**UNIVERSIDADE FEDERAL DE VIÇOSA**

# Mummies, beasts & flowers: Surviving and thriving as a predatory mite

Marcus Vinícius Alfenas Duarte
*Doctor Scientiae*

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**MARCUS VINÍCIUS ALFENAS DUARTE**

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Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

Orientador: Angelo Pallini Filho

Coorientadores: Arnoldus R. M. Janssen
Madelaine Venzon

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Assentimento:

Marcus Vinícius Alfenas Duarte
Autor

Angelo Pallini Filho
Orientador

À minha mãe Rita de Cássia e meu Pai Celmar
Ao meu irmão Matheus
A minha companheira Juliana

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## RESUMO

DUARTE, Marcus Vinícius Alfenas, D.Sc., Universidade Federal de Viçosa, janeiro de 2018. **Múmias, feras e flores: sobrevivendo e prosperando como um ácaro predador**. Orientador: Angelo Pallini Filho. Coorientadores: Arnoldus Rudolf Maria Janssen e Madelaine Venzon.

Na natureza animais encontram constantes desafios para a sua sobrevivência. Esses desafios incluem a ameaça à predação e a busca por alimentos para evitar a morte pela fome. Para lidar com essas ameaças, animais desenvolveram diversas estratégias. No presente trabalho reportamos algumas dessas estratégias adotadas por ácaros predadores. Dentre esses desafios, ser atacado por um predador pode ser imperdoável. Se algum erro é cometido talvez não haja uma outra chance. Para se proteger contra predadores, animais usam uma variedade de estruturas como abrigo. No primeiro capítulo dessa tese testamos se a presença de múmias vazias de pulgão oferecem abrigo para o ácaro predador Amblyseius herbicolus. Descobrimos que A. herbicolus sofreu menos predação e apresentou maior crescimento populacional do que quando múmias vazias de pulgão estavam presentes. Abrigo pode não estar sempre presente quando animais estão sobre ameaça de predação. Para lidar com isso, animais desenvolveram uma variedade de comportamentos para evitar predação. No segundo capítulo observamos que quando A. herbicolus estava sobre a ameaça de predação, ele apresentava um novo comportamento anti-predação onde montava e até ovipositava nas costas do percevejo predador Blaptostethus pallescens. Quando nas constas do B. Pallescens, A. herbicolus e seus ovos sofreram menos predação do que quando ao nível do solo. Para o terceiro capitulo observamos que a presença de um inimigo natural pode acelerar a floração de uma planta, flores as quais servem de alimento para esse inimigo natural. Descobrimos que quando o ácaro predador Euseius gallicus estava presente em plantas de pimentão, essas plantas produziram flores antes do que as plantas que tinham a ausência desse ácaro. Discutiremos sobre as implicações ecológicas básicas e aplicadas desses achados no decorrer dessa tese.



Palavras-chave: Phytoseiid; abrigo; canibalismo; Anthocoridae; *Amblyseius herbicolus*; *Blaptostethus pallescens*; *Capsicum annuum*

## ABSTRACT

DUARTE, Marcus Vinícius Alfenas, D.Sc., Universidade Federal de Viçosa, January, 2018. **Mummies, beasts & flowers: Surviving and thriving as a predatory mite**. Adviser: Angelo Pallini Filho. Co-advisers: Arnoldus Rudolf Maria Janssen and Madelaine Venzon.

Plants must defend against herbivory. For this, plants have developed a wide spectrum of mechanisms. Plants are able to defend by producing toxic compounds that directly affect herbivores and by recruiting and maintaining natural enemies of herbivores that attack them. These defences are thought to be costly and for this reason plants induce them when herbivores are present. The induction of indirect defences that attract natural enemies have been well studied, however the induction of provision of food for predators has not been well studied. We investigate how the presence of predators can influence the provision of food for predators by observing the flowering phenology of a plant. When the predatory mite Euseius gallicus was present, plants produced flowers earlier when compared to plants without mites. Plants with the predatory mite Amblyseius swirskii had intermediate results between plants with E. gallicus and control plants. If a plant is able to perceive the presence of natural enemies and quickening the production of flowers when predators are present, plants can offer favourable conditions to maintain these predators prior to attacks by herbivores and when herbivores arrive these plants are already protected.



Keywords: Phytoseiid; shelther; cannibalism; Anthocoridae; *Amblyseius herbicolus*; *Blaptostethus pallescens*; *Capsicum annuum*

**SUMÁRIO**

**General Introduction**

Surviving as a small arthropod, such as a predatory mite, can be very challenging. Death can come by many ways such as dissection, being eaten by a predator (which may even be your own mother!) or by starvation. To cope with all these treats predatory mites have developed a wide array of mechanism, some of which we will be addressing during this thesis.

Under natural conditions many factors, such as the availability of food or water, can limit an animal population densities (MacArthur and Levins 1967). Even thought resources such as water and food are essential for the survival. Animals that miss a meal or going without water for some time may have another chance to find food or water, however, if an animal is not able to avoid an attack by an predator once his life may end there and it may not have another chance.

Animals can use a variety of structures as shelter against predators (Persson and Eklöv 1995; Caley and St John 1996; Fukui 2001; Danks 2002). A few examples of these shelters are leaves that are rolled up by caterpillars, (Martinsen et al. 2000; Lill and Marquis 2004), shells produced by molluscs, (Vance 1972; Gutierrez et al. 2003) and webs produced by spider-mites (Lemos et al. 2015). Some plants also have domatia, which are structures that serve as shelter for arthropods (Janzen 1966; O'Dowd and Willson 198; Matos et al. 2006; Ferreira et al. 2008). When these structures produced by other animals or plants are not present in the environment, animals may need to find shelter in unconventional places, such as coconut shells (Finn et al. 2009). In the first part of this thesis, we will investigate how a predatory mite uses empty aphid mummies as shelter from cannibalism and predation.

Shelter may not away be present when organisms are under the treat of predation. Animals are also able to avoid predation by camouflage or having aposematic colorations (Edmunds 1974). Through camouflage, animals can blend into their environment making it hard for predators to see them (Stuart-Fox et al. 2006). Animals can also present aposematic coloration, which warns predators that they are toxic or distasteful (Mallet and Joron 1999), or copy those from toxic species (Joron and Mallet 1998). Another form of avoiding predators can be to simply run away from them (Ydenberg and M. Dill 1986). In the second part of this thesis we described a

completely new anti-predator behaviour displayed by a predatory mite, that instead of moving away from a predator it chooses to mount on the back of the predator and even oviposit there.

For the third and final part of this thesis, we will discuss how a predator can get food to survive and be prosperous in an environment. Prey may not always be present for predators to feed on. When prey are absent, predators that are able to feed on plant material may have an advantage over those that are strictly carnivorous. These organisms that are able to feed on prey and plant material are called omnivores (Coll and Guershon 2002) and many important natural enemies are omnivores (Symondson et al. 2002). These organisms can feed on plant provided material such as pollen and nectar and remain in the environment even when prey is absent (Wäckers et al. 2005). `In this final part, we have observed a predatory mite that when present on pepper plants, these plants produce flowers earlier then when they were not present.

**Bibliography**

Caley, M. J., and J. St John. 1996. Refuge availability structures assemblages of tropical reef fishes. Journal of Animal Ecology 65:414–428.

Coll, M., and M. Guershon. 2002. OMNIVORY IN TERRESTRIAL ARTHROPODS: Mixing Plant and Prey Diets. Annual Review of Entomology 47:267–297.

Danks, H. V. 2002. Modification of adverse conditions by insects. Oikos 99:10–24.

Edmunds, M. 1974. Defence in animals: A survey of anti-predator defences. Longman Publishing Group, Burnt Mill, UK.

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

Finn, J. K., T. Tregenza, and M. D. Norman. 2009. Defensive tool use in a coconut-carrying octopus. Current Biology 19:R1069–R1070.

Fukui, A. 2001. Indirect interactions mediated by leaf shelters in animal–plant communities. Population Ecology 43:31–40.

Gutierrez, J. L., C. G. Jones, D. L. Strayer, and O. O. Iribarne. 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. Oikos 101:79–90.

Janzen, D. H. 1966. Coevolution of mutualism between ants and acacias in Central America. Evolution 20:249–275.

Joron, M., and J. L. B. Mallet. 1998. Diversity in mimicry: paradox or paradigm? Trends in Ecology & Evolution 13:461–466.

Lemos, F., A. M. G. Bernardo, C. R. Dias, R. A. Sarmento, A. Pallini, M. Venzon, and A. Janssen. 2015. Breaking and entering: predators invade the shelter of their prey and gain protection. Experimental and Applied Acarology 67:247–257.

Lill, J. T., and R. J. Marquis. 2004. Leaf ties as colonization sites for forest arthropods: an experimental study. Ecological Entomology 29:300–308.

MacArthur, R., and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. The American Naturalist 101:377–385.

Matos, C. H. C., A. Pallini, F. F. Chaves, J. H. Schoereder, and A. Janssen. 2006. Do domatia mediate mutualistic interactions between coffee plants and predatory mites? Entomologia Experimentalis et Applicata 118:185–192.

Mallet, J., and M. Joron. 1999. Evolution of Diversity in Warning Color and Mimicry: Polymorphisms, Shifting Balance, and Speciation (Vol. 30).

Martinsen, G. D., K. D. Floate, A. M. Waltz, G. M. Wimp, and T. G. Whitham. 2000. Positive interactions between leafrollers and other arthropods enhance biodiversity on hybrid cottonwoods. Oecologia 123:82–89.

O'Dowd, D. J., and M. F. Willson. 1989. Leaf domatia and mites on Australasian plants: ecological and evolutionary implications. Biological Journal of the Linnean Society 37:191–236.

Ostreiher, R. 2003. Is mobbing altruistic or selfish behaviour? Animal Behaviour 66:145–149.

Persson, L., and P. Eklöv. 1995. Prey refuges affecting interactions between piscivorous perch and juvenile perch and roach. Ecology 76:70–81.

Stuart-Fox, D., M. J. Whiting, and A. Moussalli. 2006. Camouflage and colour change: antipredator responses to bird and snake predators across multiple populations in a dwarf chameleon. Biological Journal of the Linnean Society 88:437–446.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can Generalist Predators Be Effective Biocontrol Agents?1. Annual Review of Entomology 47:561–594.

Vance, R. R. 1972. Competition and mechanism of coexistence in three sympatric of intertidal hermit crabs. Ecology 53:1062–1074.

Wäckers, F. L., P. C. J. van Rijn, and J. Bruin. 2005. Plant-Provided Food for Carnivorous Insects: A Protective Mutualism and its Applications. Cambridge University Press.

Ydenberg, R., and L. M. Dill. 1986. The economics of fleeing from predators. Adv Stud Behav 16.

# CHAPTER 1

## Pharaoh mite: Predatory mite uses empty aphid mummy as shelter

Marcus V. A. Duarte[1]; Mateus E. Rosado[1]; Arthur V. Ribeiro[1]; Angelo Pallini[1]; Arne Janssen[1, 2]

[1]Department of Entomology, Federal University of Viçosa, Minas Gerais, Brazil

[2]Section Population Biology, University of Amsterdam, Amsterdam, The Netherlands

**Abstract**

Animals use structures for protection against unfavourable climatic conditions and predation. Modified leaves, mollusc shells as well as structures provided by plants are examples of structures that offer shelter for organisms, and can have profound effects on population dynamics of many organisms. We show that empty aphid mummies, from which a parasitoid emerged, serve as shelter for the predatory mite *Amblyseius herbicolus,* protecting them from cannibalism and predation. We found that *A. herbicolus* larvae had higher survival in the presence of cannibalistic adult conspecifics and a bug that attacks the predatory mites when empty aphid mummies were present. Empty aphid mummies also resulted in an increased growth of predator populations on plants. Whereas it has been previously reported that mites use plant-provided shelter for protection, the usage of structures that are by-product of another animal's development is a novel observation. Structures such as these are present in all habitats and could play an important role as shelter for many organisms.

Keywords: Phytoseiid, Refuge, Cannibalism, Anthocoridae, *Amblyseius herbicolus*.

## Introduction

In nature, many different resources can limit population densities (MacArthur and Levins 1967). However, in some cases a single resource, such as shelter against predation, may play such a vital role in population dynamics that it overshadows the importance of other resources (Vance 1972). Animals are known to use many different structures as shelter against unfavourable climatic conditions and predation (Persson and Eklöv 1995; Caley and St John 1996; Fukui 2001; Danks 2002). For example, caterpillars fold or roll up leaves that not only serve for their own protection, but are also used as shelter and oviposition site for other arthropods (Martinsen et al. 2000; Lill and Marquis 2004). Another example is that of mollusc shells, which are used by many other organisms after they have been abandoned by their original inhabitant (Vance 1972; Gutierrez et al. 2003). The presence of these shells results in higher species richness (Gutierrez et al. 2003), and may be limiting resources for species abundance (Vance 1972). Sometimes the structures in which species seek shelter are not made for this purpose. For example, octopus use coconut shells found at the bottom of the ocean for protection (Finn et al. 2009). Here, we report on a new type of shelter used by predatory mites.

During field observations, predatory mites were found inside empty aphid mummies (Figure 1). These aphid mummies are the result of parasitism of aphids by a hymenopteran parasitic wasp, which oviposit inside aphids. The larvae that hatch from these eggs feed on the aphids from the inside. When pupating, the parasitoid larvae produce a silk cocoon and the aphid cuticle hardens. To emerge, the adult parasitoid gnaws an exit hole in this cuticle, leaving behind a hollow structure, an empty aphid mummy. It was inside this structure that predatory mites were observed.

The species observed inside the mummies is a member of the Phytoseiidae family, which are important natural enemies and are frequently used for biological control of pests (McMurtry and Croft 1997; McMurtry et al. 2013). Members of this family are known to engage in cannibalism (Walde et al. 1992; Schausberger 2003) and to be fed upon by predatory bugs (Cloutier and Johnson 1993).

It is well known that predatory mites use shelter such as webs and domatia (O'Dowd and Willson 1989; Walter and O'Dowd 1992; Lemos et al. 2015). Domatia are plant-provided shelters, which are frequently used by ants and predatory mites (Janzen 1966; O'Dowd and Willson 1989). Acarodomatia used by predatory mites

consist of pits or tufts of hair located at bifurcation of veins at the abaxial side of leaves (O'Dowd and Willson 1989; Pemberton and Turner 1989). They serve as protection against adverse conditions, cannibalism, intraguild predation and hyperpredation (Walter and O'Dowd 1992; Grostal and O'Dowd 1994; Faraji et al. 2002; Ferreira et al. 2008, 2011).

In the present study, we tested whether empty aphid mummies can serve as shelter for predatory mites in a similar way as domatia do. We used the phytoseiid mite *Amblyseius herbicolus*, Chant (Acari: Phytoseiidae), the species found inside the empty aphid mummy in the field (Figure 1a). This predatory mite has been reported as an important natural enemy of many herbivores (Cavalcante et al. 2015; Duarte et al. 2015; Rodríguez-Cruz et al. 2017). We tested whether the presence of empty aphid mummies reduced cannibalism and predation by the predatory bug *Blaptostethus pallescens* (Hemiptera: Anthocoridae) and promoted predator persistence on plants.

**Material & Methods**

We used a mixture of two populations of the predatory mite *A. herbicolus*. The first population was provided by the Entomology lab at Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG), and was collected from chilli pepper plants in Viçosa and Oratórios (Minas Gerais, Brazil, 20° 45' 0" S, 42° 52' 0" W). The second population was collected from litchi trees located at the campus of Federal University of Viçosa (20º 45' 32.2" S; 42º 52' 05.9" W). The rearing was kept on arenas made of PVC sheets (15 x 10 cm) on top of foam pads (4 cm height), which were kept in plastic trays (29 x 14 x 4 cm) filled with water. The edges of the arenas were wrapped with wet Kleenex® paper tissue, which was suspended into the water and thus served as both barrier and water source (van Rijn and Tanigoshi 1999). A small tent-shaped piece of PVC sheet was placed on the arena to serve as shelter, and a small piece of cotton wool was put under it as oviposition substrate. *Typha* sp. and honeybee pollen were offered as food.

The predatory bug *Blaptostethus pallescens* was provided by the Integrated Pest Management lab at the Federal University of Viçosa (UFV). It was collected from tomato fields in Coimbra (20° 51' 26"S; 42° 48' 03"W) and Viçosa (20° 46' 07"S; 42° 52' 12"W) Minas Gerais, Brazil. The insects were reared in plastic pots (1.5 l) with thin mesh covering the tops. Stems of hairy beggarticks (*Bidens pilosa* L.) were used as

egg-laying substrate for the predator and were changed twice a week. The old stems containing predator eggs were transferred to smaller plastic pots (250 ml) covered with thin mesh. Both pots contained shredded paper towel to reduce cannibalism. Ample amounts of frozen eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) were added twice per week. The emerged nymphs were kept in smaller container throughout their development, and the adults were transferred to a larger glass container for mating and egg laying. The arthropods were kept at 25 ± 2 °C, 70 ± 10% RH, 12:12 h L:D.

Empty aphid mummies were collected from cabbage plants (*Brassica oleracea* var. *capitata* L.) at EPAMIG (Viçosa, Minas Gerais, Brazil). These plants were grown in 1 liter pots with a commercial substrate (Bioplant, a mixture of vermiculite and organic fertilizer). The plants were infested with *Myzus persicae*, which were naturally parasitized with *Diaeretiella rapae*. The empty aphid mummies from which the parasitoids had emerged were gently removed with a soft brush and stored in a refrigerator at 10 ± 3 $^{o}$C until they were used for experiments.

*Survival in the presence of mummies*

Two experiments were done to verify the effect of empty aphid mummies on the survival of *A. herbicolus*: in the first, we tested cannibalism of predatory mite larvae by adult *A. herbicolus*, and in the second, we evaluated predation of adults of *A. herbicolus* by a predatory bug (second instar nymph of *B. pallescens*). For the first experiment we placed 3 *A. herbicolus* larvae on a 2.7 cm (diameter) PVC disc with *Typha sp.* pollen. The discs were floating in a Petri dish (3 cm diameter) filled with water. The PVC disc had a small hole in the centre through which an entomological pin passed, which was glued to the centre of the bottom of the Petri dish to prevent the arena from drifting to the side of the Petri dish. There were four treatments with 12 replicates per treatment: a) three *A. herbicolus* (larvae) (control); b) three *A. herbicolus* (larvae) with 3 empty aphid mummies; c) three *A. herbicolus* (larvae) larvae with one cannibal (adult *A. herbicolus*); and d) three *A. herbicolus* (larvae), three empty aphid mummies and one cannibal (adult *A. herbicolus*). The second experiment was done in the same conditions as the first but instead of using *A. herbicolus* larvae we used *A. herbicolus* adults and we substituted the cannibalistic *A. herbicolus* adult for the predatory bug *B. pallescens*. The numbers of mobile larvae and adults were assessed

after 24 hours and were compared with a linear mixed-effects model (LME) with treatment as fixed factor and block as random factor (Crawley 2013). Contrasts among treatments were determined with general linear hypothesis testing (function glht of the package lsmeans in R, Lenth 2016).

*Predator densities on chilli pepper plants in the presence of mummies*

Chilli pepper seeds were sown in 1 l pots filled with a commercial substrate (Bioplant®) in a greenhouse. Plants were watered daily and fertilized weekly with a mixture of 50 g of N-P-K (20-05-20) and 100 g of simple superphosphate. Plants had 4 completely expanded leaves and were 10 cm high when used for experiments; at this stage the plants do not have well formed domatia. There were two treatments; one with empty aphid mummies and the other without (control). Plants with empty aphid mummies carried five empty aphid mummies glued with white non-toxic glue (Pritt®) to the first pair of completely expanded leaves (10 mummies/plant). Each plant of both treatments received five 10-day-old *A. herbicolus* adults. *Typha sp.* pollen (0.15 g) served as food for the predators and was added with a fine brush to the first completely expanded leaf every three days. The plants were placed in water-filled trays to avoid escape of mites and migration to other plants. On the eight day (sufficient time for a new generation of *A. herbicolus* to develop, Rodríguez-Cruz et al. 2013), the leaves were removed one by one and adult and juvenile mites were counted using a stereoscopic microscope (Zeiss® Stemi 2000-c). We were unable to count the eggs because these were frequently found inside empty aphid mummies and opening mummies would result in destroying some of them. The numbers of adult and juveniles mites were log (x + 1) transformed and compared between treatments with a linear mixed-effects model with treatment as fixed factor and block as random factor (LME, Crawley 2013). All statistical analyses were done using the computer software R version 3.1.0 (R Core Team 2014).

**Results**

*Survival in the presence of mummies*

Larval survival differed significantly among treatments in the cannibalism experiment (Figure 2 A: $\chi^2 = 42.9$, d.f. = 3, $p < 0.001$). Larval survival was significantly lower without empty aphid mummies in the presence of adult *A. herbicolus* than in the other treatments (Figure 2 A). Likewise, the number of surviving adult of *A. herbicolus*

differed significantly among treatments in the experiment with the predator (Figure 2 B: $\chi^2 = 38.1$, d.f. = 3, $p < 0.001$). *A. herbicolus* adults also had lower survival when a predatory bug was present without empty aphid mummies and the highest survival was found when empty aphid mummies were present in the absence of the predator (Figure 2 B).

*Predator densities on chilli pepper plants in the presence of mummies*

The addition of empty aphid mummies significantly increased the number of adult mites (Figure 3: $\chi^2 = 6.6$, d.f. = 1, $p = 0.01$) and immature mites (Figure 3: $\chi^2 = 10.0$, d.f. = 1, $p = 0.0015$) after 8 days on chilli pepper plants.

**Discussion**

We have shown that empty aphid mummies can offer protection against predation and cannibalism for predatory mites (Figure 1). Similar to what has been shown previously for domatia (Ferreira et al. 2008, 2011). The empty aphid mummies increased survival of predatory mites in the presence of cannibalistic adults and predators (Figure 2). The presence of empty aphid mummies on pepper plants significantly increased the densities of juveniles and adults of the predatory mites (Figure 3). Survival of juveniles on plants with empty aphid mummies was noticeably higher than of the adults, resulting in a more than six-fold increase, whereas the increase of adults was less than two-fold. Because juvenile mites are usually smaller, making them more susceptible to cannibalism (Schausberger 2003), it would be expected that the presence of a refuge would have a larger effect on juvenile than on adult survival (Figure 2). The larger number of adults found on the plants was probably a consequence of the higher survival of the first juveniles produced in the presence of the structure. Another possible cause for the lower number of juveniles on plants without empty aphid mummies is that the predatory mite adults retained theirs eggs in response to the absence of safe sites for oviposition. It is known that mites are capable of retaining their eggs when environmental conditions are not favourable for their offspring (Montserrat et al. 2007), and an environment without shelter maybe be perceived as less favourable. In any case, whether the increased density of mites was a direct effect of increased survival, an indirect effect of the presence of shelter, or both, the presence of the shelter resulted in increased predator densities. Our experiments show an

interesting and unexpected indirect interaction between predatory mites and parasitoids, where empty aphid mummies, which are a by-product of parasitism by a parasitic wasp, serves as shelter for a predatory mite.

Even though both juveniles and adults are capable of entering empty aphid mummies, we found especially larger numbers of juvenile predatory mites in the presence of empty aphid mummies. This may be due to the fact that empty aphid mummies offer more spatial structure, which decreases the negative effects of predation (Langellotto and Denno 2004; Janssen et al. 2007). Spatial structure has been shown to reduce the negatives effect of predation equally then those that completely exclude predators (Persson and Eklöv 1995).

*Amblyseius herbicolus* displayed a very peculiar behaviour in the presence of the predatory bug. Individuals, especially in the treatments without empty aphid mummies, were frequently found on the backs of the predatory bug, and sometimes even oviposited there. This behaviour will be explored in an upcoming paper (Chapter 2).

Reports on organisms that profit from shelters provided by plants (Janzen 1966; Walter and O'Dowd 1992; Grostal and O'Dowd 1994) or produced and used as shelter by other organism are common (Vance 1972; Martinsen et al. 2000; Gutierrez et al. 2003; Lemos et al. 2015). However, reports on animals using structures that were originally not produced as shelter by other organisms, but as a by-product of other processes, such as octopus using coconuts shells (Finn et al. 2009), are less common. However, many animals leave behind structures as a result of their physiology and development (such as pupae, faeces or exuviae) or corpses as result of predation or parasitism, which may serve as shelter for other organisms. Maybe due to the small size and ephemeral nature of such structures left behind by arthropods, little attention has been given to their role as shelter and refuge (Lill and Marquis 2003, 2007). We believe that the usage of such shelter deserves attention because it may have profound effects on survival and population dynamics, as we have shown here. In this sense, most life forms will inadvertently be habitat providers or ecosystem engineers (Jones et al. 1994, 1997; Gutierrez et al. 2003).

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**Bibliography**

Caley, M. J., and J. St John. 1996. Refuge availability structures assemblages of tropical reef fishes. Journal of Animal Ecology 65:414–428.

Cavalcante, A. C. C., V. L. V. dos Santos, L. C. Rossi, and G. J. de Moraes. 2015. Potential of five Brazilian populations of Phytoseiidae (Acari) for the biological control of *Bemisia tabaci* (Insecta: Hemiptera). Journal of Economic Entomology 108:29-33.

Cloutier, C. and Johnson, S.G. 1993. Predation by *Orius tristicolor* (Hemiptera: Anthocoridae) on *Phytoseiulus persimilis* (Acarina: Phytoseiidae): testing for compatibility between biocontrol agents. Environmental Entomology 22: 477–482.

Crawley, M. J. 2013. The R book. Wiley.

Danks, H. V. 2002. Modification of adverse conditions by insects. Oikos 99:10–24.

Duarte, M. V. A., M. Venzon, M. C. de S. Bittencourt, F. A. Rodríguez-Cruz, A. Pallini, and A. Janssen. 2015. Alternative food promotes broad mite control on chilli pepper plants. BioControl 60:817–825.

Faraji, F., A. Janssen, and M. Sabelis. 2002. The benefits of clustering eggs: the role of egg predation and larval cannibalism in a predatory mite. Oecologia 131:20–26.

Ferreira, J. a. M., D. F. S. Cunha, A. Pallini, M. W. Sabelis, and A. Janssen. 2011. Leaf domatia reduce intraguild predation among predatory mites. Ecological Entomology 36:435–441.

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

Finn, J. K., T. Tregenza, and M. D. Norman. 2009. Defensive tool use in a coconut-carrying octopus. Current Biology 19:R1069–R1070.

Fukui, A. 2001. Indirect interactions mediated by leaf shelters in animal–plant communities. Population Ecology 43:31–40.

Grostal, R., and D. J. O'Dowd. 1994. Plants, mites and mutualism: leaf domatia and the abundance and reproduction of mites on *Viburnum tinus*. Oecologia 97:308–315.

Gutierrez, J. L., C. G. Jones, D. L. Strayer, and O. O. Iribarne. 2003. Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. Oikos 101:79–90.

Janssen, A., M. W. Sabelis, S. Magalhães, M. Montserrat, and T. van der Hammen. 2007. Habitat structure affects intraguild predation. Ecology 88:2713–2719.

Janzen, D. H. 1966. Coevolution of mutualism between ants and acacias in Central America. Evolution 20:249–275.

Jones, C. G., J. H. Lawton, and M. Shachak. 1994. Organisms as ecosystem engineers. Oikos 69:373.

———. 1997. Positive and negative effects of organisms as physical ecosystem engineers. Ecology 78:1946–1957.

Langellotto, G. A., and R. F. Denno. 2004. Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. Oecologia 139:1–10.

Lemos, F., A. M. G. Bernardo, C. R. Dias, R. A. Sarmento, A. Pallini, M. Venzon, and A. Janssen. 2015. Breaking and entering: predators invade the shelter of their prey and gain protection. Experimental and Applied Acarology 67:247–257.

Lenth, R. V. 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software 69.

Lill, J. T., and R. J. Marquis. 2003. Ecosystem engineering by caterpillars increases insect herbivore diversity on white oak. Ecology 84:682–690.

———. 2004. Leaf ties as colonization sites for forest arthropods: an experimental study. Ecological Entomology 29:300–308.

———. 2007. 6 - Microhabitat Manipulation: Ecosystem engineering by shelter-building insects. Pages 107–138 *in* J. E. B. Kim Cuddington William G.Wilson and Alan Hastings, ed. Theoretical Ecology Series, Ecosystem EngineersEcosystem Engineers (Vol. 4). Academic Press.

MacArthur, R., and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. The American Naturalist 101:377–385.

Martinsen, G. D., K. D. Floate, A. M. Waltz, G. M. Wimp, and T. G. Whitham. 2000. Positive interactions between leafrollers and other arthropods enhance biodiversity on hybrid cottonwoods. Oecologia 123:82–89.

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

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

Montserrat, M., C. Bas, S. Magalhães, M. W. Sabelis, A. M. de Roos, and A. Janssen. 2007. Predators induce egg retention in prey. Oecologia 150:699–705.

O'Dowd, D. J., and M. F. Willson. 1989. Leaf domatia and mites on Australasian plants: ecological and evolutionary implications. Biological Journal of the Linnean Society 37:191–236.

Pemberton, R. W., and C. E. Turner. 1989. Occurrence of predatory and fungivorous mites in leaf domatia. American Journal of Botany 76:105–112.

Persson, L., and P. Eklöv. 1995. Prey refuges affecting interactions between piscivorous perch and juvenile perch and roach. Ecology 76:70–81.

Rodríguez-Cruz, F. A., A. Janssen, A. Pallini, M. V. A. Duarte, C. M. F. Pinto, and M. Venzon. 2017. Two predatory mite species as potential control agents of broad mites. BioControl 62:505–513.

Rodríguez-Cruz, F. A., M. Venzon, and C. M. F. Pinto. 2013. Performance of *Amblyseius herbicolus* on broad mites and on castor bean and sunnhemp pollen. Experimental and Applied Acarology 60:497–507.

Schausberger, P. 2003. Cannibalism among phytoseiid mites: a review. Experimental & Applied Acarology 29:173–191.

van den Bosch, F., A. M. de Roos, and W. Gabriel. 1988. Cannibalism as a life boat mechanism. Journal of Mathematical Biology 26:619–633.

van Rijn, P. C. J., and L. K. Tanigoshi. 1999. 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.

Vance, R. R. 1972. Competition and mechanism of coexistence in three sympatric of intertidal hermit crabs. Ecology 53:1062–1074.

Walde, S. J., J. P. Nyrop, and J. M. Hardman. 1992. Dynamics of *Panonychus ulmi* and *Typhlodromus pyri*: factors contributing to persistence. Experimental and Applied Acarology 14:261–291.

Walter, D. E., and D. J. O'Dowd. 1992. Leaves with domatia have more mites. Ecology 73:1514–1518.

**Figure legends**

*Figure 1*

A: Empty aphid mummy with an adult *A. herbicolus* inside; B: Empty aphid mummy with *A. herbicolus* eggs inside.

*Figure 2*

A: Average numbers of *A. herbicolus* larvae (+ SE) alive on the PVC discs with either three *A. herbicolus* larvae with 3 empty aphid mummies (Mummy), three *A. herbicolus* larvae (Control), three *A. herbicolus* larvae with an adult female *A. herbicolus* (Cannibal), or three *A. herbicolus* larvae, three empty aphid mummies and an adult female *A. herbicolus* (Cannibal + Mummy). Bars with different letters differ significantly (contrasts after LME, $p < 0.05$).

B: Average numbers of adult *A. herbicolus* (+ SE) alive on the PVC discs with either three *A. herbicolus* with 3 empty aphid mummies (Mummy), three *A. herbicolus* (Control); three *A. herbicolus* with a nymph of *B. pallescens* (Predator), or three *A. herbicolus*, three empty aphid mummies and a nymph of *B. pallescens* (Predator +Mummy). Bars with different letter differ significantly (contrasts after LME, $p < 0.05$).

*Figure 3*

Average number of *A. herbicolus* juvenile and adults (+ SE) found on chilli pepper plants without or with empty aphid mummies. Bars with different letters indicate significant difference between treatments (LME; $p < 0.05$).

## Figures

*Figure 1*

A B

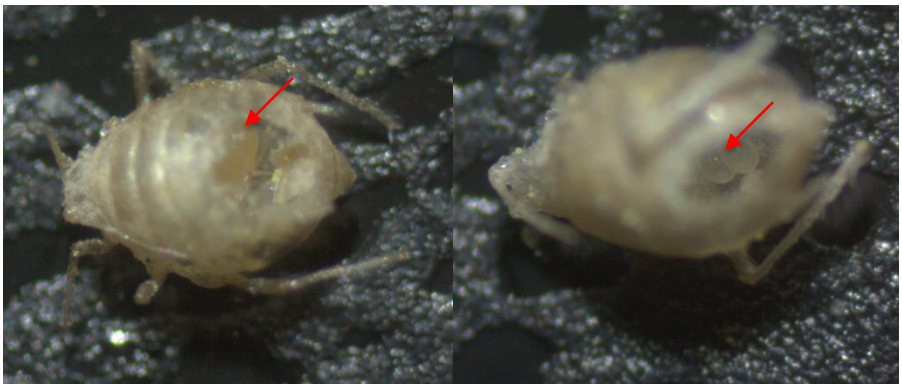
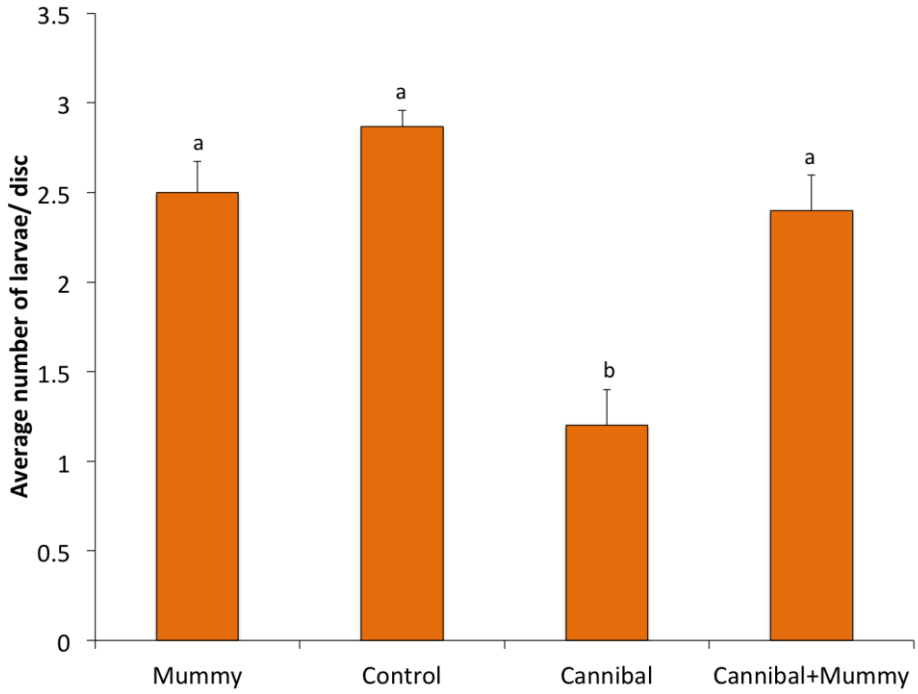


*Figure 2*

A


3.5
3
2.5
2
1.5
1
0.5
0
Average number of larvae/ disc
a
a
b
a
Mummy
Control
Cannibal
Cannibal+Mummy

B

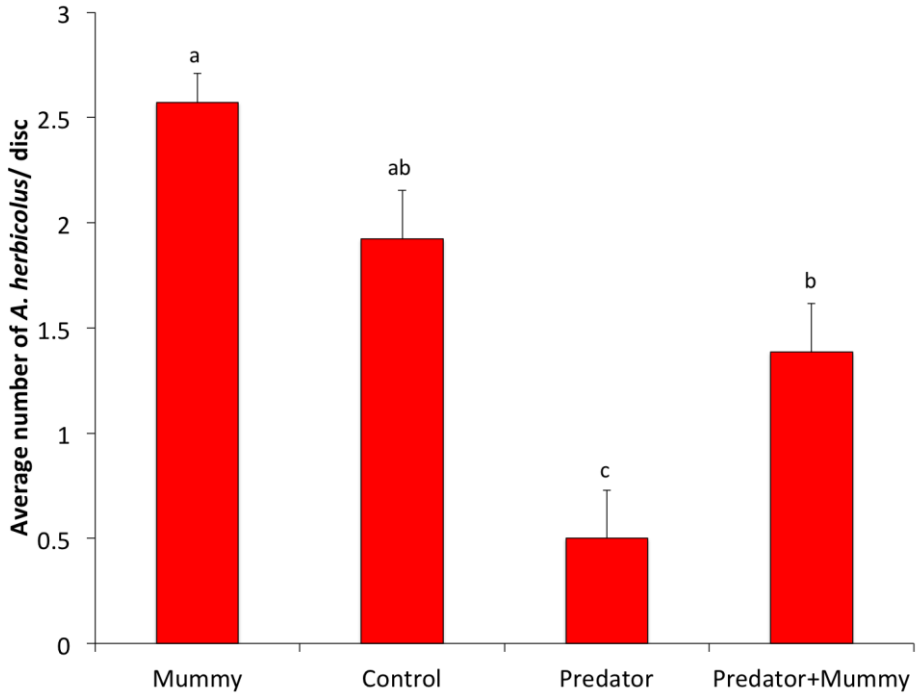
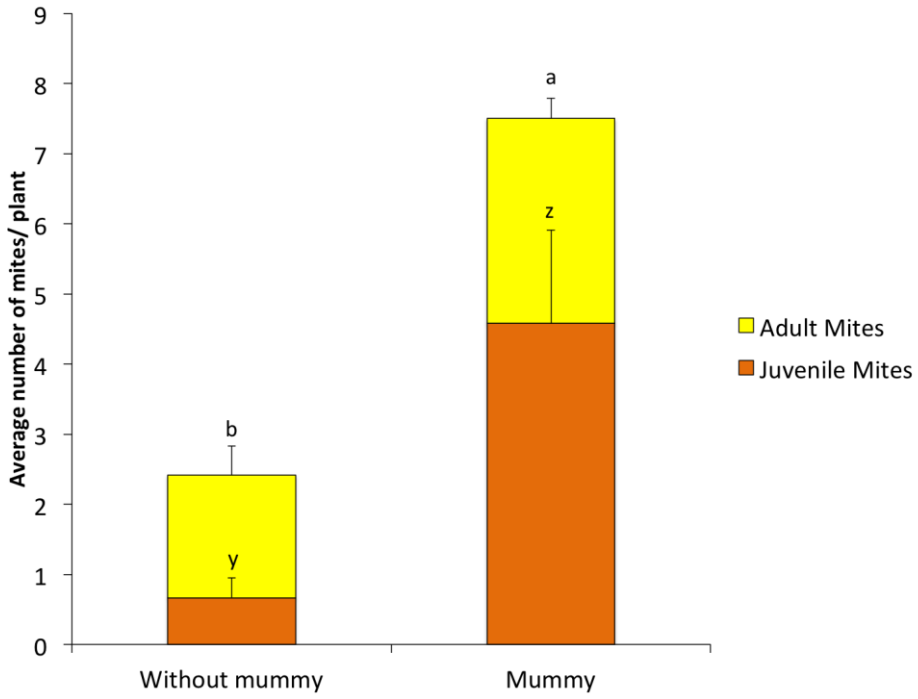

3
2.5
2
1.5
1
0.5
0
Average number of A. herbicolus/ disc
a
ab
c
b
Mummy
Control
Predator
Predator+Mummy

*Figure 3*


Average number of mites/ plant
9
8
7
6
5
4
3
2
1
0
a
z
b
y
Without mummy
Mummy
Adult Mites
Juvenile Mites

**Mounting the beast: a new antipredator behaviour**

Marcus V. A. Duarte[1]; Mateus E. Rosado[1]; Arthur V. Ribeiro[1]; Angelo Pallini[1]; Arne Janssen[1, 2]

[1]Department of Entomology, Federal University of Viçosa, Minas Gerais, Brazil

[2]Section Population Biology, University of Amsterdam, Amsterdam, The Netherlands

**Abstract**

Animals display a wide array of behaviours to avoid predation. Elaborate escapes, avoidance and defensive behaviours are a few examples of these behaviours. We show a completely new antipredator response, where the predatory mite *Amblyseius herbicolus* mounts and oviposits on the back of its predator the predatory bug *Blaptostethus pallescens* to avoid predation. When glued on the back of *B. pallescens,* the predatory mite and their eggs were able to survive more then when on the discs. We also tested if *A. herbicolus* was mounting *B. pallescens to* feed on it. We found that not even when in high densities *A. herbicolus* was not able to kill *B. pallescens*, nor did we observe any feeding from the mites. Even thought mites can display a wide array of complex antipredator behaviours mounting and ovipositing on their backs is to the best of our knowledge completely unheard of.

Keywords: *Amblyseius herbicolus,* Phytoseiidae, *Blaptostethus pallescens,* Predation.

## Introduction

Avoiding predation determines the success of individuals in nature. Animals can avoid encounters with their predator by camouflage, having aposematic colorations and protective structures (Edmunds 1974). Animals can blend into the environment through camouflage, making it hard for predators to see them (Stuart-Fox et al. 2006). Animals can also have aposematic coloration which are colour patterns that warn a predator that you are toxic or distasteful (Mallet and Joron 1999), or mimic the coloration of toxic species (Joron and Mallet 1998). Another way to avoid encounter with predators is to have protective structures or shelter, which can grant protection against predators (Olmstead and Denno 1992; Cloudsley-Thompson 1995).

Animals can also display behaviours in responses to the presence of predators, these behaviours include elaborate escape, avoidance and defensive behaviours (Barnard 1983). Flight can be an efficient strategy for a prey to distance itself from a predator (Ydenberg and M. Dill 1986). Animals are also know to gather around and harass potential predator, this strategy is called mobbing (Ostreiher 2003). Mobbing can be done to alert conspecifics, confuse potential predators, chase predators away (Ostreiher 2003). These response behaviours can be modulate depending on the perceived threat that the predator imposes (Ferrari et al. 2005). In the present work we report on new antipredator behaviour.

During previous experiments (Chapter 1) we observed a very peculiar antipredator behaviour by a mite in the presence of a predatory bug, which can prey on the predatory mites. Especially in the absence of shelter, predatory mites were frequently found on the back of the predatory bug, and sometimes even oviposited there (Figure 1).

The species that mounted was *Amblyseius herbicolus*, Chant (Acari: Phytoseiidae). This predatory mite has been reported as an important natural enemy of many herbivores (Cavalcante et al. 2015; Duarte et al. 2015; Rodríguez-Cruz et al. 2017). This mite is from the Phytoseiidae family, which are important natural enemies (McMurtry and Croft 1997; McMurtry et al. 2013). The predatory bug was *Blaptostethus pallescens* (Hemiptera: Anthocoridae) which is also an important natural enemy found in many agro-ecosystems (Sobhy et al. 2014; Ramos et al. 2017). Phytoseiid mites and Anthocorid bugs are frequently part of the same agro-ecosystems (Brødsgaard and Albajes 1999) and have been reported to engage in intraguild

predation (Gillespie and Quiring 1992; Wittmann and Leather 1997; Chow et al. 2008). Both *B. pallescens* and *A. herbicolus* have been reported to occur on tomato plants (Ramos et al. 2017; André Cardoso personal comunication).

In the present study, we tried to answer why a predatory mite would mount and oviposit on the back of a predatory bug. Mites are able to avoid predators by using shelters such as webs, domatia (which are plant provided shelter) and even aphid mummies (O'Dowd and Willson 1989; Walter and O'Dowd 1992; Lemos et al. 2015; Chapter 1). Mites can also perceive potential danger and move away from dangerous predators (Magalhães et al. 2002) and have also been show to display antipredator behaviour to protect their eggs by ovipositing away from potential egg predators, as well as increase the frequency of eggs suspended their web to avoid predation (Faraji et al. 2002*b;* Lemos et al. 2010). We hypothesize that when on the back of the predatory bug the mite could be safe from predation. Another reason could be that the mite is mounting to feed on the predatory bug. We tested whether the predatory mites and their eggs suffered less from predation when on the back of the predatory bug, then when on the ground level. We also verified whether the predatory mites mounted the bugs in order to feed on them and had the potential to kill the predatory bug.

**Material & Methods**

We used a mixture of two populations of the predatory mite *A. herbicolus*. The first population was provided by the Entomology lab at Agriculture and Livestock Research Enterprise of Minas Gerais (EPAMIG), and it was collected from chilli pepper plants in Viçosa and Oratórios (Minas Gerais, Brazil, 20° 45' 0" S, 42° 52' 0" W). The second population was collected from litchi trees located at the campus of Federal University of Viçosa (20º 45' 32.2" S; 42º 52' 05.9" W). The rearing was kept on arenas made of PVC sheets (15 x 10 cm) on top of foam pads (4 cm height), which were kept in plastic trays (29 x 14 x 4 cm) filled with water. The edges of the arenas were wrapped with wet Kleenex® paper tissue, which was suspended into the water and thus served as both barrier and water source (van Rijn and Tanigoshi 1999). A small tent-shaped piece of PVC sheet was placed on the arena to serve as shelter, and a small piece of cotton wool was put under it as oviposition substrate. *Typha* sp. and honeybee pollen were offered as food.

The predatory bug *Blaptostethus pallescens* was provided by the Integrated

Pest Management lab at the Federal University of Viçosa (UFV). It was collected from tomato fields in Coimbra (20° 51' 26"S; 42° 48' 03"W) and Viçosa (20° 46' 07"S; 42° 52' 12"W) Minas Gerais, Brazil. The insects were reared in plastic pots (1.5 l) with thin mesh covering the tops. Stems of hairy beggarticks (*Bidens pilosa* L.) were used as egg-laying substrate for the predator and were changed twice a week. The old stems containing predator eggs were transferred to smaller plastic pots (250 ml) covered with thin mesh. Both pots contained shredded paper towel to reduce cannibalism. Ample amounts of frozen eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) were added twice per week. The emerged nymphs were kept in smaller container throughout their development, and the adults were transferred to a larger glass container for mating and oviposition. The arthropods were kept at 25 ± 2 °C, 70 ± 10% RH, 12:12 h L:D.

*Survival on the back of B. pallescens*

This experiment was done to compare the survival of *A. herbicolus* and their eggs when on the back of *B. pallescens* or on the arena. We used a 2.7 cm (diameter) PVC disc floating in a Petri dish (3 cm diameter) filled with water. The PVC disc had a small hole in the centre through which an entomological pin passed, which was glued to the centre of the bottom of the Petri dish to prevent the arena from drifting to the side of the Petri dish. On this disc, we placed a third instar *B. pallescens* nymph with an *A. herbicolus* adult or *A. herbicolus* egg glued to the centre of its back with a small drop of white non-toxic glue (Pritt®). On the same disc, we also glued one *A. herbicolus* adult or egg to the disc where they were glue to the back of *B. pallescens*. Twenty-four hours later, we checked the number of *A. herbicolus* adult and eggs that had been predated. The proportions of predated *A. herbicolus* and their eggs when on the back or on the disc were compared with a generalized linear model (GLM) with a binomial distribution (Crawley 2013).

*Predation of B. pallescens by A. herbicolus*

To verify if *A. herbicolus* mounted on *B. pallescens* to feed on it, we used a square PVC sheet (2.5 X 2.5 cm) floating in cotton-soaked treads in a 5.5 cm Petri dish. A small tent-shaped piece of PVC was placed on the disc to offer shelter for the predatory mite and to reduce predator escape. On each of these arenas, a third instar nymph of

*B. pallescens* was released together with 20 adult females of *A. herbicolus*, the most voracious stage, and *Typha sp.* pollen, which served as a food source for *B. pallescens* and *A. herbicolus*. As a control we used a similar arena with only the third instar of *B. pallescens* and *Typha sp.* pollen. We registered the fate of the *B. pallescens* larva every 12 hours during 48 hours to verify whether *A. herbicolus* killed any *B. pallescens.* Mites are very small animals that feed by inserting their mouthparts into their prey and sucking the inner contents, making it difficult to see them actively feeding. However, *A. herbicolus* is a very translucent mite and it is possible to see its intestines moving when they are feeding. Their intestines also gain the colour of what they have been feeding on. We observed whether individuals mounted on the back on *B. pallescens* were feeding and checked for any pigmentation in their intestines that would point at previous ingestion of bodily fluids of the nymph.

**Results**

*Survival on the back of B. pallescens*

The predation of *A. herbicolus* was lower for individuals on the back of *B. pallescens* than for individuals on the arena *(*Figure 2: $\chi^2 = 13.0$, d.f. = 1, $p < 0.001$). Likewise, the predation on eggs of *A. herbicolus* was lower when they were on the back of *B. pallescens* than on the arena *(*Figure 2: $\chi^2 = 13.4$, d.f. = 1, $p < 0.001$).

*Predation of B. pallescens by A. herbicolus*

Throughout the experiment, no *B. pallescens* died both in the control treatment and with the *A. herbicolus.* None of the *A. herbicolus* were feeding on *B. pallescens* when they were on its back, as could be seen from movement and pigmentation of their intestines.

**Discussion**

To the best of our knowledge, we show a completely novel antipredator behaviour. Although arthropods have a wide array of antipredator mechanism such as avoiding plants with predators, hiding in plant-provided shelter and increasing the oviposition in their web when under predation risk (Nomikou et al. 2003; Lemos et al. 2010; Ferreira et al. 2011; Meng et al. 2012), mounting on a predator and ovipositing on their back is completely novel (Figure 1). When on the back of the predatory bugs, both the adults

and eggs suffered much less from predation (Figure 2). We observed once that the predatory bug used its hind legs to remove the mites from their back, which resulted in the only mite killed in all the repetitions. Our experiments show that the back of the predatory bug is much safer place then the disc.

Another explanation of why the mite was on the back of the predatory bug would be that they were trying to feed on the predatory bug. We showed that this is unlikely; the predatory bugs were never killed and no feeding of predatory mites on the bugs was observed, even with very high densities of predatory mites. The predatory mites could be mounting the predatory bug as a form of mobbing, trying to harass the predator bug, so it would chose another habitat (Ostreiher 2003). Mobbing might be a good explanation for this mounting behaviour, however it does not explain ovipositing on the back of the predator. Instead, the most parsimonious explanation is that the predatory mite mounts and oviposit on the back of the predatory bug to avoid predation.

Animals can display a wide array of antipredator behaviours (Barnard 1983). However, mounting and ovipositing on the back of their predator is something completely novel. Animals are capable of perceiving danger and triggering antipredator responses in many ways (Lima and Bednekoff 1999). The stimuli that makes a prey not move away, which is usually the safer alternative (Ydenberg and M. Dill 1986), but move close to a predator and on its back deserves future experiments.

Another possible benefit for the mites could be dispersal. Mites are know to use other arthropods to assist them in dispersing large distances, this behaviour is called phoresy (Binns 1982; M A Houck and OConnor 1991). Phytoseiidae and Anthocoridae can feed on the same prey (Gillespie and Quiring 1992; Chow et al. 2008), although there are no examples of Phytoseiidae that disperse with another arthropod, dispersing with a larger insect that could be searching for the same food could be advantageous for the predatory mite.

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**Bibliography**

Barnard, C. J. 1983. Anti-Predator Behaviour. Pages 201–217 *in*Animal Behaviour. Springer, Boston, MA.

Binns, E. S. 1982. Phoresy as migration - some functional aspects of phoresy in mites. Biological Reviews 57:571–620.

Brødsgaard, H. F., and R. Albajes. 1999. Insect and mite pests. Pages 48–60 *in*Integrated Pest and Disease Management in Greenhouse Crops, Developments in Plant Pathology. Springer, Dordrecht.

Cavalcante, A. C. C., D. Santos, V. L. Vicente, L. C. Rossi, and G. J. de Moraes. 2015. Potential of five Brazilian populations of Phytoseiidae (Acari) for the biological control of *Bemisia tabaci* (Insecta: Hemiptera). Journal of Economic Entomology 108:29–33.

Chow, A., A. Chau, and K. M. Heinz. 2008. Compatibility of *Orius insidiosus* (Hemiptera: Anthocoridae) with *Amblyseius (Iphiseius) degenerans* (Acari: Phytoseiidae) for control of *Frankliniella occidentalis* (Thysanoptera: Thripidae) on greenhouse roses. Biological Control 44:259–270.

Cloudsley-Thompson, J. L. 1995. A review of the anti-predator devices of spiders. Bulletin of the British Arachnological Society 10:81–96.

Crawley, M. J. 2013. The R book. Wiley.

Dominey, W. J. 1983. Mobbing in colonially nesting fishes, especially the bluegill, *Lepomis macrochirus*. Copeia 1983:1086–1088.

Duarte, M. V. A., M. Venzon, M. C. de S. Bittencourt, F. A. Rodríguez-Cruz, A. Pallini, and A. Janssen. 2015. Alternative food promotes broad mite control on chilli pepper plants. BioControl 1–9.

Edmunds, M. 1974. Defence in animals: A survey of anti-predator defences. Longman Publishing Group, Burnt Mill, UK.

Ferrari, M. C. O., J. J. Trowell, G. E. Brown, and D. P. Chivers. 2005. The role of learning in the development of threat-sensitive predator avoidance by fathead minnows. Animal Behaviour 70:777–784.

Ferreira, J. a. M., D. F. S. Cunha, A. Pallini, M. W. Sabelis, and A. Janssen. 2011. Leaf domatia reduce intraguild predation among predatory mites. Ecological Entomology 36:435–441.

Gillespie, D. R., and D. J. M. Quiring. 1992. Competition between *Orius tristicolor* (White)(Hemiptera: Anthocoridae) and *Amblyseius cucumeris*

(Oudemans)(Acari: Phytoseiidae) feeding on *Frankliniella occidentalis* (Pergande)(Thysanoptera: Thripidae). The Canadian Entomologist 124:1123–1128.

Joron, M., and J. L. B. Mallet. 1998. Diversity in mimicry: paradox or paradigm? Trends in Ecology & Evolution 13:461–466.

Lemos, F., A. M. G. Bernardo, C. R. Dias, R. A. Sarmento, A. Pallini, M. Venzon, and A. Janssen. 2015. Breaking and entering: predators invade the shelter of their prey and gain protection. Experimental and Applied Acarology 67:247–257.

Lemos, F., R. A. Sarmento, A. Pallini, C. R. Dias, M. W. Sabelis, and A. Janssen. 2010. Spider mite web mediates anti-predator behaviour. Experimental and Applied Acarology 52:1–10.

Lima, S. L., and P. A. Bednekoff. 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. The American Naturalist 153:649–659.

M A Houck, and  and B. M. OConnor. 1991. Ecological and evolutionary significance of phoresy in the astigmata. Annual Review of Entomology 36:611–636.

Magalhães, S., A. Janssen, R. Hanna, and M. W. Sabelis. 2002. Flexible antipredator behaviour in herbivorous mites through vertical migration in a plant. Oecologia 132:143–149.

Mallet, J., and M. Joron. 1999. Evolution of diversity in warning color and mimicry: polymorphisms, shifting balance, and speciation. Annual Review of Ecology and Systematics 30:201–233.

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

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

Meng, R.-X., M. W. Sabelis, and A. Janssen. 2012. Limited predator-induced dispersal in whiteflies. PloS one 7:e45487.

Nomikou, M., A. Janssen, and M. W. Sabelis. 2003. Herbivore host plant selection: whitefly learns to avoid host plants that harbour predators of her offspring. Oecologia 136:484–488.

O'Dowd, D. J., and M. F. Willson. 1989. Leaf domatia and mites on Australasian plants: ecological and evolutionary implications. Biological Journal of the Linnean Society 37:191–236.

Olmstead, K. L., and R. F. Denno. 1992. Cost of shield defence for tortoise beetles (Coleoptera: Chrysomelidae). Ecological Entomology 17:237–243.

Ostreiher, R. 2003. Is mobbing altruistic or selfish behaviour? Animal Behaviour 66:145–149.

Ramos, R. S., C. S. Sediyama, E. A. Queiroz, T. L. Costa, J. C. Martins, T. A. Araújo, and M. C. Picanço. 2017. Toxicity of insecticides to *Chrysodeixis includens* and their direct and indirect effects on the predator Blaptostethus pallescens. Journal of Applied Entomology n/a-n/a.

Rodríguez-Cruz, F. A., A. Janssen, A. Pallini, M. V. A. Duarte, C. M. F. Pinto, and M. Venzon. 2017. Two predatory mite species as potential control agents of broad mites. BioControl 62:505–513.

Shedd, D. H. 1982. Seasonal variation and function of mobbing and related antipredator behaviors of the American robin (*Turdus migratorius*). The Auk 99:342–346.

Sih, A. 1986. Antipredator responses and the perception of danger by mosquito larvae. Ecology 67:434–441.

Sobhy, I. S., A. M. Abdul-Hamid, A. A. Sarhan, A. A. Shoukry, N. S. Mandour, and S. R. Reitz. 2014. Life history traits of *Blaptostethus pallescens* (Hemiptera: Anthocoridae), a candidate for use in augmentative biological control in Egypt. Applied Entomology and Zoology 49:315–324.

Stuart-Fox, D., M. J. Whiting, and A. Moussalli. 2006. Camouflage and colour change: antipredator responses to bird and snake predators across multiple populations in a dwarf chameleon. Biological Journal of the Linnean Society 88:437–446.

Van Rijn, P. C. J., and L. K. Tanigoshi. 1999. 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.

Venzon, M., A. Janssen, A. Pallini, and M. W. Sabelis. 2000. Diet of a polyphagous arthropod predator affects refuge seeking of its thrips prey. Animal Behaviour 60:369–375.

Walter, D. E., and D. J. O'Dowd. 1992. Leaves with Domatia Have More Mites. Ecology 73:1514–1518.

Wittmann, E. J., and S. R. Leather. 1997. Compatibility of *Orius laevigatus* Fieber (Hemiptera: Anthocoridae) with *Neoseiulus (Amblyseius) cucumeris* Oudemans (Acari: Phytoseiidae) and *Iphiseius (Amblyseius) degenerans* Berlese (Acari: Phytoseiidae) in the biocontrol of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). Experimental & Applied Acarology 21:523–538.

Ydenberg, R., and L. M. Dill. 1986. The economics of fleeing from predators. Adv Stud Behav 16.

**Figure legends**

*Figure 1*

A: *Blaptostethus pallescens nymph* with *A. herbicolus* and an egg on its back. B: Three *A. herbicolus* on the back of a nymph of *Blaptostethus pallescens*.

*Figure 2*

Proportion of *A. herbicolus* (Mite) and eggs (+ SE) predated by *B. pallescens* when they were on the arena or on the back of *B. pallescens.* Bars with different letters differ significantly (GLM, $p < 0.05$).

## Figures

*Figure 1*

A B

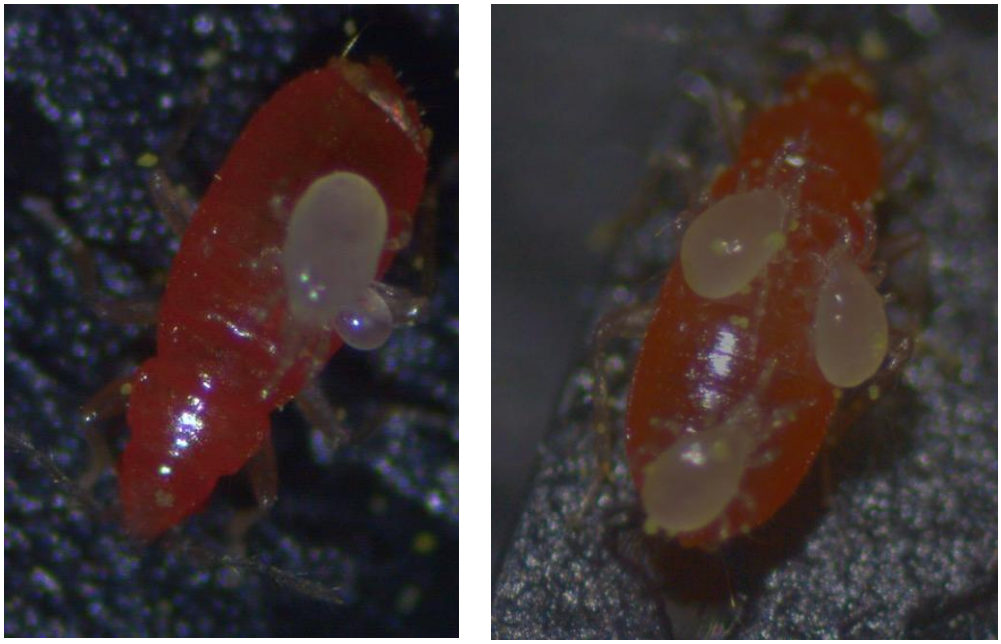
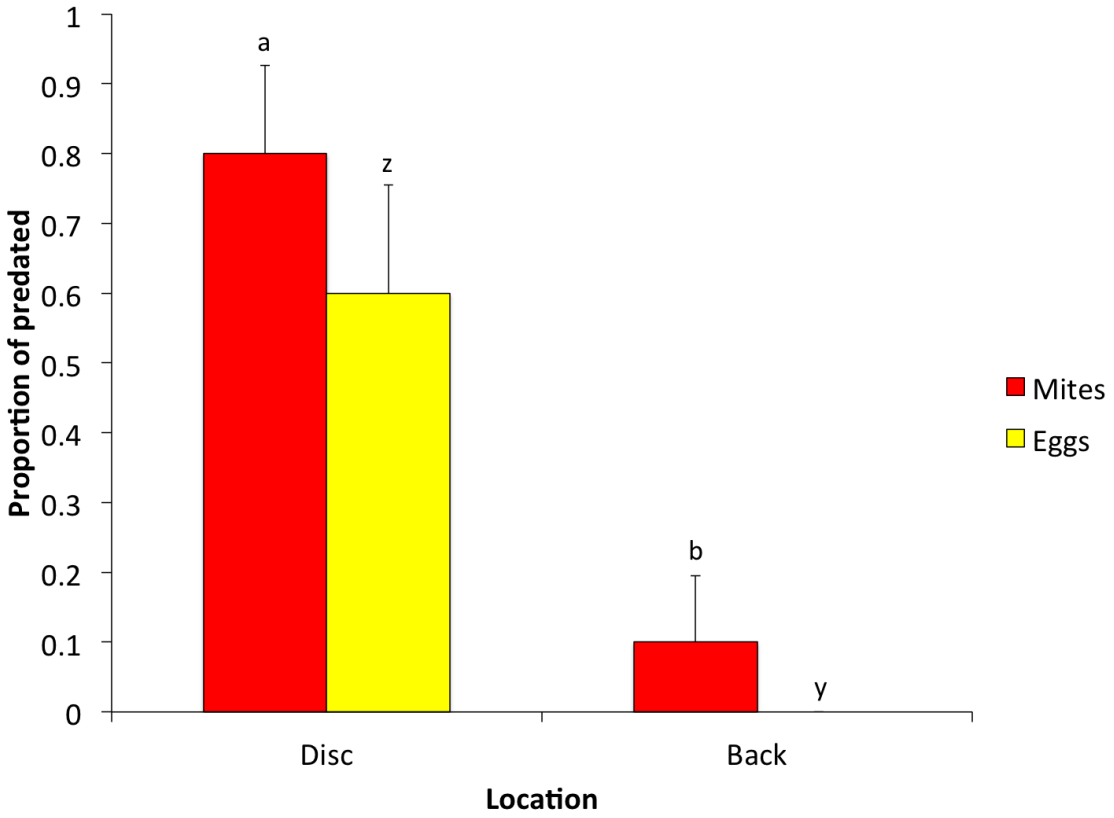


*Figure 2*


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion of predated
a
z
b
y
Disc
Back
Location
Mites
Eggs

**Predators induce flowering**

Marcus V. A. Duarte[1]; Karen Muñoz-Cárdenas[2]; Cleide R. Dias[1]; Sunny Verdonkschot[2,3]; Angelo Pallini[1]; Arne Janssen[1, 2]

[1]Department of Entomology, Federal University of Viçosa, Minas Gerais, Brazil

[2]Section Population Biology, University of Amsterdam, Amsterdam, The Netherlands

[3]Aeres University of Applied Sciences, Almere, The Netherlands

**Abstract**

Plants must defend against herbivory. For this, plants have developed a wide spectrum of mechanisms. Plants are able to defend by producing toxic compounds that directly affect herbivores and by recruiting and maintaining natural enemies of herbivores that attack them. These defences are thought to be costly and for this reason plants induce them when herbivores are present. The induction of indirect defences that attract natural enemies have been well studied, however the induction of provision of food for predators has not been well studied. We investigate how the presence of predators can influence the provision of food for predators by observing the flowering phenology of a plant. When the predatory mite *Euseius gallicus* was present, plants produced flowers earlier when compared to plants without mites. Plants with the predatory mite *Amblyseius swirskii* had intermediate results between plants with *E. gallicus* and control plants. If a plant is able to perceive the presence of natural enemies and quickening the production of flowers when predators are present, plants can offer favourable conditions to maintain these predators prior to attacks by herbivores and when herbivores arrive these plants are already protected.

Keywords: *Amblyseius swirskii, Euseius gallicus, Capsicum annuum,* sweet pepper.

## Introduction

Plants need to defend themselves against herbivores. These defences can act either directly or indirectly (Schoonhoven et al. 2005). Direct defences are those that directly influence herbivores through the production of defensive compounds such as toxins or anti-digestive substances (Chen 2008; Howe and Jander 2008). Indirect defences are mediated by mutualistic interactions between plants and natural enemies (Arimura et al. 2005; Wäckers et al. 2005; Heil 2008). Indirect defences are based on the attraction and arrestment of natural enemies by providing food or shelter (Janzen 1966; O'Dowd and Willson 1991). As a result, these natural enemies attack herbivores on the plants (Wäckers et al. 2005; Heil 2008). To attract natural enemies, plants can provide information, such as volatiles, which can assist predators in finding the hosts and prey (Dicke and Sabelis 1988; Turlings et al. 1990; Kessler and Baldwin 2001). Through the provision of food, plants are able to maintain populations of predators that act as bodyguards against pests (Dicke and Sabelis 1988; Wäckers et al. 2005; Heil 2008).

Most plant defences are thought to be costly (Kessler and Baldwin 2002), and many plant defences are therefore inducible through the actions of herbivores (Heil 2001). Induced defences can consist of an increased production of toxins or anti-digestive substances (direct defences), but also through the increment of food or production of volatiles that are attractive to natural enemies (indirect defences)(Heil 2008). Therefore, by inducing their defences plants are able to optimize and reduce the cost of defences against herbivores.

The induction of indirect defence that involves volatiles after herbivory to attract predators has been well studied (Dicke and Sabelis 1988; Turlings et al. 1990; Kessler and Baldwin 2001; Heil 2014; Ponzio et al. 2014). Not much research has been done regarding the induction of providing food for natural enemies through herbivory. Food for natural enemies comes in a variety of types, such as food bodies, extrafloral nectar, as well as pollen and nectar from flowers (Wäckers et al. 2005). Although flower nectar and pollen are important resources for natural enemies (Baggen et al. 1999), their primary function is pollination. However, even plants that self-pollinate (Schoen et al. 1996) may still produce surplus amounts of pollen, which may be used by natural enemies (Duarte et al. 2015).

Previous experiments have shown that plants can increase the secretion of nectars from extrafloral nectaries when they suffer from herbivory (Wäckers et al. 2001;

Ness 2003; Heil 2004). Plants have also been shown to increase the number of extrafloral nectaries in response to leaf damage (Mondor and Addicott 2003). Although flowers are also an important resource for natural enemies (Baggen et al. 1999; Duarte et al. 2015), the possibility of them being induced as an indirect defence has not been considered.

The major factors that control flowering induction are photoperiod, temperature and water availability (Bernier et al. 1993). Different types of stress can also influence flowering phenology (Wada and Takeno 2010). However, the influence of herbivory on flowering has been very poorly investigated. The few experiments that have been done to analyse the influence of herbivory on flowering phenology focus on herbivores that either feed on the flowers or the seeds (Pilson 2000; Freeman et al. 2003; Elzinga et al. 2007).

Many important natural enemies that protect plants from herbivores are omnivores. Omnivorous arthropods are organisms that not only feed on prey, but also on plant material, such as pollen for example (Coll and Guershon 2002; Symondson et al. 2002). Here, we report on an omnivorous natural enemy that induces flowering in sweet pepper (*Capsicum annuum*) plants.

The mite family Phytoseiidae contain many omnivorous organisms that are considered important natural enemies (McMurtry et al. 2013). In the present research we used two omnivorous phytoseiid mites: *Amblyseius swirskii* and *Euseius gallicus. Amblyseius swirskii* is frequently widely present on biological control programs, being used for the control of thrips and whiteflies (Nomikou et al. 2001; Messelink et al. 2008; Muñoz-Cárdenas et al. 2017). *Euseius gallicus* is a promising natural enemy and has already shown the potential to control thrips and whiteflies (Döker et al. 2014; Pijnakker et al. 2014, 2015).

In the present study, we tested whether the presence of the predatory mites *A. swirskii* and *E. gallicus* can influence the flowering of sweet pepper plants. These predatory mites are important natural enemy of many herbivores present on sweet pepper plants (Nomikou et al. 2001; Messelink et al. 2008; Döker et al. 2014; Pijnakker et al. 2014, 2015). We tested whether the presence of *A. swirskii* and *E. gallicus* changed the flowering phenology.

## Material & Methods

*Rearings and plants*

*Amblyseius swirskii* used for the experiments were originally collected from cotton in Redavim, Israel (Nomikou et al., 2001). They were reared on plastic arenas (8 x 15 cm), placed on a wet sponge in plastic tray containing water. Strips of wet tissue were placed along the borders of the arena to provide water. Tent-shaped pieces of plastic sheet (1–2 $cm^2$) were placed on each arena and functioned as a shelter for the mites. A few cotton wool threads were put underneath the shelter as oviposition substrates. Cultures were fed with *Typha latifolia* pollen.

*Euseius gallicus* used for the experiments were kindly given by Biobest Belgium N.V. . These mites were reared inside mite-proof cages (47.5 x 47.5 x 92 cm) on castor bean plants (*Ricinus communis*). Castor bean plants were grown in 2 litter pots filled with commercial substrate. *Typha latifolia* pollen was offered as food source. When the plants were too big for the cage they were replaced with younger plants.

Sweet pepper (*Capsicum annuum* L. Spider F1 Enza Zaden Beheer B.V., The Netherlands) plants were sown directing into 2 litre pots filled with commercial substrate. These plants were fertilized weekly with 1% NPK (10-10-10) solution. Plants were kept inside of climate chamber (25 ± 1oC, 60 – 70% RH, 16: 8 L: D) until they had 4 completely expanded leaves, at this point the plants were moved to the green house where the experiments were done.

*Flowering experiment*

In the greenhouse the pepper plants were kept individually inside mite-proof cages (47.5 x 47.5 x 92 cm). These plants were kept on top of water filled tray to avoid escape of mites. A brush tip of pollen (0.15 g) was added to the first completely expanded leaf. The plants were separated into three treatments. The first treatment was the control treatment where no mites were added, with just pollen. The second treatment received five adult *A. swiirski* females on the same leaf as the pollen and for the third treatment five adult *E. gallicus* females were also added to the same leaf with pollen. Pollen was added to the same leaf as the one in the beginning of the experiment, 3 times per week. The number of flowers produced per plant was counted beginning when the start of the first plant flowering until the fifteenth day. After these fifteen days, plants were taken to the lab and all the leaves were removed one by one and the motile stages of the predatory mites were counted with the help of a stereoscopic microscope. This experiment was done in three different occasions

(August/2015, November/2015 and November 2016), creating three independent blocks. The numbers of flowers were compared with a linear mixed-effects model (LME) with treatment as fixed factor and plant identity as a random factor to correct for repeated measures. (Crawley 2013). Contrasts among treatments were determined with general linear hypothesis testing (function glht of the package lsmeans in R, Lenth 2016)

## Results

*Flowering*

Treatment and time significantly affected the flowering of the pepper plants (Fig. 1: $\chi^2$= 12.8, df = 2, p <0.01). Plants on the treatment with *E. gallicus* produced more flowers then those on the control (Fig. 1). Plants with *A. swirskii* had intermediate number of flowers, while plants without mites had the lowest number of flowers (Fig. 1).

*Number of mites*

On average we found 43.6 ± 10.3 *A. swirskii* per plant and 209.6 ± 28.7 *E. gallicus* per sweet pepper plant (Fig. 2).

## Discussion

In the present work we have shown an arthropod that is capable of influencing a plant flowering phenology, making them flower earlier when present (Fig. 1). Although it has been previously shown that herbivores may affect other indirect defences such as volatiles and even extra-floral nectary, this is the first report of a natural enemy that accelerates a plant flowering phenology. Plants with *Euseius gallicus* changed there flowering phenology and induced flowering earlier, while *A. swirskii* had intermediate result between plants with *E. gallicus* and the control (without mites). This difference in induction between the two species can be due to the fact that these mites have different feeding habits, while *E. gallicus* feeds on plant material and must be reared on plant material (as shown in the Material & Method), *A. swirskii* said not to feed on plants (Adar et al. 2012). Another possibility for this difference in flowering induction can be attributed to the different densities that each one of the specie reached during the experiment, plants with *E. gallicus* had almost 5 times more mites then plants with *A. swirskii* (Fig. 2), which may suggest that *A. swirskii* may also be able to induce flowering if they were able to reach higher densities.

The induction of plant defences are a very well tuned mechanism, being able of distinguishing between mechanical damage and damage caused by herbivores (Baldwin 1990; Alborn et al. 1997), as well as distinguish between herbivore species (De Moraes et al. 1998; Dicke 1999; Cai et al. 2014). Since plants can notice the presence of different arthropods and display different defence induction depending on the specie, being able to notice the presence of a predator and modulating response can be very interesting for the plant and the predator.

Plants can be severely damaged by herbivores, which can influence their survival in nature (Maron and Crone 2006; Lehndal and Ågren 2015). Maintaining natural enemies that feed on the resources provided by flowers prior to herbivore attacks can be an important strategy to reduce damage caused by herbivores (Duarte et al. 2015; Kumar et al. 2015). By perceiving the presence of natural enemies and quickening the production of flowers when predators are present, plants can offer favourable conditions to maintain these predators prior to attacks by herbivores and when herbivores arrive these plants are already protected.

It has already been proposed that plants are able to modulate their deference to favour natural enemies. Kahl et al. (2000) showed that native tobacco suppresses the production of nicotine when attacked by a specialized herbivore, but increases the production of volatiles. The author hypothesizes that this could be to avoid negative indirect effects of nicotine on the natural enemies of the herbivores. Hence, it would be beneficial for the plant and the natural enemies if plants were capable of noticing their presence and modulating its defences, by inducing their flowering for example, to favour the natural enemy and consequently itself.

Plants can notice the presence of arthropods in many ways, not only when they are feed upon. Defences can be induced by oviposition by the arthropods (Hilker & Meiners, 2005) or by insect movements (Peiffer et al., 2009). Since plants are able to notice arthropods in a variety of ways, the perception of the natural enemies presence may not be restricted to plant feeding, as movement or even oviposition could play a role in the induction of flowering on these plants, the mechanism involved in the perception of the presence of these natural enemies is a question that deserves further investigation.

All of plant defence responses are closely linked to their hormone levels (Bari and Jones 2009). The plant hormonal levels and biochemical responses to the

presence of these natural enemy is also something that deserves further investigation. In the present work we have only scratched the surface when it comes to the influence of natural enemies on flowering phenology, this is a topic that certainly deserves further investigation.

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**Bibliography**

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

Alborn, H. T., T. C. J. Turlings, T. H. Jones, G. Stenhagen, J. H. Loughrin, and J. H. Tumlinson. 1997. An Elicitor of Plant Volatiles from Beet Armyworm Oral Secretion. Science 276:945–949.

Arimura, G., C. Kost, and W. Boland. 2005. Herbivore-induced, indirect plant defences. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1734:91–111.

Baggen, L. r., G. m. Gurr, and A. Meats. 1999. Flowers in tri-trophic systems: mechanisms allowing selective exploitation by insect natural enemies for conservation biological control. Entomologia Experimentalis et Applicata 91:155–161.

Baldwin, I. T. 1990. Herbivory simulations in ecological research. Trends in Ecology & Evolution 5:91–93.

Bari, R., and J. D. G. Jones. 2009. Role of plant hormones in plant defence responses. Plant Molecular Biology 69:473–488.

Bernier, G., A. Havelange, C. Houssa, A. Petitjean, and P. Lejeune. 1993. Physiological Signals That Induce Flowering. The Plant Cell 5:1147–1155.

Cai, X.-M., X.-L. Sun, W.-X. Dong, G.-C. Wang, and Z.-M. Chen. 2014. Herbivore species, infestation time, and herbivore density affect induced volatiles in tea plants. Chemoecology 24:1–14.

Chen, M.-S. 2008. Inducible direct plant defense against insect herbivores: A review. Insect Science 15:101–114.

Coll, M., and M. Guershon. 2002. OMNIVORY IN TERRESTRIAL ARTHROPODS: Mixing Plant and Prey Diets. Annual Review of Entomology 47:267–297.

De Moraes, C. M., W. J. Lewis, P. W. Paré, H. T. Alborn, and J. H. Tumlinson. 1998. Herbivore-infested plants selectively attract parasitoids. Nature 393:570–573.

Dicke, M. 1999. Are herbivore-induced plant volatiles reliable indicators of herbivore identity to foraging carnivorous arthropods? Entomologia Experimentalis et Applicata 91:131–142.

Dicke, M., and M. Sabelis. 1988. How Plants Obtain Predatory Mites as Bodyguards. Neth. J. Zool. 38:148–165.

Döker, İ., J. Witters, J. Pijnakker, C. Kazak, T. Marie-Stephane, and S. Kreiter. 2014. Euseius gallicus Kreiter and Tixier (Acari: Phytoseiidae) is present in four more countries in Europe: Belgium, Germany, the Netherlands and Turkey (Vol. 54).

Duarte, M. V. A., M. Venzon, M. C. de S. Bittencourt, F. A. Rodríguez-Cruz, A. Pallini, and A. Janssen. 2015. Alternative food promotes broad mite control on chilli pepper plants. BioControl 60:817–825.

Elzinga, J. A., A. Atlan, A. Biere, L. Gigord, A. E. Weis, and G. Bernasconi. 2007. Time after time: flowering phenology and biotic interactions. Trends in Ecology & Evolution 22:432–439.

Freeman, R. S., A. K. Brody, and C. D. Neefus. 2003. Flowering phenology and compensation for herbivory in Ipomopsis aggregata. Oecologia 136:394–401.

Heil, M. 2001. Induced systemic resistance (ISR) against pathogens – a promising field for ecological research. Perspectives in Plant Ecology, Evolution and Systematics 4:65–79.

Heil, M. 2014. Herbivore-induced plant volatiles: Targets, perception and unanswered questions. Journal of Physiology 204:297–306.

———. 2004. Induction of two indirect defences benefits Lima bean (Phaseolus lunatus, Fabaceae) in nature. Journal of Ecology 92:527–536.

———. 2008. Indirect defence via tritrophic interactions. New Phytologist 178:41–61.

Hilker, M., and T. Meiners. 2006. Early herbivore alert: insect eggs induce plant defense. Journal of Chemical Ecology 32:1379–1397.

Howe, G. A., and G. Jander. 2008. Plant Immunity to Insect Herbivores. Annual Review of Plant Biology 59:41–66.

Janzen, D. H. 1966. Coevolution of mutualism between ants and acacias in Central America. Evolution 20:249–275.

Kahl, J., D. H. Siemens, R. J. Aerts, R. Gäbler, F. Kühnemann, C. A. Preston, and I. T. Baldwin. 2000. Herbivore-induced ethylene suppresses a direct defense but not a putative indirect defense against an adapted herbivore. Planta 210:336–342.

Kessler, A., and I. T. Baldwin. 2001. Defensive Function of Herbivore-Induced Plant Volatile Emissions in Nature. Science 291:2141–2144.

———. 2002. Plant responses to insect herbivory: the emerging molecular analysis. Annual Review of Plant Biology 53:299–328.

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

Lehndal, L., and J. Ågren. 2015. Herbivory Differentially Affects Plant Fitness in Three Populations of the Perennial Herb Lythrum salicaria along a Latitudinal Gradient. PLoS ONE 10.

Lenth, R. V. 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software 69.

Maron, J. L., and E. Crone. 2006. Herbivory: effects on plant abundance, distribution and population growth. Proceedings of the Royal Society B: Biological Sciences 273:2575–2584.

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

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

Mondor, E. B., and J. F. Addicott. 2003. Conspicuous extra-floral nectaries are inducible in Vicia faba. Ecology Letters 6:495–497.

Muñoz-Cárdenas, K., F. Ersin, J. Pijnakker, Y. van Houten, H. Hoogerbrugge, A. Leman, M. L. Pappas, et al. 2017. Supplying high-quality alternative prey in the litter increases control of an above-ground plant pest by a generalist predator. Biological Control 105:19–26.

Ness, J. H. 2003. Catalpa bignonioides alters extrafloral nectar production after herbivory and attracts ant bodyguards. Oecologia 134:210–218.

Nomikou, M., A. Janssen, R. Schraag, and M. W. Sabelis. 2001. Phytoseiid predators as potential biological control agents for Bemisia tabaci. Experimental & applied acarology 25:271–291.

O'Dowd, D. J., and M. F. Willson. 1991. Associations between mites and leaf domatia. Trends in Ecology & Evolution 6:179–182.

Peiffer, M., J. F. Tooker, D. S. Luthe, and G. W. Felton. 2009. Plants on early alert: glandular trichomes as sensors for insect herbivores. The New Phytologist 184:644–656.

Pijnakker, J., Y. Arijs, A. de Souza, M. Cellier, and F. Wäckers. 2015. The use of Typha angustifolia (cattail) pollen to establish the predatory mites Amblyseius swirskii, Iphiseius degenerans, Euseius ovalis and Euseius gallicus in glasshouse crops.

Pijnakker, J., A. de Souza, and F. Wäckers. 2014. Euseius gallicus, a bodyguard for roses.

Pilson, D. 2000. Herbivory and natural selection on flowering phenology in wild sunflower, Helianthus annuus. Oecologia 122:72–82.

Ponzio, C., R. Gols, B. T. Weldegergis, and M. Dicke. 2014. Caterpillar-induced plant volatiles remain a reliable signal for foraging wasps during dual attack with a plant pathogen or non-host insect herbivore. Plant, Cell and Environment 37:1924–1935.

Schoen, D. J., M. T. Morgan, and T. Bataillon. 1996. Plant life histories: ecological correlates and phylogenetic constraints - How does self-pollination evolve? Inferences from floral ecology and molecular genetic variation. Phil. Trans. R. Soc. Lond. B 351:1281–1290.

Schoonhoven, L. M., J. J. A. van Loon, and M. Dicke. 2005. Insect-Plant Biology. OUP Oxford.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can Generalist Predators Be Effective Biocontrol Agents?1. Annual Review of Entomology 47:561–594.

Turlings, T. C. J., J. H. Tumlinson, and W. J. Lewis. 1990. Exploitation of Herbivore-Induced Plant Odors by Host-Seeking Parasitic Wasps. Science 250:1251–1253.

Wäckers, F. L., P. C. J. van Rijn, and J. Bruin. 2005. Plant-Provided Food for Carnivorous Insects: A Protective Mutualism and its Applications. Cambridge University Press.

Wäckers, F. L., D. Zuber, R. Wunderlin, and F. Keller. 2001. The Effect of Herbivory on Temporal and Spatial Dynamics of Foliar Nectar Production in Cotton and Castor. Annals of Botany 87:365–370.

Wada, K. C., and K. Takeno. 2010. Stress-induced flowering. Plant Signaling & Behavior 5:944–947.

**Figure legends**

*Figure 1*

Average numbers of flowers (± SE) per sweet pepper plant with: A. *swirskii*, *E. gallicus* or no mites (Control). Lines with different letters differ significantly (contrasts after LME, $p < 0.05$).

*Figure 2*

Average numbers (+ SE) of predatory mites: *E. gallicus* or *A. swirskii* per sweet pepper plant at the end of the experiment.

## Figures

Figure 1

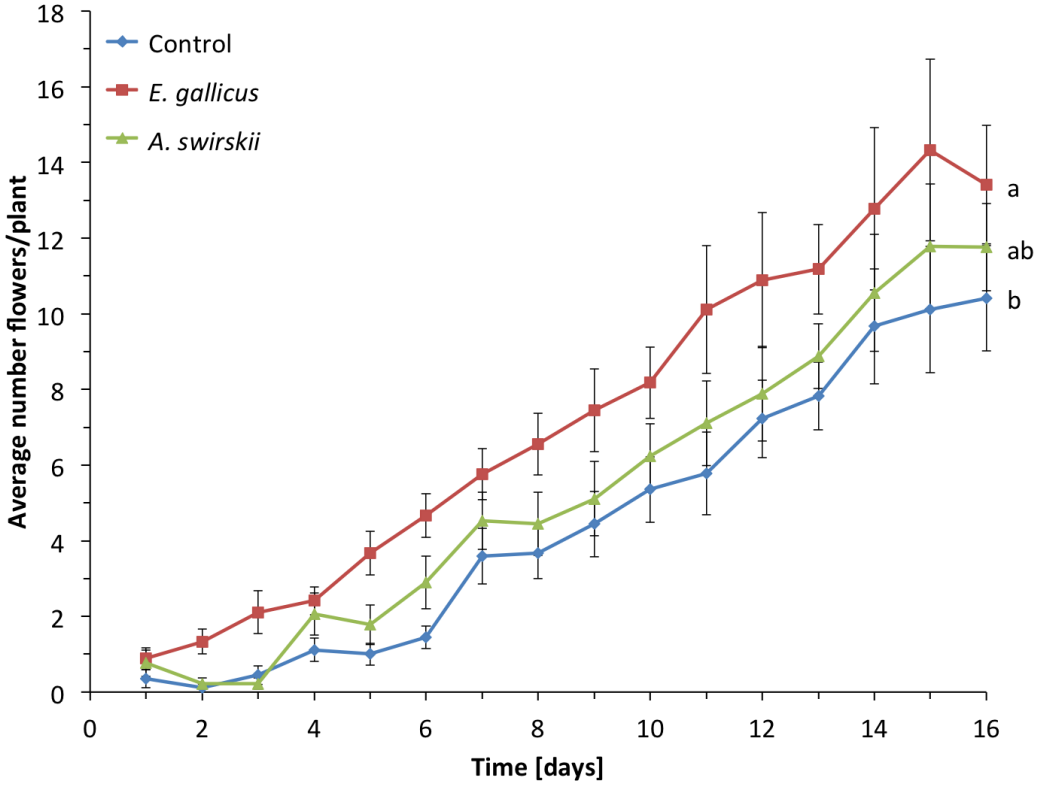
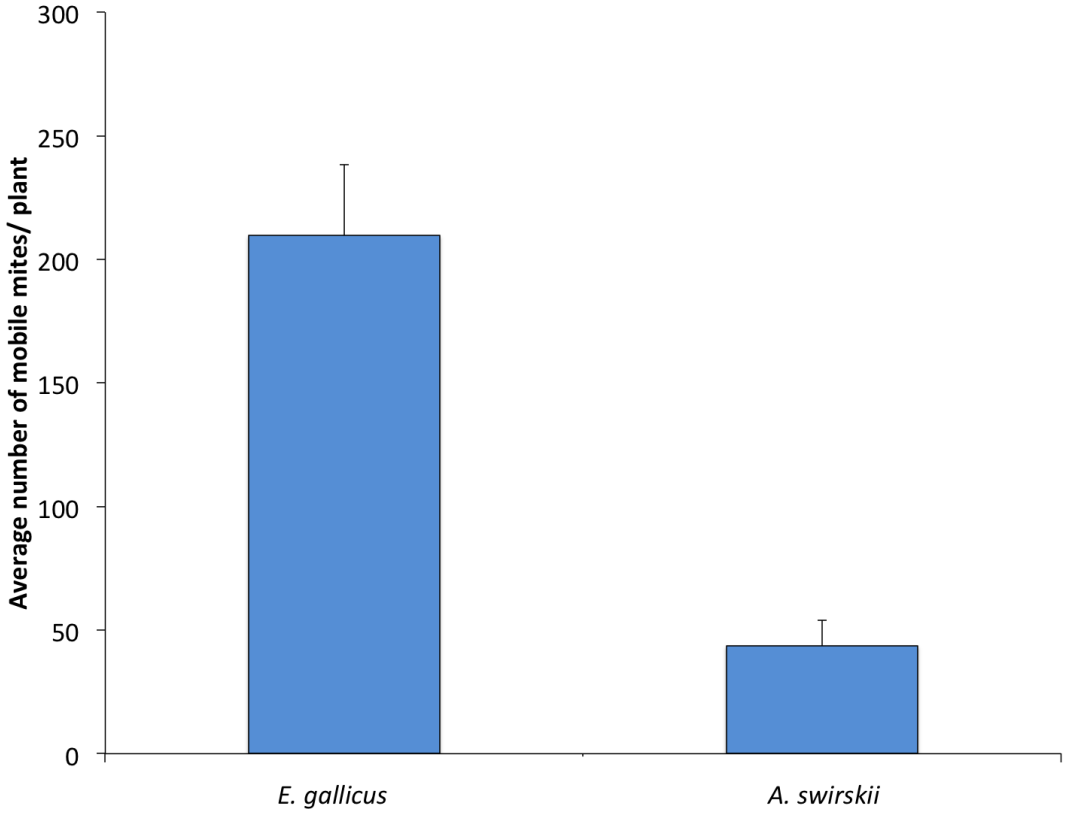


Figure 2


300
250
200
150
100
50
0
Average number of mobile mites/ plant
E. gallicus
A. swirskii

## General Conclusion

We have shown that structures such as empty aphid mummies can offer protection against predation and cannibalism for predatory mites. When present on pepper plants structures, such mummies, can significantly increased the densities of juveniles and adults of the predatory mites. We believe that the usage of unexpected structures as shelter deserve greater attention, since these may be present in many habitats and have profound effects on survival and population dynamics, as we have shown in the first chapter.

Although animals can display a wide array of antipredator behaviours, many of these, especially for small arthropods, have not been noticed yet. If greater attention is given to the behaviours of small arthropods and their predators, many other discoveries could be made. The other benefits from this mounting such as dispersion and what triggers this mounting behaviour deserves further experiment.

An arthropod that is capable of influencing a plant flowering phenology, making them flower earlier when present is something that may have profound influence in the interaction between arthropods and plant. How arthropods influence flowering phenology is a topic that has been very poorly investigated and deserves futures studies. The mechanism that arthropods induce this flowering, as well as the hormonal level of the plants also should be investigated in the future.