

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3. Professional career

2015-	Research assistent at the Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Belgium and Plant Sciences Unit, Crop Protection, Institute for Agricultural and Fisheries Research (ILVO), Merelbeke, Belgium
2011-2014:	PhD Student at the Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Belgium
Aug 2010 -Dec 2010	Laboratory assistant in Rogers Cooperations, Evergem

4. Scientific output

Publications in peer-reviewed international journals

Bonte, J., **Vangansbeke, D**., Maes, S., Bonte, M., Conlong, D. & De Clercq, P. (2012). Moisture source and diet affect development and reproduction of *Orius thripoborus* and *Orius naivashae*, two predatory anthocorids from Southern Africa. Journal of Insect Science 12: 1.

Vangansbeke D., De Schrijver, L., Spranghes, T., Audenaert, J., Verhoeven, R., Nguyen, D.T., Gobin, B., Tirry, L. & De Clercq, P. (2013). Alternating temperatures affect life table parameters of *Phytoseiulus persimilis*, *Neoseiulus californicus* (Acari: Phytoseiidae) and their prey *Tetranychus urticae* (Acari: Tetranychidae). Experimental and Applied Acarology 61: 285-298.

Nguyen, D.T., **Vangansbeke, D.,** Lü, X. & De Clercq, P., (2013). Development and reproduction of the predatory mite *Amblyseius swirskii* on artificial diets. BioControl 58: 369-377.

Vangansbeke D., Nguyen, D.T, Audenaert, J., Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. (2014). Performance of the predatory mite *Amblydromalus limonicus* on factitious foods. BioControl 59: 67-77.

Nguyen, D.T., **Vangansbeke, D**. & De Clercq, P., (2014). Artificial and factitious foods support the development and reproduction of the predatory mite *Amblyseius swirskii*. Experimental and Applied Acarology 62: 181-194.

Vangansbeke D., Nguyen, D.T, Audenaert, J., Verhoeven, R., Deforce K. Gobin, B., Tirry, L. & De Clercq, P. (2014). Diet-dependent cannibalism in the omnivorous phytoseiid mite *Amblydromalus limonicus.* Biological Control 74: 30-35.

Vangansbeke D., Nguyen, D.T, Audenaert, J., Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. (2014). Food supplementation affects interactions between a phytoseiid predator and its omnivorous prey. Biological Control 76: 95-100.

Nguyen, D.T., **Vangansbeke, D.** & De Clercq, P., (2014). Solid artificial diets for the phytoseiid predator Amblyseius swirskii. BioControl 59: 719-727.

Nguyen, D., Bouguet, V., Spranghers, T., **Vangansbeke, D**. & De Clercq, P., (2015). Beneficial effect of supplementing an artificial diet for *Amblyseius swirskii* with *Hermetia illucens* haemolymph. Journal of Applied Entomology 139: 342-351.

Vangansbeke D., Nguyen, D.T, Audenaert, J., Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. (2015). Supplemental food for *Amblyseius swirskii* in the control of thrips: feeding friend or foe? Pest Management Science. DOI 10.1002/ps.4000.

Nguyen, D.T., **Vangansbeke, D.** & De Clercq, P., (2015). Performance of four species of phytoseiid mites on artificial and natural diets. Biological Control 80: 56-62.

Vangansbeke D., Audenaert, J., Nguyen, D.T., Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. (2015). Diurnal Temperature Variations Affect Development of a Herbivorous Arthropod Pest and its Predators. PloS ONE 10 (4): e0124898.

Vangansbeke D., Audenaert, J., Verhoeven, R., Nguyen, D.T., Gobin, B., Tirry, L. & De Clercq, P. (2015). Prey consumption by phytoseiid spider mite predators as affected by diurnal temperature variations. BioControl DOI 10.1007/s10526-015-9677-0.

Meetings with oral and/or poster presentations

64th International Symposium on Crop Protection, May 22, 2012, Gent , Belgium

> Oral presentation: **Vangansbeke, D**., De Schrijver, L. , Spranghers, T., Audenaert, J. Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. The effect of alternating temperatures on life table parameters and predation capacity of three phytoseiid predators of the two-spotted spider mite)

International Symposium on Biological control of Arthropods, March 4-8, 2013, Pucón, Chili

> Poster Presentation: **Vangansbeke, D**. , Nguyen D. ,T., Gobin, B., Tirry, L. & De Clercq, P.. Alternating temperatures regimes affect development rates of *Phytoseiulus persimilis* Athias-Henriot and its target pest *Tetranychus urticae* Koch

65th International Symposium on Crop Protection, May 21, 2013, Gent, Belgium

> Oral presentation: **Vangansbeke, D.**, Audenaert, J., Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. Life history and predation rate of *Amblydromalus limonicus* Garman & McGregor (Acari: Phytoseiidae) fed on factitious foods

Fourth meeting of the IOBC meeting group "Integrated control of plant-feeding mites", September 9-12, 2013, Paphos, Cyprus

> Poster presentation: **Vangansbeke, D**., Nguyen, D.T., Audenaert, J. Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. The potential use of *Amblydromalus limonicus* to control the broad mite, *Polyphagotarsonemus latus*
>
> Oral presentation: **Vangansbeke, D**., Nguyen, D.T., Audenaert, J. Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. Implications of alternating temperature regimes for the biological control of the two-spotted spider mite, *Tetranychus urticae*. **Rewarded by Koppert Award for best student paper**

66th International Symposium on Crop Protection, May 20, 2014, Gent, Belgium

> Oral presentation: **Vangansbeke, D**., Nguyen, D.T., Audenaert, J. Verhoeven, R., Gobin, B., Tirry, L. & De Clercq, P. Food supplements for *Amblyseius swirskii* to enhance thrips control: less is more?

14th International congress of Acarology, July 13-18, 2014, Kyoto, Japan

Oral presentation: **Vangansbeke, D**., Nguyen, D.T., Audenaert, J., Verhoeven,R., Tirry, L. Gobin, B. & De Clercq, P. Diurnal temperature alternations affect dynamics between the two-spotted spider mite and its phytoseiid predators.

Poster presentation: : **Vangansbeke, D**., Nguyen, D.T., Audenaert, J., Verhoeven,R., Tirry, L. Gobin, B. & De Clercq, P. Selecting the optimal supplemental food source for thrips control with *Amblyseius swirskii*: less is more?

Conference of IOBC/WPRS Working Group on "Integrated Control in Protected Crops, Temperate Climate", September 14-18, 2014, Gent, Belgium

Poster presentation: **Vangansbeke, D**., Nguyen, D.T., Audenaert, J., Verhoeven,R., Tirry, L. Gobin, B. & De Clercq, P. Antipredator behavior of thrips towards phytoseiid eggs: Overrated or underestimated?

Oral presentation: **Vangansbeke, D**., Nguyen, D.T., Audenaert, J., Verhoeven,R., Tirry, L. Gobin, B. & De Clercq, P. Food supplements for *Amblyseius swirskii*: Supporting predator or prey populations? IOBC-WPRS Bulletin 102: 221-226.

Fifth meeting of the IOBC meeting group "Integrated control of plant-feeding mites", September 8-11, 2015, Castellon, Spain

Poster presentation: **Vangansbeke, D**., , Van Doren, E. Tirry, L., & De Clercq, P. Can we get phytoseiids to like *Echinothrips americanus?*

Poster presentation: **Vangansbeke, D**., Robyn, D., Pijnakker, J., Witters, J., Tirry, L. & De Clercq, P. *Euseius gallicus*: evidenceof thrips egg predation by a phytoseiid predator.

Oral presentation: **Vangansbeke, D**., Tirry, L. Gobin, B. & De Clercq, P. Are larger phytoseiids better biocontrol agents? **Awarded with Koppert student award for best oral communication**