The role of pollen as alternative food for predatory mites (Acari: Phytoseiidae)

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# Abbreviations

## Amino acids

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<tr>
<td>ALA</td>
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<td>ARG</td>
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<td>ASN</td>
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<td>ASP</td>
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## Chemical elements:

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1. General introduction

Biological control

Biological control is “the use of natural enemies for the reduction of pests, diseases and weeds” (sic, van Lenteren (1997), which can successfully regulate pest population densities below the economical damage threshold in greenhouses and in the field (De Clercq 2002). Compared to chemical control, biological control has many advantages, such as no pesticide residues on plant products, no waiting periods before harvest, low risk of environmental pollution, no phytotoxic effects, or high acceptance by consumers and lower costs (van Lenteren 2000ab).

Generally, practical biological control follows three general strategies: conservation of antagonists, importation, and augmentation of natural enemies, which require different scientific approaches to find candidate biocontrol agents by basic research for later implementation (van Lenteren 2012).

Conservation biological control is the first, but often neglected, strategy of biological control to improve the efficacy of natural enemies through habitat management or by modification of existing chemical control towards less or no hazardous effects on beneficials. However, this approach in biological control requires in-depth knowledge of the ecology of natural enemies and their particular target species, and the more or less complex ecological system they belong to. Besides inundation strategy, where a grower release mass-reared beneficials similar to the use of a chemical pesticide, conservation biological control is a practice which individual growers can adopt easier than classical, inoculative biological control schemes which are usually coordinated at a larger than farm scale (Rabb et al. 1976; Caltagirone 1981; van Lenteren 1988; Jonsson et al. 2008).

Importation and subsequent augmentation by inoculation, also known as “classical biological control” refers to use and establishment of allochthonous organisms, mostly to control alien, invasive species (Barbosa 1998; Bale et al. 2008; Barratt et al. 2010; De Clercq et al. 2011; Naranjo et al. 2015). Augmentation in general is distinguished by long-term aims of biocontrol and reverts to artificially mass-reared antagonists to be released: inoculative releases aim towards use of lower number of beneficials providing their offspring during season, and inundative releases aim towards rapid control by use of high numbers of beneficials released, without or low expected contribution of their offspring to suppress a pest population (van Lenteren and Woets 1988; van Driesche and Bellows 1996). However, irrespectively the strategy followed, antagonist species have to be conserved by no use of broad spectrum pesticides, by supply of ecological
requisites, e.g. alternative or supplementary food, and by establishing of optimal abiotic conditions for antagonists, if possible (Wäckers et al. 2005, 2008; Winkler et al. 2006; Zannou et al. 2005; Aguilar-Fenollosa et al. 2011; Cruz et al. 2012).

About 230 species of natural enemies are commercially available worldwide for pure biological pest control and integrated pest management in greenhouses and in the field, among which the beneficial arthropods, represented by 219 species, dominate (Cock et al. 2010; van Lenteren 2012).

In contrast to field pests, the invasion of pests into greenhouses is expected, however, not predictable by time. Thus, to ensure the establishment of an antagonist in greenhouses before pest invasion, alternative food must be provided. Otherwise, when the target prey or host species is lacking, the antagonist population will be extinct (Overmeer 1985).

The outstanding example of biocontrol agents used in inundative augmentative releases in the field and greenhouses on more than 10 Mha worldwide are egg parasitoids of the genus *Trichogramma* (Hymenoptera), parasitizing various lepidopteran pests, such as the sugarcane-borer *Diatraea saccharalis* F., the European corn borer *Ostrinia nubilalis* Hbn., or noctuid species attacking vegetables or cotton, such as *Helicoverpa armigera* Hbn. or *Heliothis virescens* F. (Smith 1996; van Lenteren and Bueno 2003; Zimmermann 2004).

Compared to field conditions, greenhouses are almost closed ecological systems with all their positive and negative aspects (Enkegaard and Brodsgaard 2005; Messelink et al. 2012). Temperature, light, and fertilizer regimes can be optimized according the crop’s specific requirements to enhance plant growth (Paulitz and Bélanger 2001). However, often these conditions are also the best conditions to let crop pests establish in greenhouses and to foster pest mass outbreaks if not controlled.

On the other hand, a closed system also allows create the best environmental conditions for bio-control agents, including subtropical or tropical antagonist species to control invasive pests, and to prevent evasion of the beneficials (van Lenteren and Woets 1988; Gerson and Weintraub 2007). Biological control gains a higher importance in greenhouse crops where the economical value of crops per area is significantly superior to field crops. Furthermore, release techniques are simpler, the number of plants is manageable, and supplementary or alternative food can be
supplied in large quantities near to the beneficials or sprayed (van Lenteren 2000ab; Coll and Guershon 2002; van Driesche et al. 2002; Opit et al. 2005; Wade et al. 2008; Huang et al. 2011; Waite et al. 2014). These frame conditions are considered the most important reasons for successful biocontrol in greenhouses on ca. 40,000 ha worldwide (van Lenteren and Woets 1988; van Lenteren 2000ab; De Clerq 2002; Bale et al. 2008).

The early approaches and practical implementations of biological control in greenhouses followed inundative release of antagonists, however, turned into long-term establishment of predators and parasitoids wherever a year-round crop production is practiced. Among the vast range of beneficial arthropods, aphid and whitefly parasitoids, predacious gallmidges, and predatory mites are the major groups of beneficials used in long-term strategies in biocontrol of arthropod pest of greenhouse crops (Huang et al. 2011; Gerson and Weintraub 2012; Parolin et al. 2012; Messelink et al. 2014). This shift from inundative to inoculative release of antagonists is only possible when alternative food/hosts or prey is offered by banker plants to let the biocontrol agents survive and maintain a stable population when the target pest is scarce or even lacking.

However, the potential of natural antagonists has not been completely exploited. Although effective antagonists are known and available, lack of uptake has been reported (van Lenteren 2012).

**Banker plants in biological control**

In the development of biological pest control, different strategies were used to improve predator efficacy. A first approach was the “pest-in-first” strategy, where the pests, expected to show up later, are artificially introduced into the greenhouses at low population densities to provide the beneficials with a starter host population to establish. However, some growers considered this strategy risky, were reluctant, and had to be convinced to release the pest attacking their valuable crops (Markkula and Tiittanen 1976; Stacey 1977; van Lenteren et al. 2000ab).

A more accepted and later adapted strategy is the provision of supplementary food sources. Sugar mites, used as alternative prey in mass rearing of predatory mites and dispersed in small sachets for “slowrelease strategy” in greenhouses, may serve as supplementary food while early establishment of predacious mites (Huang et al. 2011). However, this stock of alternative food is exploited soon and needs additional food sources during the growing season, and is not suitable for parasitoids. Additional food sources may be provided by companion or banker plants, hosting substitute prey or host species for less specialised predators or parasitoids, such as aphid species
not attacking the cash crop, also named “open rearing” to facilitate a fast and long-lasting establishment of antagonists and to stabilize predator-prey population dynamics in biological control (Stacey 1977; Landis et al. 2000; Huang et al. 2011; Parolin et al. 2012). One of the best and early examples of the positive effects banker plants in German greenhouses has been given by Albert (1995) who used wheat or maize infested with grain aphids as alternative hosts for parasitoids of cucumber aphids, which is now a well established “open rearing” system in German vegetable greenhouses and keeps the aphid infestation below the economic threshold over the complete cucumber growing season (Bünger et al. 1997, 1999; Bennison 1992; Bennison and Corless 1993).

The approach to establish supplementary or alternative food may be easier when general antagonists, depending on their life history and preferences, are facultive or opportunistic phytophagous, feeding on pollen, nectar and plant sap (Overmeer 1985; Legaspi and Legaspi 1998; Limburg and Rosenheim 2000; Lockwood et al. 2001; Coll and Guershon 2002).

Predatory bugs as commonly used predators of different pest species can establish in sweet-pepper crops in greenhouses even when prey is scarce, feeding only on pollen of these plants (van den Meiracker and Ramakers 1991; Coll and Guershon 2002; Baez et al. 2004; Brodsgaard 2004).

A likewise common “open rearing” system has been widely established for phytoseiid mites, where mostly *Ricinus communis* plants are used as banker plants providing pollen and nectar as alternative food (Ramakers and Voet 1995, 1996; van Rijn and Tanigoshi 1999ab; Pratt and Croft 2000; van Rijn et al. 2002; Hoogerbrugge et al. 2008; Nomikou et al. 2010). Xiao et al. (2012) evaluated first ornamental pepper varieties as banker plants for the predatory mite *A. swirskii* preying on *Frankliniella occidentalis* (Pergande), *Scirtothrips dorsalis* Hood, and *Bemisia tabaci* (Genn.) providing pollen as alternative food, which resulted in a significantly improved performance of this predator species.

However, depending on the particular life-style, open rearing systems are not feasible for all phytoseiid mite species used in biocontrol.
**Predatory mites as biocontrol agents**

Predatory mites of the family Phytoseiidae (Acari, Mesostigmata) are distinguished into three sub-families: Amblysiinae, Typhlodrominae and Phytoseiinae, with 84 genera in total. Estimates of species number vary from 1,600 to 2,300 species (Gerson et al. 2003; Zhang 2003; de Moraes et al. 2004; Chant and McMurtry 2007), but a more recent report lists 2,692 species (Prasad 2012). Phytoseiids are distributed worldwide from the palaearctic and nearctic to the tropical biogeographic regions. Thus, species of this family are adapted to almost all ecological conditions (Overmeer 1985; Tixier et al. 2008).

Biocontrol efficacy and their adaptation to greenhouse conditions made the phytoseiids popular in biocontrol practice and interesting for commercial production. Worldwide, twenty species are commercially available (Zhang 2003). Widely used species in Europe are *Typhlodromus pyri* (Scheuten), *Phytoseiulus persimilis* (Athias-Henriot), *Neoseiulus californicus* (McGregor), *N. cucumeris* (Oudemans), *N. barkeri* (Hughes), *Amblyseius swirskii* (Athias-Henriot), *A. aberrans* (Oudemans), *A. andersoni* (Chant), *A. potentiella* (Garman), *Amblydromalus limonicus* (Garman and McGregor), *Euseius finlandicus* (Oudemans), and *Transeius montdorensis* (Schicha) (Gerson et al. 2003; Gerson and Weintraub 2007; Cock et al. 2010; van Lenteren 2012).

**Morphology**

The body of a typical phytoseiid mite consists of two parts: the anterior gnathosoma with the chelicera mouthparts and pedipalps and the posterior idiosoma (Karg 1994; Gerson et al. 2003; Zhang 2003). Body size of males and females differs between species, with females of ca. 300-500 µm, whereas the males being usually smaller than females (Evans 1992; Houck 1994; Walter and Proctor 1999, Collyer 1982; Beard 2001).

Tixier et al. 2012 analysed 2,122 mite species of Phytoseiidae by body size and found that body size of adult females of the subfamily Amblyseiinae was superior to Phytoseiinae and Typhlodrominae. Also, life-style is assumed to determine body size as specialist mites are known to be bigger than generalist species (Chant and McMurtry 1994; Croft et al. 1999; Jung and Croft 2001). Nevertheless, also food quality may affect body size (Vangansbeke et al. 2014).

**Mouthpart morphology**

The mouthparts of predatory mites consist of two parts: chelicerae and pedipalps. The chelicerae, consisting of movable and fixed, dentate digits, serve food consumption by catching and crushing their prey, while the pedipalps hold the food. The morphology of chelicerae is correlated
with the particular life-style of a phytoseiid species. Chemosensory and thigmotactic setae on the pedipalps help localisation of prey (Flechtmann and McMurtry 1992ab; Swirskii et al. 1998). Proteolytic enzymes in the saliva, produced by salivary glands in the gnathosoma and released into the chelicerae, liquify the prey ready to imbibe into the oesophagus (Karg 1994; Swirski et al. 1998). By feeding, predatory mites change their body colour according the colour of their food source (Engel 1991; Momen and El-Saway 1993).

**Biology of predatory mites**

*Development and reproduction of phytoseiid mites*

Starting with the egg stage, predatory mites develop into the larva, followed by the proonymph and deutonymph stages, and then into adults (females and males), separated by moults between the mobile stages (Abdallah et al. 2001). Except the larva (three pairs of legs), all following stages and adults stages are eight-legged (Bonde 1989). All mobile stages need to feed, except some phytoseiid species, such as *Phytoseiulus persimilis* Athias-Henriot, *P. macropilis* (Banks), *Kampimodromus aberrans* Oudemans, *Neoseiulus longispinosus* (Evans), *N. cucumeris* (Oudemans), *N. barkeri* Hughes, and *Typhlodromus pyri* Scheuten, in which the larvae are non-feeders, being able to develop into proonymphs also without food uptake (Schausberger and Croft 1999).

After mating, fertilized females produce 1 - 3 eggs per day, depending on the food quality and on environmental conditions (Momen and El-Saway 1993; Park et al. 2010; Nguyen et al. 2013). The highest egg production is achieved in the first 10 days from start of oviposition (Ragusa et al. 2009). Eggs are oval, transparent and small in size, with about one-third to half the length of the idiosoma of the female (Karg 1994). The eggs from phytoseiid mites are very sensitive to humidity, depending from mite species with different environmental adaptation, and too high or too low humidity will cause high mortality (Sabelis 1985; Zhang 2003; Ferrero et al. 2010). Egg mass, produced by one female per day, occasionally may constitute more than 60% of their body weight (Yao and Chant 1990).

Duration of preimaginal development and adult performance is governed by abiotic conditions, mainly by temperature (Lee and Ahn 2000; Lee and Gillespie 2011; Hewitt et al. 2015) and food quality as biotic factor (Abou-Awad et al. 1992; Vantomhout et al. 2004, 2005; Lorenzon et al. 2012; Nguyen and Shih 2012).
Lifestyles of Phytoseiidae

Phytoseiids are very mobile, however differing in behaviour, dispersion, and searching activity (van de Vrie 1985; Jung and Croft 2001; Buitenhuys et al. 2010). In a take-all approach, phytoseiids can be distinguished by their life-style.

According to McMurtry and Croft (1997) and McMurtry et al. (2013), predatory mites are categorized into generalists and specialists based on their food habits and on morphological and biological traits. Specialized mites can feed only one kind of animal food, like *P. persimilis* preying on spider mites (McMurtry and Croft 1997). Generalist mites have a wide food spectrum: *viz.* mites, eggs of Lepidoptera species (Momen and El-Laithy 2007; Momen and El-Sawi 2008; Momen 2009; van Maanen et al. 2010), thrips (Sengonca et al. 2004; Messelink et al. 2006), whitefly (Nomikou et al. 2001), honeydew (James 1989; Nomikou et al. 2003), plant exudates (James 1989; Gnanvossou et al. 2005), nectar (van Rijn and Tanigoshi 1999b), pollen (Al-Shammery 2011; Kolokytha et al. 2011), and fungi (Zemek and Prenerová 1997; Pozzebon and Duso 2008). McMurtry et al. (2013) revised the lifestyles and added some new modifications concerning their adaptation to prey and habitat.

Specialist mite species are distinguished into 2 types with grouping into subtypes:

**Lifestyle type I**

According to prey suitability, subtype 1-a species are specialized on spider mites of the genus *Tetranychus* (Tetranychidae) and is best represented by *Phytoseiulus persimilis*, subtypes 1-b species are specialized to feed on web-nest producing mites (Tetranychidae), and subtype 1-c are specialized predators on tydeoids (super-family Tydeoidea).

**Lifestyle type II**

These species are, similar to the old classification by McMurtry and Croft (1997), selective predators of tetranychid mites of various genera. *Neoseiulus fallacis*, a potent antagonist of tetranychid mites in the field and greenhouses.

Generalist predators with a wide food spectrum have been separated into two lifestyles, the generalist predators and the pollen feeding generalist predators.

**Lifestyle type III**

The old type III classification, the generalist predators, was recently distinguished into 5 sub-types according to microhabitat occupancy and their morphology:
Subtype III-a species are generalist mites found on pubescent leaves, whereas generalist mite species found on glabrous leaves have been put into subtype III–b. Generalist mite species living on dicotyledonous plants belong to subtype III–c; those on monocotyledonous plants to III–d, and III–e are generalist species living in soil habitats. For example, *Kampimodromus aberrans* (Oudemans) belongs to subtype III-a, *A. swirskii* and *A. limonis* belong to subtypes III-b. Representatives of subtype III-c are *Amblyseius herbicola* (Chant) and *Euseius hibisci* (Chant), a subtype III-d species is *Neoseiulus baraki* (Athias-Henriot). *Neoseiulus barkeri* (Hughes) and *Neoseiulus cucumeris* (Oudemans) belong to subtype III-e, which occupies soil habitats, to mention only a selection of representative species.

*Lifestyle type IV*

Type IV species are generalist predators, for which pollen constitute an important part of the diet, comprising the genera *Euseius*, *Iphiseius* and *Iphiseiodes*.

Possibly further types may exist, being more specialized for certain prey, as validated by Adar et al. (2012), who proposed that the ability to feed on plants should be added as a cross type trait of phytoseiid life-style types. The discovery of a phytoseiid whose main food source is the coffee leaf rust fungus (*Oliveira et al. 2014*), suggests that more life styles may be discovered.

Lifestyle classification explained the role of predatory mites in biological control and is helpful for implementation of mite species in biological and IPM programmes on different crops against various pests (*McMurtry and Croft 1997*).

*Body size in relation to prey specificity and antagonistic potential*

The small sizes of phytoseid mites limit their predation success. Large prey may be invulnerable to predators, and small juvenile predators vulnerable to attack by prey and prey defense may be perilous. Overcoming prey defense requires specific physical capabilities and/or morphological adaptations to the prey attacked, or a kind of parental care and defense of offspring by adult predatory mites (*Bonde 1989; Walzer et al. 2004; Magalhães et al. 2005; Schausberger et al. 2010*).

Body weight, dependent on food quality, is an important feature of phytoseid mites, which directly determine survival rates, level of physical fitness, and, indirectly, predation and reproduction rates, which allows estimate success of biological pest control. Size, sex, and age of predatory mites also affect consumption rates (*Sabelis 1981; Yao and Chant 1990; Baier and Karg 1992; Cedola et al. 2001; Reis et al. 2003; Hussein and Momen 2010; Walzer and Schausberger 2011*).
Life-table parameters

Any abiotic and biotic factor may affect the individual, cohort, or population performance of predatory mites, which must be assessed and described by a standardised method. The appropriate approach to study the dynamics of animal populations is the calculation of fertility life-tables and demographic parameters (Birch 1948; Maia et al. 2000; Southwood and Henderson 2000). Demographic parameters usually estimated from fertility life-tables are: the net reproductive rate ($R_0$), the intrinsic rate of increase ($r_m$); the mean generation time ($T$); the doubling time ($D_t$), and the finite rate of increase ($l$) (Maia et al. 2000).

Although these synthetic derivatives of fertility life-tables summarize information on immature development, reproduction, and survival, duration of preimaginal development and average cohort developmental stage requires a separate calculation to assess vulnerable developmental stages to an environmental parameter. To determine the speed of development from eggs up to adults, the relative (cohort) developmental stage (rDS) is calculated (Zebitz 1984). Further parameters to estimate specific effects of test factors on adults are longevity of adults in both sexes and reproductive parameters of females, such as preoviposition period, reproduction period, and postoviposition period to assess sensitive periods in adult life of insects or mites, proved to be suitable parameters to compare the effects of environmental conditions on predatory mites (Camporese and Duso 1995; Lee and Ahn 2000; Lorenzon et al. 2012).

Research aims

Because of their omnivore character and different adaptation strategies, especially type III generalists are of particular interest for future research in biocontrol (Croft et al. 2004; Knapp et al. 2013). To improve mite performance and faster establishment, implementation of alternative diets is of great importance. Pollen as alternative or supplementary food for predatory mites is used in modern approaches of biocontrol in greenhouses (Weintraub et al. 2009; Nomikou et al. 2010), however, pollen of different plant species differ in their chemical composition (Stanley and Linskens 1974) and may also exert negative effects on predatory mites. This may probably due to poor nutritional quality or bad adaptation of mites to pollen as food source (Ragusa et al. 2009). The range of pollen as alternative food is not studied sufficiently for the majority of generalist phytoseid mites. Additionally studies on more mite species and a wider range of plant pollen are necessary for a better understanding of mite species specific food biology (van Rijn and Tanigoshi 1999a).
Thus, in this work the pollen range of the commercially available phytoseid mite species *Amblyseius swirskii* (Athias-Henriot), *Neoseiulus cucumeris* (Oudemans) and *Amblydromalus limonicus* Garman and McGregor, and their respective performance when fed with pollen was investigated to clarify the nutritional value of pollen and possible pollen food adaptations by the three mite species.

**Publications included in this thesis**


2. Suitability of different pollen as alternative food for the predatory mite *Amblyseius swirskii* (Acari, Phytoseiidae)

Abstract

The predacious mite *Amblyseius swirskii* Athias-Henriot is used as a biological control agent against various pests in greenhouses. Pollen offered as supplementary food is reported to improve their fast establishment and performance. However, the nutritional suitability of different pollens for *A. swirskii* is not sufficiently known yet.

Pollens of 21 plant species were offered to the mites as exclusive food during preimaginal development. Preimaginal mortality and developmental time have been assessed, followed by a life-table analysis of the emerged adults and a calculation of demographic parameters. *Amblyseius swirskii* can feed exclusively on pollen, but the nutritional value of the pollens differed significantly. Pollens of *Lilium martagon* and *Hippeastrum* sp. were toxic, causing 100 % preimaginal mortality, probably due to secondary plant compounds. *Hibiscus syriacus* pollen was absolutely incompatible for the juvenile and adult mites, possibly due to their external morphology, differing from all the other pollens tested and leading to 100 % preimaginal mortality also. Considering all parameters, feeding on *Aesculus hippocastanum*, *Crocus vernus*, *Echinocereus* sp. and *Paulownia tomentosa* pollens lead to the best performance of the mites. Feeding on most pollens resulted in no or low preimaginal mortality of *A. swirskii*, but affected significantly developmental time, adult longevity, and reproduction parameters. Commercial bee pollen was not able to improve life-table parameters compared to pure pollen of the plant species. Pollens of *Helianthus annuus*, *Corylus avellana* and a Poaceae mix were less suitable as food source and resulted in a poor performance of all tested parameters. Compared with literature data, 18 pollens tested proved to be a similar or better food source than cattail pollen, qualifying *A. swirskii* as a positively omnivorous type IV species. Pollens of *Ricinus communis* and *Zea mays* can be recommended as supplementary food offered as banker plants, and *A. hippocastanum* and *Betula pendula* pollen is recommended to be used as dispersible pollen in greenhouses.

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3. Life tables of *Neoseiulus cucumeris* exclusively fed with seven different pollens

Abstract

The juvenile development and survival, and demographic parameters of the predatory mite *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae) fed on pollen of castor bean, tulip, apple, *Christmas cactus*, horse-chestnut, maize, and birch were assessed under laboratory conditions. Deprivation of food and pollen of castor bean plants resulted in 100% juvenile mite mortality. Feeding mites with tulip and horse-chestnut pollen resulted in the shortest development and the highest total fecundity. Adult mites fed on birch, tulip, maize, and apple pollen lived significantly longer compared with those fed on pollen of horse-chestnut and *Christmas cactus*. The intrinsic rate of natural increase ranged between 0.1013 1/day for maize and 0.1806 1/day for horse-chestnut pollen as food. Net reproductive rate was the lowest when fed with maize pollen and highest when fed with horse-chestnut pollen. Population doubling time was highest on maize pollen and shortest on horse-chestnut pollen. Our study revealed that birch, tulip, horse chestnut, apple, and maize pollen can be used by *N. cucumeris* from early spring to late summer as a suitable alternative food in periods when prey in the field are scarce or absent.

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Abstract

The nutritional quality of pollen was assessed measuring biomass and body size of freshly emerged, 1, 3, and 30 days old adult *Amblyseius swirskii* continuously reared on pollen of *Betula pendula*, *Helianthus annuus*, *Paulownia tomentosa*, and *Zea mays*. Body weight of females was significantly higher than that of males, irrespectively both, the pollen fed and the age class. Weight of freshly emerged females was significantly superior when fed with pollen of *P. tomentosa* and *Z. mays* compared to those fed with *B. pendula* or *H. annuus* pollen. Biomass of females significantly increased until the age of 3 days, then remaining constant or decreasing until the age of 30 days. Weight gain with ageing is explained by higher food uptake by females for egg production and egg maturation. Weight gain of males also reached its maximum at day 3 of adult life, then decreasing until day 30. Congruent with biomass, adult body length and width differed significantly between sex and age showing females bigger than males and 30 day old adults bigger than 1 day old adults irrespectively the pollen fed. Both the parameters were affected by pollen, revealing *P. tomentosa* as best food source, followed by *Z. mays* and *B. pendula*. No reasonable correlation was found between body length and width, but body weight was significantly correlated with body length in females whereas there was no correlation at all in males.

5. Dietary effects on body weight of predatory mites (Acari, Phytoseiidae)

Abstract

Pollen is offered as alternative or supplementary food for predacious mites; however, it may vary in its nutritional value. Body weight appears a representative parameter to describe food quality. Thus, we assessed the body weight for adults of the generalist mites *Amblyseius swirskii*, *Amblydromalus limonicus*, and *Neoseiulus cucumeris* reared on 22, 12, and 6 pollen species, respectively. In addition, *A. swirskii* and *A. limonicus* was reared on codling moth eggs. In all mite species, female body weight was higher than that of males, ranging between 4.33 and 8.18 µg for *A. swirskii*, 2.56–6.53 µg for *A. limonicus*, and 4.66–5.92 µg for *N. cucumeris*. Male body weight ranged between 1.78 and 3.28 µg, 1.37–3.06 µg, and 2.73–3.03 µg, respectively. Nutritional quality of pollen was neither consistent among the mite species nor among sex, revealing superior quality of *Quercus macranthera* pollen for females of *A. swirskii* and *Tulipa gesneriana* pollen for males, *Alnus incana* pollen for females of *A. limonicus* and *Aesculus hippocastanum* pollen for males, and *Ae. hippocastanum* pollen for both sexes of *N. cucumeris*. The results are discussed against the background of known or putative pollen chemistry and mite’s nutritional physiology.

6. General discussion

**Biological control using predatory mites (Phytoseiidae)**

**History**

In the second half of the last century, the predatory mite family *Phytoseiidae* has been considered a successful biological control agent of numerous pest species of field and greenhouse crops (Dosse 1959; Bravenboer and Dosse 1962; van Lenteren and Woets 1988; Luh and Croft 2001; Gerson et al. 2003; Croft et al. 2004; de Moraes et al. 2004; Cock et al. 2010; Gerson and Weintraub 2007, 2012).

In early approaches, *Neoseiulus reticulatus* (Oudemans) was released to control cyclamen mites in strawberries (Huffaker and Kennett 1953, 1956). However, specialised or oligophagous phytoseiid mites, such as *Phytoseiulus persimilis* Athias-Henriot (syn. *riegeli* Dosse) gained more attention in biological control programmes of the ubiquitous two-spotted spider mite *Tetranychus urticae* Koch (Acari: *Tetranychidae*), in greenhouses on cucumbers, tomatoes and sweet pepper (Markkula and Tiittanen 1976; van Lenteren and Woets 1988; Gillespie and Raworth 2004), and on commercial strawberry in the field (Decou 1994).

Realizing the great success of *P. persimilis*, generalist phytoseiid mite species with a broader range of pests attacked and controlled were included in biocontrol programmes to complement the specialists for control of arthropod pests of minor importance or crop-specific pests, including eriophyids (Bonde 1989; Park et al. 2010, 2011), tarsonemids (Fan and Petit 1994; Stansly and Castillo 2009), and tydeids (Momen 2011), and now also insect species such as thrips or whiteflies (Ramakers 1980; Ramakers and van Lieburg 1982; de Klerk and Ramakers 1986; Gerson et al. 2003; Gerson and Weintraub 2007).

Furthermore, also phytoseiids in field crops were considered to be conserved or augmented. *Typhlodromips pyri* Scheuten, a generalist predatory mite in fruit orchards and vineyards and common in Europe and North America, Egypt (de Moraes et al. 2004), Australia, New Zealand (Schicha 1987; Collyer 1982), is object of conservational biocontrol worldwide as this species is an efficient antagonist of the European red mite, *Panonychus ulmi* (Koch) (Acari, *Tetranychidae*) (Dosse 1962; Engel and Ohnesorge 1994; Camporese and Duso 1996; Schausberger 1998; Papiaoannou et al. 1999; Marshall and Lester 2001). More than twenty years ago, *Typhlodromalus (Amblyseius) manihoti* Moraes and *Typhlodromalus (Amblyseius) aripo* de Leon represent one of the best examples for classical (inoculative) biological control of the invasive Cassava green

*Amblyseius swirskii*, *Neoseiulus cucumeris*, and *Amblydromalus limonicus* are polyphageous species adapted to subtropical and tropical climates (Zhang 2003; Zannou and Hanna 2011; Gerson and Weintraub 2007), contributing to control field pests. Notably, greenhouse temperatures of *ca.* 25 °C and high relative humidity meet the predatory mites’ claims on abiotic environmental conditions (Abou-Setta and Childers 1987; Kasap and Sekerğlu 2004; Kasap 2009; Lee and Gillespie 2011; Jafari et al. 2012; El Taj and Jung 2012; Gerson and Weintraub 2012).

Due to their environmental demands and their wide target range, these species were considered also effective antagonists of greenhouse crop pests in moderate climates. They have been commercialized to control different thrips species and whiteflies, and also some pests of minor or special importance in vegetables and ornamentals (van Houten et al. 2005; Messelink et al. 2006, 2008; Gerson and Weintraub 2007; Buitenhuys et al. 2015; Calvo et al. 2015; Hewitt et al. 2015; Leman and Messelink 2015).

Generally, quality of biological control agents is estimated by calculation and comparison of their life-table parameters as affected by biotic and abiotic factors, such as food quality and environmental regime as a semi-in vitro approach. The true quality as biocontrol agent can then only be described by greenhouse or field tests to assess the control efficacy under practice-oriented test conditions or directly under practice conditions.

**Food range of phytoseid mites and food quality**

Qualitatively the essential nutrients of arthropods are generally the same as for other animals and the main nutrients are amino acids or proteins, carbohydrates, lipids, fatty acids, vitamins, trace elements. However, the nutritional requirements of arthropod species are often rather specific and may concede only small tolerance, qualitatively as well as quantitatively.

Optimal growth, survival, and fecundity require certain protein: carbohydrate ratios, which may vary considerably among species and developmental stages and their life style. Highly active and slowly developing species need a carbohydrate-biased diet to supply energy, whereas less active and slowly developing species need a protein-biased diet to build up biomass (Behmer and Joern 1993; Gewecke 1995; Gullan and Cranston 2010). Preimaginal stages of insects and mites have higher demands in high quality food providing also sterols as precursors for steroid hormones.
and successful preimaginal development, whereas adult females have a higher demand in energy and protein for egg production (Overmeer 1981; Lundgren 2009). If nutrients in excess cannot be metabolized, excreted or defecated, the value of nutrients follows an optimum curve and and too high concentrations of a nutrient may become detrimental. A high N-content can negatively affect phytophagous mite performance, impairing longevity of both sexes and reproduction capacity of females (McNeill and Southwood 1978). Taking honeybees for comparison, an optimum of 23 % protein in the diets is reported, and higher protein contents result in a poorer performance of the honeybees, in terms of a reduced adult longevity (de Groot 1953; Herbert and Shimamuki 1977).

Comparing the two main food sources of arthropods, plants and animals, the protein content (% dry weight) in plants is significantly lower than that of animals (Mattson 1980), as reported also for diets offered to A. swirskii and N. cucumeris ranging between 0.45 and 3.86 % for cattail and maize pollen, respectively, compared to eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller with 4.56 % protein (Delisle et al. 2015ab).

Although numerous publications list target species and supplementary food sources, knowledge of the entire food spectrum of species is incomplete, and further target species and alternative food sources may be identified (Gerson and Weintraub 2007; Momen and Abdel-Khalek 2008; van Maanen et al. 2010; Park et al. 2010). Particularly, information on nutritional quality of primary food (prey) and secondary, alternative or supplementary food sources, such as pollen or any other plant material, is lacking, although this information may be useful to provide the mites with pollen by banker plants, or to improve mass rearing by offering supplementary food.

Tossed light upon the nutritional value of pollen constituents and their influence on life-table and morphological parameters of predatory mites, knowledge of their chemical composition is required. Furthermore, because nutritional physiology of mites is poorly investigated (Okuyama 2008), the approach to discuss nutritional physiology of mites basing only on knowledge in insect nutrition may be misleading. Considering the nutritional demands of phytophageous mites may also lead towards misinterpretations, except that they are closer related to predacious mites. Particularly predatory mite species of lifestyle type III and IV may correspond to the nutritional physiology and requirements of mites feeding exclusively on plants.

**Essential nutrients in arthropod diets**

Carbon-based plant compounds, such as carbohydrates and lipids, can affect the performance of insects and mites. These effects may even be contradictory between related arthropod species.
Generally it is assumed that carbon-based plant compounds provide energy in terms of carbohydrates and fats and thus may affect performance positively (Spector 1956; Neville and Luckey 1962; Gewecke 1995). However, an excess of soluble carbohydrates has also been reported to have negative effects and some sugars even may be toxic (Spector 1956; Neville and Luckey 1962; Awmack and Leather 2002).

Proteins may be considered the most important main nutrients for morphogenesis in insects, preimaginal development and growth, and egg maturation in adults (Keeley 1985; Mirth and Riddiford 2007). Protein-bound amino acids in animal or plant food, particularly in pollen, include essential amino acids required for all animal taxa (de Groot 1953; Nation 2002).

Free amino acids are equally important nutrients to synthesize de novo proteins, amino acid derived secondary products or to be directly metabolized into energy equivalents (Rodriguez and Hampton 1966; Rodriguez and Lasheen 1971).

After feeding $^{14}$C-labelled glucose to starved two-spotted spider mites, *Tetranychus urticae* Koch, and subsequent assay of labeled amino acids, 18 amino acids were detected (Rodriguez and Hampton 1966). They found that spider mites can synthesize some amino acids in high concentrations, such as ALA, ASP, CYS, cystine, GLU, GLY, PRO, SER, and THR, from glucose, thus considered not essential. In contrast, labeled ARG, HIS, ILE, LEU, LYS, MET, PHE, TYR, and VAL were found in very low concentrations and thus considered as essentials.

*Abou-Awad and Elsawi* (1992) reported a higher fitness of the predatory mite *A. swirskii* when fed with an artificial diet consisting of yeast, milk, CYS, PRO, ARG, sucrose, and glucose, mixed with *Ri. communis* pollen for six generations. Reared on this diet, adults reached the similar size as adults reared on spider mite nymphs. Only the reproduction capacity was higher when fed with animal food. However, these results may not depict a high-quality artificial diet because *A. swirskii* performs poorly when fed with spider mites.

Considering carbon-based and nitrogen-containing nutrients, the C: N-ratio seems to be more important than the absolute amount of each of these groups, because a C-biased diet diluting other nutrients may require phytophagous arthropods to increase their consumption rates to compensate (Awmack and Leather 2002).

In an approach to develop artificial diets as exclusive or additional food for rearing predacious mites as substitute for natural food sources, such as natural prey or pollen, the vitamin B complex and vitamin C as part of dietary components in a powdered artificial diet positively affected
the demographic parameters of *A. swirskii*. However, this diet was less effective than a fluid artificial diet without vitamin supplement (*Nguyen et al. 2014*). Thus, the consistency of a food source also matters in the nutritional physiology of phytoseiids.

Among the minor, but essential nutrients, sterols are of special importance because animals cannot synthesize sterols *de novo* and need precursors either from plant food as phytosteroids, or from their prey. Larvae require sterols for synthesis of ecdysteroids to undergo the moulting steps, and adults require ecdysteroids during sperm and ovariole maturation (*Nation 2002*). Cholesterol and its simple derivatives are absolutely necessary for insect and mite performance. However, not all insect and mite species are capable to convert phytosterols into innate steroid hormones. Dietary requirements of sterols vary with insect species, and some insect species have highly specific demands of sterols (*Norris and Baker 1967; Mondy and Corio-Costet 2000*).

Fatty acids, such as linolenic acid, were found to be important nutrients for development and reproduction capacity of spidermites, and starved mites had a high requirement of palmitic acid (*Walling et al. 1968*). Similar effects have been reported for the aphidophagous hoverflies *Episyrophius balteatus* (de Geer) and *Eupeodes bucculatus* (Rondani) (Diptera: *Syrphidae*), where oleic acid and linoleic acid were added to a diet consisting of powdered drone honeybee brood, which accelerated their development, enhanced the adult emergence rate and body size of syrphids (*Iwai et al. 2009*).

Besides organic nutrients, minerals are known to be important for spidermite performance. High concentrations of N accelerated preimaginal development and boosted egg production of the two-spotted spider mite, *Tetranychus urticae*. Phosphorous alone did not affect mite parameters (*Wermelinger et al. 1991*).

Na, P and Ca were found as important elements in the diet of the twospotted spider mite, *Tetranychus bimaculatus* and European red mite, *Panonychus ulmi*, although the authors could not explain their physiological role in detail (*Rodriguez 1951; Sharma and Bhardwaj 2010*).
Nutritional quality of pollen as food for predacious mites

Plant pollen

Pollen is a highly nutritive food for a wide range of insects and mite species (Stanley and Linskens 1974; Wäckers 2005; Wäckers et al. 2005; Lundgren 2009). It contains high amounts of lipids, proteins, free amino acids and carbohydrates, but differ in their composition between plant pollination type (anemophilous and entomophilous pollen) (Petanidou and Vokou 1990; Petanidou 2005; Praz et al. 2008). Generally, pollen of entomophilous plants are richer in nutrients than pollen of anemophilous plants (Roulston and Cane 2000; Hanley et al. 2008). Furthermore, pollen can differ in quantitative and qualitative composition, and thus in its quality as insect food between plant species, species genotype/ecotype, and environment (Muniategui et al. 1991; Roulston and Cane 2000; Lundgren and Wiedenmann 2004; Bogdanov 2006; Karise et al. 2006; Obrist et al. 2006; Szczeńska 2006 ab; Lundgren 2009; Dabija 2010; Nicolson 2011).

Bee pollen

Compared to plant pollen, bee pollen stripped-off at the hive entrance (gehöselter Pollen) is often a mixture of pollen of different plants. Later, this pollen package is processed into paste-like bee-bread by other Innendiensttiere of the bee colony to feed the bee brood. Since this bee-bread has not been used in the experiments with predatory mites, the nutritional ingredients and the nutritional value to honeybees is neglected for discussion of mite nutrition.

Unfortunately, the majority of authors did not distinguish between simple stripped-off pollen, pollen processed by flightless nurse bees (Innendiensttiere), and bee-bread when assessing the chemical composition, the nutritional or the pharmaceutical value of honeybee collected pollen, and a careful interpretation of data is necessary.

The chemical composition of pollen has been analyzed to assess the suitability as human or animal additional food or for medical purposes (Campos et al. 1997; Cocan et al. 2005). The main nutrients in bee pollen are proteins (25-30%), carbohydrates (30-55 %), fatty acids and sterols (1-20 %) (Campos et al. 2008; Abouda et al. 2011).

For honeybees and bumblebees, pollen mainly is considered to be a protein source for female egg production and and/or larval growth, development of sexual organs, and adult size (Pain
In addition, free amino acids are important to support or complement the nutritional value of proteins as bound amino acids to spare arthropods *de novo* synthesis when taken up with food (Cohen 2004, cited by Vanderplanck et al. 2014). However, the absolute and relative amount of free amino acids in honeybee collected pollen differs within and between plant species (Szczesna et al. 1995; Szczesna and Rybak-Chmielewska 1998, both cited by Campos et al. 2008; Yang et al. 2013).

Compared with the total protein content and total free amino acid content, bound essential amino acids are of higher importance and their concentration may serve as indicator of pollen nutritional quality (Paramas et al 2006; Stabler et al. 2015). In bee pollen, the amino acids MET, LYS, THR, HIS, LEU, ILE, VAL, PHE and TRP, essential for honeybees and bumblebees, may reach 10.4 % of total pollen protein content (de Groot 1953; Roulston and Cane 2000; Paoli et al. 2014; Stabler et al. 2015).

On average, bee pollen contains 40 % sugars of which 83% are monosaccharides (fructose 46 % and glucose 37 %), and disaccharides (8 % sucrose, 7 % maltose, 1 % trehalose), serving as energy source for honeybees (Standifer et al. 1977; Huang 2010; Szczesna 2007b).

Lipid and sterol content in bee pollen varies between 1 and 20 %, depending on the plant species the pollen was collected, and during storage the lipid content may decrease (Nicolson2011). In a detailed study, total lipid content in bee pollen has been described as 5.1 %, with an amount of essential fatty acids, such as linoleic-, γ-linoleic-, or arachidic acid of ca. 0.4 %, phospholipids with 1.5%, and 1.1 % phytosterols (Szczesna 2006b).

Particularly the unsaturated fatty acids linoleic (18:2n-6), γ-linoleic (18:3n-3), and palmitic (16:0) acids, dominate the lipids in pollen and are known to be essential for insect fitness and egg production (Canavoso et al. 2001; Manning 2001; Cohen 2004; Szczęsna et 2006b; Nurullahoglu et al. 2004; Wang et al. 2006; Khani et al. 2007).

Pollen contains different types of sterols, which regulate the cholesterol metabolism in honeybees (Marghitas 2005) among which 24-methylenecolesterol is known as most important for preimaginal development (Svoboda et al. 1980; Human et al. 2007). In combination with high
amino acid contents, the essential sterols in bee pollen, 24-methylenecholesterol, b-sitosterol, or d5-avenasterol, increased larval weight of *Bombus terrestris* L. (*Vanderplanck et al. 2014*).

**Vitamins**

Most studies on bee nutrition focus on honeybee’s requirements of protein and carbohydrates and the role of these nutrients for honeybee nutrition is well described. However, the effect of essential lipids, vitamins and minerals on brood rearing and bee development has been studied mainly using beebread and not the original pollen. It is still questionable, which ratio of all these nutritional components and which concentration is optimal for bee health (*Brodschneider and Crailsheim 2010*). Despite this restriction to estimate the role of vitamins, the vitamin B complex is of special relevance in the nutrition of all living organisms, because it cannot be synthesized and must be taken up with food. Vitamin Bs are responsible in the regulation and metabolism of fats, proteins and carbohydrates, and determine brood development success and hypopharyngeal glands in honeybees (*Pain 1956; Herbert and Shimanuki 1978; Huang 2010*).

Bee pollen contains in total about 0.7 % vitamins, distinguished into water-soluble vitamins (0.6%), represented by the vitamin B-complex and vitamin C, and lipophilic vitamins (0.1%), as represented by vitamin A and carotenoids with provitamin A function, vitamins E and D (*Asafova et al. 2001*). However, also the vitamin content differs between honeybee-collected pollen because their concentration varies between plant families (*de Almeida-Muradian et al. 2005; de Arruda et al. 2013ab*).

Besides vitamin B, vitamin A plays a crucial role in mite biology. Spidermites, and similarly, the predacious *Amblyseius potentillae* (Garman) require carotenoids in their diet to enter diapause (*Overmeer and van Zon 1983*). Vitamin A and provitamin A were essential in the induction of diapause in the eyeless *Amblyseius potentillae* Garman (*Veerman et al. 1983*).

**Minerals**

Total ash amounts to 2 – 6.5 % of pollen dry weight of stripped-off bee pollen (*Herbert and Shimanuki 1978; Campos et al. 2008*), with a high amount of minerals such P, K, Ca, Mg, Zn, Fe, Mn and Cu (*Yang et al. 2013*). Macroelements in pollen, such as calcium, phosphorus, magnesium, sodium, and potassium made up 1.6%, and microelements (iron, copper, zinc, manganese,
silicon, and selenium) are found at a concentration of 0.02% of pollen dry weight (Campos et al. 2008; 2010; Kędzia and Holderna-Kędzia 2012).

In a direct comparison of macro- (P, K, Ca, Mg) and micro-elements (Fe, Zn) of pure bee-collected stripped-off Helianthus annuus L. and Salix sp.-pollen with hand-collected pollen from these plant species, mineral composition and concentration differed between plant species and collection method. The amount of all mineral elements was superior in hand-collected than in bee-collected pollen (Stanciu et al. 2011).

However, mineral content in bee pollen varies with plant species and geographical origin (Szczesna 2007b; Stanciu et al. 2011).

These studies showed that pollen is rich in minerals, which can satisfy the demand for minerals in arthropods. The three major minerals occurring in higher amounts in pollen, potassium, phosphate, and magnesium, are considered highly valuable for honeybees. However, some minerals, such as sodium, sodium chloride and calcium, may exert negative effects and even being lethal (Huang 2010). The negative effect of mineral elements may be concentration-dependent, as the amendment of 1% of pollen ash to an artificial diet had a positive effect on bee brood, but 2% was a too high concentration leading to detrimental effects (Herbert and Shimanuki 1978; Gergen et al. 2006).

Depending on the environmental pollution of the plant’s habitat, pollen may be contaminated by heavy metals, which can negatively affect pollen-feeding organisms. Toxic heavy metals have been found in bee pollen in considerable concentrations: As (≤0.06), Pb (≤ 0.8 mg/kg), Cd (≤ 0.03 mg/kg), Hg (≤ 0.01 mg/kg), Se, Cr, Ni, Al (> 100 mg/kg) (Gergen et al. 2006; Campos et al. 2008; Roman 2009; Hladun et al. 2011; Hladun et al. 2013 ab; Yang et al. 2013). In contrast Fe, Mn, Cu and Zn are useful elements, however toxic if higher concentrations (Gergen et al. 2006).

Selenium found in bee pollen ranks among the very toxic elements for honeybees if the concentration in pollen is very high. Greenhouse-grown Brassica juncea (L.) Czern. And Raphanus sativus L. produce pollen with a concentration of 710 – 1,700 mg Se / kg (Hladun et al. 2011; Hladun et al. 2013 ab). Honeybee larvae suffered from a 30-50 times higher mortality when fed with selenoamino acids and inorganic forms of Se than adults (Hladun et al. 2013a).
Toxic compounds

Sugars in nectar and pollen, such as galactose, stachyose, glucuronic acid, galacturonic acid, polygalacturonic acid, and pectin mannose, arabinose, xylose, melibiose, raffinose and stachyose are known to be toxic to arthropods (Staudenmayer 1939; Barker and Lehner 1974; Barker 1977). Especially mannose causes bee mortality, however if 50% sucrose is added artificially to nectar, the toxic effect can be reduced (Barker 1977; Huang 2010).

Secondary compounds like alkaloids, phenolics, and non-proteinogenic amino acids may also be incorporated in plant pollen (Stanley and Linskens 1974), however, their role in insect nutrition is equivocal. Some studies report positive effects of nectar and pollen containing secondary compounds, attracting pollinators and improving their performance, as given by the guild of pyrrolizidinalkaloid-attracted pollinators (Manson et al. 2010; Boppré et al. 2005). Some secondary compounds were found to exert negative effects when consumed, generally causing lethal effects (Detzel and Wink 1993) or reduced mobility (Cook et al. 2013; Manson et al. 2013), ovary development (Manson and Thomson 2009), and survivorship (Detzel and Wink 1993; Singaravelan et al. 2006; Köhler et al. 2012; Sedivy et al. 2012).

High amounts of secondary compounds in nectar and pollen may be extremely toxic to bees (Adler 2000; Arnold et al. 2014; Eckhardt et al. 2014), dependent on specialization in pollinating particular plant families, genera, or species, found in monolectic or oligolectic bee species. Thus, metabolization of toxic compounds in the specialized bee species must be given and preimaginal development on pollen of non-specialized plants is often not possible when fed to the brood (Praz et al. 2008; Müller and Kuhlmann 2008; Sedivy et al. 2011).

Phenolic and polyphenolic compounds

Phenolics in apple leaves had a negative effect on spidermite fecundity and $r_m$-values (Wermeling et al. 1991). Probably phenolics if abundant in pollen may explain why predatory mites consuming bee pollen rich in phenolics suffered from low egg production and a prolonged preimaginal development of A. swirskii in our experiments.

The majority of pollen contains phenolic xenobiotics, which are highly toxic for bees (Barker 1977; Wiermann 1968; Bonvehi et al. 2001; Johnson et al. 2012; Johnson 2015). Analysis of bee collected and stripped-off pollen revealed significant amounts of flavonoids, such as kaempferol,
quercetin, and isorhamnetin (1.4 %), and phenolic acids, predominantly chlorogenic acid (0.2% ) (Asafova et al. 2001; Negri et al. 2011). However, as found for other pollen constituents, the amount of secondary compounds varies in concentration between plant species (Stanley and Linskens 1974). Although quercetin is a protease-inhibitor, honeybees are able to metabolize high concentrations of quercetin (Mao et al. 2009), and quercetin and other flavonoids are considered valuable for bees as antioxidant and antimicrobial components (Treutter 2005). However, the role of quercetin and other flavonoids in the nutrition of carnivorous arthropods is not known.

Alkaloids

Alkaloids are nitrogen-containing anti-herbivory compounds produced by plants, accumulated in higher concentrations in leaves, flower petals and sepal, and seeds than in the nectar and pollen (Detzel and Wink 1993). Nectar and pollen containing alkaloids (pyrrolizidine alkaloids, caffeine, nicotine, D-lupanine) exerted negative effects on insect fitness (Emrich 1991; Bennet and Wallsgrove 1994; Adler 2000; Singaravelan et al. 2005, 2006; Johnson et al. 2012; Arnold et al. 2014). Angiosperm plants produced pyrrolizidine alkaloids (PAs), which are highly toxic to mammals and insects. According to Boppré et al. (2005) pollen contains 14,000 mg/kg PAs, however, found not being harmful to honeybees, whereas the amount of 20,000 mg/kg PA was found toxic for bees (Reinhard et al. 2009). Also the toxicity of PAs to honeybees must be seen on the species level because 60% of 119 samples of pollen packages collected by honeybees) were PA-positive (Dübecke et al. 2011), obviously not harming the honeybees. On the other hand, predatory mites may not be adapted to PA-containing pollen and, thus, suffer from a bad performance when fed with this kind of pollen.

Four pollen of the plant family Asteraceae, known to contain alkaloids (Anthemis cotula L., Conyza bonariensis (L.) Cronquist, Lactuca serriola L., and Taraxacum officinale (Weber) ex F.H. Wigg.) were absolutely toxic for the predatory mite T. pyri (Bermúdez et al. 2009).

Terpenoids

Pollen of sunflower contain twenty-four different terpenoids (Ukiya et al. 2003), which probably explains the unsuitability or less suitability for most insect and particularly predator species.
7. Conclusion and outlook

The suitability of different pollen as alternative or supplementary food differs between predatory mite species and pollen species, indicating different adaptations to plant pollen. Preimaginal development, life-table and demographic parameters, and body weight and size are indicators for food suitability in predacious mites. The differences in the species’ performance may be seen in different enzymatic equipment to utilize pollen nutrients or to metabolize detrimental secondary plant compounds.

Bee pollen is known as pollen of low quality for mostly predatory mite species, probably of low quality of all ingredients, containing in this pollen in comparison to hand collected pollen.

Also, commercial bee pollen is a mixture of different plant species and differ in pellet colour, probably contain secondary compounds, which may be the reason of their low quality for the mite species tested.

This study contributes to the wider knowledge in the biology of phytoseiid mite species, particularly the use of pollen as alternative or supplementary food. This may be found either on banker plants in greenhouses to improve the performance of these beneficials in biocontrol, or in the field when introduced species may escape and try to establish in a new environment.

However, this study also revealed that knowledge in the chemical composition of pollen is poor or even lacking and detailed pollen chemical analyses are required. Protein, amino acid and fatty acid composition, sterols, mineral composition, vitamins, secondary compounds, or even heavy metals should be assessed, because not only the plant species determines the composition of nutrients and detrimental substance, but also the environment and the ecotype of the plant may affect the pollen constituents.

Looking at the nutritional physiology of predatory mites, pollen preference and suitability of pollen, studies in this field are lacking. In future studies, the same or more pollen sources should be chosen to assess their suitability to other predatory mite species, to find compatible food sources for most mite species for practical reasons, such as offering pollen as supplementary food in greenhouse biocontrol.

To determine the nutritional needs of a predatory mite species and the nutritional value of single nutrients, not only supplied by pollen ingredients but also by other food sources, descriptive fitness parameters, such as life-table or demographic parameters, must be correlated with nutrient absolute and relative amounts in the particular food.
This information will allow to select plant pollen that better meets predatory mite’s nutritional demands. With knowledge of their nutritional needs, we will be able to prepare optimal artificial diets to maximize their reproductive potential and development. Additionally, artificial diets should be tested on their suitability for predatory mite species, maybe species-specific artificial diets have to be shaped. Greenhouses experiments should be conducted to find the practical relevance of the best pollen and/or artificial diet and the control efficacy of predatory mites.

Summarizing, the following specific questions should be answered in future:

(i) which are the major nutrients, distinguished by macro- and micronutrients, determining phytoseiid mite performance;

(ii) at which relative proportion do the nutrients occur in the diets;

(iii) which elements are toxic for mite species;

(iv) how can predatory mites adapted to less suitable or partly toxic food metabolize detrimental or toxic food compounds;

(v) is it feasible to compose an optimal artificial diet for all predatory mite species reared for practical biocontrol to improve mass rearing by commercial producers;

(vi) which requirements to food quality have different predatory mite species.
8. Summary

Predatory mites of the family Phytoseiidae (order Acari) are important biological control agents of various greenhouse pests. Their successful establishment in greenhouses depends on abiotic and biotic factors, and on different adaptation levels of the different mite species, which must be considered before practical implementation.

Phytoseiid mites also differ in control efficacy of different pest species, particularly depending on the species-specific predation capacity and searching behavior. Besides, differences in size between predatory mites and the potential target pests, but also their prey and alternative/ supplementary food preferences and food quality are major factors for successful biological control programs. Thus, understanding their qualitative food requirements supports successful implementation in greenhouses. Release of predatory mites into new habitats may exert a certain stress if the amount of food is limited and, thus, can weaken their fitness. However, generalist predatory mites can also feed on pollen of different plants as alternative food, which enhances their survival ability when the target prey is scarce or lacking. In practice, pollen is supplied by banker plants, such as Castor beans (Ricinus communis), or artificially in form of pollen sprays or dusts. Suitability of pollen as food differs, however between plant species and mite species, making it necessary to investigate the performance of phytoseiids when fed with different pollen. Lifetable and demographic parameters are considered the best descriptors of arthropod performance under different abiotic and biotic conditions.

In this study, the suitability and effect of pollen as alternative food was assessed for the predatory mites A. swirskii, A. limonicus, and A. cucumeris (Acari, Phytoseiidae). Besides lifetable and demographic parameters, body weight and size was included into the descriptive parameters, which has not been done before to obtain more detailed information on pollen quality for these mite species.

In the first study (Goleva and Zebitz 2013), the suitability of pollen of 21 plant species as alternative food for A. swirskii was tested. Preimaginal mortality and developmental time have been assessed, followed by a life-table analysis of the emerged adults and a calculation of demographic parameters. Amblyseius swirskii was able to feed on 18 pollens, but the nutritional properties of the pollen differently affected mite performance. Pollen of Lilium martagon and Hippeastrum sp. were found to be toxic for the mites, probably due to secondary plant compounds in this pollen. Amblyseius swirskii was absolutely not adapted to feed on Hibiscus syriacus because of the external morphology of pollen grains, leading also to 100 % preimaginal mortality. The best pollen resulting in superior mite performance in all parameters tested were: Aesculus hippocasta-
num, Crocus vernus, Echinocereus sp. and Paulownia tomentosa. No or low mortality was observed when mites fed on other pollen tested. Developmental time, adult longevity, and reproduction parameters, were significantly affected, probably because of differences in pollen nutrient or non-toxic secondary compound composition. Commercial bee pollen was of very poor quality for the mites leading to low egg production, which excludes this pollen for practical use. Pollen of Helianthus annuus, Corylus avellana and a Poaceae-mix were of inferior quality, causing high mortality rates, low egg production and short adult longevity. For practical implementation in greenhouses, pollen of Ricinus communis, Zea mays, A. hippocastanum and Betula pendula pollen appeared suitable to improve predatory mite performance, either provided by banker plants or in form of dispersible pollen.

The objective of the second study (Ranabhat et al. 2014) was to assess the suitability of seven pollen (castor bean, tulip, apple, Christmas cactus, horse-chestnut, maize, and birch) for Neoseiulus cucumeris (Acari: Phytoseiidae), and to scrutinize potential effects of these pollen on reproduction and life history parameters of this mite. Neoseiulus cucumeris accepted six pollen sources as alternative food, pollen of castor bean plants, however, caused 100 % mortality. Highest fecundity was observed when feeding on pollen of tulip and horse chestnut, resulting in the shortest preimaginal development compared with all pollen tested. Pollen of birch, tulip, maize, and apple had a positive effect on longevity, whereas pollen of horse-chestnut and Christmas cactus was significantly inferior.

Our study suggests that birch, tulip, horse-chestnut, apple, and maize pollen may serve as alternative food in the field, and birch and maize pollen have a good potential in practical use as banker plants or dusts in the greenhouse to guarantee mite establishment in periods of prey scarcity.

The aim of the third study (Goleva et al. 2014) was to investigate the role of pollen of differing quality (tested previously in first study (Goleva and Zebitz 2013) on size and weight of A. swirskii. These parameters were considered from the physical aspect of predator-prey relations because predatory mites are not able to conquer prey individuals bigger than themselves and any deviation from normal size may negatively affect predation success. Biomass and body size of freshly emerged, 1, 3, and 30 days old adult A. swirskii continuously reared on pollen of B. pendula, H. annuus, P. tomentosa, and Zea mays revealed, regardless of pollen source, that females were bigger than males. Both parameters weight and size were affected by pollen tested. Females fed on pollen of P. tomentosa and Z. mays were significantly bigger than on B. pendula or H. annuus pollen. Increase of female biomass was observed until the adult age of 3 days, remaining
constant or slightly decreased until the age of 30 days. This can be explained by particularly high nutritional requirements of females for egg production especially in the beginning of their reproduction period. Males also reached their weight maximum at day 3 of adult life, then decreasing until day 30. Congruent with biomass, adult body length and width differed significantly depending on sex and age showing females bigger than males and 30 day old adults bigger than 1 day old adults irrespective of the pollen fed. While sunflower pollen was of poor quality for *A. swirskii*, causing small size and low weight of both sexes, pollen of *P. tomentosa* was significantly superior in both parameters. No reasonable correlation was found between body length and width, but body weight was significantly correlated with body length in females whereas there was no correlation at all in males.

In a **fourth study** (Goleva et al. 2015), adult body weight of the generalist mites *A. swirskii*, *A. limonicus*, and *N. cucumeris* reared on 22, 12, and 6 pollen species, respectively, was assessed. In addition, *A. swirskii* and *A. limonicus* were reared on codling moth (*Cydia pomonella*) eggs. In all mite species, female body weight was higher than that of males, ranging between 4.33 and 8.18 µg for *A. swirskii*, 2.56–6.53 µg for *A. limonicus*, and 4.66–5.92 µg for *N. cucumeris*. Male body weight ranged between 1.78 and 3.28 µg, 1.37–3.06 µg, and 2.73–3.03 µg, respectively. Nutritional quality of pollen was neither consistent among the mite species nor among sex, revealing superior quality of *Quercus macranthera* pollen for females of *A. swirskii* and *T. gesneriana* pollen for males, *Alnus incana* pollen for females of *A. limonicus* and *Ae. Hippocastanum* pollen for males, and *Ae. Hippocastanum* pollen for both sexes of *N. cucumeris*.

Pollen affected predatory mite species in our studies in various ways. Besides lifetable parameters, pollen also affected adult weight and size of predatory mites and these parameters should therefore be considered additionally in future studies to obtain more exhaustive information on nutritional biology and physiology of predatory mites.

The differences in the parameters assessed and the mite species performance may be explained by different adaptations of mite species to pollen compounds. Knowledge in the chemical composition of pollen, however, particularly the nutritive substances, is poor or even lacking. Any correlation of performance parameters with nutrients is more or less theoretical unless the pollen constituents are known. Even then, nutritional physiology of predatory mites and their adaptation to various food sources must be improved and include mite’s enzymatic activity to metabolize secondary plant compounds in pollen. The results presented in this thesis open the door to future research on mite nutritional biology.
9. Zusammenfassung


Werden Raubmilben in einem neuen Lebensraum, z. Bsp. in Gewächshauskulturen, freigelassen, so können die Tiere einem gewissen Stress unterworfen sein falls die Nahrungsmenge durch geringes Schaderregeraufkommen begrenzt ist und der daraus resultierende Hunger ihre Fitness beeinträchtigt.

Generalisten unter den räuberischen Milben können jedoch auch Pollen verschiedener Pflanzen als Alternativnahrung aufnehmen, wenn Beutetiere rar sind oder fehlen. So könenn sie nicht nur überleben, sondern sich auch fortpflanzen und stabile Populationen aufbauen. In der Praxis wird daher Pollen durch sogenannte „banker plants“, beispielsweise Rizinus (Ricinus communis), oder durch künstliche Ausbringung (Sprühen bzw. Stäuben) bereitgestellt.


In dieser Studie wurden die Eignung und die Wirkung von Pollen als alternative Nahrungsquelle für die räuberischen Milben A. swirskii, A. limonicus und A. cucumeris (Acari, Phytoseiidae) geprüft.
Neben demographischen Daten und Lebenstafeln gehörten das Körpergewicht und die Größe zu den berücksichtigten Parametern. Eine solche Untersuchung zur Gewinnung genauerer Informationen über die Qualität von Pollen für diese Milbenarten wurde bisher noch nicht durchgeführt.


Pollen von Birke, Tulpe, Mais und Apfel hatten eine positive Wirkung auf die Lebensdauer; dagegen waren Pollen von Roßkastanie und Weihnachtskaktus signifikant ungünstiger.

Unsere Untersuchung legt nahe, daß Pollen von Birke, Tulpe, Roßkastanie, Apfel und Mais im Freiland als alternative Nahrungskonsorten dienen könnten und Birken- oder Maispollen als Futtermittel oder verstäubt eine praktische Möglichkeit darstellen, im Gewächshaus eine Ansiedlung der Milben in Phasen der Beuteknappheit zu gewährleisten.


In einer vierten Arbeit (Goleva et al. 2015) wurde das Körpergewicht von erwachsenen Milben der Arten *A. swirskii, A. limonicus* und *N. cucumeris*, gezogen auf 22, 12, beziehungsweise 6

Pollenarten beeinflussten räuberische Milbenarten in unseren Arbeiten unterschiedlich. Neben den Lebenstafelparametern wirkten sie sich auch auf das Gewicht und die Größe der adulten räuberischen Milben aus; diese zusätzlichen Parameter sollten daher in zukünftigen Studien einbezogen werden um umfassendere Informationen über die Ernährungsbiologie und Physiologie räuberischer Milben zu erhalten.

Die Unterschiede in den erhobenen Parametern und der Leistungsfähigkeit der Milben können erklärt werden durch unterschiedliche Anpassung der Milbenarten an Polleninhaltsstoffe. Das Wissen über die chemische Zusammensetzung verschiedener Pollen, insbesondere der Nährstoffe, ist lückenhaft oder fehlt gänzlich.

Jedwede Korrelation von Leistungsparametern mit Nährstoffgehalten bleibt weitgehend theoretisch solange die Bestandteile des Pollens nicht bekannt sind. Selbst dann müssen die Kenntnisse der Ernährungsphysiologie räuberischer Milben und ihrer Anpassung an unterschiedliche Nahrungsquellen verbessert und um solche der enzymatischen Aktivität zur Verdaunung sekundärer Pflanzeninhaltsstoffe in Pollen erweitert werden.

Die Ergebnisse dieser Dissertation schaffen die Voraussetzung zu zukünftiger Forschung über die Ernährungsbiologie von Milben.
10. References (for chapters 1 and 6)


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