Université de Montréal

Are predatory mites efficient dispersal agents of entomopathogenic fungi? Understanding the process of disease transmission from predators to prey for biological control

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Thèse présentée à la faculté des arts et des sciences

en vue de l'obtention du grade de doctorat

en sciences biologiques

Mai 2019

Page d'identification du jury

Université de Montréal

Faculté des Études Supérieures et Postdoctorales

Cette thèse est intitulée:

Are predatory mites efficient dispersal agents of entomopathogenic fungi: understanding the process of disease transmission from predators to prey for biological control

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Résumé

Les acariens prédateurs et les champignons entomopathogènes sont couramment utilisés dans les programmes de lutte biologique contre le thrips des petits fruits, *Frankliniella occidentalis*. Les acariens prédateurs peuvent localiser les thrips même lorsque ceux-ci se cachent, mais ne consomment que le premier stade larvaire. Les champignons entomopathogènes, quant à eux, peuvent infecter tous les stades, mais leur dispersion est passive. Dans cette thèse, nous avons évalué le potentiel des acariens prédateurs comme agents de dispersion des champignons entomopathogènes dans les colonies de thrips. Le travail expérimental de cette thèse a été divisé en trois sections.

Dans la première section, nous avons évalué la pathogénicité de la souche ANT-03 du champignon entomopathogène *Beauveria bassiana* pour chacun des stades de thrips ainsi que pour trois espèces d'acariens prédateurs: deux espèces principalement actives sur les plantes (*Amblyseius swirskii* et *Neoseiulus cucumeris*) et une espèce active dans le sol (*Stratiolaelaps scimitus*). Nous avons (1) établi que la souche ANT-03, les acariens prédateurs et les thrips forment des associations fonctionnelles d'agent pathogène, de vecteurs et d'hôte, (2) démontré que des spores mélangées aux substrats d'élevage des acariens s'accumulent sur leurs corps au fil du temps, et (3) mis au point une méthode d'application permettant aux acariens de disséminer les spores de *B. bassiana* directement à partir des substrats d'élevage.

Dans la deuxième section, à l'aide d'enregistrements vidéo, nous avons déterminé comment les acariens prédateurs délogent les spores de leurs corps en examinant la relation entre le nombre de spores restant sur les acariens et le temps alloué au toilettage ou à la marche. Nous avons comparé les comportements des acariens prédateurs avec ou sans spores. Nous avons montré que la marche, est plus efficace le toilettage pour déloger les spores. Les acariens prédateurs peuvent percevoir la présence des spores sur leur corps et augmenter le temps alloué à la marche.

Dans la troisième section, nous avons déterminé la capacité des acariens prédateurs à acheminer les spores jusqu'aux colonies de thrips. *Amblyseius swirskii* et *N. cucumeris* ont été chargés de spores et relâchés sur des plantes infestées de thrips de premier stade et

regroupés sur les feuilles. Nous avons caractérisé la distribution spatiale de chaque organisme, calculé l'empiètement spatial entre les spores et les thrips et estimé la proportion de thrips portant des spores. Les deux acariens ont dispersé une quantité similaire de spores sur les plantes, mais *A. swirskii* a distribué plus de spores sur les feuilles infestées de thrips et a donc augmenté le taux de rencontre entre le pathogène et les thrips. Les différences observées entre les espèces d'acariens prédateurs résultent de leurs différents comportements de chasse.

En comprenant le processus de transmission des spores de champignons entomopathogènes des acariens prédateurs vers les proies, nous fournissons une base théorique pour identifier quels prédateurs feraient de bons candidats comme agents de dispersion de spores. Ainsi, nous pourrons augmenter la capacité des acariens prédateurs à réduire les populations de thrips en combinant la prédation et la dispersion d'entomopathogènes dans un contexte de lutte biologique.

Mots clés: dispersion de spores fongiques, comportement de toilettage, empiètement spatial, interactions tritrophiques, *Frankliniella occidentalis*, *Beauveria bassiana*, *Amblyseius swirskii*, *Neoseiulus cucumeris*, *Stratiolaelaps scimitus*

Abstract

In biological control programs, predatory mites and entomopathogenic fungi are commonly used against western flower thrips, one of the most challenging pests in food and ornamental crops. Predatory mites can locate thrips even when thrips hide in plant crevices, but they only consume first instar larval thrips. Entomopathogenic fungi can infect all other stages, but their dispersal is passive and host encounter is therefore random. This thesis examines the potential role of predatory mites as dispersal agents of entomopathogenic fungi to thrips colonies. The experimental work has been divided into three sections. In the first section, we evaluated the pathogenicity of the entomopathogenic fungus *Beauveria bassiana* strain ANT-03 to all stages of the western flower thrips and to three species of predatory mites: *Amblyseius swirskii* and *Neoseiulus cucumeris* that are active on plants and *Stratiolaelaps scimitus* that is active in soil. We established that *B. bassiana* ANT-03, predatory mites and thrips form appropriate pathogen-vector-host associations. We also developed a commercially applicable method for predatory mites to collect *B. bassiana* spores directly from the rearing substrates and transport them to the environment. We demonstrated that spores did accumulate on predatory mites over time in the substrates. In the second section, using video recordings, we described how predatory mites dislodged spores by linking the number of spores remaining on a mite to the time spent grooming and walking. We compared behaviors of the predatory mites with and without spores following their release from rearing substrates. Using low-temperature scanning electronic microscopy, we visualized the spore distribution on mites. We showed that walking primarily contributed to predatory mites dislodging spores in our experimental arena, whereas grooming was insufficient. When bearing conidia, all three species of predatory mites extended their walking periods. The duration of grooming behavior was not affected for *A. swirskii* and *N. cucumeris*, and was even reduced for *S. scimitus*. For the third section, we determined the capacity of predatory mites to deliver spores to thrips colonies. *Amblyseius swirskii* and *N. cucumeris* were loaded with spores and released on plants that had been previously infested with first instar thrips clustered on leaves. We carefully characterized the spatial distribution of each organism on plants, calculated the spatial co-occurrence index of spores and thrips, and estimated the proportion of thrips with spores. Both mites

dispersed similar amounts of spores per plant, but *A. swirskii* delivered more spores to thrips infested leaves and thereby played a significant role in spreading the fungal disease to thrips populations. The observed differences between predatory mite species resulted from different foraging activity patterns. By understanding how the pathogens can be transferred from foraging predatory mites to prey, we provided a theoretical basis for identifying candidate predators as efficient fungal dispersal agents. These methods, if validated in commercially representative settings, could increase the capacity of predatory mites to suppress thrips populations by combining predation and dispersion of entomopathogens for biological control.

Key words: fungal dispersal, grooming behavior, spatial co-occurrence, tritrophic interactions, *Frankliniella occidentalis*, *Beauveria bassiana*, *Amblyseius swirskii*, *Neoseiulus cucumeris*, *Stratiolaelaps scimitus*

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List of symbols and abbreviations

ANOVA − One-way analysis of variance

ANT-03 − Name of a *Beauveria bassiana* strain

ARS − Agricultural Research Service

BARC − Beltsville Agricultural Research Centre

BC − Before Christ

BMP − Bursary in a Practice Environment

BORIS − Behavior observation research interactive software

CA − California

CABI − Centre for Agriculture and Bioscience International

CFU − Colony-forming units

df – Degrees of freedom

DOI – Digital object identifier

DWV − Deformed wing virus

et al. − and colleagues

GHA − Name of a *Beauveria bassiana* strain

INRS − Institut national de la recherche scientifique

IOBC − International Organization for Biological Control

IPM − Integrated pest management

IRBV − Institut de Recherche en Biologie Végétale

KBV − Kashmir bee virus

LED – Light-emitting diode

LT-SEM − Low temperature scan electron microscope

MVOC − Microbial volatile organic compound

n – Sample size

QQ – Quantile-Quantile

RH − Relative Humidity

SE − Standard Error

TGP − Technical grade powder

TX − Texas

UK − United Kingdom

USA − the United States of America

USDA − the United States Department of Agriculture

UV − Ultraviolet

UVB − Ulraviolet B

Acknowledgements

Now at the end of my PhD, I feel like I own it! However, I'm not the only one who deserves credits, because it involved the contribution and support of so many people. I wouldn't have been able to complete my PhD without these people.

First of all, thanks to Silvia Todorova for giving me the opportunity to start a PhD project and kindly to introduce me to her company Anatis Bioprotection Inc. It was fun working with Félix Longpré, Alexandre Tanguay, Dominik Bergeron, Silvie Bergeron, Jorge Fernandez and Martin Nadeau. They are kind and fascinating people who made me feel welcomed and they all tried their best to help me with my project. Without Silvia, I wouldn't have met my research director Jacques Brodeur. Jacques is like an ocean, open and supportive. With him, I don't feel limited, but encouraged to explore. I don't remember him ever telling me what to do or not to do. He asked me questions and guided me to find the answers myself. Our relationship stays professional, but I'm sure Jacques has a fun and crazy side. It creeps out from time to time. Once he told me, 'Sometimes you have to be crazy', and we went to Amsterdam to meet the man I admire.

At the beginning of my PhD, I kept running into several articles on interactions between predatory mites and thrips. I learned that thrips counterattack predatory mites, which confirmed my observations when I was helping growers control thrips with predatory mites. From time to time, I saw predatory mites cadavers within thrips colonies. Why are they dead while thrips remain still alive? They are thrips predators! All these articles about wars between predatory mites and thrips fascinated me and a name was commonly associated with these studies: Dr. Maurice Sabelis.

When came the time to select my PhD committee members, Jacques asked me if I had thought about it. I asked, "Can we ask Maurice Sabelis from the University of Amsterdam?" Jacques hesitated and said, "One of our common friends told me Maus is very sick and may not recover." I remember during that week, on a winter day, I was suddenly feeling very sad in my living room for Maurice, a person who I had never met. I had this crazy idea of flying to Amsterdam to meet him. Later that summer, when I told this story to Roy Norton, the oribatid mite specialist in Ohio Acarology program, he said,

“Yes, you should go and see him.” Roy told me that when he was a graduate student, he admired François Grandjean, but hesitated to meet him because at the time Roy thought of himself as nobody. Roy waited until his first paper was published to introduce himself to Grandjean, but that year Grandjean passed away. A month after the Ohio program, I was lucky to attend the International Congress of Acarology in Kyoto, Japan, following the invitation of my parents. They are both Acarologists. When I told my mom I love Maurice, she said her PhD supervisor Dr. Yutaka Saito is a friend of Maurice. Dr. Saito visited us for collaboration from time to time when I was a child. The night when I arrived in Japan, my mom said, “Dr. Saito has arranged a diner tomorrow with everyone from his lab and Maurice’s lab.” I said, “But I heard Maurice is very sick. How can he be in Japan?”

The next day at the conference, the cell phone of a person sitting in front of me rang during a presentation and a bunch of people looked at me. I was shaking my head, crossing my arms and mouthing, “It’s not me!” Amongst the people who turned around to look, a kind-looking old man was smiling at me. I asked Frederic Beaulieu, who was sitting next to me at the time, who was that man. Frederic said, “The big boss: Maurice Sabelis.” I was struck by lightening! Later that evening, we went to a Japanese restaurant. People slowly showed up. When Maurice and his wife Izabela Lesna arrived, I waved at them, “Sit here!” That was my first dinner with Maurice and we had pleasant conversations. It was like a dream for me. At the conference, he gave amazing presentations. I was fascinated by how he made complex interactions so easily understandable. It was so clear, even to my dad whose English is very limited. During the last day of the conference, I had the courage to ask him, “Maurice, would you be a member of my PhD committee?” It took him half a second to say, “Yes, I will help you as long as my health allows me.” I went back to Montreal and told the story to Jacques. Jacques said, “We have to go and see him.” I replied, “It’s crazy.” He added, ‘Sometimes you have to be crazy.”

At the University of Amsterdam, I presented my proposal to Maurice with Jacques sitting by my side. He made many valuable comments that helped me throughout my PhD. Later that afternoon, Maurice got very tired so we thanked him and said goodbye. He told us he

just needed to nap for half an hour and insisted to invite us for supper. We went to a restaurant where we sat on sheepskins. In that restaurant, I felt like I was in a dream again because I still suffered from jetlag and couldn't believe I was spending precious time with Maurice. We hugged goodbye, I didn't think I would see him again. During the following three months, we continued to exchange emails until the week before he passed away. He kept his promise and helped me as long as he could. Moved by his marvelous gesture, I want to help others and make them feel the way in which Maurice made me feel.

During my PhD, I had the opportunity to meet with many other researchers who were also kind and patient enough to discuss my studies for hours. Special thanks to Drs. Svetlana and Vladimir Gouli for letting me, a stranger, to show up at their home in Vermont: they prepared a big meal for me and introduced me to the techniques involved in working with thrips and entomopathogenic fungi.

Thank you Dr. Yutaka Saito for the discussions about his fascinating mite behavior studies and for his suggestions concerning my project.

I'm also lucky to have met Dr. Eric Palevsky in Beijing. We later crossed paths in Fuzhou and Montréal and I've always enjoyed the discussions with him on ecology and behavior of predatory mites. Eric and I have a common friend, Ronald Ochoa.

How I met Ronald Ochoa feels like destiny. He was teaching one of the mite courses in the Ohio Acarology Summer Program and his presentation about mites was a visual and auditory feast. I could feel his passion and couldn't help but fall in love with mites. One day, he asked me where I was from, I replied, "Fuzhou China." He asked me if I happen to know Dr. Lin, a Tarsonemid mite taxonomist. "Yes, he is my father" I said. I then saw Ronald's jaw drop as if he was in a Spanish drama. Ronald and my father had exchanged letters for years, but had never met. I introduced them to each other in Kyoto, Japan.

Thanks to Ronald, I had the opportunity to work at the United States Department of Agriculture (USDA) last spring for three weeks and took gorgeous LT-SEM photos of predatory mites bearing spores with Gary Bauchan. Thank you Gary and sorry for

pushing you to work so late every opportunity I got. You and Ronald made me feel right at home at the Electron and Confocal Microscopy Unit, thank you again for the great experience.

I also want to thank all my peers from Jacques and Guy Boivin's labs. First, I'd like to thank Paul Abram. When I first joined the lab, he kindly offered to discuss my project and develop ideas together. He has always been there to answer my questions, especially on statistics. Later, before he graduated, he told me he had to wean me off his statistical breastfeeding. That's when I started to become independent in statistical analyses. Paul and I have a common friend, Jean-Philippe (JP) Parent. He has been a very important person during my PhD studies. He is wonderfully strange and incredibly kind. When I was at the worst time of my PhD, he was there to discuss with me and helped me with my experiments. We travelled in Japan and China together. Because of him and Maurice, my trip in Japan is probably one of the best trips of my life. Even though JP moved away to Ontario for work, he still gave me advice on my data analysis and helped me put the stories together. Paul and JP made me realize how wonderful it is to have students supporting each other in a lab. I'm so lucky to have overlapped with them in Jacques' lab.

I would also like to thank Josée Doyon, Julie Augustin, Mathilde Gaudreau, Alexandre Leblanc from Jacques' lab, and visiting students Letizia Martorana, Valeria Bertoldi, Deborah Kapantaidaki for their support and friendship.

I'd like to thank Alexandre Tanguay and Sean di Paolo for helping me conduct my experiments. It's not easy to work with me or work with predatory mites. Thank you for your dedication. I remember, once, Alex stayed until midnight in the lab and said to me, "I want to go home." I told him, "You started it and you have to finish it." He is such a great person to work with. He often said, "Give me responsibilities, not jobs." We collaborated twice. Every time after he left, I couldn't help but breakdown and cry. Alex and I later became very good friends. Thank you Sean for being such a good helper. If it weren't for you, the experiments wouldn't have gone so smoothly.

I would also like to thank my friend Mo Wang for volunteering to help me with my experiments from time to time. I'm happy that I met you in statistics class and continued

to share our stories with each other throughout my PhD. Over the past years, I feel we've grown together.

I want to express my special gratitude to Dr. Claude Guertin for allowing me to work for three years in his lab at Institut national de la recherche scientifique (INRS). It started with me borrowing a growth chamber for three months. He extended my stay again and again, even after he moved to a new lab. I also want to thank him for being available when I needed advice. I want to thank Narin Srei from Claude's lab for showing me all the microbiology lab work techniques. I would also like to thank Dr. Étienne Yergeau from INRS for lending me a room for growing plants. Thanks also to his lab and Claude's lab for participating in my protocol development and giving me valuable suggestions.

At the INRS, I met a special friend, Mr. Itumeleng Moroenyane. He's very critical, therefore a great person to discuss about science. His powerful flaw-finding brain is amazing at pointing out problems in a project. Thank you for the wakeup call in the middle of my PhD. One day he said to me, 'You are not doing science, you are just moving insects.' After his comments, I rethought about my plan, focused on answering my research questions and got rid of as much unnecessary work as possible. After we discussed about my thesis for six hours, it took me a two-hour nap to recover. I hate you and I love you at the same time.

Thank you Dr. Anne-Lise Routier for lending me your new lab space to conduct my experiments at Institut de Recherche en Biologie Végétale (IRBV).

I would like to thank my mom and dad for their financial support and giving me the opportunity and freedom to explore the world of biological control and Acarology. My mom is such an inspiring and resourceful woman. Because of her perseverance and hard work, she has helped growers in every province of China applying biological control and she continues to expand and push the biological control industry. I want to make her work easier by improving the efficiency of production and application of biological control agents and continue to build the kingdom of biological control.

I am grateful to my ex-husband Jamie Wilt for his love and support. He once told me that I changed because of my PhD. He wondered if I had gone crazy. I think I started questioning myself more, which led to making changes in my life.

I would like to thank Félix Longpré. Thank you for your curiosity, your creativity and patience. I've been able to share my passion and struggle with you. You've participated in the development of my work for the past two to three years, from designing 3D-printed mite filming arena, to text editing. We are still developing projects together beyond my PhD. It has been a pleasure bouncing ideas back and forth with you. Mostly, I want to give special thanks for your emotional support.

Achieving a PhD has been a difficult process. I think it was well worth because of time spent with all the great people I mentioned above. Therefore, I made peace with the anxiety and stress that came with my PhD. I believe with good intellectual and emotional support and, if conditions allow, with a good psychological therapy, PhD studies can be an extraordinary positive (otherwise traumatic) life transforming experience.

Finally, I would like to thank institutions where I conducted my experiments: INRS, IRBV and USDA. I am thankful for generous financial supports from my funding agencies and scholarship providers: Bursary in a Practice Environment (BMP) Innovation Research Scholarship Program from Anatis Bioprotection Inc., Natural Sciences and Engineering Research Council of Canada and Fonds de Recherche Nature et Technologies du Québec; International student fee exemption bursary from University of Montréal; bourse Jacques-Rousseau from IRBV, student travel awards from International Organization for Biological Control (IOBC), bourse Pehr Kalm from Les Amis du Jardin, Montréal Botanical Garden, and the best student oral presentation award from XV International Congress of Acarology.

Preface

In June 2013, the year before I started my PhD, there was a conference entitled the International Entomophagous Insects Conference held in Orford, Canada. I presented the work conducted by my mother about using a predatory mite to deliver entomopathogenic fungi to Asian citrus psylids. The study showed that the entomopathogenic fungus was infectious to the pest but not to the predatory mites. Following the release of predatory mites dusted with entomopathogens on citrus twigs, almost 100% psylids died of infection after six days. I naively thought that biological control researchers and practitioners would be enthusiastic about this approach and started applying it in biological control programs, but this was not the case. Five years later, no one has used predatory mites to deliver entomopathogenic fungi at a commercial scale. Now I understand why, the available information was not convincing enough that the approach could work in a realistic situation. There was a lack of proof of concept: the impact on pest populations had been evaluated in small test tubes where the probability for pest to encounter the entomopathogen was high despite the presence of predatory mites. Another study followed. This time, two species of predatory mites covered with entomopathogenic fungi were released on a potted orange Jessamine tree (Zhang et al. 2015b). The pest infection rate was compared not only with control treatment, but also with the pathogens sprayed all over the plant. The result showed that one species of predatory mites covered with pathogen induced a higher infection rate in prey populations than when the same quantity of pathogen was sprayed all over the plant, the other predatory mite species induced the same level of prey infection rate. The submitted manuscript was rejected a few times for several reasons. The proof of concept was not comprehensive: one predatory mite species induced 100% mortality in prey population, but how did it happen? How did predatory mites respond to the presence of pathogens on their bodies? How did predatory mites dislodge and disperse the pathogens? Why did two predatory mites induce different infection rate in prey population? Is it possible to apply it commercially? All these unanswered questions led to the beginning of my PhD: trying to understand the nature of each organism, their interactions and the process of disease transmission.

Chapter 1: Introduction

1.1 Literature review

Fungi, plants and animals are the three major groups of multicellular eukaryotes in terrestrial ecosystems (Freeman and Hamilton 2005). Plants are autotrophs, using solar energy and inorganic compounds to develop and reproduce. Fungi and animals are heterotrophic, consuming other organisms in a food chain. Fungi can develop as parasite, saprophyte or mutualist (Roper and Seminara 2019). Once fungi come into contact with a suitable resource, they undergo intense proliferation. When the resource is depleted, they need to find and colonize new substrates in order to persist (Malloch and Blackwell 1992).

"Fungi cannot walk or run, but some can swim, most can soar, a few can jump, and some must be carried" (Kendrick 1985).

Fungi have evolved specific structures for dispersal (e.g. spores, hyphal fragments, sclerotia, soredia, sporangia, peridioles), spores being the main dispersal units (Magyar et al. 2016). Spores can be disseminated by dispersal agents such as wind, water and animals (Aylor 1990; Magyar et al. 2016). They are small enough to be carried by even weak wind (Roper and Seminara 2019). Since air is relatively stationary at the surface of a solid, especially above a leaf surface (Nobel 1999), an initial kick can facilitate spores to reach the dispersive airflow (Magyar et al. 2016). Spores can be violently discharged by osmosis pressure and released in many ways: from an apical pore rupture, by surface tension catapult or cavitation (forcing the cell walls snapping back between spores and conidiophore) reviewed by Roper and Seminara (2019). Some fungi from the phylum Basidiomycota grow long stalks, so the mushroom caps can reach the layer of air turbulence before releasing spores (Magyar et al. 2016). Mechanisms of initial spore dispersal are active and well understood. However, once spores reach the atmosphere, their fate remains unknown and highly unpredictable: some are dispersed locally, others over thousands of kilometers (Komonen and Müller 2018). Rain brings spores to the ground and dense vegetation facilitates spore deposition (Magyar et al. 2016). Spores can

also be washed from top leaves to a lower canopy. Leaf structures (e.g. trichomes) and sticky substances (e.g. plant sap and honeydew) enhance spore retention (Magyar et al. 2016; Malloch and Blackwell 1992). The spores that are not securely attached to a suitable substrate re-suspend in the air after they are dried by airflow and continue their journey (Magyar et al. 2016).

"*Do fungi surrender to uncertainty? Can they strategize to exert some control of the fate of their progeny?*" (Roper and Seminara 2019)

Fungi can established commensalistic, mutualistic and parasitic relationships with animals, including arthropods, gastropods, mammals and birds, that purposefully move from one place to another (Peck 1999), thereby greatly reduce the randomness in encountering their host (Magyar et al. 2016). One of the routes for fungi to reach their substrates is by attaching to animals while the animals feed on the fungi or fungal substrates. For example, *Sporothrix* fungi that infect flowers and fruits of *Protea* trees in Africa are essential food for several species of mites. These fungi are dispersed as mites move from fruits to developing flowers via tree branches. Some species of mites are phoretic to pollinating beetles and nectar feeding Cape sugarbirds, which allow further dispersal of *Sporothrix* fungi between *Protea* trees even when the trees are separated by mountains. Cape sugarbirds directly disperse the spores when they feed on fungus contaminated nectar and pollen (Theron-De Bruin et al. 2018). In the region where *Protea* trees grow, natural fire cycles occur every 5-50 years. Following a fire, it takes at least three years for *Protea* trees to re-start producing flowers. The complex, diverse and close associations between *Sporothrix* fungi, mites and birds allow the fungi to persist under such harsh conditions (Roets et al. 2009).

Fungi adhere to the surface of their dispersal agents or sometimes survive through their digestive tracts (Magyar et al. 2016). Some insects and mites evolved specific morphological structures, for example, the mycangia and the sporothecae, which function as "pockets" to store fungal spores for propagation. Fungi not only provide nutrients for their dispersal agents, in bark beetles for instance, fungi also transform phloem content of pine tree to essential nutrients for the beetles. In return these arthropods allow spores to

be dispersed within pine trees (Moser et al. 1995). Amongst animals, arthropods have the most complex, unique and close relationships with fungi (Magyar et al. 2016).

Commonly, fungi never reach their host/substrates before their dispersal agents die. Some fungi have evolved the ability to exploit arthropod cadavers. They penetrate arthropod chitin-rich cuticles (Humber 2008) and degrade arthropod proteinaceous exoskeleton (Vilcinskas 2010), known as soil-borne saprophytic fungi. Some fungi are able to exploit the resource of their dispersal agents while they are still alive, known as entomopathogenic fungi. Entomopathogenic fungi are common antagonists to arthropods. Amongst all insect orders, 65% are known to be infected by fungi (Araújo and Hughes 2016). The capacity of fungi to infect insects has evolved in several fungal phyla: including Ascomytcota, Zygomycota, Deuteromycota, Chytridiomycota and Oomycota, with the highest proportion and greatest diversity reported in Ascomycota (Mora et al. 2017; Shah and Pell 2003).

The infection process is summarized as follows: conidia adhere to the host cuticle, germinate, penetrate in the host by enzymatic and mechanical processes and then reproduce by exploiting host hemolymph and various host tissues (Askary et al. 1999; Boomsma et al. 2014; Valero-Jiménez et al. 2016). The transition from saprophytic to parasitic lifestyle has involved several mechanisms in order to conquer arthropod immune response, including budding and growing rapidly in host hemocoel in a yeast-like mode, avoiding detection by host hemocytes by having cell walls lacking of an antigenic compound (e.g. chitin and galactomannan), escaping hemocyte phagocytosis by growing a germ tube out of phagocyte encapsulation, and/or suppressing the host immune system by producing toxins (Valero-Jiménez et al. 2016).

Dispersal of entomopathogenic fungi

Once host nutrients are depleted, the fungus breaches the cuticle from the inside out and usually sporulates in large numbers outside the host cadaver in order to disperse (Hajek and St. Leger 1994; Roper and Seminara 2019; Shah and Pell 2003; Valero-Jiménez et al. 2016). In some cases, huge numbers of fungal spores can be discharged even when the host is alive (Shah and Pell 2003). For example, *Entomophthora thripidum* which infects

abdominal organs of onion thrips, keep its host alive while sporulating between abdominal integuments like a fountain (Shah and Pell 2003). After *E. thripidum* stopped ejecting mature spores, fungal hyphae and thrips died at the same time (Shah and Pell 2003). Like for any other fungus, spores of entomopathogenic fungi can be carried by wind, rain and animals to reach suitable hosts (Shah and Pell 2003). Transmission of entomopathogenic fungi has been observed in closely-interacting species, for example, from phoretic tarsonemid mites to bark beetles, from phoretic *Macrocheles* mites to pales weevils, and from aphids to ants (Novgorodova and Kryukov 2017; Schabel 1982; Tkaczuk et al. 2011). Within host species, fascinating host-pathogen interactions have evolved to reduce the randomness in disease transmission. Entomopathogens can benefit from altering host behavior of infected individuals to increase disease transmission (Baverstock et al. 2010). For example, ants infected by *Ophiocordyceps* fungi display a series of atypical behaviors (Hughes et al. 2011). They cannot walk normally; convulsion and negative geotaxis keep them from falling off the plants and climb up to approximately 25 cm above ground, which is ideal for fungal development and dispersal. Infected ants bite into the primary or secondary vein of the abaxial leaf to secure the attachment of cadavers. Following ant mortality, a fungal stalk grows from behind the ant head and spores are released from the stalk. These complex host behavior modifications allow fungi to complete their development, spread and ultimately reach other ant colonies. Entomopathogenic fungi can also manipulate host courtship and mating behaviors by making infected hosts attractive or easier to copulate with for conspecifics than uninfected individuals. Male periodical cicadas infected by *Massospora cicadina* produce the same song frequency of sexually available female cicadas and therefore attract healthy males to copulate (Cooley et al. 2018). When healthy males copulate with infected male cicadas, they contract spores. In some instances, vigorous copulation by healthy males can cause infected males to lose their posterior abdominal segments and the release of spores contained inside the abdomen. These cicadas with open abdomens can remain alive for some time, continue to spread disease by leaving trails of spores behind as they walk and attempting to copulate with other cicadas (Cooley et al. 2018). *Entomophthora muscae* infected houseflies attach to the tip of a grass blade with their extended proboscis, spread their wings and shower the habitat with spores at night. Even

though these females are dead the next morning, they attract male flies to mate and transmit the disease (Gryganskyi et al. 2017). In fact, male houseflies are more attracted to dead females than to healthy females because of their infected swollen abdomens (Möller 1993). Curiously, fungi without brains can control the behavior of their hosts which do posses brains (Hughes et al. 2016). These 'neuroengineers' are the products of natural selection (Hughes et al. 2016).

Do arthropods perceive the presence of entomopathogenic fungi on their bodies and in the environment?

Arthropods can perceive the presence of fungi on their body through their physical weight and/or microbial volatile organic compound (MVOC) emission. For small organisms like arthropods, the accumulation of even tiny particles on their body surface can affect their movement due to overwhelming weight (Amador and Hu 2015). For example, water droplets attached to mosquitoes can weigh up to 80 times the mosquito body mass, which makes it impossible to fly (Amador and Hu 2015). Entomopathogenic fungi such as *B. bassiana*, *M. anisopliae* and *Isaria fumosorosea* (=*Paecilomyces fumosoroseus* Wize) (Ascomycota: Clavicipitaceae) produce an array of MVOC that can be detected by the termite *Coptotermes formosanus* Shiraki (Blattodea: Rhinotermitidae) through olfaction (Davis et al. 2013). MVOC of entomopathogens contained alkanes: the highly virulent entomopathogens produce straight-chain alkanes while the less virulent produce branched and cyclic alkanes (Hussain et al. 2010). It was found that the degree of repellency was linked to the virulence of the entomopathogen species: the higher the virulence, the more repellent it is to the termites. In fact, the most virulent entomopathogen caused the least mortality in termite populations, and vice-versa partially because they are actively avoided. (Hussain et al. 2010).

Are arthropods doomed after they come into contact with entomopathogenic fungi?

When hosts detect entomopathogenic fungi on their body, they may respond with behaviors that negatively affect disease transmission, such as intensive self-grooming, chemical disinfection and removal of infected conspecifics in gregarious or social species (Roy et al. 2006; Tragust et al. 2013). Grooming was described as the use of legs to clean

the body while arthropods stay stationery (Wekesa et al. 2007). Increased grooming following exposure to entomopathogenic fungi has been observed in several arthropod species such as predatory mites, African Tephritid fruit flies, ants and termites (Dimbi et al. 2009; Konrad et al. 2015; Reber et al. 2011). After predatory mites *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) were exposed to leaves treated with the entomopathogenic fungus *B. bassiana* Balsamo, they spent 1.88 min more time grooming over a 10 min observation window (Morelos-Juárez et al. 2010; Reber et al. 2011). *Phytoseiulus longipes* Evans (Arcari: Phytoseiidae) spent 1.4 min longer grooming over the first 15 min after being exposed to leaves with entomopathogenic fungus *Neozygites floridana* (Zygomycetes: Neozygitaceae) capilliconidia (Wu et al. 2018). Ants *Lasius japonicus* exposed to spores of entomopathogenic fungi increased duration and frequency of grooming and improved survival (Okuno et al. 2012). Grooming towards others, known as allogrooming, contributes to removal of entomopathogens. Invasive garden ants exposed to entomopathogenic fungi *Metarhizium brunneum* Petch (Ascomycota: Hypocreales) mechanically removed the spores and applied antifungal chemicals (formic acid secreted from their acidopore) to disinfect the brood (Tragust et al. 2013). An ant queen would bite co-founding dead queen's corpse to pieces to prevent disease from developing in the corpse following by burying or removing the pieces of corpse. In case the fungi started sporulation on the corpse, the ant queen would not perform these disease prevention behaviors, but rather avoid the fungi (Pull and Cremer 2017). Ants perform hygiene behavior not only for conspecifics but also towards mutualists that are contaminated with entomopathogens. Some ants in *Formica* species throw aphids that are infected by entomopathogens off the plants (Novgorodova and Kryukov 2017). These changes in behaviors imply that arthropods can perceive the presence of entomopathogenic fungi and actively reduce pathogen density on the individual level and in the colony level thereby compromise disease transmission (Tragust et al. 2013).

The fungal infection process can also be interrupted by the arthropod's innate immune system. Unlike vertebrates that have evolved an immune system relying on pathogen-specific receptors and immune memories, invertebrates defend against foreign invaders with different mechanisms (Christensen et al. 2005). The first line of defense operates directly at the level of the cuticle. For instance, red flour beetles produce quinones in

their cuticle, which function as both strong physical barrier and toxins suppressing fungi such as *B. bassiana* (Leal 2015). Once the cuticle is breached, localized hemolymph coagulation and melanization can occur following the production of reactive intermediates of oxygen and nitrogen (Christensen et al. 2005), hemocytes encapsulating fungal blastospores and antimicrobial peptides acting synergistically to kill invading fungi (Hoffmann 1995). Different antimicrobial peptides can be induced depending on the type of parasites (Rolff and Schmid-Hempel 2016), because arthropods have receptors recognizing different microbial patterns (e.g. glucans on fungal cell walls) and virulence factors (e.g. protease) of entomopathogens (Gottar et al. 2006). Some genes encoding antimicrobial peptide are constitutively expressed in arthropods to inhibit the growth of blastospores, thereby contributing to the host surviving from *B. bassiana* (Maistrou et al. 2018). Furthermore, insects can increase their body temperature to maintain hemocyte populations, enhancing defense-related enzyme activities (such as lysozyme and phenoloxidase) to suppress pathogens, known as behavioral fever (Kryukov et al. 2018; Ouedraogo et al. 2003). Even though infection cannot be cleared with behavior fever, insects could still survive long enough to develop into adults and reproduce (Elliot et al. 2002). These anti-pathogen defense mechanisms reduce the mortality rate at the host population level.

The use of entomopathogenic fungi in biological control

Entomopathogenic fungi have been used in biological control against a large variety of insect pests: mosquitoes, aphids, thrips, flies, caterpillars, beetles, scale insects, whiteflies, grasshoppers, locusts and others (Shah and Pell 2003). At least 12 species have been commercialized world wide, such as *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales), *Beauveria brongniartii* (Saccardo) Petch, *Lagenidium giganteum* Schenk (Oomycota: Lagenidiales), *Lecanicillium lecanii* (=*Verticillium lecanii* Zimmerman) (Ascomycota: Cordycipitaceae), *Metarhizium anisopliae* (Metschn.) Sorokin (Ascomycota: Clavicipitaceae) and *Isaria fumosorosea* (=*Paecilomyces fumosoroseus* Wize) (Ascomycota: Clavicipitaceae) (Lacey et al. 2015; Shah and Pell 2003). Entomopathogenic fungi have several advantages compared to other biological control agents. They can be produced on culture media and stored for a longer period, for

example, with more than 90% viability after one year in refrigeration and viable up to ten years in -80 °C freezer (Lacey et al. 2001). They can be applied with a conventional pesticide sprayer (Lacey et al. 2001). Recent findings demonstrate that some species are beneficial to plants as endophytes, plant pathogen antagonists, rhizosphere colonizers and plant growth promoters (Lacey et al. 2015). To attain successful biological control using entomopathogenic fungi, practical efficiency, profitability, sustainability and public safety remain important challenges (Shah and Pell 2003). Two main approaches have been adopted for their commercial applications: classical and augmentation biological control (Shah and Pell 2003). Examples of classical biological control are found in the order entomophthorales, which are mainly classified as specialist fungal pathogens (Shah and Pell 2003). In comparison to generalist entomopathogenic fungi, specialist species/strains provide the following advantages: (i) narrow host range with minimal detrimental effects on non-target organisms, (ii) only few conidia[1] are required to cause rapid infection, (iii) ability to induce epizootics (Navon and Ascher 2000). However, specialist fungi are rarely commercialized for biological control because they are difficult to culture on artificial media and they produce relatively small number of conidia. In addition, it is costly and labor intensive to collect the source of inoculum, propagate them on the host, store the propagules and release them back to the environment. In 1860s, the gypsy moth was accidently introduced from Europe to the Boston area and spread rapidly through northeastern USA affecting a wide variety of trees (Liebhold et al. 1992). *Entomophthora maimaiga* Humber, Shimazu & Soper (Entomophthorales: Entomophthoraceae) was found effective against gypsy moth populations, however, the application of *E. maimaiga* requires hand-collecting and redistribution of infected moth cadavers and soil containing resting spores in forests with gypsy moth outbreaks (Shah and Pell 2003). In contrast, generalist fungi, such as many species of entomopathogenic Hyphomycetes, have a wide range of arthropod hosts and more importantly they can grow as saprotrophs (Navon and Ascher 2000). The later characteristic allows them to be mass-produced on organic matter (e.g. barley and rice) at a high production rate and low cost, therefore, they can be developed into profitable products for augmentative biological control (Lacey et al. 2015).

[1] Conidia and spores are interchangeable hereafter. Conidia are asexual form of spores.

Strategies for enhancing infection rates of generalist entomopathogens used for biological control

Entomopathogenic Hyphomycetes such as *Beauveria* and *Metarhizium* species have been commercialized worldwide to control arthropod pests (Meyling and Eilenberg 2007; Zimmermann 2007). Infection rate has always been a constraint to their efficacy. Based on population dynamic models of infectious diseases, the infection rate is directly linked to pathogen density, host density and the transmission coefficient (Anderson and May 1981). Accordingly, mainly five strategies have been applied to increase infection rate: increasing pathogen densities, increasing the persistence of conidia in the environment by adding protectants and nutrients in a formulation, increasing conidial dispersal, targeting conidia to host aggregation sites, attracting hosts to semiochemical inoculation traps (auto-dissemination) (Shah and Pell 2003), and using beneficials such as bees and predatory mites to disseminate conidia (Al-Mazra'awi et al. 2006).

Growers can spray large amount of conidia at intervals to increase the density of entomopathogenic Hyphomycetes in their crops (Jaronski 2010). For example, a dose of 10^{13}-10^{14} *M. anisopliae* conidia per hectare is recommended to suppress pest in the field (Wraight et al. 2001). Spraying provides a means of dispersing entomopathogens. However, it cannot provide thorough coverage of plants due to spray runoff and drift and also because all plant surfaces cannot be reached (Courshee 1960). When the plant structure is complex, the host contact is challenging. For example, western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) inhabiting impatiens flowers acquired less *B. bassiana* conidia when flowers were partially closed than when they were fully open. Spraying *B. bassiana* reduced thrips populations by approximately 30%, but increasing the dose of application from $4.9x10^{13}$ to $2x10^{14}$ conidia per ha did not significantly increase the infection level nor reduce the thrips populations, suggesting that fungal transmission is the limiting factor (Ugine et al. 2007). Furthermore, sprayed conidia have a short window of time to contact their host before dying, mainly from UV exposure. *Beauveria bassiana* conidia without UV protectants lost 95% viability 15 minutes after being applied with water on a leaf surface (Inglis et al. 1995). Commercially, conidia can be formulated with protectants to prolong their viability, with

adhesives to improve attachment to plant surface, with humectants to stimulate rapid germination and with nutrient source for regeneration (Wraight et al. 2001).

Another strategy for growers to increase host contact is to apply conidia on specific zones in a crop where pests aggregate (Jaronski 2010). For example, with hydraulic nozzles pointed upward, the quantity of *B. bassiana* conidia was increased between 6 to 30 times on the underside of the leaves, where whitefly pests are mostly located (Byrne and Bellows Jr 1991). Similarly, when *B. bassiana* was sprayed specifically on soil near sugar beet maggot oviposition sites, the amount of *B. bassiana* conidia deposited on the soil surface was increased five times compared to conventional broadcast spray (Jaronski 2010). However, inundative application of entomopathogenic fungi does not guarantee successful biological control. For example, researchers have attempted to control termites with entomopathogenic fungi for more than 50 years. After 279 published studies on the topic, termite control by fungi still ends up in failures (Chouvenc et al. 2011). Several reasons have been identified (Chouvenc et al. 2011).

Termites live in cryptic habitats and within a nest, it contains many galleries. Therefore spores are unlikely to reach the host when sprayed on the external surface of the nest. For species having a central nest, the inundative approach is costly but still possible. For species that have extended underground nests, it remains impossible to access the entire nest with current application methods. Unfortunately, the inundative application of entomopathogenic fungi would have a larger impact on non-target species than on termites (Chouvenc et al. 2011).

Researchers have attempted to use bait to control populations of underground species, however, the proportion of termites trapped was far from enough to impact the whole colony. The reasons became obvious after termite behaviors were understood: termites can perceive the presence of entomopathogenic fungi and actively avoid them. Termites can also acquire ‘learned-avoidance’ to harmful fungi. In this case, the pathogen-host encounter doesn’t follow the principle of mass action: the probabilities of a host moving in different directions are not equal, especially when pathogens are present. Under such conditions, increasing pathogen density is useless for increasing the infection rate.

'Artificial' vectors or disease dispersal agents have been developed and tested to enhance the transmission of entomopathogens in the context of biological control. For example, when foraging within an aphid colony, the aphid predator *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae) inoculated with the entomopathogenic fungus *Erynia neoaphidis* Remaudiere & Hennebert (Entomophthorales: Entomophthoraceae) induced 10% of infection in pea aphid populations under laboratory conditions (Pell et al. 1997). Commercially mass-produced beneficials such as bumblebees, honeybees and predatory mites have been tested for entomopathogenic fungus dissemination for insect control (Al-Mazra'awi et al. 2006; Kapongo et al. 2008; Zhang et al. 2015a; Zhang et al. 2011). More than 90% of flowers and leaves contained detectable *B. bassiana* conidia and 30-40% of pest individuals were infected when bumble bees were used to disseminate *B. bassiana* to control the tarnished plant bug *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) and *F. occidentalis* on greenhouse sweet pepper (Al-Mazra'awi et al. 2006). However, the limitations of this system are the following: 1) It can only be applied to flowering crops; 2) Being phototactic positive, bees do not avoid UV (which is detrimental to *B. bassiana*) but use UV signals to locate flowers or to escape into an open space (Heiling et al. 2005; Menzel and Greggers 1985). Nonetheless, the pollinator-vectored entomopathogen dissemination system opened a new field of study in biological control.

Mites have been tested for their capacity as dispersal agents of entomopathogenic fungi. Laboratory experiments have been conducted to test the ability of phoretic *Macrocheles* mites to transfer conidia of *M. anisopliae var. major* to their host, the pales weevils *Hylobius pales* (Herbst) (Coleoptera: Curculionidae). Mites dusted with conidia were isolated with one weevil larva in a Petri dish and, within 24 h all beetles carried at least one mite. Eighty percent of the beetles carrying the phoretic mites died of infection after six weeks (Schabel 1982).

Could predatory mites be efficient dispersal agents of entomopathogenic fungi?

Several characteristics of predatory mites make them potential dispersal agents of entomopathogenic fungi. Phytoseiid mites are small and therefore have a large

surface/volume ratio for spores to be attached. Predatory mites are periodically released in large numbers in cropping systems. For example, *N. cucumeris* is released at a rate of 200-500 mites/m^2 bi-weekly or monthly (as recommended by Applied Bio-Nomics Ltd., Canada). Under these conditions, mites inoculated with entomopathogenic fungi can regularly bring large amount conidia to the plant. Secondly, predatory mites are actively searching for prey on plants. They are highly mobile and have a relatively fast walking speed relative to their small size. For example, *Phytoseiulus* species have a walking speed between 0.3-1.8 mm/s at 25°C (Coombs and Bale 2013). Thirdly, phytoseiid mites have the capacity to respond to herbivore-induced plant chemical cues to locate their prey, which would bring conidia to areas where prey aggregate (Dicke and Sabelis 1987). The prey finding capacity is critical for controlling pests with a cryptic nature, such as those dwelling in secluded sites on plants. Fourthly, phytoseiid mites and entomopathogenic fungi often have a similar preference for habitats characterized by high humidity and low UV intensity. Many phytoseiid species are vulnerable to ultraviolet radiations (Onzo et al. 2010; Tachi and Osakabe 2012), low humidity (Perring and Lackey 1989) and high temperature (Montserrat et al. 2013). For instance, *Neoseiulus californicus* (=*Amblyseius californicus* McGregor) (Acari: Phytoseiidae) actively avoids UV radiation and visible light (Tachi and Osakabe 2012). *Typhlodromalus aripo* De Leon (Acari: Phytoseiidae) avoids UV exposure and low humidity by hiding in plant apex during the day and actively foraging in the dark (Onzo et al. 2009). *Amblydromalus manihoti* Moraes (Acari: Phytoseiidae) and *Euseius fustis* Pritchard & Baker (Acari: Phytoseiidae) spend most of the time on the abaxial surfaces of leaves, because UVB exposure is highly lethal to them, especially to the eggs (Onzo et al. 2010).

The capacity of predatory mites to transmit disease to Asian citrus psylid populations was tested by Zhang et al. (2015b). They showed that *Neoseiulus cucumeris* (=*Amblyseius cucumeris* Oudemans) (Acari: Phytoseiidae) loaded with *B. bassiana* spores induced the same mortality in psylid populations as aerial spraying of the fungus, which means phytoseiid predatory mites could be explored as an alternative way to apply entomopathogenic fungi in biological control. *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) performed even better and completely eliminated psylid populations. Why did one predatory mite outperform the other? What criteria make a predatory mite an

efficient disease dispersal agent? The proof of concept needs to be comprehensive in order to develop a viable biological control tool.

1.2 Objectives

To understand the process of fungal disease transmission from predatory mites to prey and to develop a theoretical basis for such a method;

To develop a predatory mite-mediated entomopathogenic fungi dispersal system as a biological control tool that is easy to use and ready to be validated for greenhouse or field trials.

1.3 Research questions

Can predatory mites enhance the probability of encounter between their prey and entomopathogenic fungi? If yes, what are the ecological mechanisms involved?

Specifically,

1. Can predatory mites perceive the presence of entomopathogenic fungi on their bodies? If yes, what are their behavioral responses?
2. How do predatory mites dislodge fungi from their bodies to the environment? Is it by grooming?
3. Do the spatial distributions of fungal spores differ when they are dispersed by different species of predatory mites? Does it influence the pathogen-host contact rate?
4. To what extent the foraging behavior of predatory mites loaded with fungal spores determine the spatial distribution of the fungus on a plant?

1.4 Hypothesis

Our general hypothesis is the following: Predatory mites loaded with fungal spores

increase the encounter rate between entomopathogenic fungi and their prey. This capacity of predatory mites as dispersal agents is linked to their foraging activities. More specific hypotheses are described in each of the following chapters.

1.5 The study system

The biological system under study consists of the entomopathogenic fungus *Beauveria bassiana*; two species of plant-dwelling phytoseiid predatory mites *N. cucumeris* and *A. swirskii* and one species of soil-dwelling predatory mites *Stratiolaelaps scimitus* (=*Hypoaspis miles* Berlese) (Acari: Laelapidae) as potential fungal dispersal agents; and the western flower thrips *Frankliniella occidentalis*, a key pest species in agriculture, as a resource for both the fungus and the predators. These species share similar habitats (i.e. plants supporting thrips populations) and can coexist in commercial greenhouses applying biological control programs.

Beauveria bassiana is a generalist entomopathogenic fungus that exploits over 200 host species from most insect orders, with some isolates showing a high degree of specificity (Brodeur 2012; Uma Devi et al. 2008). The pathogenicity of *B. bassiana*, especially the registered strains, against beneficial insects, mammals and human is rarely reported (Zimmermann 2007). Therefore, they are considered as safe biological control agents. Conidia are responsible for infection and naturally dispersal by air movement because of their small size (1-3 μm) (Shimazu et al. 2002), by contact with infected hosts or via a dispersal agent (Baverstock et al. 2010; Fuxa and Tanada 1987; Vega et al. 2000). Conidia adhere to the host cuticle, germinate, penetrate in the host by enzymatic and mechanical processes and next reproduce by exploiting host hemolymph and various host tissues (Askary et al. 1999; Boomsma et al. 2014; Valero-Jiménez et al. 2016). Once host nutrients are depleted, the fungus breaches the cuticle from inside out and sporulates in large numbers (Valero-Jiménez et al. 2016). Commercial strains of *B. bassiana* are used for the control of arthropod pests in biological control programs. They are typically sprayed over the crops like pesticides and the probability of contact with the host depends on the spatial distribution of the pests (Brodeur 2012; Ugine et al. 2007). For our study, we used the *B. bassiana* strain ANT-03, which has been registered in North America for greenhouse biological control of thrips, aphids and whiteflies.

Western flower thrips is a cosmopolitan and highly polyphagous insect that feeds on almost every plant part, from leaves to flower and pods (De Jager et al. 1993; Trichilo

and Leigh 1988; Zhi et al. 2005), it can also vector a number of plant viruses (Reitz 2009). Thrips are difficult to control with contact insecticide because of their (i) thigmokinetic behaviour (hiding in concealed parts of plants, such as plant crevices and flowers, where insecticide cannot reach), (ii) propupal stage occurring below ground, also escaping from direct exposure to foliar pesticide, and (iii) rapid development of pesticide resistance (Broadbent et al. 2003; Jensen 2000).

The two phytoseiid predatory mites are generalist predators that actively search for prey (McMurtry et al. 2013). Foraging phytoseiid mites typically respond to chemical cues emitted by plants when attacked by herbivores and move towards infested areas (Dicke et al. 1998). They are both commercialized and released on vegetable and ornamental crops to control insect pests, including thrips (McMurtry et al. 2013). They mostly attack first instar thrips larvae because larger prey successfully counterattack predatory mites (Bakker and Sabelis 1986). Small and large thrips larvae live together in colonies on plant parts and larger larvae can protect their younger siblings from predation (de Bruijn et al. 2014). Below ground, the distribution of thrips propupae and soil-dwelling predatory mites remains poorly understood. Under greenhouse conditions, most *F. occidentalis* propupae were found within the first two centimeters in soil (Deligeorgidis and Ipsilandis 2004). Soil predatory mites such as *S. scimitus* has the capacity to reduce thrips population below ground and can induce up to 77% thrips mortality within a week (Berndt et al. 2004a; Saito and Brownbridge 2016). However, *S. scimitus* alone is usually not efficient for suppressing *F. occidentalis* populations (Berndt et al. 2004b). For this polyphagous soil predator, the ability to locate *F. occidentalis* and availability of alternative prey such as nematodes and collembola may limit its capacity for controlling thrips below acceptable levels (Berndt et al. 2004b; Wiethoff et al. 2004). When *S. scimitus* was combined with the phytoseiid mite *N. cucumeris*, control was not better than applying *N. cucumeris* alone due to competition and intraguild predation (Pochubay and Grieshop 2012; Wiethoff et al. 2004). It is reasonable to combine other biological control agents that have no negative interactions with *S. scimitus* in order to achieve satisfactory level of thrips control.

1.6 How did we answer the questions using our study system?

The main results of my thesis are structured in three scientific papers. In the first one, we developed a commercially applicable method for predatory mites to collect and transport spores of entomopathogenic fungi. We mixed *B. bassiana* into commercial rearing substrates of the predatory mites, extracted the mites after different hours and evaluated the number of spores on mite bodies. We also showed that *B. bassiana* strain (ANT-03), predatory mites and thrips form a suitable pathogen-vector-host association, meaning that the fungus is pathogenic against thrips, but benign towards the dispersal agents. In the second paper, we used video recordings to categorize and compare behaviours of the predatory mites with or without spores. We studied how mites dislodged spores by linking their time spent on different behaviours to the number of spores remaining on their bodies. Using low-temperature scan electronic microscopy (LT-SEM), we visualized the distribution of spores on mites. Finally, in the third study, plant-dwelling predatory mites were released on plants that had been previously infested with first instar thrips clustered on leaves. We examined each plant section to characterize the spatial distribution of each organism. We compared the performance of two species of predatory mites by calculating the spatial co-occurrence of spores and thrips and by measuring the proportion of thrips bearing spores. For the soil-dwelling predatory mites, we loaded the mites with spores and released them in soil infested with thrips propupa. We compared the number of emerging adult thrips and the proportion of adult thrips bearing spores across different experimental treatments. However, the results from the soil system are incomplete and therefore inconclusive. This part has been excluded from this thesis.

1.7 Contribution of the author and co-authors

Being the first author and the corresponding author of one paper and two manuscripts (Chapter 2-4), I formed the research questions, conceived and designed the experiments, organized lab meetings to discuss and optimize experimental protocols, performed the experiments, collected the data, analyzed the data, wrote the manuscripts, revised the manuscripts and responded to peer review. Being the last author, my supervisor Dr. Jacques Brodeur played a crucial role in guiding me through every process. Not only that, but he also hired a student for me every time I needed help for my experiments. Being the second last author, my co-supervisor Dr. Silvia Todorova participated in all the experimental designs and provided her professional and editorial advice. She also provided me with the predatory mites *N. cucumeris* and the means of producing technical grade powder of *B. bassiana* (strain ANT-03) for my experiments.

Below I will describe how each of my other co-authors contributed to the three Chapters.

Chapter 2: Lin, G., Tanguay, A., Guertin, C., Todorova, S. & Brodeur, J. 2017. A new method for loading predatory mites with entomopathogenic fungi for biological control of their prey. *Biological Control* **115**, 105-111

Alexandre Tanguay participated in the protocol development and performed the experiment with me. Dr. Claude Guertin participated in the experimental design and provided the lab space for the experiment. They both reviewed the manuscript.

Chapter 3: Lin, G., Di Paolo, S, Bauchan, G., Ochoa, R., Todorova, S. & Brodeur, J. Walking is the primary behavioral mechanism for predatory mites to dislodge fungal conidia from their bodies Manuscripts in preparation.

Sean-Anthony Di Paolo performed the experiment with me and analyzed the video. Dr. Gary Bauchan and Dr. Ronald Ochoa were leading the LT-SEM observations and edited the manuscript.

In Chapter 4: Lin, G., Guertin, C., Di Paolo, S., Todorova, S. & Brodeur, J. Phytoseiid predatory mites can disperse entomopathogenic fungi to prey patches. Revision has been submitted to *Scientific Reports* on April 5th, 2019.

Dr. Claude Guertin participated in the experimental design and provided editorial advice. Sean-Anthony Di Paolo performed the experiment with me. They both reviewed the manuscript.

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Chapter 2: A new method for loading predatory mites with entomopathogenic fungi for biological control of their prey

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Published as: Lin, G., Tanguay, A., Guertin, C., Todorova, S. & Brodeur, J. 2017. A new method for loading predatory mites with entomopathogenic fungi for biological control of their prey. *Biological Control* **115**, 105-111

2.1. Highlights

- Predatory mites can be loaded with fungal conidia added to mite commercial rearing substrates.
- Conidia loading capacity of soil mites increases with exposure time in the substrate.
- Overall, *Beauveria bassiana* (Strain ANT-03) had a limited effect on survival of the predatory mite species included in the study
- *Beauveria bassiana* showed stage-specific virulence to *Frankliniella occidentalis*.
- Predatory mites are potential vectors of entomopathogenic fungi in biological control.

2.2 Abstract

Movement of invertebrates can promote contact between entomopathogenic fungi and their hosts. In biological control programs, foraging predatory mites have the capacity to increase disease transmission rates and can potentially be used as fungal vectors. In this study, a method has been developed for predatory mites to collect and transport conidia of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) directly from the commercial rearing substrate. Increasing the duration of exposure (2-24 hours) to contaminated substrate significantly increased the number of conidia retained on the body of a soil predatory mite, *Stratiolaelaps scimitus* (=*Hypoaspis miles* Berlese) (Acari: Laelapidae). However, this was not observed in two phytoseiid species, *Neoseiulus cucumeris* (=*Amblyseius cucumeris* Oudemans) (Acari: Phytoseiidae) and *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae). These results suggest that upon receiving predatory mites from a supplier of biocontrol agents, conidia can be mixed into the substrate and, for the soil predatory mite, the length of time between mixing and release can be manipulated to determine the conidia load. Furthermore, the *B. bassiana* strain ANT-03 showed low virulence towards *N. cucumeris*, and had no significant effect on survival of *A. swirskii* or *S. scimitus*. However, stage-specific virulence was observed with their shared prey, the western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). Exposure to *Beauveria bassiana* (10^7 conidia ml^{-1}) significantly reduced survival of adults, propupae and 2^{nd} instar larvae, but not 1^{st} instar larvae. This biological model fits the profile of a suitable pathogen-vector-host association, where the pathogen uses vectors as dispersal agents and the host as a resource for reproduction.

Key words fungal dispersal, conidia load, *Beauveria bassiana*, *Stratiolaelaps scimitus*, *Neoseiulus cucumeris*, *Amblyseius swirskii*

2.3 Introduction

The probability that an individual becomes infected by a pathogen depends on various factors, including the pathogen's dissemination capacity. The rate of transmission of a disease is typically proportional to the rate of encounter between the host and the free-living infective stages of a pathogen (Anderson and May, 1980). Entomopathogenic fungi move between hosts by means of horizontal transmission among conspecifics, through conidia disseminated in the habitat, or via a vector (Fuxa and Tanada, 1987; Vega et al., 2000). The vector, a living organism that disseminates infectious agents (Timmreck, 2002), may transmit the pathogen passively without being infected by the infectious agent. The role played by vectors in entomopathogenic fungal epidemiology is complex, and in less studied vector-host systems such as arthropods, many aspects remain unclear.

The capacity of mites and insects to carry fungal pathogens of arthropod pests is a potentially desirable trait that could be promoted in biological control programs. This strategy would be of particular interest when pests cannot effectively be reached by a biopesticide (meaning formulated entomopathogens in this case) sprayed in a culture. Commercially mass-produced arthropods, either pollinators or predators, have recently been tested for vectoring entomopathogenic fungi to agricultural pests (Baverstock et al., 2010). For example, foraging bumblebees loaded with conidia of *B. bassiana* were released on sweet pepper plants in greenhouses where they transmitted the disease to the western flower thrips, *F. occidentalis*, and the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Al-Mazra'awi et al., 2006). Similarly, two phytoseiid predatory mite species *N. cucumeris* and *A. swirskii* showed the capacity to disseminate *B. bassiana* to their prey, the Asian citrus psyllid *Diaphorina citri* Kuwayama (Homoptera: Psyllidae) in potted citrus plants (Zhang et al., 2015b). These results suggest that arthropod vectors can contribute to increasing the rate of encounter between entomopathogenic fungi and arthropod pests.

In nature, *B. bassiana* is an opportunist pathogen. It has been used for augmentative biological control with single or multiple applications in crops when climatic conditions facilitate interaction with the targeted pests (Brodeur, 2012; Waage, 1995). However,

transmission rate and conidia persistence in the habitat can dramatically affect the efficacy of an entomopathogenic fungus. In soil, the viability of *B. bassiana* (strain GHA) conidia decreased by 30% and by 75% one and seven weeks after application, respectively (Świergiel et al., 2015). When exposed to UV radiations on a leaf surface, *B. bassiana* (Strain GHA) conidia viability is reduced by 95% after only 15 minutes (Inglis et al., 1995). Typically, microbial biopesticides are applied like chemical pesticides as they are sprayed over a crop. The probability of contact with the targeted pest depends on the spraying technique and the spatial distribution of the host. In greenhouse impatiens crops, spraying *B. bassiana* reduced thrips populations by 30%, but increasing the concentration of application from $4.9x10^{13}$ to $2x10^{14}$ conidia per ha did not significantly increase the infection level, suggesting that host contact is the limiting factor (Ugine et al., 2007). Contacting the host when conidia remain viable is therefore crucial for the persistence and efficacy of entomopathogenic fungi as biocontrol agents.

For a pathogen-vector-host association to be sustainable in a biological control program, the pathogen has to be benign towards the vector and virulent against the host; the vector conveys the pathogen to the host which is then used as a resource for growth and multiplication of the pathogen (Ewald, 1994). The present study is part of a research program aiming to explore ways to increase transmission rates of the entomopathogen *B. bassiana* to western flower thrips above and below ground using predatory mites as vectors. Under experimental conditions, predatory mites have been shown to acquire conidia either by walking on sporulating *B. bassiana* in a Petri dish (Zhang et al., 2015b) or being sprayed with a conidia suspension (Wu et al., 2016). However, these methods are not applicable for commercial use because mass-reared predatory mites are contained within rearing substrates such as bran or mixture of bran vermiculite and sphagnum moss (Freire and de Moraes, 2007).

Frankliniella occidentalis was the target host for this study. It is a highly polyphagous insect with a short generation time and high reproductive rate, especially when it feeds on flowers (Jager et al., 1993). The pest is difficult to control with contact insecticide because of its (i) thigmokinetic behaviour (hiding in concealed parts of plants, such as flowers, where insecticide cannot reach), (ii) propupal stage occurring below ground, also

escaping from direct exposure to foliar pesticide, and (iii) rapid development of pesticide resistance (Broadbent et al., 2003; Jensen, 2000).

Three generalist predatory mite species were used as potential vectors of *B. bassiana*: two phytoseiids foraging on plants *N. cucumeri* and *A. swirskii* and the soil-dwelling *Stratiolaelaps scimitus* (=*Hypoaspis miles* Berlese) (Acari: Laelapidae). *Neoseiulus cucumeris* and *A. swirskii* typically feed on 1st instar *F. occidentalis* while *S. scimitus* can attack *F. occidentalis* larvae and propupae (Gerson and Weintraub, 2007; Wu et al., 2014a). All three species are commercialized and have been used successfully to control populations of *F. occidentalis* in vegetable and ornamental crops worldwide (Van Lenteren, 2012). Above ground, phytoseiid mites respond to chemical cues emitted by plants and move toward areas infested with thrips (Midthassel et al., 2016). Phytoseiid mites and thrips both include pollen in their diet with predatory mites spending a large proportion of time in flowers where thrips aggregate (Faraji et al., 2002). Below ground, the distribution of thrips propupae and soil-dwelling predatory mites remains poorly understood. Under greenhouse conditions, most *F. occidentalis* propupae were found within the first two centimeters in soil (Deligeorgidis and Ipsilandis, 2004). *Stratiolaelaps scimitus* has the capacity to reduce thrips population below ground (Berndt et al., 2004).

In this study, we first tested the susceptibility of all developmental stages of *F. occidentalis* to *B. bassiana* (strain ANT-03). We next developed a method for one ground-dwelling (*S. scimitus*) and two foliar (*N. cucumeris* and *A. swirskii*) predatory mite species to acquire conidia by exposing them to *B. bassiana* technical grade powder in their respective rearing substrates. Finally, we examined conidia load and tested survival of these potential predatory mite vectors after different exposure durations in contaminated rearing substrates.

2.4 Materials and Methods

2.4.1 Arthropod colonies and fungal inoculum

A laboratory colony of *F. occidentalis*, obtained from Anatis Bioprotection Inc. was reared on French bean plants *Phaseolus vulgaris* L. (Fabaceae) in a growth chamber at 25°C, 70% RH, 16:8 h (L:D). Apple pollen (Firman Pollen Co., (Yakima, WA)) was supplied *ad libitum* on a weekly basis. To obtain cohorts of *F. occidentalis* of a given stage for experiments, 10 day-old *P. vulgaris* seedlings were placed in a *F. occidentatlis* rearing cage for 24 h for females to oviposit in leaf tissues. Leaves with eggs were excised, sustained with water from a glass bottle, and isolated in a plastic container with meshed ventilation on the lid. First instar larvae, 2nd instar larvae, propupae and adults were obtained 5, 7, 9 and 12 d following oviposition.

Neoseiulus cucumeris, provided by Anatis Bioprotection, Canada, were maintained on a factitious prey *Aleuroglyphus ovatus* Toupeau (Acari: Acaridae) and apple pollen. *Amblyseius swirskii*, purchased from BioBest Canada, were reared on a diet mixture containing *Carpoglyphus lactis* (Acari: Carpoglyphidae), *Tyrophagus putrescentiae* Schrank (Acari: Acaridae) and apple pollen. Both species were reared at 25°C, 70% humidity and under 14L: 10D light cycle. In the experiments, we used adult females that were less than one week old. Cohorts were produced by isolating ovipositing females on plastic sheets spread on wet sponges in plastic trays containing water. Larvae and protonymphs were collected and transferred onto another arena and supplied with their diet mixture. Wheat bran was supplied as shelter. After 4-5 d, most individuals of both species developed into adults.

Stratiolaelaps scimitus, purchased from Applied Bionomics, Canada, were maintained on a mixture of wheat bran, vermiculite and sphagnum moss (2:2:1) with the factitious prey *T. putrescentiae* (Freire and de Moraes, 2007). To standardize the age of tested adult females, larvae were isolated on moistened plaster-charcoal (7:1) in plastic containers with meshed ventilation (Enkegaard et al., 1997) and reared at 25°C, 70% humidity and under dark. Predatory mites were fed on *T. putrescentiae* and *A. ovatus*, and moist sphagnums moss and wheat bran were supplied as shelter. After approximately 10 d,

most individuals developed into adults (Enkegaard et al., 1997; Ydergaard et al., 1997). Less than one-week-old adult *S. scimitus* females were used for the experiments.

We used *B. bassiana* strain ANT-03 for this study. It has been registered as a pest control agent for greenhouse vegetable and ornamental crop production in North America. The technical grade powder (TGP) containing $5x10^{10}$ to $1.4x10^{11}$ conidia per gram (depending on the batch) and manufactured by Anatis Bioprotection, was used for the experiments. The viability of conidia was tested within a week before each experiment. A minimum germination rate of 90% was required. TGP was stored at 4°C.

2.4.2 Stage-dependent susceptibility of F. occidentalis to B. bassiana

Beauveria bassiana TGP was suspended in 0.05% Tween 80 and adjusted to an intermediate concentration 10^7 conidia per ml with a hemocytometer. This intermediate concentration was chosen to increase the probability of detecting effects: at high concentrations, thrips may die too rapidly for differences to be observed among life stages; at low concentrations, thrips may die too slowly for differences between control and conidia treatment to be detected within a 12-day period (Vestergaard et al., 1995). For each life stage of *F. occidentalis* (1^{st} instar larva, 2^{nd} instar larva, propupa and adult female), a cohort of 20-30 individuals was isolated in a Solo cup (29.6 ml) and 10 ml conidia suspension was poured into the cup. Thrips were immersed for 5 s and then transferred onto a black filter paper (Ø 9 cm, Thomas Scientific) in a Buchner funnel. The excess liquid was evacuated through a vacuum and thrips were individually transferred onto a *P. vulgaris* leaf disc (Ø 25 mm) placed upside down on a filter paper (Ø 42.5 mm, Fisher Scientific) soaked with 1 ml of distilled water. A small amount of apple pollen (≤0.001 g) was brushed on the leaf disc. The leaf disc and the pollen were replaced every 3-4 days. Each leaf disc was kept in one tightly closed Falcon Petri dish (Ø 50 mm) with a meshed ventilation hole (Ø 6 mm) at the top and observed daily at the same hour (1:30 pm) for survivorship over a 12 day period (Vestergaard et al., 1995). *Frankliniella occidentalis* treated with 0.05% Tween 80 served as control. The Petri dishes were kept in a growth chamber maintained at 25°C, 70% RH, 16:8 h (L:D). The entire test followed a randomized complete block design and was repeated three times

(temporal blocks) using the same batch of *B. bassiana* TGP (stored at 4°C, the inoculum was freshly prepared at the day of each experiment) and different generations of *F. occidentalis*. Thrips that escaped from the funnel and arena or accidentally crushed during experimental manipulation were eliminated. Within each block, 16-20 *F. occidentalis* of a given stage were tested.

2.4.3 Conidia loading capacity of predatory mites

The substrate used to assess the capacity of predatory mites to acquire *B. bassiana* conidia on their body over time was prepared as follows: medium-sized wheat bran (1-5 mm in flake size, Farinex, Canada) was placed in an oven at 65°C for 4-7 d until it was completely dry. Distilled water was sprayed onto the dry wheat bran to reach 25% water content (w:w). *Beauveria bassiana* TGP was next mixed to 50 g moistened wheat bran to reach $1.3x10^8$ conidia g^{-1} substrate. It was mixed by hand and with a vortex mixer at 3200 rpm for five minutes. Apple pollen was also added to the wheat bran (0.01 g^{-1} substrate) to as food for the phytoseiid mites. For *S. scimitus*, the substrate contained wheat bran, vermiculite and sphagnum moss (2:2:1), together with the same *B. bassiana* concentration. No food was provided for *S. scimitus* in the tube because its prey *T. putrescentiae* is known as a fungivorous mite (Canfield and Wrenn, 2010), possibly consuming conidia mixed in the substrate.

To expose mites to conidia, the experimental arena consisted of a modified 1.5-mL Eppendorf tube (Fisherbrand®, Fisher Scientific, USA). The tip of the tube was cut to make an opening of 5 mm in diameter. The opening was sealed with a fine mesh (23 µm-size polyester mesh 508®, Clever©, Japan) and connected to a vacuum tube. A 5-mL pipette tip (Brand®, Germany) was cut and fit into the other opening of the Eppendorf tube. Before introducing the mites in the arena, substrate (0.05 g) without conidia was placed in the Eppendorf tube, serving as a buffer to minimize physical damage to the mites when vacuuming. Mites were vacuumed from the pipette tip into the Eppendorf tube. The substrate (0.15 g) mixed with *B. bassiana* conidia at $1.3x10^8$ conidia g^{-1} was placed into the Eppendorf tubes. Therefore, the final concentration of conidia in each tube was adjusted to 10^8 conidia g^{-1} substrate. The tubes were flicked for 5 s, so that the

substrates with and without conidia were mixed within the tube. For each predatory mite species, 25 individuals were introduced per tube, and 1-2 tubes were prepared for each exposure period (Figure 2-1). Tubes were kept in a growth chamber set at 25°C, 70% humidity and 14:10 h (L:D) until mite extraction.

Mites were extracted from experimental arenas 2, 8, 20 and 24 h following exposure to conidia. Mites isolated in the substrate without conidia for 24 h were checked for potential contamination from rearing, substrates, and experimental manipulations. At the end of the test, tubes were emptied and their content spread onto a 1 mm meshed screen placed on a glass funnel (5 cm in diameter at the top). A 40-watt tungsten light bulb was placed 1 cm above the mesh and turned on for 10 min. An arena similar to the ones used for phytoseiid mite oviposition (see *Arthropod colonies and fungal inoculum*) was placed below the funnel to capture mites that escaped from light and heat. Mites were picked up from the arena with fine brushes and placed inside a 1.5-mL Eppendorf tube containing 0.5 ml of 0.05% Tween 80 solution. The tubes were then stirred twice for 30 s at 2000 rpm (Dromph, 2001). This allowed the mites to remain intact while most of the conidia on their cuticles were washed into the suspension. The suspension (0.1 ml) was plated onto media selective for *B. bassiana* containing: oatmeal agar medium (Difco, Detroit, MI) amended with 0.55% Dodine, 0.005% chlortetracycline and 0.01% crystal violet in Petri dishes (Chase et al., 1986). They were kept in darkness at 25°C for 8 d, after which colony-forming units (CFU) were counted and recorded (Al-Mazra'Awi et al., 2007). The experiment followed a randomized complete block design and was repeated four times (temporal blocks) using different batches of *B. bassiana* TGP and mites for different generations. Within each block, 7-11 individual mites of each species were used to evaluate their conidia loading capacity per exposure duration.

2.4.4 Survivorship of predatory mites loaded with conidia

A subsample from the previous experiment was used to test for mite survivorship following extraction from the substrate. For each exposure duration, mites were individually transferred to a glass tube arena (Zhang et al., 2015a) and kept at 25°C, 70% RH, 16:8 h (L:D). Their survival was checked daily over a period of 10 days. The entire

test followed a randomized complete block design and was repeated four times (temporal blocks). Within each block, 11-14 *N. cucumeris*, 18-22 *S. scimitus* and 11-15 *A. swirskii* per exposure duration were tested. Mites isolated in the substrate without conidia for 24 h were served as control for the effect of exposure to *B. bassiana* for 24 h.

2.4.5 Statistical analyses

Susceptibility of *F. occidentalis* to *B. bassiana* (time until death) was analyzed using Cox proportional hazards survival model with mixed effects. For each *F. occidentalis* life stage, the death hazard rate was the response variable, the presence of conidia was a categorical fixed factor, and block was a categorical random factor. The package 'coxme' was used for the analysis, carried out using R version 0.99.896 (R, 2013).

To estimate the conidia loading capacity of predatory mites, the number of CFU per mite (response variable) was analyzed using a generalized linear mixed-effect model with negative binomial distribution, with exposure duration as a continuous fixed factor and block as a categorical random factor. The package 'glmmADMB' was used for the analysis, carried out using R version 0.99.896 (Fournier et al., 2012; R, 2013).

Predatory mite survival was first compared between controls (individuals isolated in clean substrate for 24 h) and individuals that had been exposed to conidia mixed in substrate for 24 h. The death hazard of the treatment and control was analyzed using a Cox mixed effects survival model with the presence of conidia (present/absent) as a fixed categorical factor and block as a categorical random factor. When exposure to conidia significantly increased predatory death hazard rate, we next tested the effect of exposure duration. The death hazard was analyzed using mixed effect Cox model with exposure duration as a continuous fixed factor and block as a categorical random factor. We tested if the association between exposure duration to *B. bassiana* and time to death was significant. The package 'coxme' was used for the analysis, carried out using R version 0.99.896 (R, 2013).

2.5 Results

2.5.1 Susceptibility of F. occidentalis to B. bassiana

Frankliniella occidentalis displayed stage-dependent susceptibility to *B. bassiana* strain ANT-03 when exposed to a conidia concentration of $10^7 ml^{-1}$ (Figure 2-2). Within a period of 12 d, the strain reduced the survival of adults (χ^2=88.58, p<0.001), propupae (χ^2=12.00, p<0.001) and 2nd instar larvae (χ^2=8.83, p=0.003). However, the strain did not induce additional mortality of 1st instar larvae (χ^2=2.01, p=0.156) compared to the control treatment.

2.5.2 Conidia retention of predatory mites

The duration of exposure to *B. bassiana* conidia in the substrate had no significant effect on conidia retention by *N. cucumeris* (z=0.64, p=0.520, Fig. 2-3a) or *A. swirskii* (z=0.9, p=0.370, Fig. 2-3b). Numbers of CFU recorded were 122±21 (Mean ± S.E.) for *N. cucumeris* and 53±5 for *A. swirskii*. For both species, no CFU were observed in control treatments.

A different pattern was observed for *S. scimitus*, with a significant increase in *B. bassiana* conidia retention with increasing time spent in the substrate (z=9.09, p<0.001 Fig. 2-3c). By 24 h, each *S. scimitus* was loaded with an average of 1079±93 CFU. No CFU were observed in the control treatment.

2.5.3 Survival of predatory mites exposed to B. bassiana

Exposure to *B. bassiana* strain ANT-03 did not affect the survival of *A. swirskii* (χ^2=0.30, p=0.584) or *S. scimitus* (χ^2=0.38, p=0.536), but reduced the probability of *N. cucumeris* survival (χ^2=6.31, p=0.012) (Fig. 2-4). Relative to control, the death hazard of *N. cucumeris* is 3.14 times higher when exposed to the strain (p=0.018). However, exposure duration to conidia in the substrate did not significantly affect *N. cucumeris* survival (χ^2=0.16, p=0.688, Fig. 2-5).

2.6 Discussion

Our study describes a biological system that fits the profile of a suitable pathogen-vector-host association, where the pathogen uses vectors as dispersal agents and the host as a resource for reproduction. Furthermore, from a biological control perspective, we provide a simple and operational method for predatory mites to collect and transport fungal conidia directly from the commercial rearing substrate to the crop.

Mortality of *F. occidentalis* exposed to *B. bassiana* (strain ANT-03) was stage-specific; generally, thrips susceptibility increased with life stage. This pattern concurs with the study by Vestergaard et al. (1995) where adult *F. occidentalis* had the highest mortality (near 100%) 6 days following inoculation with *Metarhizium anisopliae*, followed by propupae (41.3%) and larvae (27%). As suggested by Vestergaard et al. (1995), the underlying mechanism behind this stage-dependent susceptibility to entomopathogenic fungi likely relates to the probability of host molting before conidia can successfully penetrate the cuticle and invade the host tissues. First instar *F. occidentalis* shed their exuviae faster than other stages (Zhang et al., 2007) and this may prevent successful infection by *B. bassiana*. Adult *F. occidentalis* are the most vulnerable stage to *B. bassiana* infection, and the question remains as to whether phytoseiid mites can actually deliver conidia to adult thrips on the plant. This is likely to occur since both thrips (especially the adults) and phytoseiid mites tend to aggregate in flowers where they feed on pollen (Faraji et al., 2002; Hulshof et al., 2003). However, the spatial co-occurrence of phytoseiid mites and thrips of different stages on plant structure, as well as the potential of predatory mites as vectors of *B. bassiana* need to be further investigated.

In our biological system, *B. bassiana* (strain ANT-03) was not pathogenic to *A. swirskii* or *S. scimitus* and showed low virulence towards *N. cucumeris*. Similar results were obtained for *S. scimitus* when infected by *B. bassiana* (BotaniGard) or *Metarhizium brunneum* (Met52) (Saito and Brownbridge, 2016). Differential susceptibility to *B. bassiana* of predatory mites compared to thrips suggests that predatory mites, especially *A. swirskii* and *S. scimitus*, could potentially be used as vectors to disseminate the fungus to the host pest. Reproduction of the fungus can occur on the three stages of *F.*

occidentalis susceptible to *B. bassiana*. The outcomes of these complex interactions remain to be examined at the population level to determine whether the pathogen and the vectors would interact positively in suppressing thrips populations under field or greenhouse conditions. For example, the relative contribution of above and below ground predatory mites in vectoring fungal conidia to different thrips developmental stages needs to be further explored. In past greenhouse experiments, applying the predatory mite *Neoseiulus barkeri* coated with *B. bassiana* did not result in increasing suppression of *F. occidentalis* populations (Wu et al., 2014b). The combined treatment (pathogen + predatory mite) provided better control of thrips than introducing the predatory mite alone, but the level of control obtained was not better when the pathogen was used alone. Although the *B. bassiana* strain SZ-26 is not pathogenic to *N. barkeri*, it was suggested that predation rate was reduced because *N. barkeri* exposed to *B. bassiana* spent a considerable amount of time grooming (Wu et al., 2016). On the other hand, the pathogen and the vector may interact positively, for example, *B. bassiana* delivered by *A. swirskii* caused higher mortality of *Diaphorina citri* than when *B. bassiana* was sprayed onto potted citrus plants, suggesting the predatory mite as a vector can enhance pathogen-host encounter rate (Zhang et al., 2015b).

Beauveria bassiana conidia accumulated on the body of the three tested species of predatory mites. For both *N. cucumeris* and *A. swirskii*, the maximum conidia load was attained rapidly (within 2 h) when mites were introduced into the substrate and remained constant thereafter. In contrast, the number of conidia that accumulated on *S. scimitus* increased over time between 2 h and 24 h. This might arise from differences in adhesion force between conidia and mite associations. For example, *M. anisopliae* conidia had higher adhesion force to the cuticle of mealworm than to the cuticle of mosquito larva or glass (Greenfield et al., 2014). The level of adhesion between conidia and substrates may vary as well. Conidia may be more readily dislodged from the sphagnum/bran/vermiculite medium than from bran alone. Differential movement of the mites within the two substrates, or differences in mite activity levels may influence the rate at which conidia are acquired. The phytoseiid mites may spend more time moving outside of the substrate compared to the soil mite. In commercial packages, phytoseiid

mites are often seen on the lids but not soil mites (G. Lin, personal observations). It is also possible that phytoseiid mites have a better capacity than the soil mite to detect and remove fungal conidia from their body. Grooming may prevent mites from accumulating conidia in their rearing substrates. Red imported fire ants *Solenopsis invicta* Buren (Hymenoptera: Formicidae) exposed to *M. anisopliae* increased self- and allo-grooming activities leading to a significant decrease of conidia on their cuticles over time (Qiu et al., 2014). As for our system, the role of grooming behavior in conidia accumulation on predatory mite body remains to be examined.

The current study is a part of a project testing if predatory mites can promote the encounter rate of their prey with an entomopathogen that is virulent against the prey. In the following two chapters, we will describe how predatory mites dislodge conidia and the spatial distribution of conidia dispersed by predatory mites in lab settings. If validated in large-scale greenhouse and/or field trials, the methodology developed here can have direct applications to biological control programs targeting thrips. Upon receiving commercially packed predatory mites, growers can mix *B. bassiana* conidia into rearing substrates. *Neoseiulus cucumeris* and *A. swirskii* can then be released after two hours with the maximum conidia load. For *S. scimitus*, the length of time between mixing and releasing can be manipulated to attain the desired conidia load.

As for the practical application of this method, conidia can be mixed into bulk products instead of slow-release sachets, because sprinkling conidia with the substrates directly onto crops would likely increase pathogen dispersal. The fate of conidia spread onto crops remains to be examined. Furthermore, fungivorous prey mites, such as *T. putrescentiae*, that are present in commercial predatory mite products can potentially carry and disperse *B. bassiana*, because *B. bassiana* has frequently been associated with *T. putrescentiae* in stored products (Abdel-Sater and Eraky, 2002). If further studies show predatory mites to be efficient in delivering conidia to pests in commercial productions, the approval of such an application by regulatory agencies should not be a major concern. For instance, in Canada, the strain ANT-03 was granted full registration in Canada in 2014. Furthermore, we are suggesting mixing predatory mites and *B. beauveria* conidia within commercial substrates/carriers that have already been applied in greenhouses and

fields for years and have not showed negative impacts on public health, environment or food safety. This approach consists of a simultaneous application of two already approved pest control products. Once validated with efficacy test in commercial settings, registration as a new product can be considered.

2.7 Acknowledgements

We thank Yutaka Saito, Jean-Philippe Parent, Svetlana Gouli, Vladimir Gouli, Mo Wang, Jamie Wilt, Narin Srei, Dominik Bergeron and Philippe Constant for advice and technical assistance; Paul Abram for help on statistical analyses. This study was supported by BMP Industrial Innovation Scholarship Program from Natural Sciences and Engineering Research Council of Canada, Fonds de Recherche Nature et Technologies du Québec and Anatis Bioprotection Inc.

Declaration of interest

The authors declare there is no conflict of interest.

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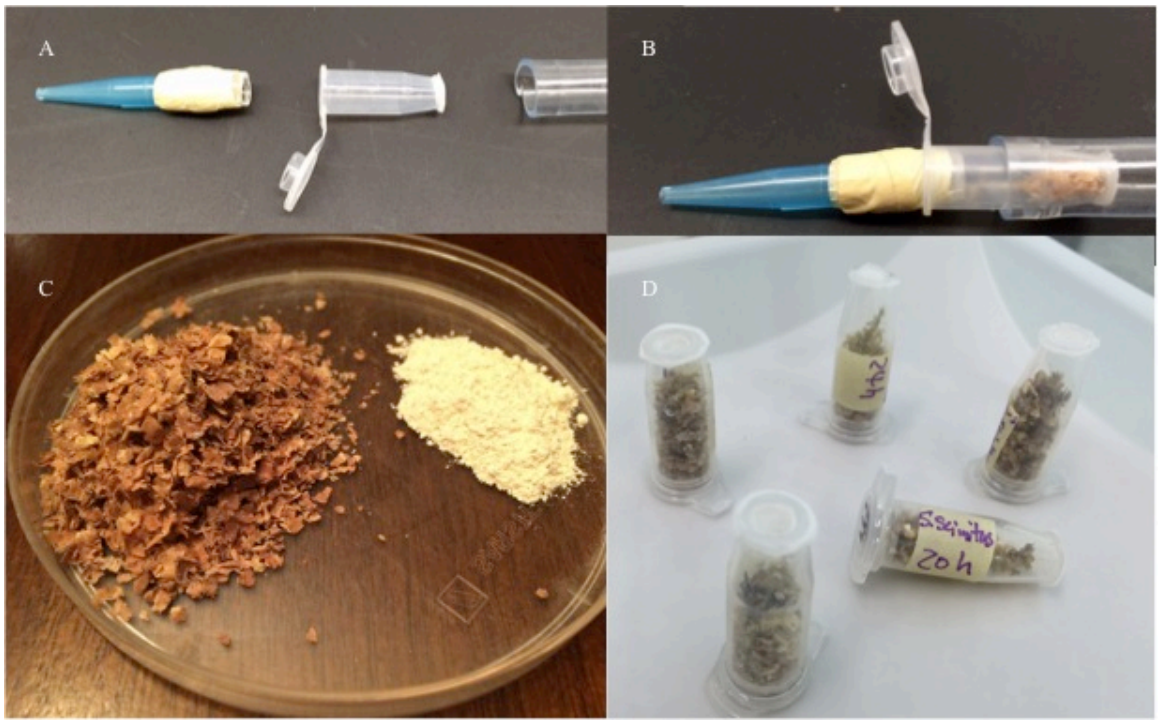

Figure 2-1. Experimental setups showing how predatory mites were exposed to *Beauveria bassiana* in rearing substrates: (A) connecting parts from left to right: a pipette tip with base wrapped with masking tape, a modified Eppendorf tube, a tube connecting to vacuum; (B) three connected parts with 0.05 g substrate in the tube as a buffer, mites were then vacuumed into the modified Eppendorf tube; (C) wheat bran substrate (left) and *B. bassiana* technical graded powder (right) for mixing; (D) mites were exposed to *B. bassiana* contaminated substrates in modified Eppendorf tubes.

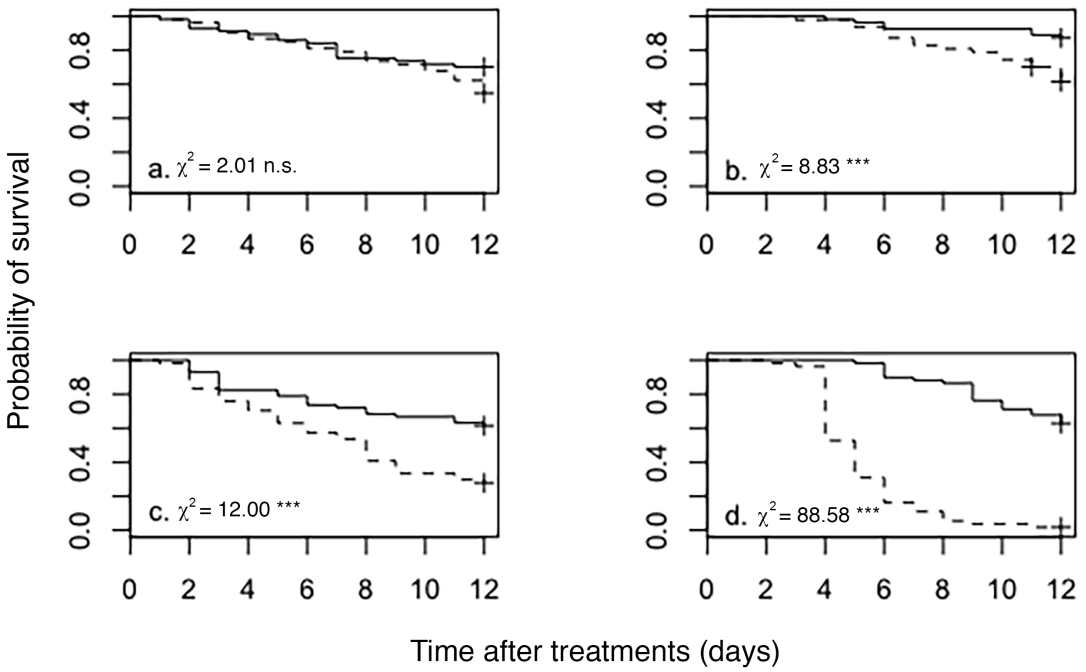

Figure 2-2. Survival probability of 1st instar larvae (a), 2nd instar larvae (b), propupae (c) and adult female (d) *Frankliniella occidentalis* treated with 0.05% Tween-80 suspension (Control; solid line) and *Beauveria bassiana* conidia suspension at 10^7 per ml of 0.05% Tween-80 (dashed line). The asterisks indicate significant differences between treatments: n.s. = not significant ($p > 0.05$), *** = $p < 0.0001$ (Cox proportional hazards mixed effect model).

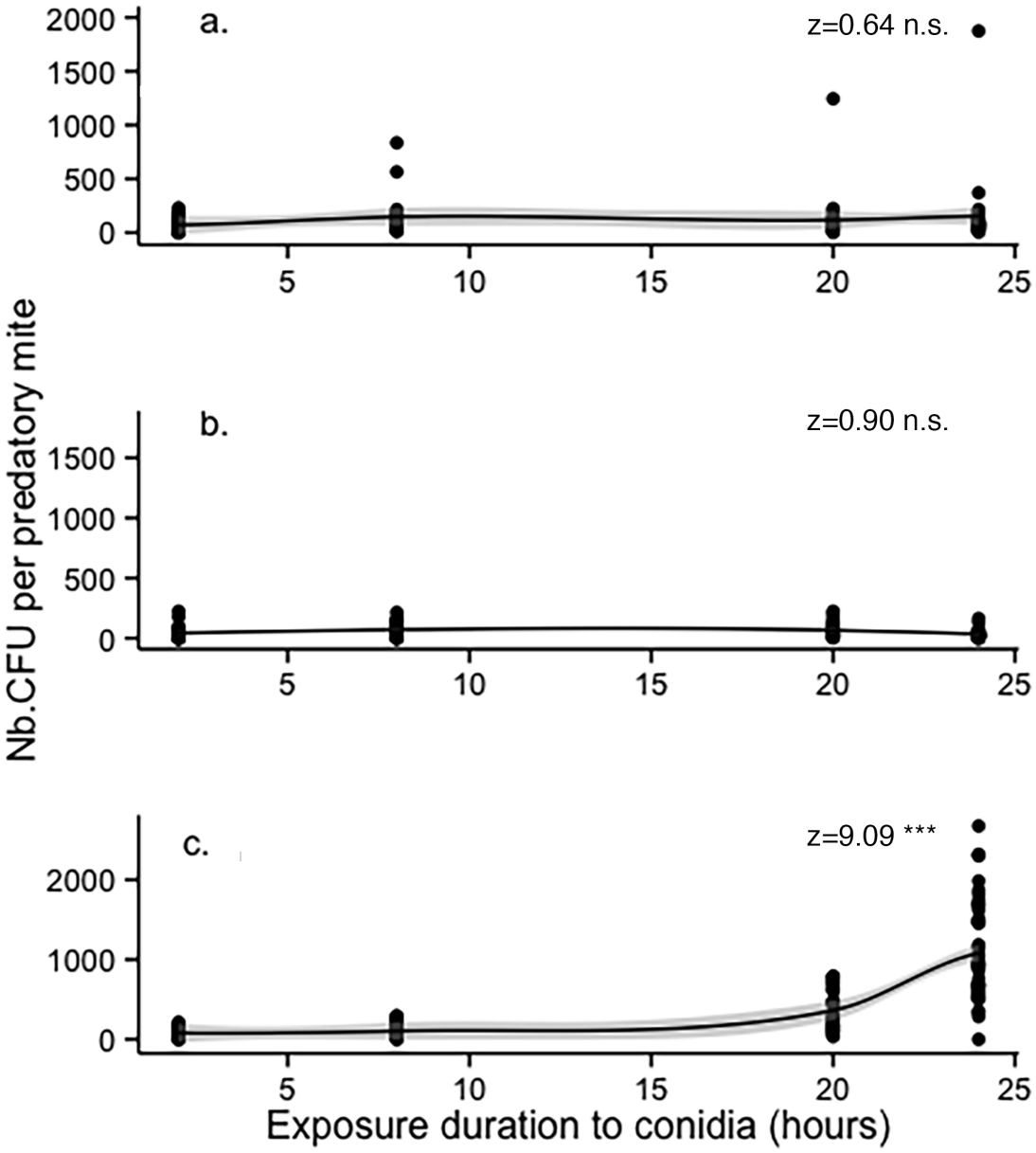

Figure 2-3. Number of colony-forming units (CFU) washed off three species of predatory mites after exposure to *Beauveria bassiana* conidia (10^8 g^{-1}) in the substrate for 2, 8, 20 and 24 h. (a) *Neoseilus cucumeris*, (b) *Amblyseius swirskii*, (c) *Stratiolaelaps scimitus*. The asterisks indicate significant differences between treatments: n.s. = not significant ($p > 0.05$), *** = $p < 0.0001$ (Generalized linear mixed-effect model). The grey area demonstrates 95% confidence intervals predicted by 'smooth' function from ggplot2.

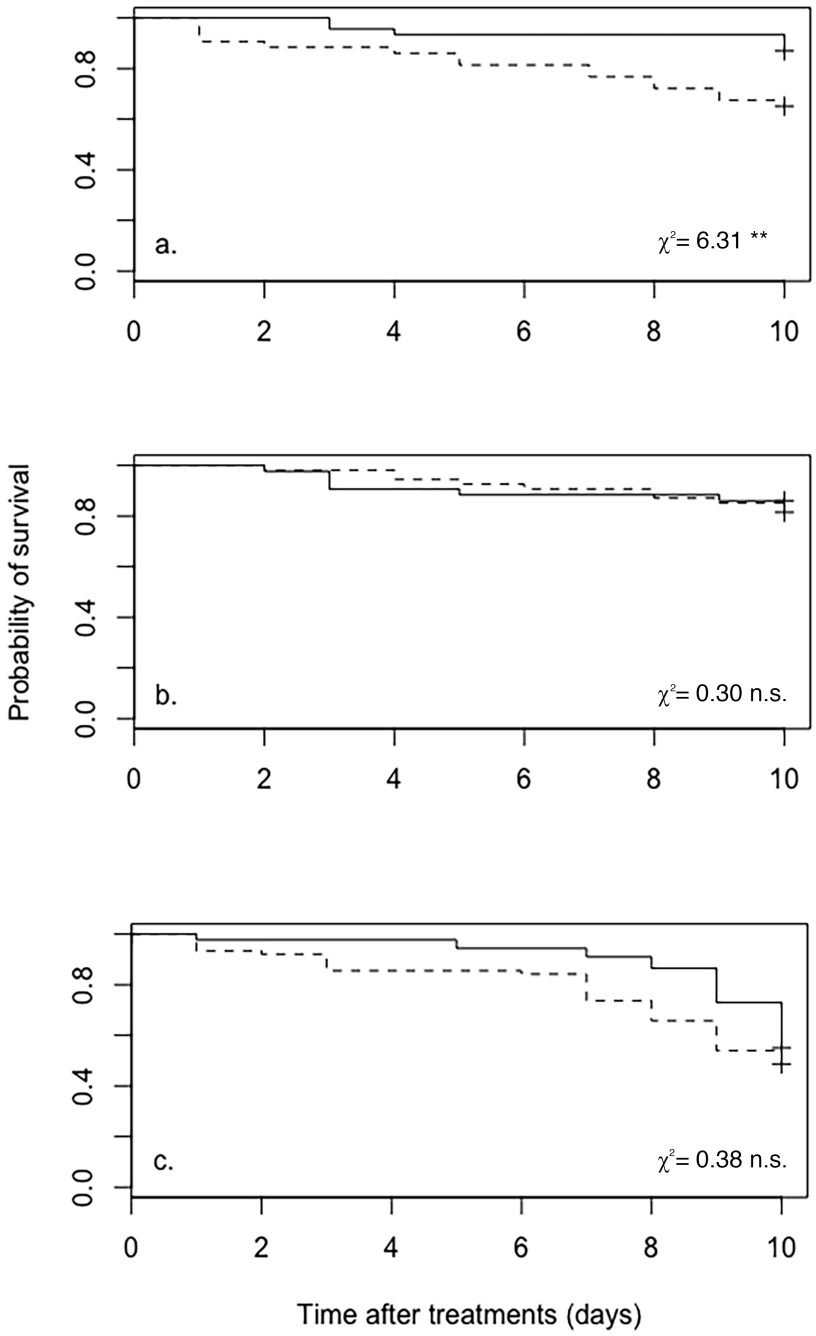

Figure 2-4. Probability of survival of predatory mites over 10 days after exposure to *Beauveria bassiana* conidia (dashed line) in the substrate for 24 h. For controls (solid line), predatory mites were isolated from the substrate without *B. bassiana* conidia after 24 h. Exposure to conidia significantly reduced the survival of *Neoseiulus cucumeris* (a), but had no significant effect on survival of *Amblyseius swirskii* (b) or *Stratiolaelaps scimitus* (c). The asterisks indicate significant differences between treatments: n.s. = not significant ($p > 0.05$), ** = $p < 0.01$ (Cox proportional hazards mixed effect model).

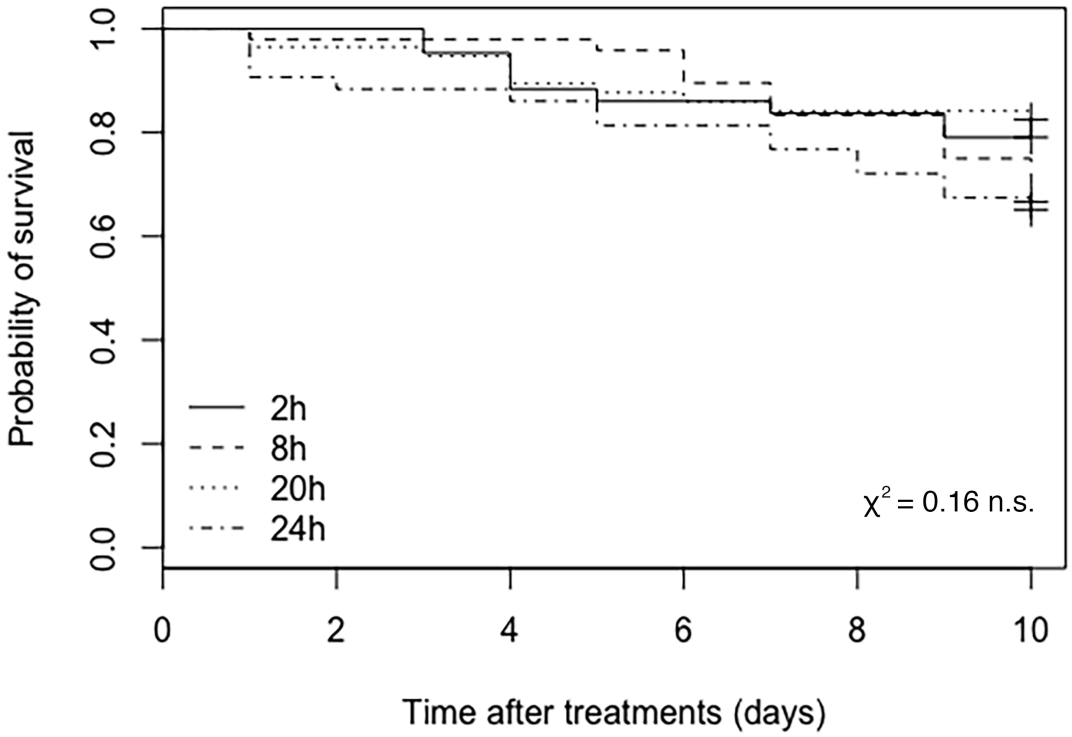

Figure 2-5. Probability of survival of *Neoseiulus cucumeris* over 10 days after exposure to *Beauveria bassiana* conidia in the substrate for 2, 8, 20 and 24 h. Exposure duration had no significant effect on *N. cucumeris* survival, n.s. = not significant ($p > 0.05$) (Cox proportional hazards mixed effect model).

Chapter 3: Walking is the primary behavioral mechanism for predatory mites to dislodge fungal conidia from their bodies

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Manuscript in preparation.

3.1 Abstract

Beneficial arthropods can be used to deliver fungal conidia to pest populations for biological control programs. A method was developed for predatory mites to acquire conidia of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) from their commercial rearing substrate before they are released into the crop. Under laboratory conditions, conidium-unloading patterns of predatory mites were characterized by (i) linking the number of conidia remaining on the mite to the time spent grooming and walking and (ii) observing their behavior when loaded or not with conidia using video recordings. Three predatory mite species were tested: *Neoseiulus cucumeris* (= *Amblyseius cucumeris* Oudemans) (Acari: Phytoseiidae), *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) and *Stratiolaelaps scimitus* (= *Hypoaspis miles* Berlese) (Acari: Laelapidae). Walking rather than grooming was the most important factor for predatory mites to dislodge conidia in our experimental arena. When bearing conidia, all three species of predatory mites spent more time walking. The duration of grooming behavior was not affected by conidia for *A. swirskii* or *N. cucumeris*, and was even reduced for *S. scimitus*. Using low-temperature scan electronic microscopy, it was confirmed that grooming was only successful to clean spores off the lateral surfaces where legs can reach. A prolonged period of walking could increase *B. bassiana* dispersal by predatory mites and thus favor disease transmission in prey populations, which would be of benefit for biological control measures.

Key words: mite-microbial interaction, entomopathogenic fungi, walking, grooming, fungal dispersal, low-temperature scanning electron microscopy, biocontrol

3.2 Introduction

The accumulation of particles on the body surface of small organisms like arthropods can impair their movement due to overwhelming weight (Amador and Hu 2015). For example, water droplets attached to the body surface of a mosquito can weigh up to 80 times of its body mass and render flight impossible (Amador and Hu 2015). The consequences can be worse when infective units of entomopathogens, i.e. conidia, attach to the arthropod cuticle. Animals therefore respond to the presence of foreign elements on their exoskeleton with various grooming behaviors such as scratching, licking, preening and rubbing against a surface to remove particles and parasites (Amador and Hu 2015; Hart and Hart 2018; Spruijt et al. 1992; Zhukovskaya et al. 2013). Grooming in arthropods has mainly been described as the use of legs to clean parts of the body while remaining stationery (Takano-Lee and Hoddle 2002). Ants exposed to entomopathogenic fungal conidia increase the duration and frequency of self-grooming and allo-grooming to increase individual survival (Morelos-Juárez et al. 2010; Okuno et al. 2012; Reber et al. 2011) and to slow down disease transmission through the colony (Hart and Hart 2018; Zhukovskaya et al. 2013). Self-grooming for mites has been frequently observed and assumed to contribute to the removal of entomopathogenic conidia. For example, *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) exposed to leaves treated with the entomopathogenic fungus *B. bassiana* spent more time grooming than conspecifics foraging on non-contaminated leaves (Wu et al. 2018). A similar pattern was observed for *Phytoseiulus longipes* Evans (Arcari: Phytoseiidae) when exposed to leaves bearing capilliconidia of the entomopathogenic fungus *Neozygites floridana* (Weiser and Muma) Remaud and Kellar (Zygomycetes: Neozygitaceae) capilliconidia (Wekesa et al. 2007).

We investigated the capacity of predatory mites to deliver fungal conidia to pest populations, thus spreading the disease and thereby improving biological control by combining both predation and infection. The dispersal of fungal conidia is relatively passive (Magyar et al. 2016) while the movement of predatory mites is active and purposeful (Sabelis et al. 1984). Our study involves three species of predatory mites: *A. swirskii*, *N. cucumeris* and *S. scimitus*. These species have been commercialized and

successfully released on vegetable and ornamental crops to control insect pests, including the western flower thrips *F. occidentalis* (McMurtry et al. 2013; Okuno et al. 2012). Therefore, the movement paths of the predatory mites should overlap to some extent with the movement paths or location of the thrips. Previously we developed a method for these predatory mites to collect and transport *B. bassiana* conidia directly from the mite rearing substrates (Lin et al. 2017). The capacity of predatory mites as dispersal agents to influence prey infection rates would depend on their behavior: how and when they dislodge conidia and whether foraging is affected when bearing conidia. For instance, the distribution pattern of conidia dispersal will vary depending on the predatory mites dislodging conidia by grooming or by walking, thus spreading conidia along the path of the predatory mites. Furthermore, any change in predatory mite behavior induced by conidia could affect their navigation and motion capacity, thereby changing their movement paths (Nathan et al. 2008).

In this study, behaviors of the predatory mites were investigated to determine how conidium dispersal occurs by predatory mites. Under laboratory conditions, we 1) categorized behavioral observations that may link to conidium dislodgement with video recordings, ie. grooming and walking; 2) linked conidia dislodgement to behaviors of predatory mites by counting the number of conidia remaining on a predatory mite prior to and after several periods grooming and walking on an arena; 3) determined if grooming was an efficient way to dislodge conidia by visualizing the presence and absence of conidia where predatory mites scrubbed their bodies using a low temperature scan electron microscope (LT-SEM); 4) examined changes in predatory mite behavior over 60 min when bearing conidia.

3.3 Materials and Methods

3.3.1 Arthropod colonies and fungal inoculum

Amblyseius swirskii, purchased from BioBest Canada (Guelph, Ontario, Canada), were reared on a diet containing *Carpoglyphus lactis* Linnaeus (Acari: Carpoglyphidae) and cherry pollen (Firman Pollen Co., Yakima, WA). *Neoseiulus cucumeris*, provided by Anatis Bioprotection Inc. (Saint-Jacques-le-Mineur, Québec, Canada), was maintained on a factitious prey *Aleuroglyphus ovatus* Toupeau (Acari: Acaridae). *Stratiolaelaps scimitus*, purchased from Applied Bionomics Ltd. (Victoria, British Columbia, Canada), was reared on a mixture of wheat bran, vermiculite and sphagnum moss (2:2:1) (Freire and de Moraes 2007) and fed with the factitious prey *A. ovatus* (Lin et al. 2017). The predatory mites used for LT-SEM observations were purchased in USA, from IPM Laboratories Inc. (Locke, New York, United States). All three species were reared at 25°C, 60-70% humidity and under 14L:10D light cycle. Only adult females were used in the experiments.

Beauveria bassiana is a generalist entomopathogenic fungus that exploits over 700 species from most insect orders, with some isolates showing a high degree of specificity (Rohrlich et al. 2018). *Beauveria bassiana* strain ANT-03 is registered in North America for greenhouse biocontrol of thrips, aphids and whiteflies. We used the technical grade powder produced at Anatis Bioprotection Inc., which contains $5x10^{10}$ conidia per gram for all experiments.

3.3.2 Loading predatory mites with B. bassiana *conidia*

Adult female predatory mites were exposed to *B. bassiana* conidia in the commercial rearing substrates ($2.5x10^{9}$ conidia g^{-1} substrate) in a modified Eppendorf tube for 20 hours to ensure coverage of *B. bassiana* conidia on their body surface (Lin et al. 2017). Each tube contained 25 mites and cherry pollen was supplied as food (0.05 g^{-1} substrate) (Lin et al. 2017).

3.3.3 Linking behavior to conidium removal

Predatory mites were extracted from *B. bassiana*-contaminated substrate with a modified Berlese funnel (Lin et al. 2017) onto a plastic sheet spread on wet sponges in plastic trays containing water. The mites were transferred individually to experimental arenas and filmed using a Dino-lite digital microscope (Model AM-4012NZT, Torrance, CA) (Fig. 3-1). The experimental arenas were conceived by F. Longpré, London Research and Development Center, Agriculture and AgriFood Canada, (London, Ontario, Canada) and made using a 3D printer. They were designed to accommodate both the size of the mites and the limited depth of field of the camera. The behavior of the phytoseiid mites, *A. swirskii* and *N. cucumeris,* was observed on arenas of 1.8 mm in length x 1.3 mm in width x 0.8 mm in height. The arena size for *S. scimitus*, which is 4 times larger than the two other mites (from top perspective view), was 4 mm in length x 3 mm in width x 1 mm in height. Lightning was provided by the built-in LED light of the Dino-lite digital microscope. We characterized predatory mite behavior as walking, grooming and resting. Walking is defined as locomotion, often accompanied by waving the first pair of legs (leg 1, nearest to the gnathosoma) in the air (Fig. 3-2). Resting is defined as standing motionless. Grooming is defined as scrubbing between legs, between legs and idiosoma, and between legs and palps (Fig. 3-2).

Mites were washed immediately after released from the substrate to estimate the initial conidia load or filmed for 10, 15, 20, 40 or 60 min and washed immediately after filming to assess the number of remaining conidia on a mite. For each mite species, 9-13 individuals used per block were observed per block and the experiment was repeated in 3 temporal blocks for *A. swirskii* and *S. scimitus* and 4 blocks for *N. cucumeris*. The mites were picked up with fine brushes and placed inside a 1.5-mL Eppendorf tube containing 0.5 ml of 0.05% Tween 80 solution. Each tube was stirred twice for 30 s at 2000 rpm (Dromph 2001), which allowed the mites to remain intact while most of the conidia on their cuticles were washed into the suspension. The suspension (0.1 ml) was plated onto a medium selective for *B. bassiana*, containing oatmeal agar medium (Difco, Detroit, MI) amended with 0.55% Dodine, 0.005% chlortetracycline and 0.01% crystal violet in Petri dishes (Chase et al. 1986). The plates were kept in the dark at 25°C for 8 d, after which

colony-forming units (CFU) were counted and recorded (Al-Mazra'Awi et al. 2007). Discernment of the relative contribution of grooming and walking to conidium numbers on the mites was determined by studying videos of each mite and the calculation of the duration of grooming and walking was ascertained using the behavior observation research interactive software (BORIS) (Friard and Gamba 2016). Relationships were established between the number of conidia remaining on a mite and the duration of walking or grooming.

3.3.4 Observation of conidium distribution on predatory mites using LT-SEM

Visualization of whether conidia were removed by predatory mites grooming their bodies was realized by examining the conidium distribution pattern on the predatory mite with the use of a LT-SEM. Predatory mites were exposed to *B. bassiana* in their rearing substrates for 20 hours and subsequently released on plastic sheets spread on wet sponges in plastic trays containing water. Cherry pollen was supplied as food. Mites were randomly sampled at time 0, 30 and 60 min. Two to four specimens were observed using the LT-SEM as described in Bolton et al. (2014). Briefly, live predatory mites were secured to 15 mm x 30 mm copper plates using ultra smooth, round (12 mm diameter), carbon adhesive tabs (Electron Microscopy Sciences, Inc., Hatfield, PA, USA). The specimens were frozen conductively in a Styrofoam box, by placing the plates on the surface of a pre-cooled (-196 °C) brass bar whose lower half was submerged in liquid nitrogen (LN_2). After 20-30 s, the holders containing the frozen samples were transferred to a Quorum PP2000 cryo-prep chamber (Quorum Technologies, East Sussex, UK) attached to an S-4700 field emission scanning electron microscope (Hitachi High Technologies America, Inc., Dallas, TX, USA). The specimens were etched inside the cryo-transfer system to remove any surface contamination (condensed water vapor) by raising the temperature of the chamber to -90 °C for 10-15 min. Following etching, the temperature inside the chamber was lowered to below -130 °C, and the specimens were coated with a 10 nm layer of platinum using a magnetron sputter head equipped with a platinum target. The specimens were transferred to a pre-cooled (-130 °C) cryostage in the SEM for observation. An accelerating voltage of 5kV was used to view the specimens. Images were captured using a 4pi Analysis System (Durham, NC). Individual

images were re-sized and placed together to produce a single figure using Adobe® Photoshop CS 5.0 (Friard and Gamba 2016).

3.3.5 Comparing behavior of predatory mites with and without spores

Predatory mites exposed or not exposed to *B. bassiana* were released from the rearing substrates and encaged individually in the arena and filmed using a Dino-lite digital microscope for 60 min (Fig. 3-1). The proportions of time spent grooming, walking and resting were calculated every 5 min and compared between treatments. Observations were made of 3-5 individuals per treatment in each block and the experiment was repeated three times (3 temporal blocks) for a total of 9-15 individuals per treatment.

3.3.6 Statistical analyses

In order to investigate the relationship between behaviors and the possible dislodgement of conidia, the numbers of conidia remaining on mites was analyzed with the grooming duration, the walking duration and the resting duration as independent continuous variables with mixed-effect generalized linear model with negative binomial distribution. Multicollinearity was tested by analyzing one behavior duration with another behavior duration as the independent variable with generalized linear mixed-effect model. When multi-collinearity occurred between two behaviors (for example, resting and grooming), they were not included in the same model. To optimize the model, non-significant factors were removed. The model generated an equation providing the predicted number of conidia remaining on a mite across the entire range of grooming duration, walking duration and/or resting duration. Determination of the effect of exposure to *B. bassiana* on predatory mite behaviors over 60 min was obtained by analyzing the duration of each behavior using a generalized linear mixed-effect model with exposure to *B. bassiana* as a categorical factor and time after released from the rearing substrate as a continuous factor. Kruskal-Wallis tests were used when residuals were not normally distributed, following a Normal QQ-plot test (Kozak and Piepho 2018). Non-significant factors were removed to optimize the model following a log-likelihood ratio test. All statistical analyses were carried out with R version 1.0.143 (R 2013).

3.4 Results

3.4.1 A detailed description of grooming behavior

The grooming behavior of all three predatory mite species was similar. The four pairs of legs and mouthpart, including chelicerae and palps, were involved in grooming. Adjacent legs of a given body side (either left or right) can scrub each other from proximal to distal direction. The second pair of legs (leg 2) can scrub legs 1, 3 and mouthparts laterally. When leg 2 scrubbed leg 1, it was mostly accompanied with 'head ducking' behavior: mouthparts of the mite bending downwards to avoid being hit. Leg 1 was scrubbed between two palps. Multiple scrubbings can occur simultaneously, for example, left legs 2 and leg 3 scrubbing each other while right leg 1 scrubbing between two palps. The femur of legs 3 and 4 can scrub the lateral surface of the body when legs were bended. The tibia and tarsi of leg 4 can scrub the body, specifically the posterior lateral and ventral opisthosoma, but leg 1, 2 and 3 were not able to reach the posterior end of opisthosoma. Leg 4 can scrub from the lateral to the ventral surface, never the opposite direction.

There were small variations in grooming behaviour among the three predatory mite species. For *N. cucumeris* and *S. scimitus*, both the left and right leg 2 can simultaneously scrub ventral gnathosoma in an alternating manner, whereas *A. swirskii* used one leg 2 at a time to scrub the ventral gnathosoma. *Neoseiulus cucumeris* and *S. scimitus* used both the right and left leg 4 to scrub posterior lateral and ventral idiosoma but cannot reach the dorsal shield. *Amblyseius swirskii* can stretch the furthest and reach the Z4 and Z5 setae located along the edge of posterior dorsal shield, possibly due to its longest leg-to-opisthosoma ratio. *Stratiolaelaps scimitus*, displayed a distinct feature in which their chelicera can extend far from the base of the gnathosoma, for approximately 0.45 of body lengths, thus as the chelicera extended it was scrubbed by the palps extensively, followed by both the right and left leg 1 or leg 2.

3.4.2 Behaviors contributing to removal of conidia from mite bodies

There was multicollinearity between grooming and resting durations in *A. swirskii*

(χ^2=5.36, df=1, p=0.021). In addition, the time spent resting did not influence the number of conidia remaining on *A. swirskii* (χ^2=0.23, df=1, p=0.629); it was therefore excluded from the model. Grooming and walking durations were significant predictors of the number of conidia remaining on *A. swirskii* (grooming: χ^2=10.37, df=1, p=0.001; walking: χ^2=5.28, df=1, p=0.022). There was a significant interaction between grooming and walking durations (χ^2=13.60, df=1, p<0.001). In simplified models where either grooming or waking was analyzed as the only variable, walking was a significant predictor of the number of conidia remaining on *A. swirskii* (χ^2=9.74, df=1, p=0.002), but grooming was not (χ^2=1.29, df=1, p=0.257), implying walking is the most important behavioral mechanism for dislodging conidia. To understand the interaction term, the initial mite cohort was subsampled across the entire range of grooming duration. In the subsamples where grooming lasted between 74 to 1527 seconds, grooming was a significant predictor of the number of conidia remaining on *A. swirskii*. When grooming was less than 74 seconds, there were not enough data points to construct the model. When the subsamples included individuals that spent more than 1527 seconds grooming, grooming became an insignificant predictor, because the mites that spent excessive time grooming were not likely to remove additional conidia and they are likely to devote less time walking.

There was multicollinearity between the time spent grooming and resting in *N. cucumeris* (χ^2=4.79, df=1, p=0.029). In addition, resting duration was not a significant main effect on *N. cucumeris* remaining conidia (χ^2=0.13, df=1, p=0.715). Therefore, resting duration was excluded from the model. Walking duration was a significant predictor of the number of conidia remaining on *N. cucumeris* (χ^2=5.08, df=1, p=0.024), but grooming was not (χ^2=1.39, df=1, p=0.238) nor the interaction (χ^2=1.51, df=1, p=0.219). The model was then simplified with walking duration as the only independent variable and walking significantly reduced the number of conidia remaining on *N. cucumeris* (χ^2=4.17, df=1, p=0.041).

There was multicollinearity between grooming and walking durations (χ^2=18.32, df=1, p<0.001) and between grooming and resting durations in *S. scimitus* (χ^2=6.12, df=1, p=0.013), therefore, the number of remaining conidia was analyzed with walking,

grooming or resting duration, each as an independent variable. Grooming duration (χ^2=14.66, df=1, p<0.001, Fig. 3-3), walking duration (χ^2=17.65, df=1, p<0.001) and resting duration (χ^2=7.85, df=1, p=0.005) significantly reduced the number of conidia remaining on *S. scimitus*.

3.4.3 Predatory mite behavior with and without conidia

Three behaviors that could be important to determine a predatory mite's capacity as a dispersal agent of fungal conidia were quantified over 60 min: grooming, walking and resting. For *A. swirskii*, exposure to *B. bassiana* in their rearing substrate did not affect the time spent grooming (Fig. 3-4), but significantly increased time spent walking by 11.05±2.66% (χ^2=17.26, df=1, p<0.001) and reduced time spent resting by 9.24±3.60% (χ^2=6.61, df=1, p=0.010). The length of time following the release from the substrate affected the proportion of time *A. swirskii* spent grooming and resting. Grooming was reduced (χ^2=73.47, df=1, p<0.001), resting was increased (χ^2=68.27, df=1, p<0.001) and walking remained the same over the course of 60 min.

For *N. cucumeris*, exposure to *B. bassiana* in their rearing substrate did not affect the time spent grooming (Fig. 3-5), but significantly reduced resting time by 12.214±4.481% (χ^2=7.43, df=1, p=0.006). For walking time, there was a significant interaction between treatment and time (χ^2=12.17, df=2, p=0.002) only for the first 25 min; exposure to *B. bassiana* significantly increased *N. cucumeris* walking time. The length of time since *N. cucumeris* had been released from the substrate reduced grooming (χ^2=80.43, df=1, p<0.001), increased resting (χ^2=22.80, df=1, p<0.001) while walking remained constant over the course of 60 min.

For *S. scimitus*, upon exposure to *B. bassiana* in their rearing substrate, they reduced time spent grooming by 2.994±1.393% (χ^2=4.62, df=1, p=0.032, Fig. 3-6), increased walking time by 3.18±1.471% (χ^2=4.665, df=1, p=0.030), but did not modify resting time. The length of time since *S. scimitus* had been released from the substrate increased the proportion of time they spent resting (χ^2=5.561, df=1, p=0.018) over the course of 60 min, but not grooming or walking.

3.5 Discussion

Our results demonstrated that walking is the primary behavioral mechanism for all three species of predatory mites to dislodge conidia. Grooming helps remove conidia, but it's insufficient. Three predatory mite species showed slightly different behavioral patterns in dislodging conidia. Grooming contributed to dislodge conidia from the body of *A. swirskii* and *S. scimitus*. As revealed by LT-SEM images, the lateral surface and the legs of the mites became relatively clean 30-60 min following release from the substrates (Fig. 3-8 B, 3-8F). However, grooming was insufficient to completely clean predatory mites, with large numbers of conidia still remaining on the dorsum (Figs. 3-8B, 3-8D, 3-8F). For *A. swirskii*, more than 25 min of grooming is necessary to clean the lateral surface and the legs. These mites spending excessive time grooming were likely to devote less time walking; therefore conidia were not efficiently dislodged. For *N. cucumeris*, grooming mostly led to condium translocation from the lateral surface to the legs (Fig. 3-8D). Walking is further required to dislodge these conidia.

Walking could create mechanical disturbance and air movement at the mite body surface that favor the detachment of conidia. Fungal spores can secrete mucilage that further secures the attachment to the host and the adhesion force between a conidium and the arthropod cuticle varies among species (Askary et al. 1999; Qu et al. 2017). *Metarhizium anisopliae* conidia attach very well to the host *Tenebrio molitor*, but fail to attach to the host *Aedes aegypti* larvae (Greenfield et al. 2014). In our study, mucilage at the conidium-cuticle interface was not observed using LT-SEM images, as shown by Qu et al. (2017). It might be that *B. bassiana* ANT-03 conidia do not strongly attached to cuticles of *A. swirskii*, *N. cucumeris* or *S. scimitus*. The effect of walking on removing conidia would be larger than grooming when predatory mites walk on leaf surfaces because trichomes and other plant structures create a 'jungle-like' habitat that increases physical contacts with foraging predatory mites (Fig. 3-8A). Hence, leaf surface characteristics also influence the number and distribution of conidia deposited on a plant.

Observational studies showed that predatory mites loaded with fungal conidia modify their behavior, mostly by extending walking periods. Prolonged periods of walking could

increase *B. bassiana* dispersal by predatory mites and thus favor disease transmission in prey populations, thereby benefiting biological control. Since walking, rather than grooming, is the primary behavioral mechanism to dislodge conidia, it is not surprising that when loaded with conidia, *A. swirskii* and *N. cucumeris* did not increase grooming time. As for *S. scimitus*, grooming duration even decreased.

Our results revealed that predatory mites without *B. bassiana* conidia already spend considerable amount of time grooming, with *A. swirskii* and *N. cucumeris* devoting half of their time cleaning themselves upon release from the substrate. Predatory mites use their legs and mouthparts to clean accessible segments of the body while remaining stationary. Grooming helps remove accumulated substrate particles on the mite cuticle that could impair their movement or be a threat to their life, such as entomopathogen infective units (Zhukovskaya et al. 2013, Amador and Hu 2015, Hart and Hart 2018). As shown in other arthropods, grooming also contributes to clean sensory organs. In crustaceans, the cousin taxon of predatory mites, the Caribbean spiny lobster *Panulirus argus* Latreille (Decapoda: Palinuridae) uses mouthpart appendages, the third maxillipeds, to wipe olfactory sensilla on their attenules (Wroblewska et al. 2002). American cockroaches, *Periplaneta Americana* Linnaeus (Blattodea: Blattidae) maintain olfactory sensitivity by regularly grooming their antenna to remove excessive cuticular hydrocarbons and foreign chemicals (Böröczky et al. 2013). Similarly, predatory mites use grooming to clean their sensors to better perceive cues in the environment. In fact, legs and mouthparts of Acari contain multiple sensory organs (sensilla) that function as mechano- and chemoreceptors (De Bruyne et al. 1991; de Lillo et al. 2005). For *A. swirskii*, we observed with LT-SEM that legs frequently interact with mouthparts during grooming bouts and that a liquid substance may be involved (Fig. 3-7). This unknown substance from the mouth could function as a cleaning solution (saliva) to help remove foreign particles or neutralizing pathogen infective units.

The grooming responses of *N. cucumeris*, *A. swirskii* and *S. scimitus* towards fungal conidia differ from those of two other predatory mites, *P. persimilis* and *P. longipes*, which spent more time grooming when foraging on leaves treated with entomopathogenic fungi than when foraging on clean leaves (Wekesa et al. 2007; Wu et al. 2018). Such a

divergent pattern might arise from differences in experimental conditions. The intensity of grooming in the presence of pathogens might depend on the predatory mite species, the identity and virulence of the pathogen, the relative burden of the infective units, the abundance of other foreign particles, the type of experimental arena, including the nature of the substrate, and so forth. The process of loading conidia on predatory mite bodies might also influence the frequency and duration of grooming behavior. In our experiments, *B. bassiana* conidia were loaded on mites by exposing them in a three-dimensional contaminated substrate that results in the entire body being potentially covered by conidia. In contrast, in the studies of Wekesa et al. (2007) and Wu et al. (2018), predatory mites became contaminated with conidia when walking on a leaf. It might therefore be that self-grooming is effective enough to remove relatively small numbers of conidia mostly located on the legs. Additional work is needed to elucidate these differences in grooming patterns.

We also noticed significant reductions of viable conidia on *S. scimitus* even when mites were resting. This was not the case for *A. swirskii* or *N. cucumeris*. Chemical substances and bacteria on *S. scimitus* cuticles could reduce the viability of conidia. As revealed in LT-SEM images, *S. scimitus* cuticles were covered with sticky substance and the embedding conidia appeared deflated (Fig. 3-9D) compared to the conidia sampled in the technical grade powder (Fig. 3-9A), on *A. swirskii* (Fig. 3-9B) or on *N. cucumeris* (Fig. 3-9C). These substances could be cuticular compounds (e.g. lipids, aldehydes, salicylaldehyde, iridoid monoterpene epi-chrysomelidial, free fatty acides) that have been identified from various insect species exhibiting antifungal activities (Ortiz-Urquiza and Keyhani 2013). Bacteria are commonly observed on *S. scimitus* cuticles (Fig. 3-10A), possibly originating from the rearing substrates (Fig. 3-10B), and may have antifungal activities. The mechanism of *B. bassiana* degradation on the surface of *S. scimitus* remains to be investigated. In any case, such degradation is likely to impede *S. scimitus* capacity as an efficient *B. bassiana* dispersal agent.

Understanding predatory mite behaviors and their conidia dislodging mechanisms helps predict their potential as dispersal agents of entomopathogens for biological control purposes (Lin et al. 2017). Conidia are expected to be dispersed along the path of

foraging predatory mites and eventually reach insect prey (pest) colonies. Whether a prolonged period of walking affects their regular foraging behavior and predatory capacity remains to be explored.

3.6 Acknowledgements

We thank Jean-Philippe Parent and Félix Longpré from Agriculture and Agri-Food Canada for fruitful discussion and generous support; Connor Gulbronson and Joseph Mowery from Electron and Confocal Microscope Unit, United States Department of Agriculture, Agricultural Research Service and Gregory Evans for their technical assistance and comments. We thank Arne Janssen for his editorial advice. This study was supported by the Practice Environment Innovation Research Scholarship Program from Natural Sciences and Engineering Research Council of Canada, the Fonds de Recherche Nature et Technologies du Québec and Anatis Bioprotection Inc. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA; USDA is an equal opportunity provider and employer.

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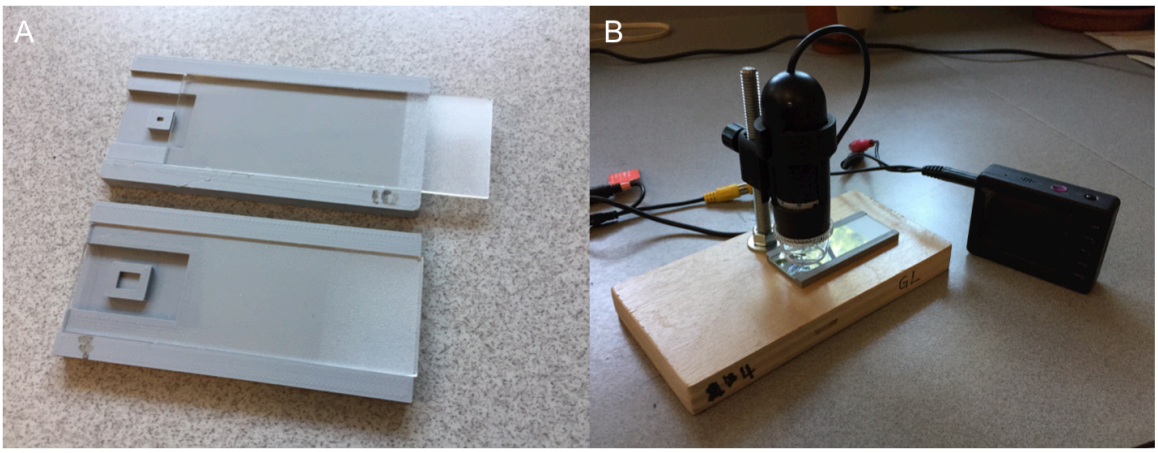

Figure 3-1. Apparatus used for filming predatory mite behavior. (A) Two 3D-printed filming areas, the top one was used for *Amblyseius swirskii* and *Neoseiulus cucumeris* (1.8 mm x 1.3 mm) and the bottom one was used for *Stratiolaelaps scimitus* (4 mm x 3 mm); The tested individual was enclosed in the inner well covered with a glass slide; moat around the well is designed for aeration. (B) An arena placed under a Dino-lite digital microscope camera.

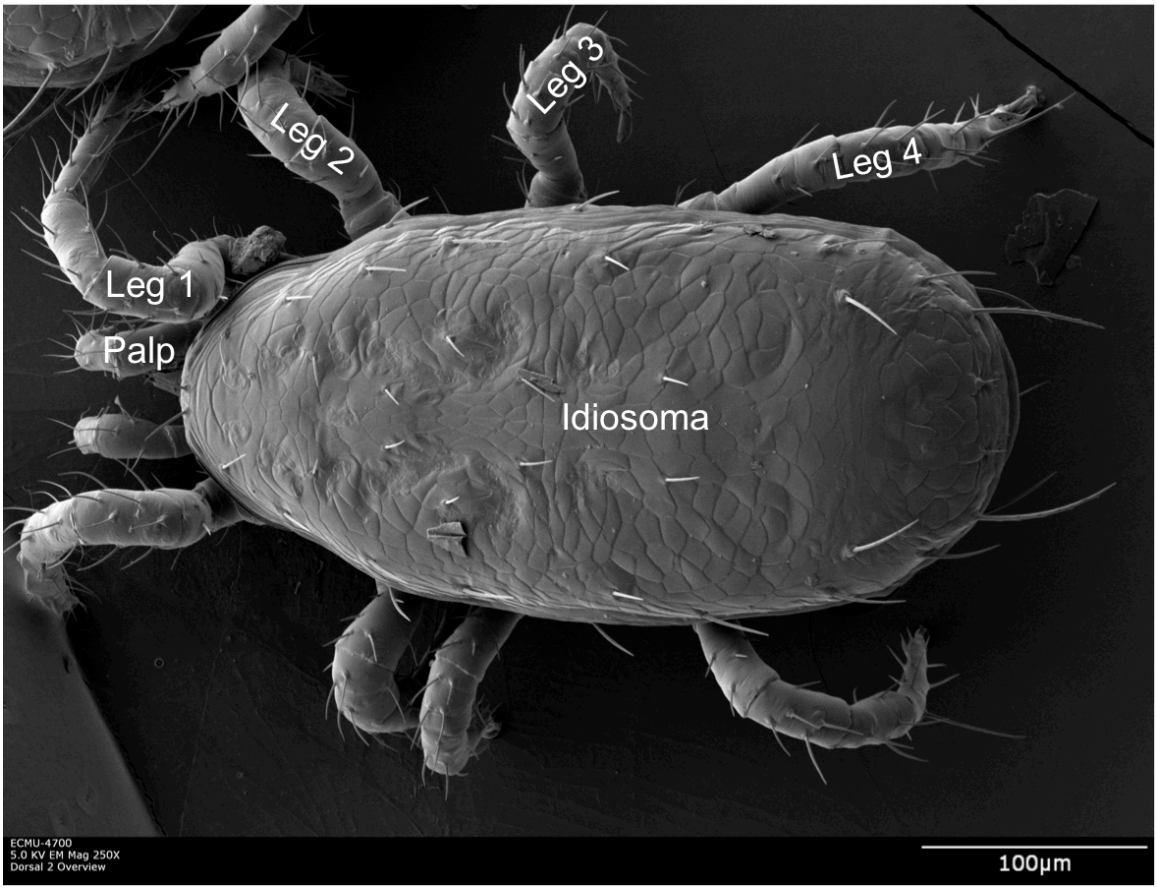

Figure 3-2. *Neoseiulus cucumeris* released from the rearing substrates with no *Beauveria bassiana* conidia. Idiosoma and the right set of palps and legs were indicated in the photo. The specimens were observed using a low temperature scan electron microscope (LT-SEM) as described in Bolton et al. (2014).

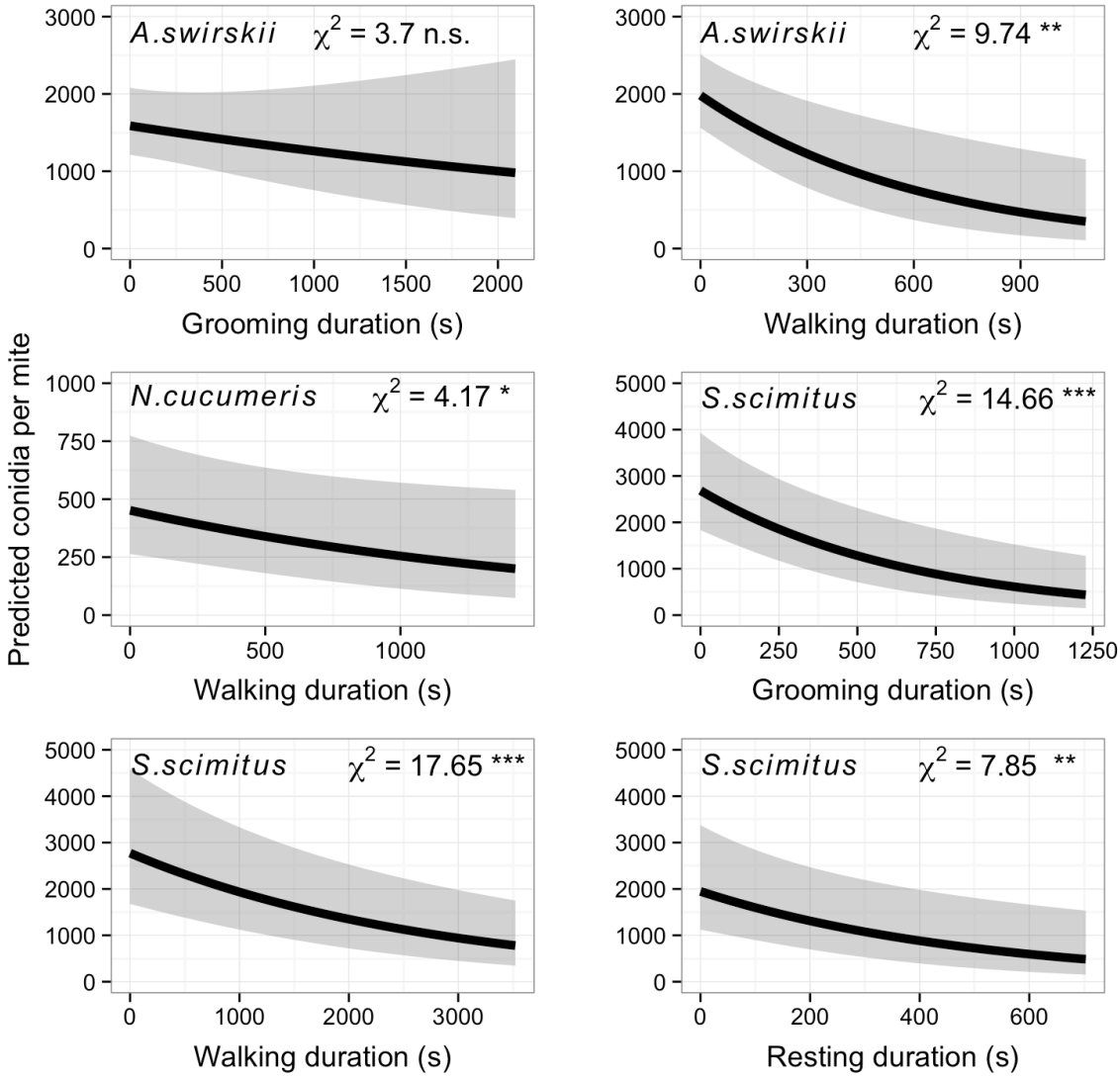

Figure 3-3. Figure 3-3. The predicted number of conidia remaining on the body of *Amblyseius swirskii*, *Neoseiulus cucumeris* and *Stratiolaelaps scimitus* across the range of grooming duration, walking duration or resting duration along with 95% confidence intervals (grey area). Stars indicate significant predictors of number of spores on a mite: n. s. = $p > 0.05$, * = $0.05 < p < 0.01$, ** = $0.001 < p < 0.01$, *** < 0.001; generalized linear mixed-effect model with negative binomial distribution.

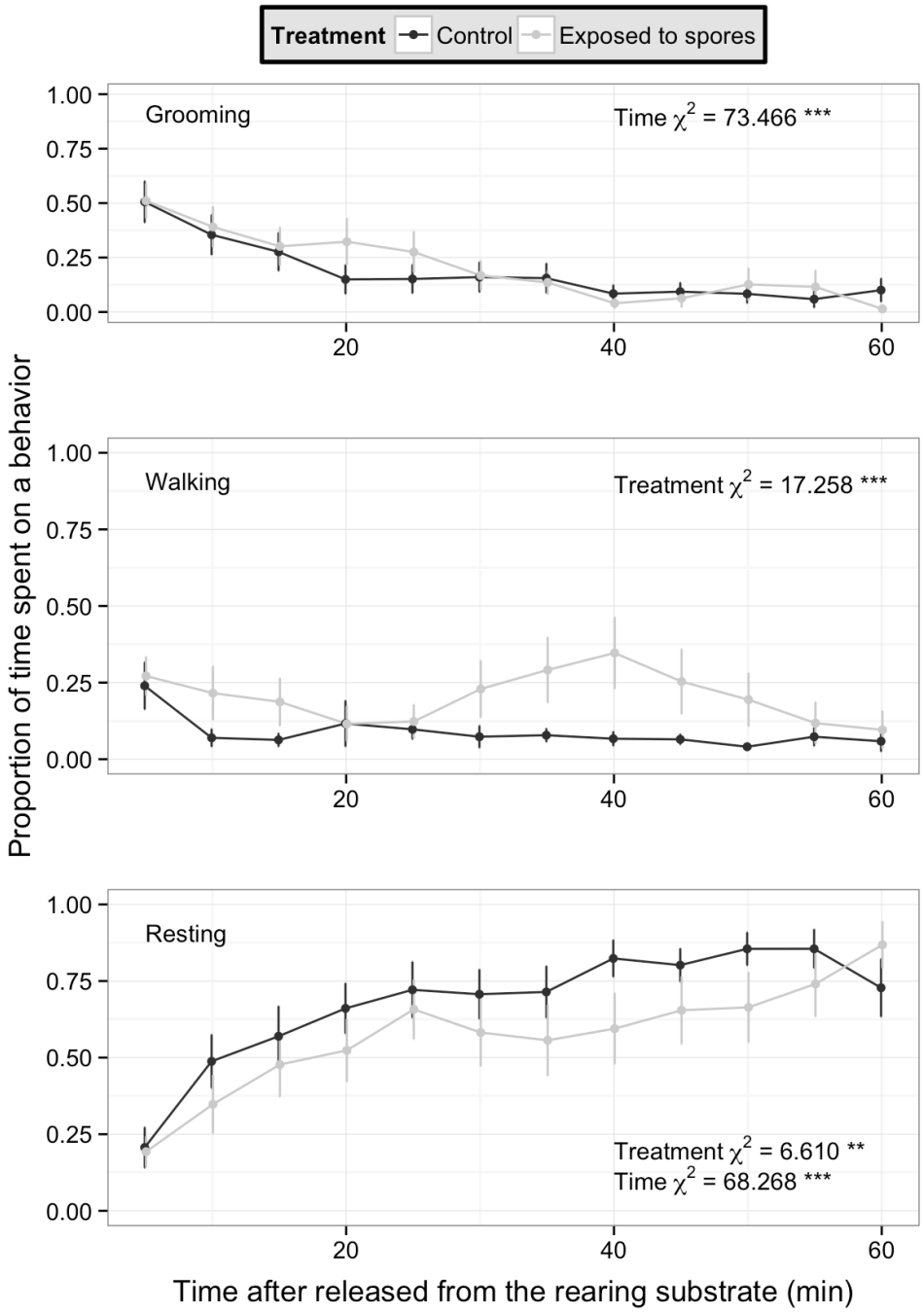

Figure 3-4. Proportion of time (mean ± S.E.) *Amblyseius swirskii* spent grooming (top), walking (middle) and resting (bottom) following release from *Beauveria bassiana* contaminated (grey curve) or non-contaminated (black curve) substrate. Stars indicate significant predictors of the proportion of time *A. swirskii* spent on a behavior: ** = $0.001 < p < 0.01$, *** < 0.001; generalized linear mixed-effects model.

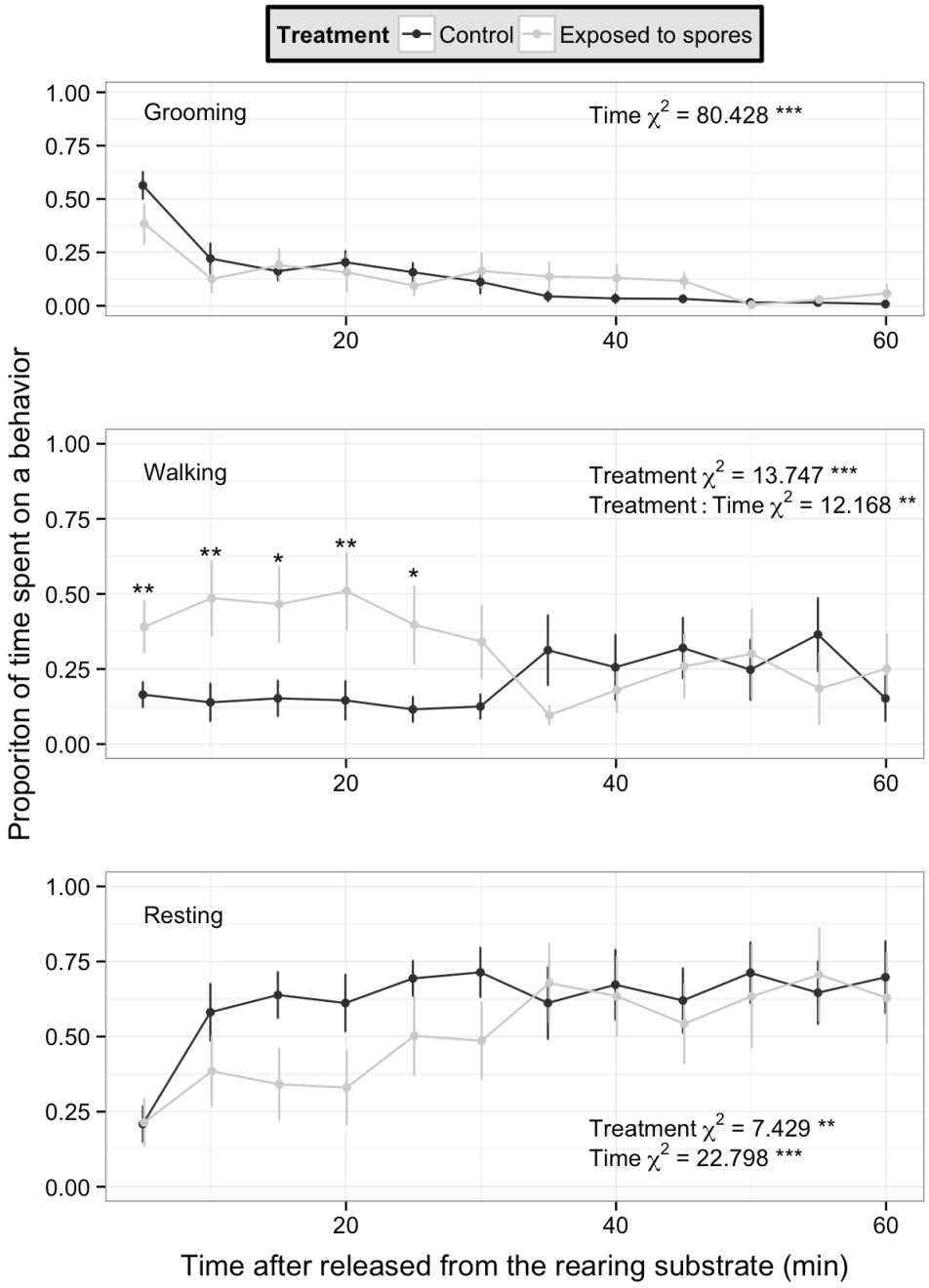

Figure 3-5. Proportion of time (mean ± S.E.) *Neoseiulus cucumeris* spent grooming (top), walking (middle) and resting (bottom) following released from *Beauveria bassiana* contaminated (grey curve) or non-contaminated (black curve) substrate. Stars indicate significant predictors of the proportion of time *N. cucumeris* spent on a behavior: ** = $0.001 < p < 0.01$, *** < 0.001; generalized linear mixed-effects model.

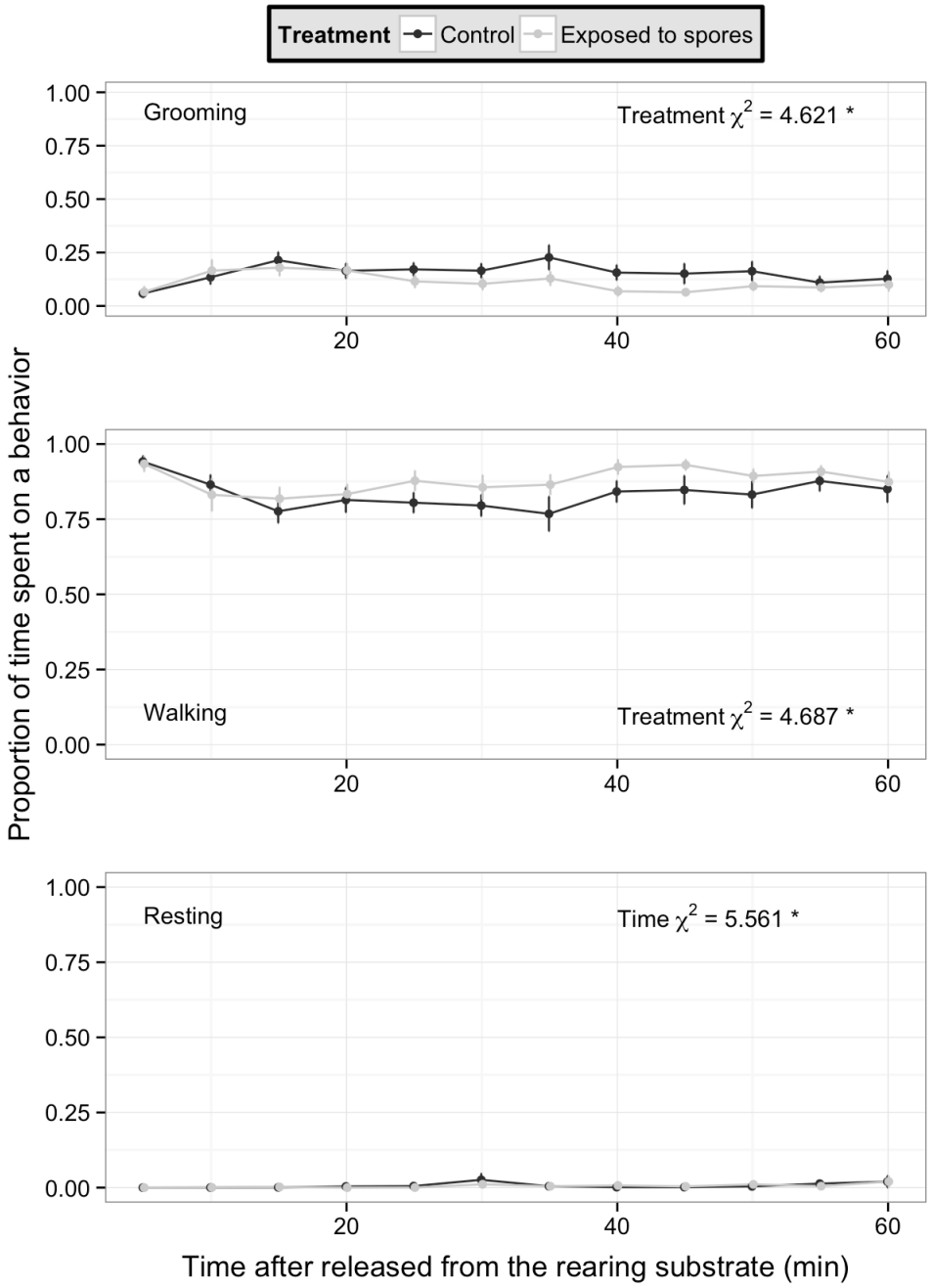

Figure 3-6. Proportion of time (mean ± S.E.) *Stratiolaelaps scimitus* spent grooming (top), walking (middle) and resting (bottom) following released from *Beauveria bassiana* contaminated (grey curve) or non-contaminated (black curve) substrate. Stars indicate significant predictors of the proportion of time *S. scimitus* spent on a behavior: * = $0.01 < p < 0.05$; generalized linear mixed-effects model.

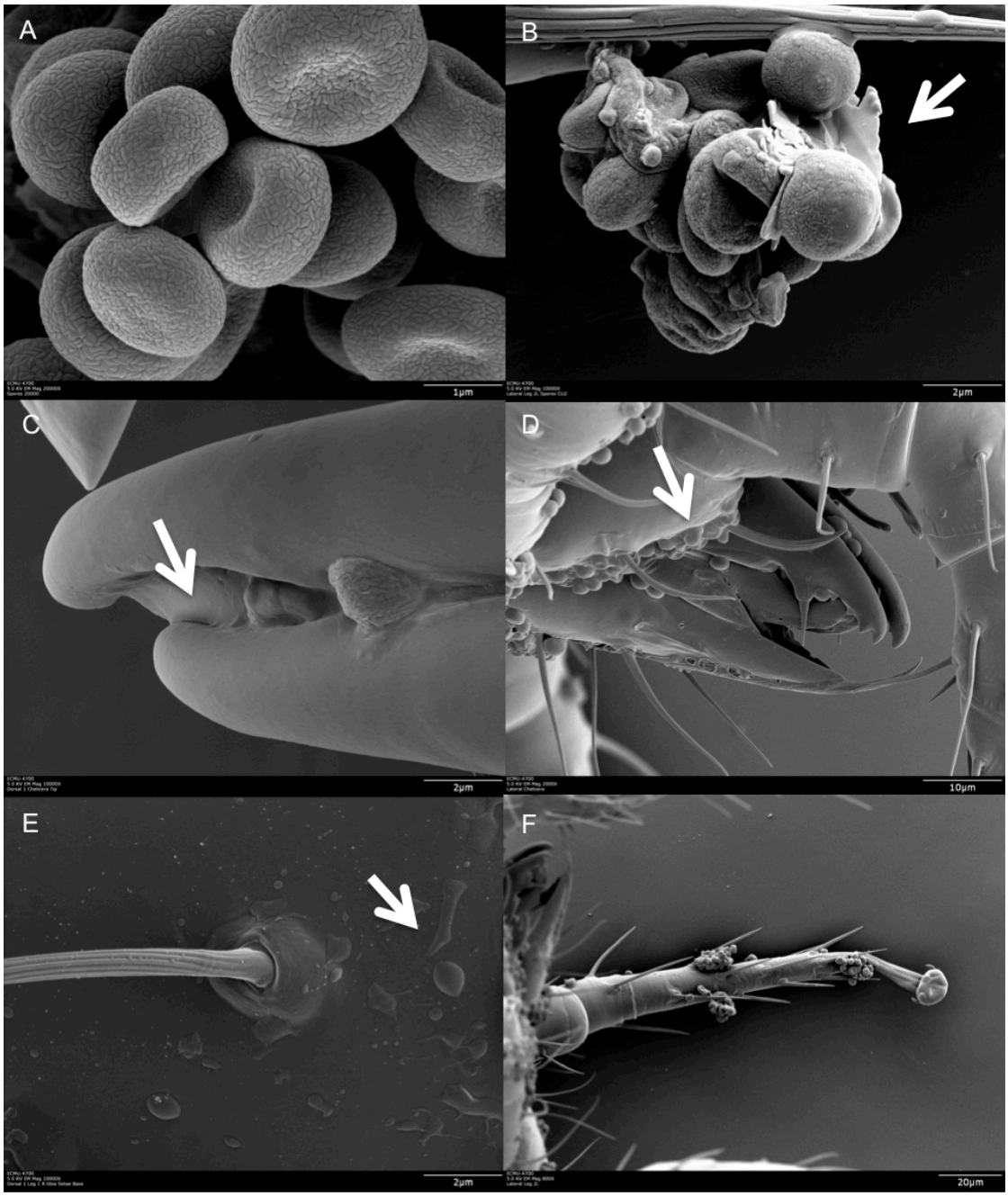

Figure 3-7. Multiple conidia mixed with possibly mite saliva observed on *Amblyseius swirskii* mouth and legs. Arrows are pointed to examples of multiple conidia. (A) *Beauveria bassiana* conidia before being loaded onto *A. swirskii*. (B) Conidia attached to an *A. swirskii* seta on leg 2, covered with multiple conidia. (C) Dorsal view of chelicerae with multiple conidia between chelicerae. (D) Lateral view of chelicerae with conidia covered with multiple conidia on the mouth opening. (E) Conidia near a seta on leg 1. (F)

Conidia in clusters on seta bases of leg 2. The specimens were observed using LT-SEM as described in Bolton et al. (2014).

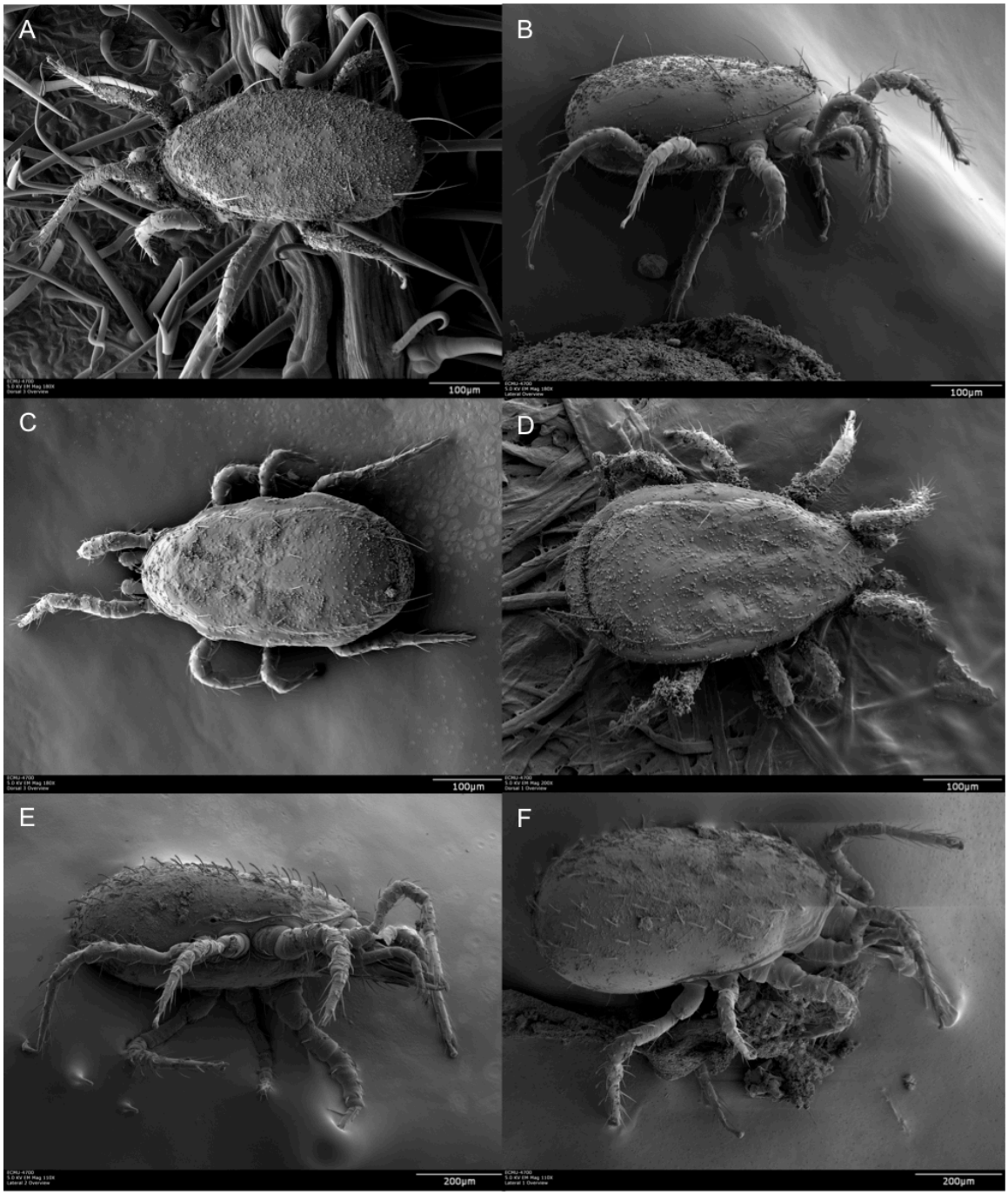

Figure 3-8. Predatory mites bearing conidia of *Beauveria bassiana*. (A) Dorsal view of *Amblyseius swirskii* placed on a bean leaf immediately following released from *B. bassiana* contaminated substrate. The stringy structures are trichomes. (B) Lateral view of *A. swirskii* at 30 min following release from the substrate. (C) Dorsal view of *Neoseiulus cucumeris* immediately following release from *B. bassiana* contaminated

substrate. (D) Dorsal view of *N. cucumeris* at 60 min after it was released from the substrate. (E) Dorsal view of *Stratiolaelaps scimitus* immediately following release from *B. bassiana* contaminated substrate. (F) Dorsal-lateral view of *S. scimitus* at 60 min after it was released from the substrate. The specimens were observed using LT-SEM as described in Bolton et al. (2014).

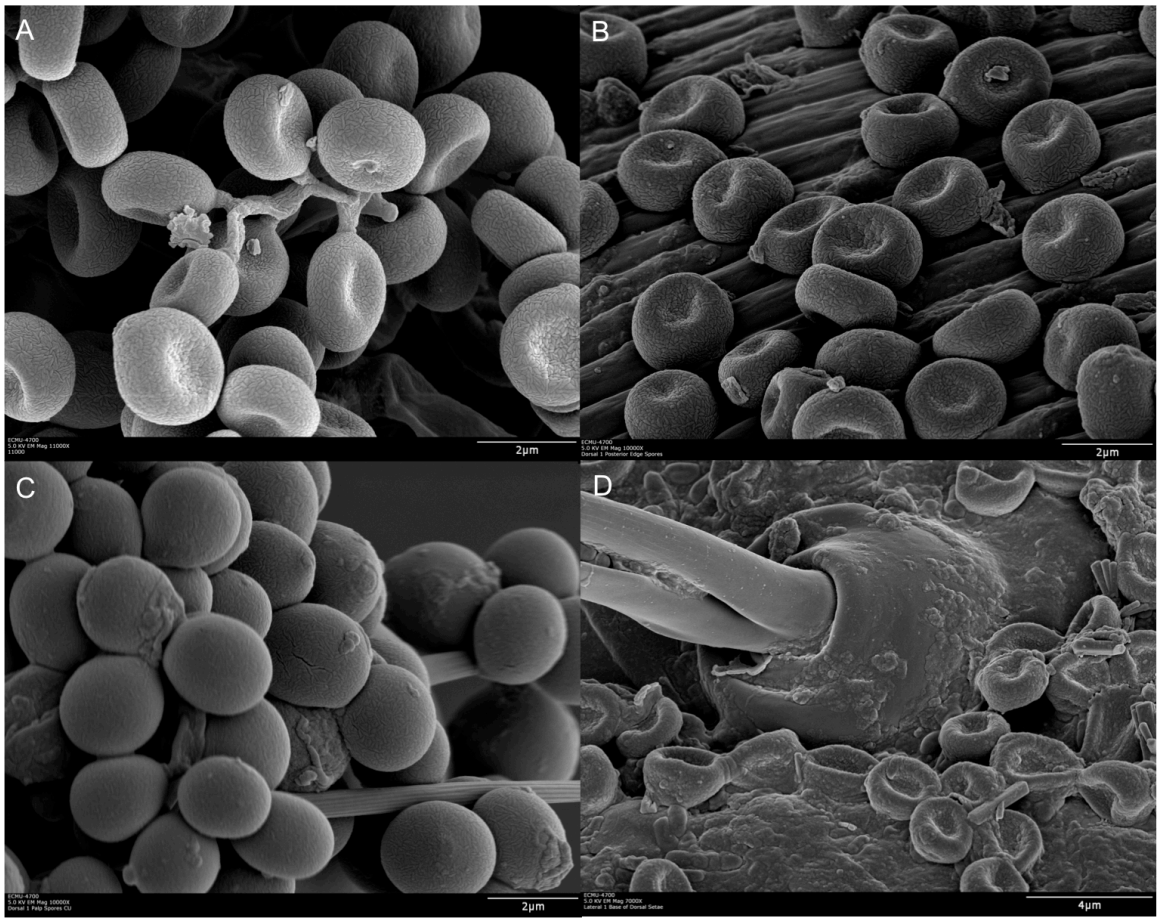

Figure 3-9. Multiple *Beauveria bassiana* conidia. (A) Conidia in technical grade powder. (B) Conidia on *Amblyseius swirskii* cuticle 60 min following release from the substrate. (C) Conidia on *Neoseiulus cucumeris* cuticle 60 min following release from the substrate. (D) Conidia on *Stratiolaelaps scimitus* cuticle 60 min following release from the substrate. The specimens were observed using LT-SEM as described in Bolton et al. (2014).

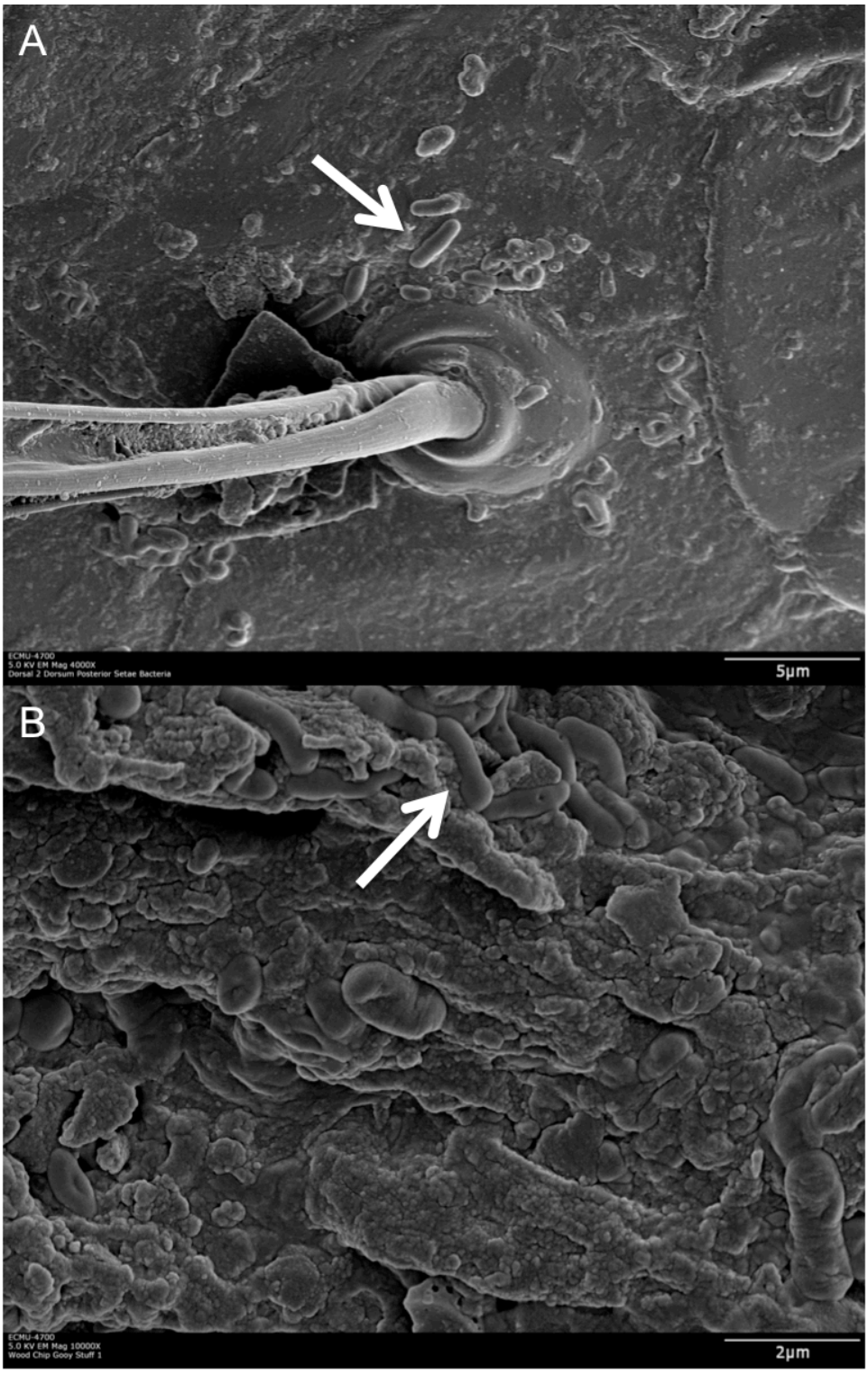

Figure 3-10. Presence of bacteria on (A) *Stratiolaelaps scimitus* cuticle and (B) *S. scimitus* rearing substrate. The specimens were observed using LT-SEM as described in Bolton et al. (2014).

Chapter 4: Phytoseiid predatory mites can disperse entomopathogenic fungi to prey patches

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In review at *Scientific Reports*.

4.1 Abstract

Recent studies have shown that predatory mites used as biocontrol agents can be loaded with entomopathogenic fungal conidia to increase infection rates in pest populations. Under laboratory conditions, we determined the capacity of two phytoseiid mites, *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) and *Neoseiulus cucumeris* (=*Amblyseius cucumeris* Oudemans) (Acari: Phytoseiidae) to deliver the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) to their prey, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). Predatory mites were loaded with conidia and released on plants that had been previously infested with first instar prey clustered on a bean leaflet. We examined each plant section to characterize the spatial distribution of each interacting organism. Our results showed that *A. swirskii* delivered high numbers of conidia to thrips infested leaves, thereby increasing the proportion of thrips that came into contact with the fungus. The effect was larger when thrips infestation occurred on young leaves than on old leaves. *Neoseiulus cucumeris* delivered less conidia to the thrips infested leaves. These patterns result from differences in foraging activity between predatory mite species. *Amblyseius swirskii* stayed longer on plants and had a higher predation rate than *N. cucumeris*. Our study suggests that loading certain predatory mite species with fungal conidia can increase their capacity to suppress thrips populations by combining predation and dispersing pathogens.

Key words: Disease transmission, spatial co-occurrence, dispersal agents, biological control, *Amblyseius swirskii*, *Neoseiulus cucumeris*, *Frankliniella occidentalis*, *Beauveria bassiana*

4.2 Introduction

Pathogens have evolved several ways to disperse and increase the probability of encountering their host. A pathogen can be transferred directly from an infected individual to an uninfected individual, indirectly when the host encounters the free-living infectious stage of the pathogen in the environment, or via a vector [1,2]. The rate of disease transmission within a host population is strongly influenced by the spatial distribution, temporal activity pattern and foraging behaviour of interacting species (i.e. pathogens, uninfected hosts, infected hosts, vectors) [3-5].

A growing number of studies have shown that arthropods can act as dispersal agents and transmit pathogens passively to potential hosts without becoming themselves infected [6-9]. For example, in the soil environment, collembolans can facilitate fungal dispersion by carrying conidia attached to their bodies or located in their guts [10,11]. In honeybees, phoretic Varroa mites have been identified as common vectors of viruses and fungi contributing to mortality and colony collapse [12,13]. Arthropod vectors therefore have the potential to shape direct and indirect interactions between a microorganism and its host and consequently influence their population dynamics, as well as the structure and stability of communities [8]. Although such interactions should be common in nature, the role of arthropod dispersal agents in pathogen epidemiology remains poorly understood.

From an applied perspective, insect pollinators and arthropod biological control agents can be used for dispersing pathogens to agricultural pests [14], weeds [15] and antagonists to plant diseases [16]. For example, in addition to pollinating greenhouse tomato and sweet pepper, bumble bees have the capacity to co-disseminate two fungi *B. bassiana* and *Clonostachys rosea* (Link) Schroers, Samuels, Seifert, and Gams (Ascomycota: Hypocreales) for control of insect pests (greenhouse whitefly and tarnished plant bug) and grey mould, respectively [17]. Similarly, some species of commercially mass-produced predatory mites have shown potential for dispersing entomopathogenic fungi to insect pests. Under laboratory conditions, two phytoseiid species, *N. cucumeris* and *A. swirskii* facilitated the dissemination of *B. bassiana* conidia to their prey, the Asian citrus psyllid *Diaphorina citri* Kuwayama (Homoptera: Psyllidae), a major pest of citrus [18]. Such

findings stimulated research on techniques to load arthropod dispersal agents with optimal doses of infective fungal conidia before releasing them in the crop where they can disseminate the pathogen to the targeted pests [19-21].

While the role of host and non-host arthropods in facilitating entomopathogenic fungi dispersal in the environment has been identified [1,14], the underlying ecological and behavioural mechanisms still need to be examined. Arthropods can mediate the rate at which a disease is horizontally transmitted to susceptible hosts through either direct physical contact (e.g. during a predation or a parasitism attempt by natural enemies) or indirectly by releasing infective propagules (fungal spores) in the habitat. In such cases, when there is a close association between the dispersal agent and the host susceptible to the pathogen, the encounter between interacting species is not a random event. For example, the capacity of a predator to disperse fungal conidia to its prey will primarily depend on how conidia are dislodged from the cuticle (either by grooming or walking) and its foraging behaviour (e.g. habitat location, area-restricted searching behaviour, numerical response) that contribute to increasing the spatial co-occurrence with the prey [22].

This study aimed to investigate the capacity of two species of predatory mites commonly used as biological control agents in dispersing conidia of an entomopathogenic fungus to their prey. We predict that foraging predatory mites artificially loaded with conidia will move close to their prey, thereby increasing the spatial co-occurrence between the fungus and the prey and, predictably, the fungal infection rate. Under laboratory conditions, we examined the (i) spatial distribution of conidia on plant parts when unloaded from predatory mite bodies, as well as their co-occurrence with prey, (ii) predation rates and (iii) proportion of prey bearing conidia on their body. These data provide valuable insights into mechanisms involved in dispersing fungal conidia when transported by an arthropod predator that is not harmed by the fungi. They also inform the biological control community of researchers and practitioners about the potential of predators to induce fungal epizootics in pest populations.

4.3 Methods

4.3.1 The study system

The biological system under study consisted of the entomopathogenic fungus *B. bassiana*, two species of predatory mites *A. swirskii* and *N. cucumeris* as potential fungal dispersal agents and the western flower thrips *F. occidentalis* as a resource for both the fungus and the predators. These species share similar habitats (i.e. plants supporting thrips populations) and can coexist in commercial greenhouses applying biological control programs. In a previous study, we showed that *B. bassiana* strain ANT-03 is virulent to thrips (all stages, except first instar larva), slightly virulent to *N. cucumeris* and avirulent to *A. swirskii* [20]. This system thus perfectly fits the profile of a suitable pathogen, vector and host association, in which the pathogen is virulent against host and benign towards the vector [23].

Beauveria bassiana is a generalist entomopathogenic fungus that exploits more than 200 species from most insect orders, with some isolates showing a high degree of specificity [24,25] . Conidia are responsible for infection and naturally dispersed by air movement because of their small size (1-3 μm) [26], by contact with infected hosts or via a dispersal agent [1,14,19]. Conidia adhere to the host cuticle, germinate, penetrate in the host by enzymatic and mechanical processes and reproduce by exploiting host hemolymph and tissues [27-29]. Once host nutrients are depleted, the fungus breaches the cuticle from inside out and sporulates in large numbers [28]. Commercial strains of *B. bassiana* are used for the control of arthropod pests in biological control programs. They are typically sprayed over the crops like pesticides and the probability of contact with the host depends on the spatial distribution of the pests [25,30].

The two phytoseiid species are generalist predators that actively search for prey [31]. Foraging phytoseiid mites typically respond to chemical cues emitted by plants when attacked by herbivores and move towards infested areas [32]. They are both commercialized and successfully released on vegetable and ornamental crops to control insect pests, including thrips [31]. They both mostly attack first instar thrips larvae because larger prey successfully counterattack predatory mites [33]. Small and large thrips larvae live together

in colonies on plant parts and larger larvae can protect their younger siblings from predation [34].

Frankliniella occidentalis is a cosmopolitan and highly polyphagous insect that feed on almost every plant parts, from leaves to flower and pods [35-37], and it can also vector a number of plant virus [38]. Their eggs are laid in plant tissues and nymphs develop for two instars before pupating in the soil. Mobile stages can hide in concealed parts of plants where pesticide cannot reach, and they rapidly develop resistance to chemicals [38,39].

4.3.2 Arthropod colonies and fungal inoculum

A colony of *N. cucumeris*, provided by Anatis Bioprotection Inc., was maintained on a factitious prey *Aleuroglyphus ovatus* Troupeau (Acari: Acaridae) while *A. swirskii*, purchased from BioBest Canada, was reared on a diet mixture containing *Carpoglyphus lactis* L. (Acari: Carpoglyphidae) and cherry pollen (Firman Pollen Co., Yakima, WA). *Frankliniella occidentalis* was obtained from a lab colony in Anatis Bioprotection Inc. and reared on California red kidney bean plants *Phaseolus vulgaris* L. (Fabaceae), with cherry pollen supplied *ad libitum* on a weekly basis. All colonies were maintained at 25°C, 60-70% relative humidity and under a 14L: 10D light cycle.

Beauveria bassiana strain ANT-03 has been registered in North America for greenhouse thrips control. We used the technical grade powder produced by Anatis Bioprotection Inc. containing $5x10^{10}$ conidia g^{-1} for all experiments.

4.3.3 Prey patch establishment on a plant

To test the capacity of predatory mites to deliver *B. bassiana* to thrips, we first established a spatial structure combining plant parts infested or not by thrips. To standardize the structure of a plant, we first trimmed bean plants (approximately 20 cm in height) to two sets of trifoliate leaves (Fig. 4-1). To create a clumped distribution of thrips larvae on the plant, we enclosed 25 ovipositing female thrips for 24 hours in a clip cage on a single leaflet. The clip cage was designed by F. Longpré, London Research and Development Center, Agriculture and AgriFood Canada, and made using a 3D printer. During the oviposition period, female thrips were assumed to have fed and left olfactory

cues on the leaflet that can further be used by predatory mites to locate the prey [40,41]. Based on the control treatment (see below), 27.3 ± 2.8 (mean ± S.E.) thrips larvae were produced per plant, which is the unit of replication. To avoid potential experimental bias related to leaf age or position, half of the plants had thrips on leaflet 2, the middle leaflet of the old trifoliate leaf, while the second set of plants had thrips on leaflet 5, the middle leaflet of the young trifoliate leaf (Fig. 4-1). Following oviposition, the clip cage and female thrips were removed from the plant. Four days later, when most eggs had developed into first instar larvae, the suitable prey stage for predatory mites, we released predatory mites loaded with *B. bassiana* on the plant.

4.3.4 Releasing predatory mites loaded with B. bassiana conidia

Adult female predatory mites of various age were exposed to *B. bassiana* conidia in the commercial rearing substrate ($2.5x10^9$ conidia g^{-1} substrate) for two hours to obtain maximum conidia load on their body (Fig. 4-2) [20]. In a modified Eppendorf tube, we put 25 predatory mites with 0.2 g of *B. bassiana* contaminated rearing substrate [20]. The tube was attached on the stem, at equal distance to the base of the petiole of the two trifoliate leaves (Fig. 4-1). To control for dispersal of *B. bassiana* conidia by air and potential mechanical disturbance during experimental manipulations, a tube containing 0.2 g of *B. bassiana* contaminated rearing substrate was attached to the plant (Control treatment). Each plant was isolated in a paper cylinder and the inner walls and the bottom of the paper cylinder were coated with rings of Tanglefoot® glue to prevent conidia and predatory mites dispersal between experimental units. For each set of plants (leaflet 2 *vs.* leaflet 5), there were three treatments: control, *B. bassiana* dispersed by *N. cucumeris* and *B. bassiana* dispersed by *A. swirskii*. The experiment was repeated nine times (temporal blocks, n=9) at 25°C, 60-70% relative humidity and under a 14L: 10D light cycle. The two blocks where all thrips had been consumed were excluded from the analyses of spatial co-occurrence index and of proportion of thrips bearing *B. bassiana* because these parameters cannot be estimated in absence of thrips. Inter-block variation was low and repeating the experiment 9 times was sufficient to reach significant conclusions.

At the beginning of the experiment, it was not possible to load the two predatory mite species with a similar number of conidia because of significant differences in their size, *A. swirskii* being bigger than *N. cucumeris* (dorsum length 452 ± 6 (mean ± S.E.) vs. 426 ± 4 μm, generalized linear model, χ^2=12.262, n=12, p<0.001, Lin et al. unpublished data), and their capacity to retain conidia on their body, due to dissimilarities in morphological structure such as the texture of the tegument. As a result, *A. swirskii* can carry a higher number of conidia on their body than *N. cucumeris* (1526 ± 96 (mean ± S.E.) vs. 396 ± 31; Lin et al. unpublished data) when exposed to *B. bassiana* conidia in the rearing substrate. This substantial difference in conidia carrying capacity between species was considered when interpreting results.

4.3.5 Recovery of predators and prey

Forty-eight hours after the release of phytoseiid mites, plants were carefully examined to establish the number and spatial distribution of surviving predators and prey. Each of the nine plant parts were collected and placed in a 2 oz black solo cup with lid. The cup was filled with carbon dioxide from SodaStream® to stop movement of thrips and predatory mites for the ease of handling and to avoid fungal cross-contamination between individuals. The number of mites and thrips found alive on each plant part was recorded. Thrips mortality was assumed to result from predation since *B. bassiana* conidia cannot germinate and invade thrips tissues within a 48 h period [20,42].

4.3.6 Recovery of B. bassiana conidia from prey and plant parts

To detect the presence or absence of *B. bassiana* on thrips that survived from predation, thrips were individually picked with a sterilized toothpick or clean fine brush (sterilized with 75% ethanol and rinsed with 0.1% Tween-80 between samples) and placed in a small Petri dish (Ø 35 mm) containing 2.5 ml of an oatmeal selective media for *B. bassiana* [43]. Petri dishes were examined 10 days later when colony-forming units (CFUs) can be visualized. The proportion of thrips bearing conidia was recorded.

To assess the number of conidia on each plant part following arthropod removal, leaves and stems were cut into small pieces (<2 cm in width or length) and put back into the

solo cup. Conidia were washed off by adding 5 ml of 0.1% Tween-80 into each solo cup and putting the cups on a rotary shaker for 2 hours at a speed of 125 rpm [44]. Next, one aliquot of a 0.5 ml suspension was transferred onto the selective media for *B. bassiana* [43] and CFUs were counted 9 days later. For each plant, we noted the sum of CFUs delivered to the entire plant and, more specifically, the quantity of CFUs on the leaflet where thrips females laid their eggs.

4.3.7 The spatial co-occurrence between B. bassiana and thrips induced by predatory mites

Since a large proportion of thrips (40.3±3.8% S.E.) moved away from the oviposition leaflet following hatching, we calculated a co-occurrence index between the fungus and thrips on bean plants. The proportion of *B. bassiana* CFUs and thrips that share the same plant part was calculated in the following equation (1) [45]:

$$\phi = \sum_{s=1}^{S} pn$$

where S is the number of plant parts (9 in our case), p is the proportion of conidia on part s, and n is the proportion of thrips on part s. The higher the proportions of *B. bassiana* and thrips sharing the same plant part, the higher is the co-occurrence index.

4.3.8 Statistical analyses

Our experimental design includes two categorical factors: treatment (3 levels: control, *N. cucumeris* and *A. swirskii*) and leaflet where eggs were laid (2 levels: leaflet 2 and leaflet 5). When either factor was not identified as a significant predictor of the dependent variable following a log-likelihood test, it was eliminated from the initial statistical model to optimize the final model. The number of predatory mites remaining on the plants was analyzed using generalized linear models with negative binomial error distribution and with species as a factor. The number of thrips remaining on the plants was analyzed with generalized linear models with negative binomial distribution and with treatment a factor. The proportion of thrips bearing *B. bassiana* was analyzed using generalized linear

models with treatment and oviposition leaflet as factors. The number of conidia delivered to the entire plant was analyzed with generalized linear models with negative binomial distribution and with treatment and oviposition leaflet as factors. The number of conidia on the thrips oviposition leaflet was analyzed with both generalized linear models with negative binomial distribution and Kruskal-Wallis tests, depending on whether the residuals were normally distributed or not, determined by Normal QQ-plot [46]. The co-occurrence index was analyzed using generalized linearized models with treatment as factor. When either factor (treatment or oviposition leaflet) was significant, multiple comparisons were performed with the package 'multcomp' with 'glht' function and Tukey's all-pair comparisons method. Kruskal-Wallis multiple comparison tests were performed to compare differences among means when residuals were not normally distributed. All the statistical analyses were carried out with R version 1.0.143 [47].

4.4 Results

4.4.1 Number of B. bassiana conidia delivered by predatory mites and spatial co-occurrence between B. bassiana and thrips

The number of CFUs recovered from the entire plant significantly differed among treatments (treatment χ^2=26.88, df=1, p<0.001, Fig. 4-3). Both *A. swirskii* (z=5.60, p<0.001) and *N. cucumeris* (z=4.48, p<0.001) contributed to increase total CFUs on plants compared to control. Both predatory mites delivered the same quantity of CFUs to the plant (z=1.14, p=0.489). One plant from the control treatment was excluded from the analysis because extremely high number of conidia (~19,800) landed on a single leaflet (most likely due to error in experimental manipulation); this outlier was more than three times of absolute deviation above the median [48].

There was an interaction between the thrips oviposition leaflet and treatment (interaction χ^2=31.47, df=5, p<0.001; Fig. 4-4). Simple effects (the effect of each independent variable within each level of the other independent variable) were examined. For *A. swirskii*, this effect is greater when thrips eggs were laid on the young leaflet than on the old leaflet (z=2.32, p=0.020). *Amblyseius swirskii* increased the number of CFUs

recovered from the thrips oviposition leaflet compared to control, but *N. cucumeris* did not (Kruskal-Wallis test, when thrips eggs were laid on old leaflet: treatment simple effect χ^2=19.81, df=2, p<0.001; when thrips eggs were laid on young leaflet: treatment simple effect χ^2=18.55, df=2, p<0.001).

The co-occurrence index varies among treatments (χ^2=13.14, df=2, p=0.001; Fig. 4-5) with *A. swirskii* increasing the co-occurrence of *B. bassiana* and thrips on a given plant part compared to control (z=3.49, p<0.001), but not *N. cucumeris* (z=0.97, p=0.594).

4.4.2 Proportion of thrips contacting B. bassiana delivered by predatory mites

The proportion of thrips coming into contact with *B. bassiana* was significantly affected by both treatment and thrips oviposition leaflet (treatment χ^2=22.37, df=2, p<0.001; oviposition leaflet, df=1, χ^2=10.78, p=0.001; Fig. 4-6), as well as by their interaction (χ^2=8.00, df=2, p=0.018). For *A. swirskii*, this effect was much greater when thrips laid eggs on the young leaflet rather than the old leaflet (z=3.029, p=0.002).

4.4.3 Predatory mites and thrips remaining on the plant

Forty-eight hours following predatory mite release, higher numbers of *A. swirskii* (8.21±0.88 X±S.E.) were recovered from the plants than *N. cucumeris* (2.86±0.63 S.E.) (z=5.00, p<0.001, generalized linearized model with negative binomial distribution). The numbers of thrips recovered on plants at the end of the experiment also varied between treatments (χ^2=20.29, df=2, p<0.001, Fig. 4-7). *Amblyseius swirskii* significantly reduced thrips number on plants (z=-4.47, p<0.001; Fig. 4-7) compared to control, but not *N. cucumeris* (z=-1.24, p=0.427).

4.5 Discussion

Our results demonstrate that *A. swirskii* and *N. cucumeris* both have the capacity to disseminate *B. bassiana* conidia on plants when foraging. Although *N. cucumeris* was initially transporting three to four times fewer conidia than *A. swirskii* (Lin et al., unpublished data), the number of spores recovered on plant surfaces after two days was

similar for both species, as well as the proportion of thrips carrying conidia on their body. However, *A. swirskii* is more efficient than *N. cucumeris* in delivering higher proportions of conidia to thrips colonies, as revealed by the analysis of the spatial co-occurrence between thrips and *B. bassiana*.

There are mainly two ways in which conidia can be dislodged from the predatory mite body and be dispersed on the plant. They can either be actively groomed off by mites or rubbed off on the plant surface when predatory mites move along (Lin et al., unpublished data). Grooming, the use of legs to clean the body, has been observed in phytoseiid mites when they encounter potentially pathogenic fungi [49,50]. However, grooming is not efficient to remove all conidia from a mite, especially those located on the dorsal sections of their body. We further more showed that *A. swirskii* and *N. cucumeris* mostly dislodged conidia from their body when walking on the plant surface. Indeed, the duration of walking is correlated to conidia removal for both species (Lin et al., unpublished data). Trichomes and other structures associated with the surface of bean leaves are likely to facilitate the dislodgement of conidia when mites are walking (Fig. 4-2B). Foraging predatory mites thus actively disperse *B. bassiana* conidia in the environment.

When mediated by predatory mites, transfer of conidia to thrips can either be a passive or an active process. As seen above, conidia are unloaded on plant surfaces and can subsequently passively attach to thrips cuticle when thrips forage on a contaminated substrate. Alternatively, conidia can be directly transferred from predatory mites to thrips during an unsuccessful predation event involving a physical contact between the two protagonists. Thrips are aggressive prey that display counterattack behaviours. They can swing their abdomen to 'slap' predatory mites [51] or secrete irritating anal fluid which causes predatory mites to withdraw [33]. Moreover, the presence of predatory mites in the vicinity of a thrips colony can affect their behaviour [52]. Following detection of predators, thrips may switch state from stationary feeding to escaping, thereby increasing the probability of coming into contact with spores disseminated on plant surfaces [14].

The observed differences between *A. swirskii* and *N. cucumeris* in their capacity to disseminate *B. bassiana* conidia to thrips colony might arise from differences in predator foraging patterns. *Amblyseius swirskii,* a more robust predator [53,54], seems to be better adapted to detect thrips colonies and subdue this type of prey than *N. cucumeris*, as shown by *A. swirskii* having a higher predation rate than *N. cucumeris* in our experimental setup. The difference in co-occurrence values between thrips and the two predatory mite species attest to the better capacity of *A. swirskii* to exploit thrips on bean plants. In another study system, it showed that the predatory mite *Neoseiulus* (*Amblyseius*) *barkeri* Hughes (Acarina: Phytoseiidae) did not increase *B. bassiana* transmission to thrips population [55]. *Neosiulus barkeri* is a less voracious thrips predator than *N. cucumeris* with a relatively low capture success when attacking first and second instar larvae of *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) [51]. Furthermore, the level of foraging activity of *N. barkeri* is lower than *N. cucumeris* [53]. Therefore, it is normal that *N. barkeri* did not efficiently deliver *B. bassiana* to thrips colonies. These results suggest that the foraging capacity of a predator and the strength of its interaction with a prey would be essential determinants of its potential efficiency as a dispersal agent of entomopathogens.

The rate at which *B. bassiana* contacts its host is crucial in the context of biological control, not only because it is directly linked to the infection rate but also because the viability of conidia is very sensitive to environmental conditions such as UV and humidity [56,57]. Typically, entomopathogens are used like pesticides with single or multiple applications of large quantities of pathogens in crops. However, in some instances, aerial applications are not effective to reach targeted pests. For example, due to its thigmokinetic behaviour, Western flower thrips are often concealed in plant crevices and flower buds [58]. As a result, spraying fungal pathogens has little effect on thrips infection level [30]. In such circumstances, the capacity of predatory mites in delivering pathogen to thrips colonies could increase disease transmission. In our experiment, the thrips mortality after contacted with pathogen was not evaluated. Further experiments are needed to evaluate the thrips mortality. Nevertheless, it was shown that LD50 is relative low for technical grade powder of *B. bassiana* strain: approximately 50 conidia per 2nd instar *F. occidentalis* larva and only 5 per adult [59].

Our findings about the relative potential of *A. swirskii* and *N. cucumeris* in dispersing *B. bassiana* conidia to thrips are consistent with the conclusion drawn by Zhang, et al. [18] who studied a similar biological system on the tropical shrub, *Murraya paniculata* (L.) Jack (Rutaceae), infested by the Asian citrus psyllid, *Diaphorina citri*, in the laboratory. Higher mortality in *D. citri* populations was achieved when *B. bassiana* was delivered by *A. swirskii* rather than by *N. cucumeris*, and compared to *B. bassiana* being sprayed evenly onto plants. We can thus conclude that under our experimental conditions, *A. swirskii* is a better biological control agent because it both preyed more on thrips and transmitted conidia to a larger number of thrips escaping from predation. However, *N. cucumeris* could show good potential both as a predator and an entomopathogen dispersal agent when used in a different crop-pest association. For example, in greenhouses from temperate regions, it has been shown that *N. cucumeris* showed similar performance as *A. swirskii* as a thrips biocontrol agent under simulated winter conditions [60].

Finally, how can we apply such a system in a biological control program? Growers periodically release predatory mites and spray *B. bassiana* onto crops to control thrips. The strategy we proposed does not require two separate applications, but solely a premix of *B. bassiana* conidia (technical grade powder) into commercially available predatory mite package (if approved by regulatory agencies) [20]. The predatory mites would likely increase disease transmission rate to concealed pests. The overall quality of a predatory mite species as a pathogen dispersal agent would depend on its capacity to be loaded with conidia, its capacity to resist pathogenic infection and, as shown by the present study, its foraging activity. Predatory mites should be closely associated to the target pest and have the ability to search for, locate and engage interactions with the pest on the plant, so they can disperse spores on the plant like little pebbles strewn about by Tom Thumb.

4.6 Acknowledgements

We thank members from laboratories of Jacques Brodeur, Claude Guertin, Étienne Yergeau for assistance and fruitful discussions. We also thank Félix Longpré and Jean-Philippe Parent from Agriculture and Agri-Food Canada for generous support and

technical assistance. Finally, we thank Ronald Ochoa and Gary Bauchan from Electron and Confocal Microscope Unit, United States Department of Agriculture, Agricultural Research Service for helping me take LT-SEM mite photos together. This study was supported by Bursary in a Practice Environment (BMP) Innovation Research Scholarship Program from Natural Sciences and Engineering Research Council of Canada, Fonds de Recherche Nature et Technologies du Québec and Anatis Bioprotection Inc.

Author contributions statement

GL and JB conceived and designed the experiments. CG and ST participated in the experimental design and provided editorial advice. GL and SDP performed the experiments. GL analyzed the data. GL and JB wrote the manuscript. All authors reviewed the manuscript.

Competing interests statement

The project is partially supported by Anatis Bioprotection Inc.. There is no other financial competing interests or any non-financial competing interest.

Additional information

Canadian Council on Animal Care does not regulate the use of insects or mites.

Data availability statement

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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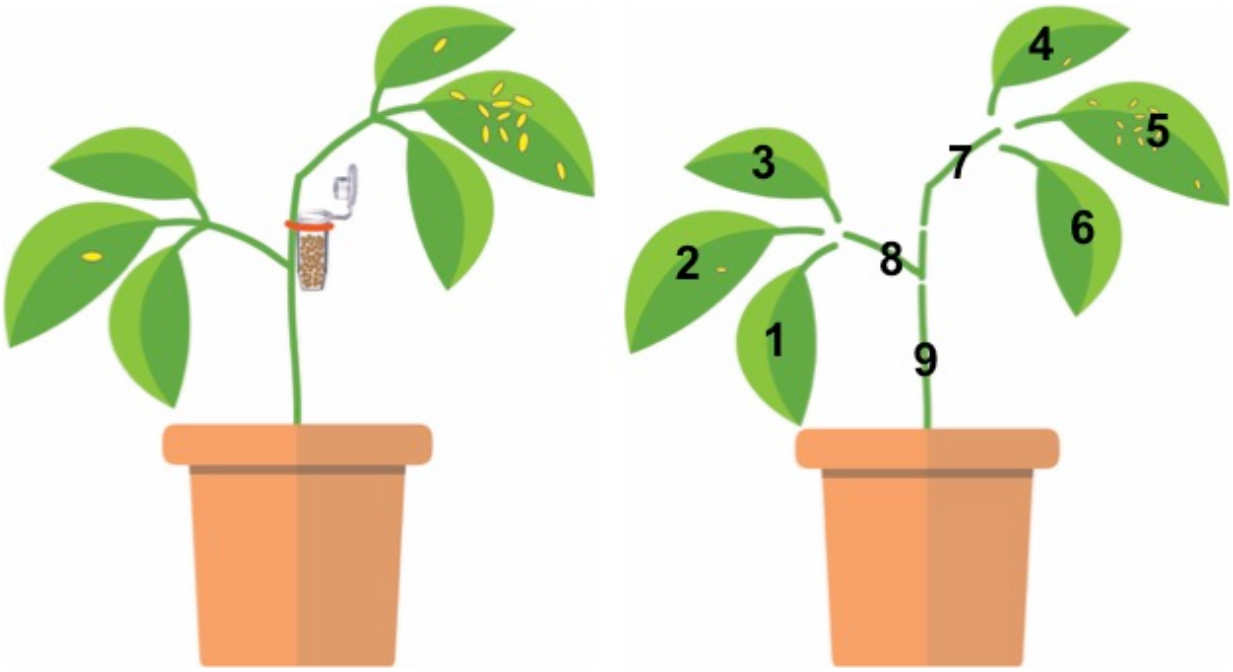

Figure 4-1. Schematic drawing of the bean plant structure after being trimmed (left). Plant parts (leaflet and stem) are each identified by a number (right). An Eppendorf tube containing predatory mites and fungal conidia to be released was attached in position 8. An example of the spatial distribution of larval thrips is illustrated using yellow oval spots - in this case, thrips are mostly clumped on the oviposition leaflet 5. Drawn by G. Lin with the software Adobe Illustrator.

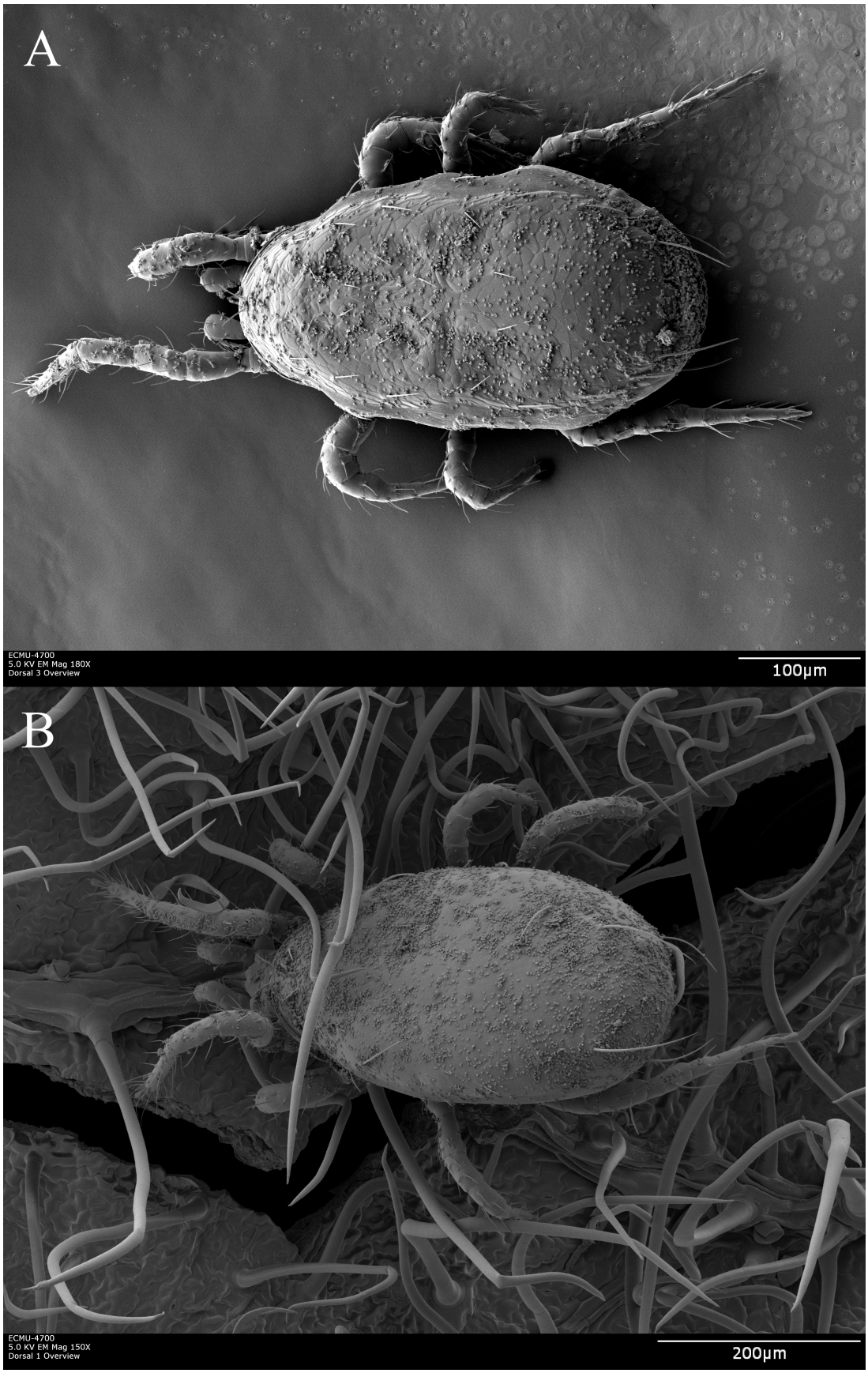
A
ECMU-4700
5.0 KV EM Mag 180X
Dorsal 3 Overview
100µm
B
ECMU-4700
5.0 KV EM Mag 150X
Dorsal 1 Overview
200µm

Figure 4-2. (A) *Neoseiulus cucumeris* bearing *Beauveria bassiana* conidia. (B) *Amblyseius swirskii* bearing *B. bassiana* conidia, released on a bean leaflet. The hair-like structures are dense bean trichomes. We observed and took photos of the specimens using a low temperature scan electron microscope (LT-SEM) with the same method described in Bolton, et al. (2014).

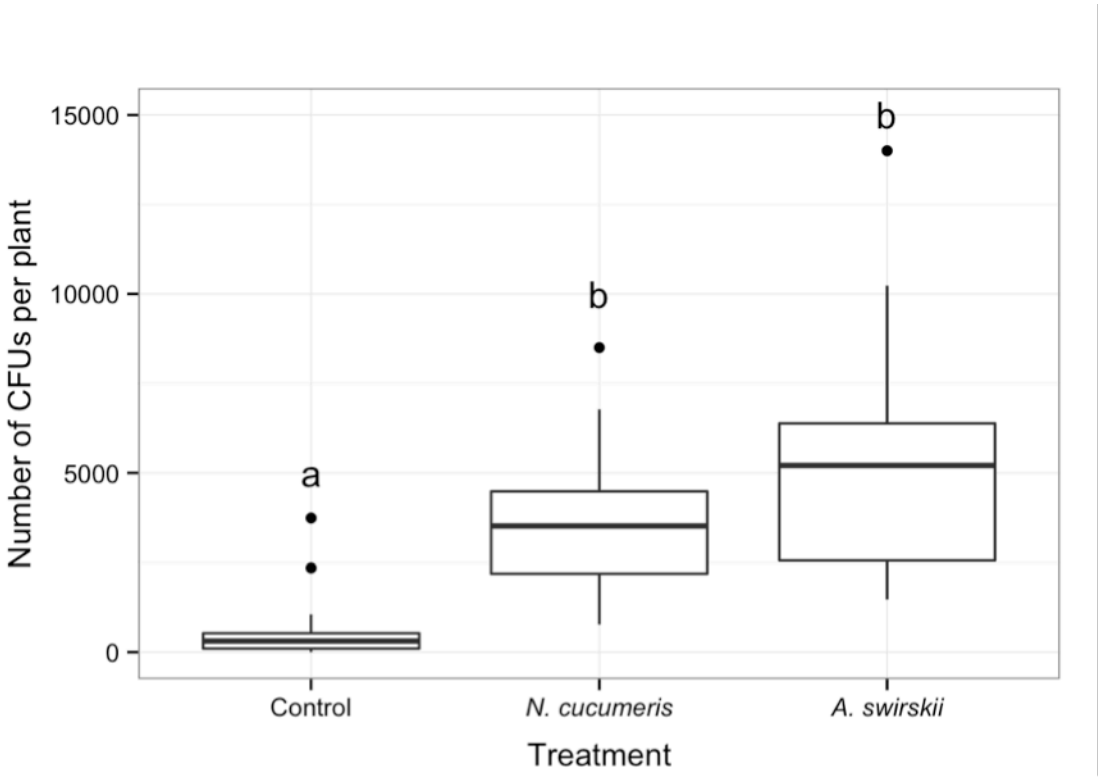

Figure 4-3. Number of *Beauveria bassiana* colony-forming units (CFUs) recovered on a plant 48 hours after the beginning of the experiment on plants without (control) and with predatory mites, *Neoseiulus cucumeris* or *Amblyseius swirskii*. Different letters indicate a significant treatment effect ($p<0.05$ generalized linear model with negative binomial distribution, multiple comparisons with 'glht' function, Tukey method). Dots identify outliers (values exceeding 1.5 interquartile range) as defined by ggplot2.

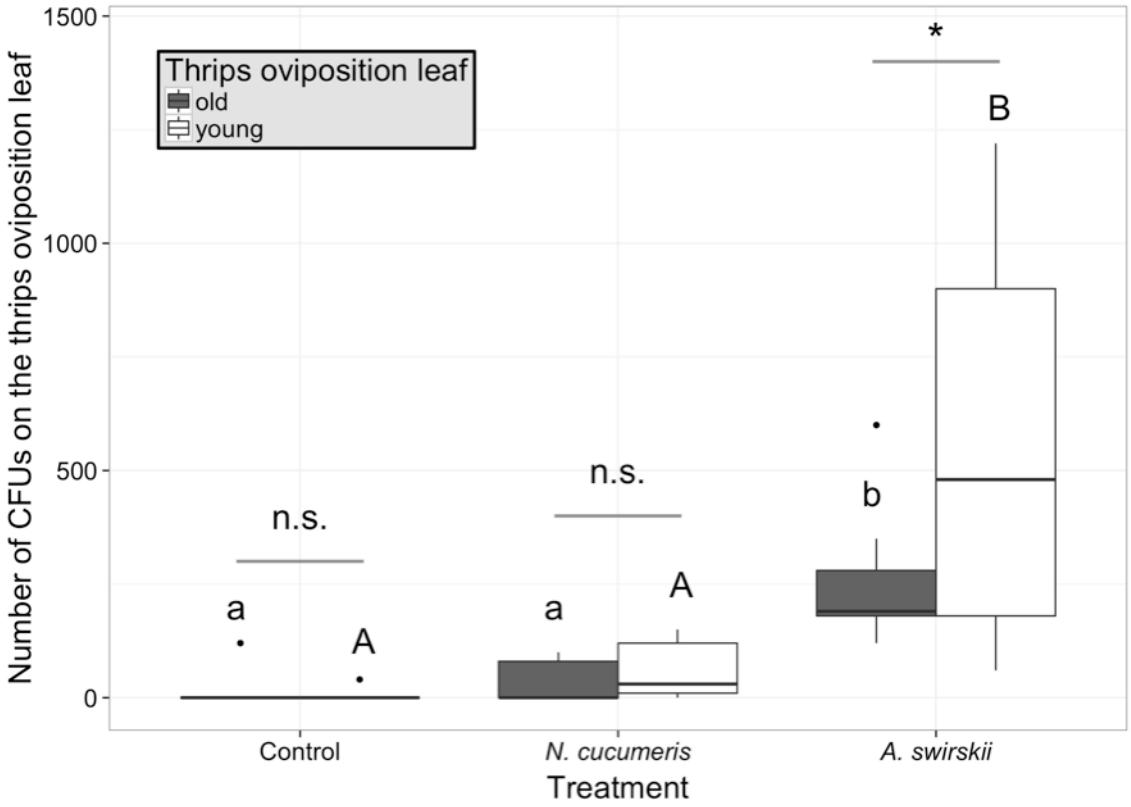

Figure 4-4. Number of *Beauveria bassiana* colony-forming units (CFUs) recovered from the thrips oviposition leaflet (young *vs.* old) 48 hours after the beginning of the experiment on plants without (control) and with predatory mites, *Neoseiulus cucumeris* or *Amblyseius swirskii*. Thrips oviposition leaflet refers to the leaflet where thrips females were caged for 24 hours to lay eggs prior to treatments. Dots identify outliers (values exceeding 1.5 interquartile range) as defined by ggplot2. Different capital and lower case letters indicate significant treatment effect for young and old leaflet, respectively ($p<0.05$, Kruskal-Wallis test with multiple comparisons). The asterisk indicates a significant difference ($0.05 < p < 0.01$) between thrips oviposition leaflet: n.s. = not significant (Kruskal-Wallis test for plants in treatment 'control' and treatment 'cucumeris', generalized linear model with negative binomial distribution for plants in treatment 'swirskii').

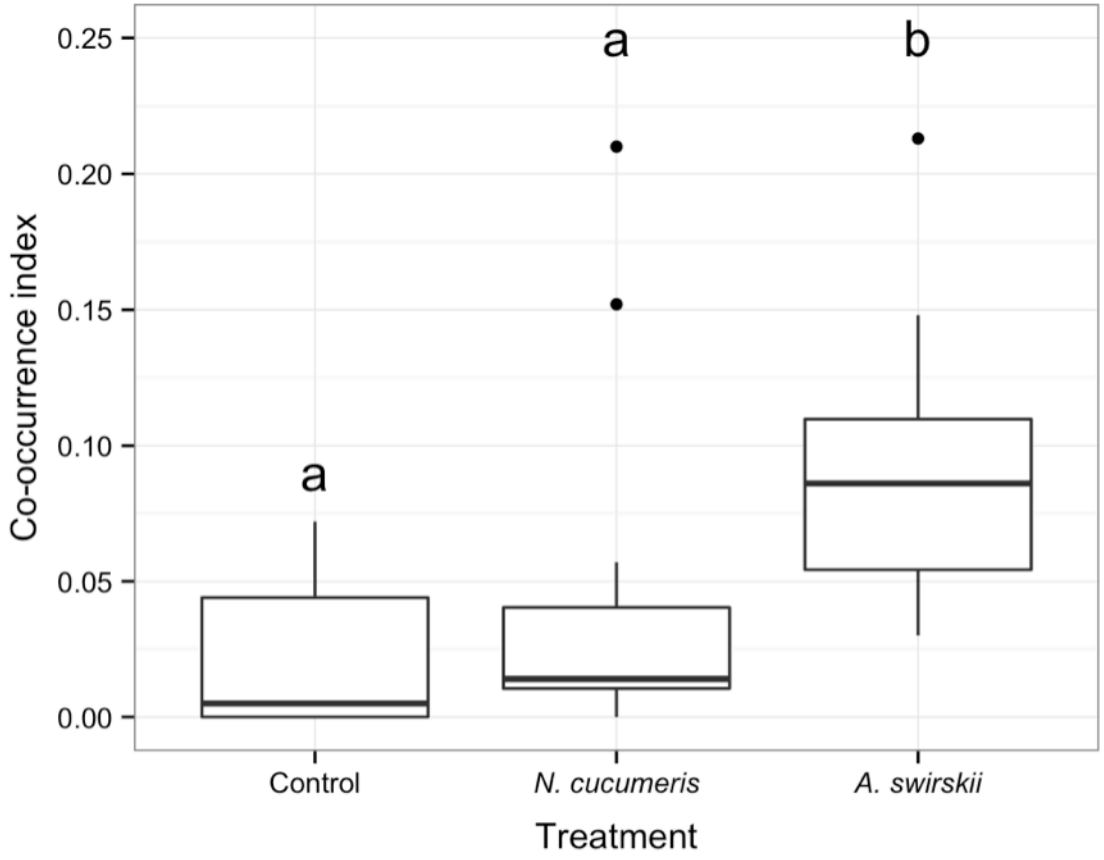

Figure 4-5. Co-occurrence index between thrips and *Beauveria bassiana* delivered to plants passively (control) and by predatory mites *Neoseiulus cucumeris* and *Amblyseius swirskii*. Different letters indicate significant differences between treatments ($p<0.05$, generalized linear model, followed by multiple comparisons with 'glht' function, Tukey method). Dots identify outliers (values exceeding 1.5 interquartile range) as defined by ggplot2.

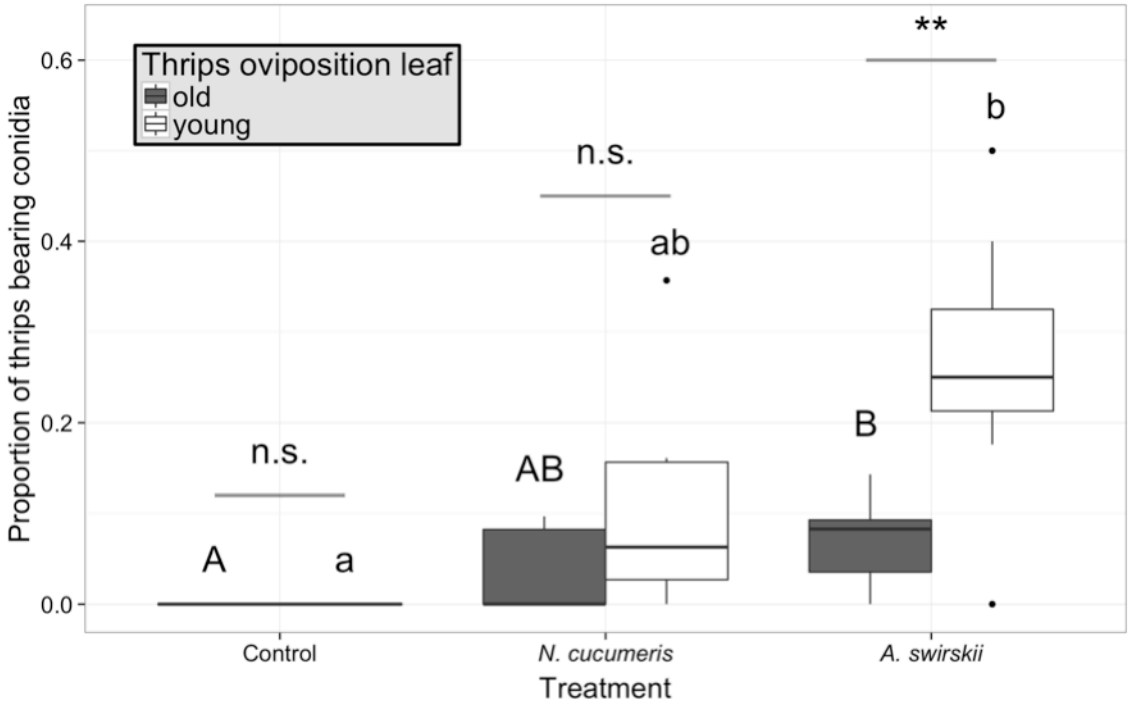

Figure 4-6. Proportion of thrips bearing *Beauveria bassiana* 48 hours after the release of *B. bassiana* on plant parts without (control) and with predatory mites, *Neoseiulus cucumeris* or *Amblyseius swirskii*. Thrips oviposition leaflet refers to the leaflet where thrips females were caged for 24 hours to lay eggs prior to treatments, (D) leaflet No. 5, young: leaflet No. 2. Different capital letters indicate significant treatment simple effect in plants where thrips eggs were laid on the young leaflet ($p<0.05$, generalized linear model, followed by multiple comparisons with 'glht' function, Tukey method) while different lower case letters indicate significant treatment simple effect in plants where thrips eegs were laid on the old leaflet ($p<0.01$, generalized linear model, followed by multiple comparisons with 'glht' function, Tukey method). Differences between thrips oviposition leaves within a treatment are shown above bars: n.s. = not significant ($p > 0.05$), ** = $0.001 < p < 0.01$ (generalized linear model). Dots identify outliers (values exceeding 1.5 interquartile range) as defined by ggplot2.

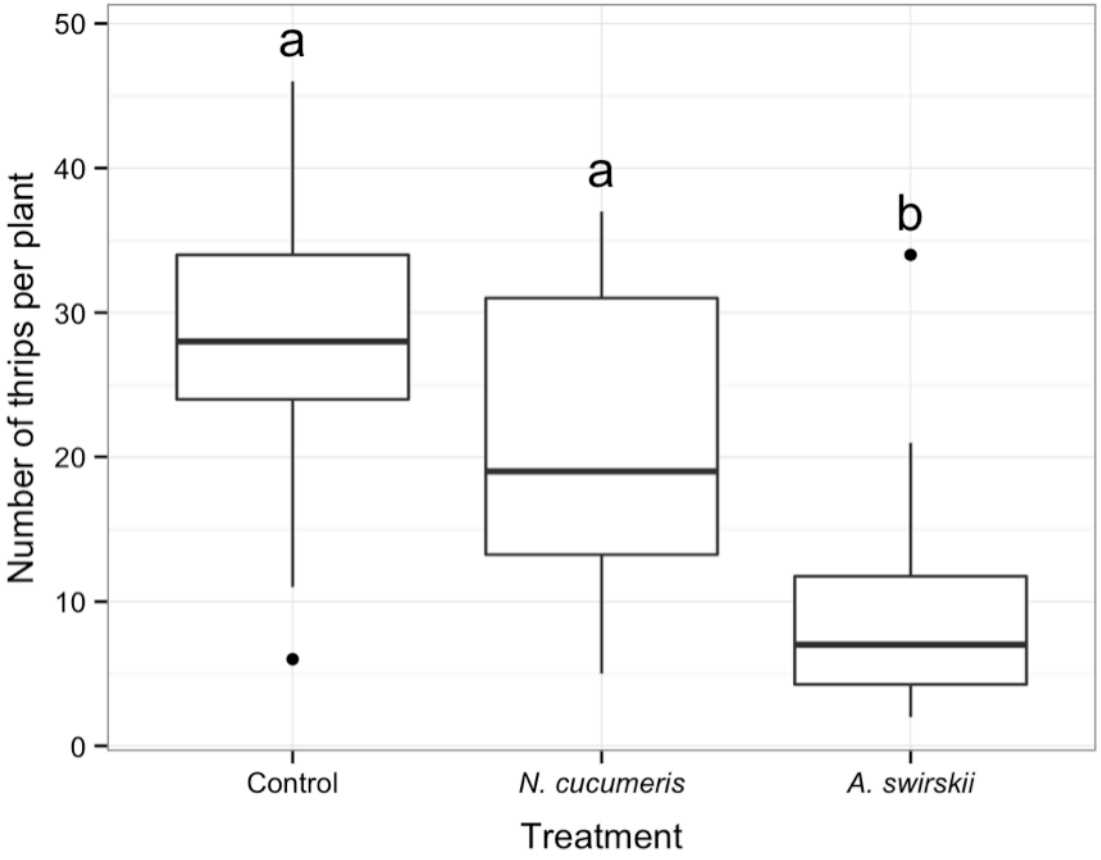

Figure 4-7. Number of thrips recovered on plant 48 hours after the beginning of the experiment on plants without (control) and with predatory mites, *Neoseiulus cucumeris* or *Amblyseius swirskii*. Different letters above bars indicate significant differences between treatments ($p<0.05$, generalized linear model with negative binomial distribution, followed by multiple comparisons with 'glht' function, Tukey method). Dots identify outliers (values exceeding 1.5 interquartile range) as defined by ggplot2.

Chapter 5: Discussion

Our studies show that, under our experimental conditions, predatory mites can be efficient dispersal agents for entomopathogenic fungi. They collected conidia from their rearing substrates and increased walking duration to dislodge conidia. The species that spent sufficient time walking in prey colonies increased disease transmission in the prey populations. In this discussion, we will answer the specific research questions posed in the introduction, demonstrate how our results from the three chapters support the answers, and make suggestions and predictions for future research.

5.1 We have made it easy to use for biological control

We developed a method for fungal dispersal by predatory mites that would be user-friendly for growers and the biological control industry. In future, to test the efficacy of the method in greenhouse or field trials, growers can simply mix conidia with predatory mites in the commercial rearing substrates, wait a short time for conidia to accumulate on mites and then release predators in the crops using their usual practices (i.e. sprinkling mites or using mite blowers). Biological control companies could mix conidia into mite rearing substrates before packing them into slow-release sachets. We have observed that conidia remain intact after eight days spent in the rearing substrates between 20-25 °C (Fig. 1). If the conidium infectivity is not significantly reduced, biological control companies can prepare the mix and growers will receive viable products if the standard shipping time (normally less than a week for predatory mites) is respected.

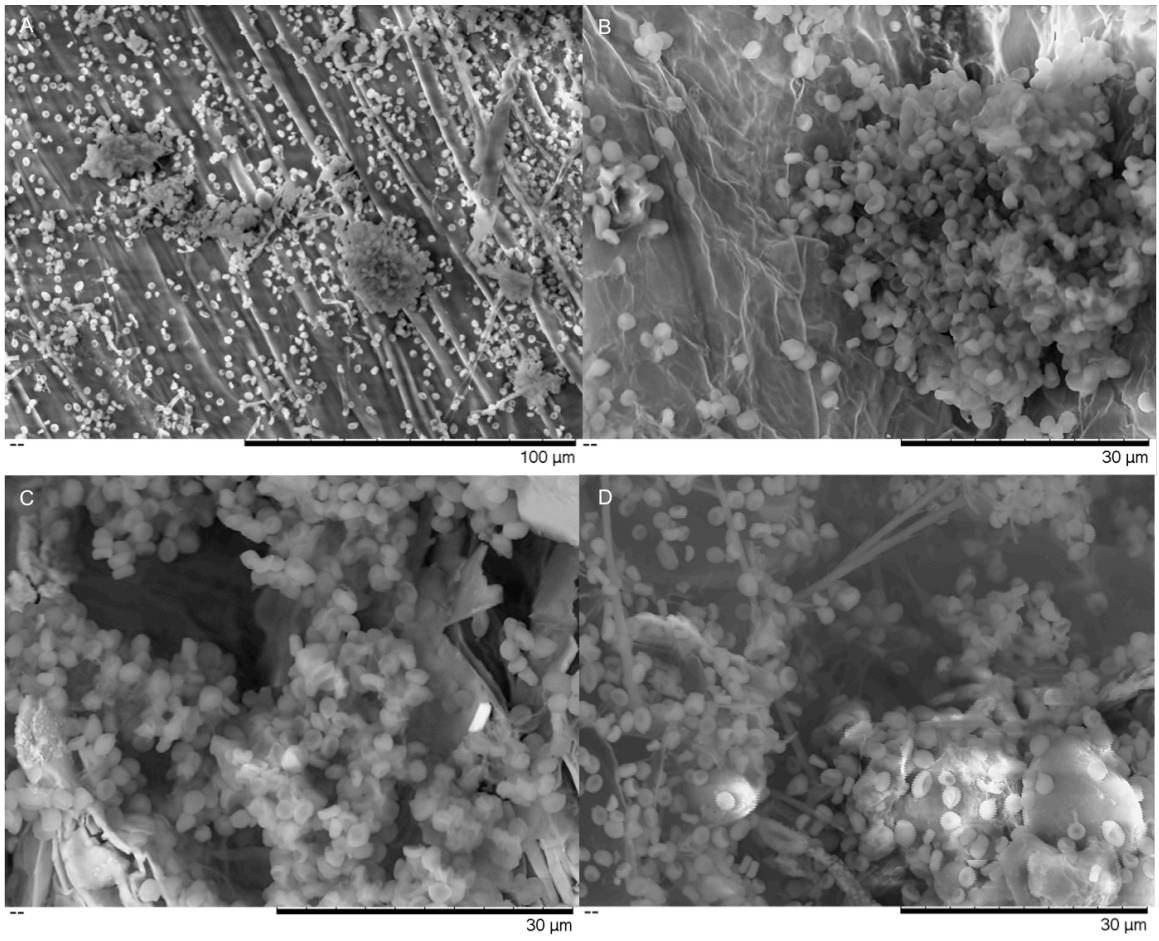

Figure 5-1. Conidia contained within mite rearing substrates after eight days (A) in rearing substrate of *Amblyseius swirskii*, (B) and (C) in rearing substrate of *Stratiolaelaps scimitus*, and (D) on dead feeder mite in the rearing substrate of *Neoseiulus cucumeris*. The specimens were observed using Hitachi tabletop TM3030 scanning electron microscope equipped with Deben Cold Stage Deben TM-3000 Coolstage (Deben UK Ltd., Suffolk, UK) as described in Otero-Colina et al. (2018).

We have demonstrated that using this method under our experimental conditions, conidia are transferred from the rearing substrates to the plants and reach thrips via *Amblyseius swirskii*. The efficacy of this method in reducing thrips populations should be validated in greenhouse or field trials before it can be registered as a product. The ecological factors that may promote or impede the efficacy of this method should be further investigated in order to determine if the method is suitable for specific settings. Furthermore, the method could be particularly interesting for specialist entomopathogen species because their

dispersal within a crop remains challenging (Butt 2002). Instead of spreading infected host cadavers, it would be possible to mix host cadavers bearing sporulating entomopathogens into predatory mite rearing substrates so that conidia could accumulate on mites and be transferred to the prey/host colonies.

5.2 Desirable traits of predatory mites as successful fungal dispersal agents

The availability of predatory mite species varies depending on the regions, so how do we select the species as promising fungal dispersal agents? The rapidity at which the prey patches are detected and the duration of residence time within prey patches would determine disease transmission rate, because predatory mites dislodge conidia primarily by walking. The less 'detours' predatory mites make to find prey patches, the more conidia will be left to contaminate prey colonies. Therefore, the nature of the predator-prey association is important to determine the capacity of predatory mites as efficient dispersal agents of entomopathogenic fungi. Disease transmission in prey population is likely to be favored by a close co-evolved link between the predator and its prey, for example, when predatory mites include the target pest in their diet. Zhang et al. (1992) showed that the foraging time allocation of oligophagous predatory mite *P. persimilis* depends on the initial density of its prey spider mites, but it was not the case for the polyphagous predatory mites *Typhlodromus occidentalis* or *Amblyseius andersoni*. They proposed that both polyphagous predatory mites randomly encountered prey patches, but the narrow polyphagous predator *T. occidentalis* is likely to stay longer in prey patches because they turn back more frequently when walking away from the prey patch compared to the broadly polyphagous predator *A. andersoni*. As the degree of feeding specialization increases, randomness of walking reduces: prey patch entry becoming faster and patch leaving becoming slower (Zhang et al. 1992), and it is likely that the entomopathogen spreading capacity in prey populations will increase accordingly.

The searching efficiency varies even within a species of specialist predatory mite, because of intraspecific variation in its attraction to prey (Margolies et al. 1997). Margolies et al. (1997) selected lines of *P. persimilis* that were more attracted to spider mite induced plant volatiles and such attraction level can be passed on to at least three

generations. Therefore, within a specialist predatory mite species, it might be possible to further increase their capacity as dispersal agents by selectively maintaining lines that respond strongly to volatiles (Lommen et al. 2017). Interestingly, the *P. persimilis* population with the strongest response (+ line population) had shorter residence time compared to control (base line population) (Margolies et al. 1997). This means that within a given time, the population from the + line is likely to exploit more prey patches than the base line population. This trait could favor the spread of the fungi and allow predatory mites to induce higher infection in prey populations.

Rearing conditions influence the attraction of predatory mites to prey as well (de Boer and Dicke 2006). Predatory mites reared on cucumber plants infested with spider mites find spider mites much faster on the same species of host plant than when reared on spider mite infested lima bean plants. Herbivore-induced plant volatile cues differ between plant species and predatory mites can develop a preference to a plant-prey association through associative learning (de Boer and Dicke 2006). An improvement in prey searching efficiency would be desirable when using predatory mites as dispersal agents of fungi because timing is crucial: all conidia are dislodged within two days. However, these predictions are based on the assumption that conidia do not influence the path of predatory mites. In fact, *B. bassiana* conidia did affect predatory mite behavior.

5.3 Fungal conidia prolonged predatory mite walking behavior

Predatory mites with conidia spend more time walking. Such a behavioral could promote predatory mites as promising fungal dispersal agents because movement has a positive relation with disease transmission (Sumner et al. 2017). Indeed, both *A. swirskii* and *N. cucumeris* dispersed conidia from the rearing substrates to the plants.

We expected conidia to be dispersed along the path of foraging predatory mites and reach thrips colonies. We found that only *A. swirskii* dispersed *B. bassiana* to thrips colonies. It is likely that *A. swirskii* walked towards thrips colonies but *N. cucumeris* did not. This is unexpected, because *N. cucumeris* has been well recognized as a common biocontrol agents of western flower thrips (Messelink et al. 2006) and they are attracted to plant

volatiles induced by thrips feeding, which increases the probability of *N. cucumeris* walking towards thrips infested leaves (Tatemoto and Shimoda 2008). It might be that the amount of conidia dispersed to thrips colonies by *N. cucumeris* is too little to be detected. Another possible explanation is that conidia might impede *N. cucumeris* prey finding capacity. We found that conidia drastically reduced palp activity in *N. cucumeris* but not in *A. swirskii* (Fig. 3-2). Palp activity is alternating vertical movement of palps. This could be a behavior linked to predatory mite searching efficiency. Palps of predatory mites such as *P. persimilis* contain porous sensilla that may be involved in chemosensing, i.e. gustation (Jagers et al. 1985). Predatory mites respond to steep gradients of prey kairomone and circulate around the odor source (Sabelis et al. 1984). Do predatory mites perceive the cues from palp activity, 'tasting' the molecules on the leaf? Do *B. bassiana* disrupt their perception by reducing palp activity and make *N. cucumeris* run around like a decapitated chicken? Future studies can answer this question by testing the effect of *B. bassiana* on *N. cucumeris* searching efficiency.

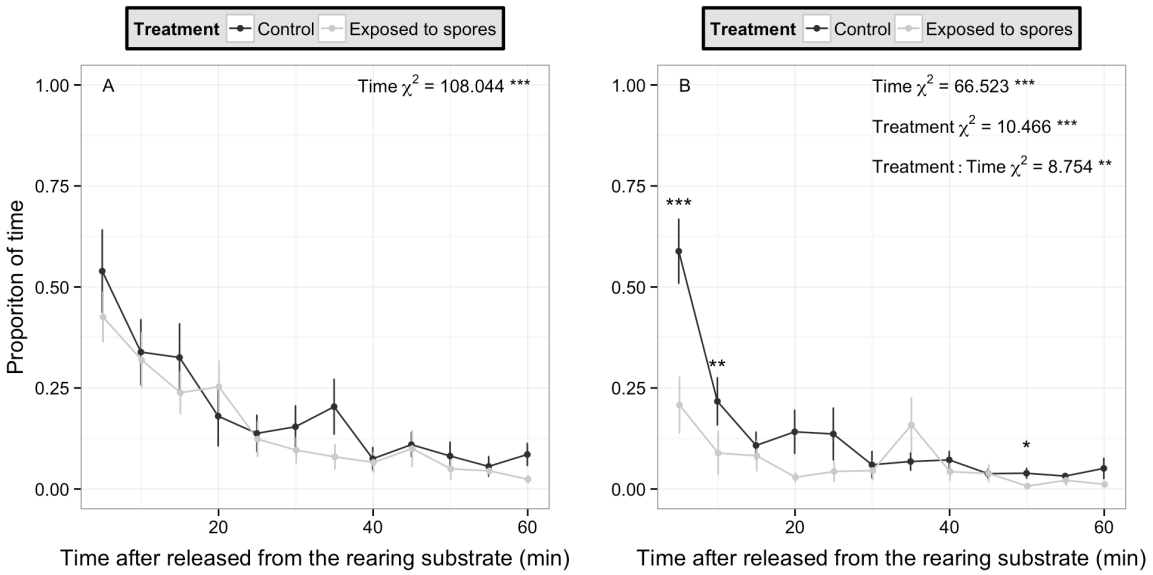

Figure 5-2. Proportion of time (mean ± S.E.) predatory mites spent on palp activity: (A) *Amblyseius swirskii* and (B) *Neoseiulus cucumeris* following release from *Beauveria bassiana* contaminated (grey curve) or non-contaminated (black curve) substrate. Stars beside the factors indicate significant predictors of the proportion of time spent on a behavior (beside the factors) and stars above the curve indicate significant difference

between treatments: * = 0.01 < p <0.05, ** = 0.001 < p < 0.01, *** < 0.001; generalized linear mixed-effect model.

5.4 Grooming is not simply a behavior for preventing infection

We studied the effect of *B. bassiana* conidia on predatory mite behavior because we were concerned that mites would spend more time grooming, which would remove the conidia before they could be transferred to the prey colonies. To our surprise, this is not an important issue. Plant-dwelling predatory mites (*A. swirskii* and *N. cucumeris*) did not spend more time grooming when loaded with conidia. Grooming duration of soil mite *S. scimitus* was even reduced. Several studies claimed that grooming removes entomopathogens from arthropod bodies (Wekesa et al. 2007; Yanagawa et al. 2018). However, their conclusion could be false, as they did not disentangle the time spent on walking or grooming but linked the number of conidia remaining on the body directly to the time after conidia application. *Drosophila melanogaster* responded to musty odor of *B. bassiana* with an increased walking or running but not grooming (Yanagawa et al. 2018), which is in agreement with what we have observed.

Amblyseius swirskii and *N. cucumeris* without *B. bassiana* conidia spent half of their time grooming, scrubbing their bodies and mouthparts with their legs, where most known sensilla are located (de Lillo et al. 2005). These sensilla function as mechanoreceptors and chemoreceptors (de Lillo et al. 2005). Using electrophysiological recording, De Bruyne et al. (1991) found that the sensilla located on first leg tarsi of *P. persimilis* respond to methyl salicylate, a key component of spider mite induced plant volatile (De Bruyne et al. 1991). Therefore, grooming could be a maintenance behavior to keep the pores on sensilla clear in order to maximize chemical molecule detection. In crustaceans, a cousin taxon of predatory mites, grooming is actually a behavior to keep olfactory organs sensilla clean. Caribbean spiny lobster *Panulirus argus* uses mouthpart appendages called the third maxillipeds to wipe sensilla on their attenules (Wroblewska et al. 2002). When their prey extract was released in water, it elicited grooming behavior. After sensilla were ablated, the grooming behavior was reduced. Similarly, predatory mites might use grooming to clean their sensors in order to perceive various cues in the

environment. If this is true, we expect that the sensory capacity of *S. scimitus* involving sensilla on the legs can be disrupted by *B. bassiana,* but not of *N. cucumeris* or *A. swirskii,* because exposure to *B. bassiana* reduced grooming duration in *S. scimitus*, but not in the other two species.

5.5 Does the susceptibility of predatory mites to the entomopathogen influence fungal dispersal capacity?

We did not address this question with the experiments described in this thesis. We showed that *N. cucumeris* is susceptible to ANT-03, but not *A. swirskii*. Our data demonstrated that the behavior response of *N. cucumeris* is stronger than *A. swirskii* towards *B. bassiana* (strain ANT-03): with reduced palp activity and with a larger increase in activity. In fact, the amplitude of host response can be influenced by the virulence of a pathogen, because virulent and non-virulent pathogens emit different MVOC profiles that can be distinguished by host via olfaction (Davis et al. 2013). The wildtype *D. melanogaster* could perceive musty odor of *B. bassiana* and responded with an increased activities (prolonged walking or running duration), but a mutant with olfactory deficiency did not show any behavior change. Can the virulence of a pathogen influence navigation capacity of its dispersal agents? The question remains open for further work.

5.6 Conclusion

Our study demonstrated that, 1. Predatory mite increased *B. bassiana* conidia dispersal, 2. Conidia increase predatory mite movement, 3. Time of predatory mites spent in prey patches is likely to predict the pathogen-host encounter rate. We have provided theoretical basis for identifying candidate predators to become efficient fungal dispersal agents. Last but not least, we have made this system easy to use for greenhouse or field trials for biological control.

5.7 References

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The finishing touch

Towards the end of my PhD, I decided to combine my two passions together: burlesque and biological control. Here is the link to an eight-minute burlesque video that interprets my PhD. I danced as a predatory mite and my friends danced as thrips.

https://www.youtube.com/watch?v=N-UT5engHRc

Yes, sometimes we have to be crazy.