A ‘MITEY’ INFLUENCE?
LIFE HISTORY, IMPACTS AND DISTRIBUTION OF A GALL-INDUCING ARTHROPOD IN A TEMPERATE FOREST CANOPY

by

RAJIT PATANKAR

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Department of Ecology and Evolutionary Biology
University of Toronto

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ABSTRACT

Mature forest canopies worldwide sustain an enormous diversity of arthropods, many of which are specialist natural enemies. However, with the exception of species that exhibit massive outbreaks, host-specific canopy arthropods are thought to have relatively little influence on tree health and overall forest productivity. My thesis examines the role of one such arthropod in a temperate forest stand in central Ontario. The maple spindle gall mite *Vasates aceriscrumena* (Riley) (Acari: Eriophyoidae) is a host-specific canopy parasite that induces galls on leaves of sugar maple *Acer saccharum* Marsh. I examine three diverse topics related to this host-parasite system: 1) the seasonal phenology of this mite in mature sugar maple canopies, 2) the impacts of galling on host physiology and growth and 3) the distribution of this mite across host ontogeny and within the broader context of the local forest community. With respect to phenology, I document a previously unobserved interaction between the gall-inducer and a gall-invading mite (ubiquitous in the canopy but new to science) and consequences of this on the bionomics of
galling mite populations. This work is also the first to examine differential physiological responses to galling across two distinct stages in the ontogeny of the host. Infected leaves in mature trees show drastic reductions in gas-exchange processes (photosynthesis, stomatal conductance and water use efficiency) while infected sapling leaves show no such detectible responses. Further, I find a significant negative correlation between radial increment growth in mature trees and levels of mite galling, as well as significantly increased galling frequency in mature trees compared with understory saplings. Finally, I explore the relationship between galling abundance and abiotic and biotic variables within a large mapped forest plot and show that gall densities are most strongly correlated with local species diversity and less so with host densities. Overall, my research provides a new perspective on the influence of host-specific, dispersal-limited canopy arthropods as major drivers of ‘age-dependent’ reductions in physiological performance and growth of older trees and as natural enemies that are strongly associated with local forest community distribution patterns.
DEDICATION

In loving memory of my father, Subhash Amarnath Patankar (1946-1996)

To my mother Meera Raj and my lovely wife Anjali Karve
ACKNOWLEDGMENTS

My journey through graduate school has been an amazing roller-coaster ride, with all the requisite twists and turns of a spicy Bollywood matinee! I would first like to begin with a sincere note of thanks and gratitude to my two supervisors Sean Thomas and Sandy Smith, without whom my journey as a Ph.D. scholar at University of Toronto might have ended prematurely. I met Sean and Sandy during what was the most tumultuous part of my graduate school experience, having just fallen out with a former advisor. I spent a good measure of summer 2006 running from pillar to post in search of potential advisors who were willing to ‘adopt’ a recently orphaned student, and was fortunate to land a meeting with Sean which I still remember vividly. After spending a while talking about various ongoing projects in the lab, Sean queried “So, what do you think about mites?” and four years on, here I am! Sandy was unrelenting in dealing with the complex issues of adopting a student from another lab, and it is thanks in large part to her that I was able to continue my degree in the department. I also thank them for placing faith in my abilities in spite of little prior experience in this area of research. Finally, it is with the deepest gratitude that I thank them both for their guidance and support these past few years. I received the best of both worlds with the two of them as my guides: Sean always encouraged me to think ‘big’ while Sandy always kept my feet on the ground.

A natural extension of advisors is the graduate committee, and once again I owe a lot of thanks on this front. Frederic Beaulieu (Canadian National Collection, Ottawa) was the ‘resident expert’ on mites, and on whom I relied (and continue to rely) on heavily with respect to mite life history work. Thank you for taking the time from your work to teach me all that I know about mite taxonomy and for those amazingly detailed manuscript edits. Peter Kotanen and James Thomson were fantastic committee members, genuinely curious about mites and galls, and always providing insightful ideas at meetings. Your timely comments and suggestions have helped define this dissertation.

The field component of my thesis would not have been possible without the help of a few key people. I would like to thank Peter Shleifenbaum and the people at Haliburton Forest and Wildlife Reserve for permitting me to ‘run wild’ in their forest. I’m also grateful to John Caspersen for allowing me use of the canopy lift, without which no remote-canopy physiology work would have been possible (tree-climb with a LiCor 6400 anyone?). I had fantastic assistance for two field seasons and for this I am heavily indebted to Jessie Colgan and Moe Luksenber. In addition, my fellow ‘Bone Lakers’ and graduate researchers Erin Mycroft and Shaik Hossain provided invaluable time and assistance in the field.

I had a small army of undergraduates that helped with all manner of data collection and analyses in the lab. In particular I would like to thank Fernando Gomez-Sanchez, Sarah Shujah, Maciej Jamrozik, Laura Fernandez, Heather Mcleod, Irena Nikoloska, Nabila Rahman and Hamsha Pathmanathan. Heartfelt thanks to all of you who contributed your efforts in the lab towards my research. Additionally I
thank Henry Hong and Audrey Darabie for assistance with producing scanning electron microscopy images of mites.

The members of my lab helped me through the trials of grad school, and hence I owe them a huge debt of thanks. Mike Fuller and Erin Mycroft transcended from ‘lab mates’ to very close and supportive friends. A special thanks to Mike, who worked so hard to help me with R coding, and for prolonged, thoroughly enjoyable discussions on all things science and non-science. I am glad our collective daydream of collaborating on research has come to fruition. And thanks to Erin for all the encouragement when things seemed to fall apart (literally and figuratively) in the field: you had my back, and I’ll miss our summer antics at Bone Lake. Liz (‘Lizbot’) Nelson was amazing as an officemate and equally amazing as an avid and enthusiastic reader/editor of my written work. I also thank Adam Martin for reviewing my grant proposals and Michael Drescher for insightful discussions on ecology.

During my years as a graduate student, I had a lot of support from fellow grads outside the lab. Specifically, Catherine Febria, Suddhasheel Sen, Anu Mohan and Anindo Hazra: thanks for sharing the ups and downs with me and listening to how ‘cool’ mites really are. I would also like to thank my ‘non-academic’ friends in Toronto who, despite often not having a clue about galls and other such ‘drivel’, were genuine well-wishers in my pursuit of a Doctorate degree. One non-human entity that helped me through a lot of tough school days is my dearest rascally feline, Passepartout. Thanks for keeping me company through all those gloomy writing days and for generous heaps of ‘mudhus’. Although you will never know it, you were an important and integral part of this journey.

My mom Meera Raj has been a phenomenal stalwart, fan, and a major source of inspiration over the years, especially since overcoming a major illness recently with such renewed vigour, grace and ‘oomph’! It is an understatement to say that you remain a constant source of inspiration to those who are fortunate to know you. Thank you so much for your love, kindness, support and encouragement and also for putting up patiently with questions like ‘Is he still studying?’ and ‘No grandkids yet?’ from friends and relatives back home in India. I also thank my family members and friends from India for their words of encouragement.

Every part of this amazing ride was witnessed and shared by my best friend, wife and soul mate Anjali Karve. You have put up with all the frustrations, disappointments, and resulting tantrums that come with the role of a graduate student. For this, I owe you my deepest gratitude, love and appreciation. As well, thank you for being ‘right there’ when I needed you most, collecting leaves from wet forest floors, digging up saplings, editing written work and other such sundry tasks. Enough cannot be said about the part you have played in all of this. You have been the rock, the fan, the shoulder, the love, and of course, one hell of a sugar mamma (oh, how I’ll miss this!). You are my everything; I could not have done it without you.
CHAPTER ACKNOWLEDGMENTS

This thesis is comprised of three co-authored manuscripts that are either submitted or in preparation for submission to peer-reviewed journals (Chapters 2-4). Experimental design, analyses, and manuscript preparation were all carried out by the principal author and PhD candidate. Co-authors of the chapters contributed their expertise in experimental design, manuscript editing, taxonomy, computer programming and financial and/or in-kind support. Hence, with the exception of the general introduction (Chapter 1) and general conclusion (Chapter 5), the chapters in this thesis remain in first person plural instead of first person singular. Chapter 1 provides the motivation for this research, and Chapter 5 summarizes the major findings and discusses the significance of this study.


3. **Patankar, R.**, Thomas, S.C., Fuller, M.M. and Smith, S.M. 2010. *In preparation.* The distribution of a host-specific canopy parasite is linked with local species diversity in a northern temperate forest. Oikos. (Chapter 4)
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CHAPTER 1: INTRODUCTION
1.1 **HOST-SPECIFIC ARTHROPODS IN FOREST COMMUNITIES**

Forests worldwide are home to a diverse array of natural enemies, a large number of which are phytophagous arthropods. Among these, several thousands of species are highly specialized, with the ability to exploit only single (monophagous) or a limited number of host tree species. Such host-specific arthropods figure prominently in current ecological theories pertaining to forest ecology and dynamics. For instance, host-specific arthropods, along with pathogens and other host specific natural enemies are thought to be responsible in part for the maintenance of tree species diversity in both tropical (Janzen 1970, Connell 1971, Wright 2002) and temperate forests (Humphrey and Swaine 1997, Packer and Clay 2000, Wada et al. 2000) through density-and distance-dependent mechanisms (i.e. the Janzen-Connell effect). Host-specific arthropods also play vital roles as prominent enemies of forest health; some of the best-known examples of temperate forest destruction are caused by specialized outbreak species such as the Douglas fir tussock moth *Orgyia pseudotsugata* (McDunnough) and mountain pine beetle *Dendroctonus ponderosae* Hopkins (Mattson and Addy 1975). More recently, actions by such outbreak species have led to vital shifts in temperate forests from being carbon sinks to carbon sources (Kurz et al. 2008). Hence the importance of specialist arthropods in shaping forest communities is undeniable. However, one guild of arthropods that continues to receive little attention, especially in the context of forest health and dynamics, are gall-inducing insects and mites.
1.2 GALL-INDUCING ARTHROPODS

Gall-inducing arthropods (hereafter GIA), along with leaf miners, are amongst the most specialized group of phytophagous arthropods (Gaston et al. 1992). GIA are generally thought be relatively benign, as gall formation rarely results in host mortality (a rare example being the galling wasp *Quadrastichus erythrinae* that causes mortality in coral trees (Yang et al. 2004). A recent study estimated that species richness of GIA globally ranged from anywhere between 21,000 - 210,000 species and averaged around 132,000 species (Espirito-Santo and Fernandes 2007).

GIA influence host plant growth and physiology in a number of interesting ways. For example, galling induces significant change in plant gas exchange, from increases (Fay et al. 1993b, Bagatto and Shorthouse 1996, Dorchin et al. 2006) to decreases (Larson 1998, Florentine et al. 2005) in photosynthesis. Similarly, plant architecture (Larson and Whitham 1997), shoot growth (Vuorisalo et al. 1990), nutrient allocation (McCrea et al. 1985), and whole plant growth and survival (Hakkarainen et al. 2005) may all be dramatically affected by galling. Additionally, GIA manipulate host plant histology and chemistry to access undifferentiated nutritive tissue and to avoid host defenses respectively (Kane et al. 1997, Nyman and Julkunen-Tiitto 2000). While much has been learnt about interactions between GIA and their hosts, results from most studies have largely been based on examinations in agricultural, horticultural, manicured or common garden systems. Studies pertaining to GIA in natural forests have mainly focused on estimates and correlates of GIA species richness (Fernandes and Price 1988, Price et al. 1998, Abrahamson et al. 2003). Although fairly common in forest canopies (Ribeiro and
Basset 2007), very little is known about the basic life history of such ‘chronic parasites’, their distribution within forests, or their potential impacts in forest ecosystems.

1.3 **GALL INDUCING ERIOPHYOID MITES**

Gall-inducing mites (Acari: Eriophyoidea), in particular, are highly host-specific but are considered to have little or no impacts on forest tree species. The process of gall induction by eriophyoid mites differs fundamentally from that of familiar insect inducers such as members of the Cecidomeiidae or Cynipidae wherein galls are initiated by developing larvae within host tissues. Mite galls are initiated when adult females pierce host plant cell walls using their cheliceral stylets (mouth parts) (Westphal 1992). This puncture leads to the swelling of the cell nucleus, followed by the denaturation of nuclear material in the cell, and the formation of nutritive tissue by the surrounding cells. The mite then moves to surrounding cells to induce growth of tissue that compensates for the necrosis of injured cells. In order to further induce differentiation of nutritive tissue and gall formation, the mite continues to feed on cells repeatedly, and the formation of galls can take anywhere from a few hours to a few days (Westphal 1992). Eggs are oviposited into galls while galls are still developing and gall development is complete within approximately 10-15 days. The founding female remains within the gall and continues to feed on gall tissue (i.e. an undifferentiated nutritive tissue layer, see Figure 1.4). Gall tissues are maintained by the continuous feeding of the various life stages of the inducer (i.e. founding female, larvae, nymphs, new adults) and galls begin to senesce and die when feeding is arrested (Westphal 1992). New adults exit galls through the exit holes (ostioles) and disperse to other suitable habitats to either a) overwinter (for mites that gall
temperate deciduous hosts) or b) initiate new galls (for mites that gall evergreen hosts) (Manson and Oldfield 1996).

Although around a quarter of the ~ 4000 described species of eriophyoid mites induce galls, their roles in forest communities are not known. Below I present a brief review on what is currently known about the influence of galling mites on forest trees.

Galling by mites can lead to alteration, not just in basic physiological processes at the leaf level, but also in the size of leaves and shoots on host plants. For example, galling by *Phytoptus emarginatae* (Keifer) (Acari; Eriophyidae) on leaves of wild plum (*Prunus americana* Marsh) leads to significantly reduced shoot length in heavily galled plants when compared to plants with low levels of leaf-galling; additionally it appears that smaller leaves are associated with high levels of galling by this mite (Willson and Odowd 1990). Similarly, heavily galled leaves in *Alnus glutinosa* L. (black alder) species have significantly smaller leaf areas than ungalled leaves in short shoots, and these leaves suffer from slower growth rates (Vuorisalo et al. 1990). Other effects of high galling levels include reductions in assimilation, growth retardation and leaf loss (e.g. Vaneckova-Skuhrava 1996).

The effect of mite galling on photosynthetic rates in trees has not been examined extensively, but results from one prior study have shown decreases in net photosynthesis in galled versus ungalled leaves. Photosynthetic rates were significantly less (> 50%) in galled leaves of wild cherry (*Prunus serotina* (Ehrh)) and in neighbouring ungalled leaves (by almost 25%) compared to ungalled leaves on control shoots (Larson 1998). The leaves of *P. serotina* are host to an eriophyid galler, *Phytoptus cerasicrumena* Walsh. Whether arthropod gall inducers in general tend to increase or decrease photosynthesis is still
debated and seems to depend on the type of gall inducer (see Dorchin et al. 2006).

Eriophyid gall mites are capable of inducing differential impacts between sexes and hybrids of hosts. Verdu et al. (2004) looked at the effects of the galling mite *Aceria macrorhynchus* (Nalepa) on male, protandrous and protogynous morphs of the Mediterranean tree, *Acer opalus* Mill. Their five-year study found that there was a significant relationship between galling intensity and morph type, with males being the most heavily infested. Further, photosynthetic rates decreased the most in leaves of male morphs (compared to appropriate control leaves) when compared to the other morphs in the species. Although there was no significant difference in length between galled and respective control shoots in trees, there was a difference in shoot length between morphs, with males having the smallest shoots. Further, it appears that hybrids between some tree species appear significantly more susceptible to gall mite infestations. Kalischuk et al. (1997) observed that hybrids of three cottonwood species stands (narrow leaf, prairie and balsam poplar) in Alberta had higher levels of infestation by the poplar bud gall mite *Aceria parapopuli* (Keifer) than in trees of the three species. Moreover, artificially planted hybrids also showed the heaviest galling levels, confirming that intersectional hybrids were most vulnerable to herbivory.

It is clear that gall-inducing mites have profound effects on their hosts locally at the site of gall formation, and that this further extends to shoots and whole trees. GIA, particularly eriophyid mites, are often integral components of host tree canopies that have the potential to shape the outcome of both host and community dynamics. However, what little is known about their impacts is restricted to forest understories (Aldea et al.)
2006), and much remains to be understood about their interactions with their hosts, especially within the context of large forest trees.

1.4 THESIS OBJECTIVES

This thesis examines the role of a gall-inducing mite (Acari: Eriophyoidae) on canopies of its host species in a temperate mixed forest. The host species sugar maple *Acer saccharum* Marsh is commonly a dominant tree in temperate deciduous forests of eastern North America. The maple spindle gall mite *Vasates aceriscrumena* (Riley; hereafter SGM) induces finger-like galls on leaves of sugar maple (Figure 1.1) and appears to be the most common aboveground herbivore on sugar maple, with a single infected sugar maple tree estimated to contain $10^4$-$10^5$ galls. The primary objective of my thesis is to examine the influence of this dispersal-limited canopy arthropod as a natural plant parasite on its host. My work is encompassed within a broader framework on the role of host-specific natural enemies in forest communities. In order to determine the influence of this mite, I address three broad questions:

1. What is the (seasonal) phenology of *Vasates aceriscrumena* during the growing season of its host sugar maple?

2. What are the impacts of galling on the physiology and growth of sugar maples?

3. What is the spatial distribution of *Vasates aceriscrumena* in relation to biotic and abiotic factors in a forest stand?

In Chapter 2, I address questions related to the seasonal phenology of this little-known mite, including the role of a frequently occurring gall-invading tarsonemid mite (Acari; Tarsonemidae). The life history of *V. aceriscrumena* is predicted to follow a pattern
similar to other eriophyid species that form galls on deciduous hosts in temperate forests. As the life cycles of GIA, specifically eriophyid gall inducers, are intimately associated with the phenology of their hosts, a close examination of parasite life history will provide a more inclusive explanation of this host-parasite system. Further, given the likely impacts of mite-galling on sugar maple, a better understanding of SGM life history would help elucidate the relationships between the mite and sugar maple’s growth, yield, and physiological response to variations in mite galling intensity through time or space. In addition, I examine the role of the gall-invading tarsonemid as a natural enemy within SGM galls. In Chapter 3, I examine the influence of galling on host physiological functioning and growth. Specifically, I test the prediction that mite-galling will have significant negative impacts on gas-exchange processes (photosynthetic capacity, water use efficiency and stomatal conductance) in infected versus uninfected foliage. Further, I test the prediction that heavily infected trees might exhibit reduced (radial incremental) growth, analogous to responses in other tree species exposed to dispersal-limited canopy parasites such as dwarf mistletoes. Additionally the hypothesis that mature trees and understory saplings will show differential responses to galling due to dissimilar requirements is tested (Boege and Marquis 2005), both in terms of the distribution and abundance of galls and in gas-exchange processes. In Chapter 4 I address the spatial distribution of *V. aceriscrumena* in the context of a suite of biotic and abiotic stand variables at the scale of a 13.5 ha mapped forest stand. Here I test the hypothesis that gall densities are strongly correlated with host densities as proposed by the Resource Concentration Hypothesis (RCH; Root 1973) which states that herbivore densities are expected to be higher in patches of higher host densities. Further the association of *V.*
aceriscrumena gall densities with forest stand variables (species diversity, overall stem density, other tree species) and edaphic factors (slopes, aspects and elevations) is examined. Hence the role of V. aceriscrumena as a host-specific parasite within the context of tree species composition and diversity is explored. Finally in Chapter 5 I summarize my main findings and discuss the ecological and applied significance of this work.

1.5 THE ACER-VASATES STUDY SYSTEM

1.5.1 The host - Acer saccharum Marsh.: 

Acer saccharum is a shade-tolerant deciduous hardwood species that is often dominant in forest communities across its range in eastern North America. In central Ontario, sugar maple grows in the presence of several other trees, including yellow birch (Betula alleghaniensis Britton), American beech (Fagus grandifolia Ehrh.), white ash (Fraxinus americana L.), red maple (Acer rubrum L.), red oak (Quercus rubra L.), black cherry (Prunus serotina Ehrh.), Eastern white pine (Pinus strobus L.), and Eastern hemlock (Tsuga canadensis (L.) Carr.). Invertebrate natural enemies that attack sugar maple foliage in the study area include leaf rollers, leaf skeletonizers, miners, leaf cutters and gall midges (Thomas et al. 2009). Acer saccharum is a valuable commercial species, both as a source of lumber and as the primary source species in the maple sugar industry.

1.5.2 The gall inducer - Vasates aceriscrumena (Riley):

The maple spindle gall mite (V. aceriscrumena) belongs to the family Eriophydae within the superfamily Eriophyoidae (Subclass Acari; Order Acariformes) that comprises over 1000 species of gall inducers. This mite is host-specific, inducing galls only on leaves of
sugar maple. Galls are formed on the upper surface of leaves, while the ostiole (exit hole) is on the underside. Prior mention of the gall-forming habit of this mite on maple is found in two books: 1) Jeppson et al. (1975) and 2) Keifer et al. (1982). Jeppson et al. (1975) briefly describe differences in morphology between *V. aceriscrumena* and a closely related species *Vasates quadripedes* (Shimer) that forms galls on leaves of red (*Acer rubrum* L.) and silver maple (*Acer saccharinum* L.). Keifer et al. (1982) provide a brief description of the mite and the appearance of galls, but wrongly associate ‘tufts of unicellular hair’ with the exit holes of SGM galls.

1.6 DESCRIPTION OF STUDY AREA

All fieldwork was conducted within the Haliburton Forest and Wildlife Reserve Ltd., a 26,700-ha privately owned forest in the Haliburton Highlands of central Ontario (45°15’ N, 78°35’ W). The hardwood forest is dominated by *Acer saccharum* and is also comprised of associated forest species such as American beech (*Fagus grandifolia* Ehrh.), yellow birch (*Betula alleghaniensis* Britton) and hemlock (*Tsuga canadensis* (L.) Carr.).

1.6.1 Study Site 1

All fieldwork related to mite life history (Chapter 2) and physiological impacts (Chapter 3) were conducted in an uneven-aged forest stand dominated by *A. saccharum* (Figure 1.3A). Access trails that passed through the forest at this site enabled me to make use of a mobile canopy lift (Scanlift SL240; Kesla Oyj, Finland; Figure 1.2) to access maple canopies. The maximum working height of the canopy lift is around 24 m, just above the tallest maple canopies at this site.
1.6.2 Study Site 2

Fieldwork related to mite spatial distribution (Chapter 4) was conducted within a recently established forest mega-plot (13.5 ha) within Haliburton Forest. The plot is situated near the west-central shoreline of a lake within the forest, and is part of the mega-plot system established by the Center for Tropical Forest Science (CTFS). The site supports mixed hardwood forest of the Great Lakes - St. Lawrence forest type. Tree species include sugar maple (*Acer saccharum* Marsh), yellow birch (*Betula alleghaniensis* Britton), American beech (*Fagus grandifolia* Ehrh.), white ash (*Fraxinus americana* L.), red maple (*Acer rubrum* L.), red oak (*Quercus rubra* L.), black cherry (*Prunus serotina* Ehrh.), Eastern white pine (*Pinus strobus* L.), and Eastern hemlock (*Tsuga canadensis* (L.) Carr.). All trees >1 cm dbh (diameter at breast height) have been identified, geo-referenced and assigned a permanent id as part of the first CTFS census, conducted prior to my study. In total, there are 7211 stems ≥ 10 cm dbh in the entire plot. Sugar maple is the dominant species here with 2545 stems comprising 35.3% of stems ≥ 10 cm dbh. The plot is divided into 367 20x20 metre grids with permanent grid-posts marking the Northwest corner of each 20m² quadrat (Figure 1.3A,B).
1.7 Figures

Figure 1.1 *Vasates aceriscrumena* galls on *Acer saccharum* (sugar maple) leaves in Haliburton Forest, Ontario. (A) Early season galls (induced in May) appear as tiny red protuberances on newly flushed leaves in the canopy. (B) Fully developed galls typically exhibit a characteristic ‘spindle-like’ appearance on mature leaves later in the season. (Photo © Rajit Patankar).
Figure 1.2  The mobile elevating platform (MEP; Scanlift SL240) in operation within a sugar maple stand in Haliburton Forest, Ontario. The use of this MEP (with a maximum height of 24 m) enabled data collection on *V. aceriscrumena* life history, impacts and distributions from remote locations in the canopy. (Photo © Rajit Patankar).
Figure 1.3  A) The two study sites in Haliburton Forest, Ontario. B) Map highlighting the 8.8 ha study plot used for the SGM gall density study (Chapter 4), part of the 13.5 ha Haliburton Forest mega-plot. The mega-plot is bordered by lake margins on two sides. Access to the plot is via canoe.
Figure 1.4  Transverse sections of Vasates aceriscrumena (SGM) galls. A-D: Parenchymatic galls from the upper canopy of a mature sugar maple tree. A) General view. B) Mites in the inner chamber of the gall. C) Cortex. D) Vascular bundle. Notice undifferentiated tissue surrounding the vascular bundles. S= sclerenchyma; p= phloem; x= xylem. Bars: A, B = 50 µm; C,D = 20 µm. Reproduced with kind permission from, and is part of a collaborative study with, professor Rosy Mary dos santos Isias (Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.)
1.8 REFERENCES


CHAPTER 2: THE SEASONAL PHENOLOGY OF *VASATES ACERISCRUMENA* AND GALL-INVADING PREDATOR (TARSONEMIDAE: *TARSONEMUS SP.*) IN A SUGAR MAPLE CANOPY
2.1 ABSTRACT

Eriophyoid mites (Acari: Eriophyoidea) are among the most ubiquitous gall-inducing arthropods on a broad diversity of plants, but their life histories remain poorly studied, especially in natural habitats such as forests. We examined the seasonal phenology of a leaf-galling eriophyoid mite, *Vasates aceriscrumena* Riley, or the maple spindle gall mite, in naturally occurring stands of sugar maple, *Acer saccharum* (Marsh), in south-central Ontario in 2007 and 2008. Galls are first induced in the spring (mid May) and are devoid of mites by late August. SGM appears to be a bivoltine species with overwintering, deutogyne females that initiate galls in spring and give rise to an F1 generation of protogyne (primary) females, and then an F2 generation of future overwintering deutogyne females. In addition, a gall-invading mite, *Tarsonemus* sp. (Acari: Tarsonemidae) was discovered in ~ 40% of SGM galls examined. As much as 95.4% of galls in 2007 (124/130) and 97.4% in 2008 (37/38) that contained tarsonemid larvae did not contain SGM eggs suggesting that these juveniles feed opportunistically on SGM eggs. Gall ostiole morphology (‘open’ versus ‘closed’ entrances) appeared to influence both SGM and *Tarsonemus* densities within galls. It is unknown what the adult tarsonemid feeds on, but this species is likely an important regulator of SGM populations.
2.2 INTRODUCTION

Gall-inducing arthropods are specialized parasites that have evolved intimate associations with their hosts. Gall-inducing mites (Acari: Eriophyoidea) in particular, are highly host-specific and are known to have dramatic impacts on host plants. Among the ca. 4000 described species of eriophyid mites that occur worldwide, over a quarter of them induce galls (including erinea) on the leaves, buds, twigs, or flowers of their host plant (Jeppson et al. 1975, Oldfield 2005). While the role of gall-inducing eriophyid mites as disruptors of plant growth and physiology (such as gas-exchange processes) (Anderson and Miznell 1987, Larsson 1998), and as plant virus vectors (Oldfield and Proeseler 1996) have been documented to some extent, studies on the life history of these mites remain scarce outside agricultural and horticultural systems. Recently eriophyid life histories have received attention in the field of invasive plant management (Ozman and Goolsby 2005). Eriophyids induce galls on a variety of tree species in tropical and temperate forests, but their minute size, complex taxonomy, and the remote locations of galls in forest canopies are likely reasons for the dearth of life history examinations in natural settings.

Here we study the seasonal phenology of a commonly occurring gall mite on canopies of its dominant deciduous host, sugar maple (Acer saccharum Marsh), in a temperate mixed forest. Acer saccharum is inhabited by six species of eriophyoids, which are mostly confined to that host (Baker et al. 1996, Amrine et al. 2003). Among those, Vasates aceriscrumen (Riley & Vasey), the maple spindle gall mite (hereafter SGM), is a ubiquitous species on sugar maple, at least in certain regions of the host’s geographic distribution. SGM induces ‘spindle’ or ‘finger’ galls on the leaves of its host (Jeppson et
al. 1975b) and is likely the single most common ‘macroscopic’ animal present in sugar maple canopies in eastern Canada: one typical mature tree contains $10^4$-$10^5$ SGM galls (Thomas et al. 2010). SGM individuals show preference for the upper canopy (>20 m) leaves of older trees (>30 cm diameter) and more importantly, cause drastic declines (up to 50%) in photosynthetic capacity in the leaves of these trees, which likely causes concomitant reductions in radial tree growth (Patankar et al. in review). However, in spite of its ubiquity and profound impact on the performance of sugar maple – a regionally dominant tree and a valuable timber species – the biology of this mite and of its natural enemies is poorly known.

Given the impacts of SGM on whole-tree growth of sugar maple, a better understanding of SGM life history would help elucidate the relationship between SGM and sugar maple’s growth, yield, and physiological response to variations in mite galling intensity through time or space. We asked the following questions with respect to SGM phenology during the growing season: 1) Does *V. aceriscrumena* show a bivoltine life history with overwintering (deutogyne) and primary (protogyne) females present in galls? (2) What is the timing of life-history events of the species (gall formation, egg-laying, etc)? (3) What are the (average and peak) densities of various SGM life stages? (4) Are there other arthropods that commonly occur within SGM galls, and if so, what role do they play (competitors, natural enemies, or another role) in relation to *V. aceriscrumena*? Using a mobile elevating platform, we address these four questions related to SGM phenology in remote canopies of its host *A. saccharum*. 
2.3 METHODS

2.3.1 Study site and canopy access

The study site was located in the Haliburton Forest and Wildlife Reserve in Ontario, Canada (45°13’ N 78°35’ W), where *Acer saccharum* accounts for ~60% of tree basal area. We accessed tree canopies using a mobile elevating work platform (Scanlift 240, Kesla Oyj, Kesälahti, Finland; Figure 2.1A) that enabled us to sample up to 24 m high (slightly above the tallest sugar maples in the area). Galled leaves were collected at least once weekly (but up to three times a week initially) from when the first galls began to develop in Spring (around mid May) up until galls were completely devoid of mites (mid August).

2.3.2 SGM gall morphology, development and frequency of formation

To determine temporal development of galls, we measured gall dimensions (height and width in mm) in a subset of galls (n = 298) collected across the summer (18 June - 17 August) in 2007. We detected noticeable variation in gall entrances and hence scored ostiole (gall entrance) condition on the above galls to examine whether or not this correlated with the presence of inquilines or predators within galls. Ostioles were of two discrete types: cavernous, easily accessible gall entrances were scored as ‘open’ and entrances covered by gall/leaf tissue were scored as ‘closed’ (Figure 2.9). To determine whether or not new galls were initiated later in the summer, 39 galled and 25 un-galled canopy leaves (at a height ~ 20 m) from four mature trees were randomly selected and tagged on 19 May 2007 and gall development was followed until galls were devoid of mites (18 August 2007).
2.3.3 SGM phenology

Leaves were sampled from the canopy and galls were then cut out from sampled leaves and dissected on the same day or stored at ~4°C until dissection within two days. We found that the most efficient way for counting mites was to extract entire galls along with the surrounding leaf tissue and then to adhere galls horizontally onto double-sided foam tape. This enabled us to make a single longitudinal incision, thereby causing minimal damage to the contents (Figure 2.11). For each sampling date, galls were scored for founding adults, eggs, immatures (larvae or nymphs), new deutogynes and for the presence of any other arthropods, under a dissecting stereoscope (Wild, Heerbrugg, Switzerland) with an additional magnification of 1.5X objective lens. It was difficult or impossible to distinguish larvae from nymphs under a dissecting scope and hence these were scored together as ‘immatures’. Founding female adults (deutogynes that induced galls in Spring) were easily distinguishable from other stages based on external appearance and location within the gall: founding females were larger, distinctly orange-brown and were usually found at the apical tip of the gall chamber. Protogynes females were larger but had similar colour as the immature stages (pale white). New deutogynes (F2 generation) were similar to founding deutogynes and arrived later in the summer.

In 2007, leaves were collected from 19 May to 18 August (at least once a week) from two mature sugar maple trees approximately 15 m apart. To test for variation between years, in space and across host ontogenetic stages, leaves were randomly collected from 12 May to 29 August 2008 from several understory saplings and trees of varying heights and sizes. For a given date, we calculated mean number of founding adults, eggs, immatures and new (F2) deutogyne adults per gall.
2.3.4 Occurrence and phenology of a gall-invading tarsonemid mite

In 2007 we discovered a frequently occurring mite within SGM galls identified as *Tarsonemus* sp. (Tarsonemidae). Its phenology was studied in 2007 and densities were estimated similar to SGM densities (see below). We excluded phenology from 2008 due to the haphazard nature of our sampling, since different maple individuals were sampled for SGM galls on different dates, and often we found no tarsonemids in galls. This was likely due to their absence from leaves of that particular host. This led to large gaps in tarsonemid phenology. Nevertheless, total numbers of tarsonemid eggs, larvae and adults were scored from both years.

2.3.5 Statistical analyses of SGM and Tarsonemid phenology

Peak densities in eggs, immatures, adults and progeny were estimated by fitting a smoothing spline function to the SGM (2007 and 2008) and Tarsonemid (2008) phenology data (‘fields’ package in R (version 2.11.1), The R Foundation for Statistical Computing). Cubic smoothing splines provide a nonparametric interpolation of univariate data based on a flexible smoothing parameter known as $\lambda$ (lambda). Controlling the value of $\lambda$ enables one to control between the ‘smoothness’ of the resulting curve and the goodness-of-fit to the data (see Eubank 1999). We performed non-parametric analyses (Kruskal-Wallis test) and Dunn’s multiple comparison tests to check for differences in median numbers of life-stages between years and locations.
2.4 RESULTS

2.4.1 SGM gall morphology and development

SGM galls appeared at the beginning of leaf flush, in mid-May. They began as nascent red protuberances on the adaxial surface of leaves, and attained their maximum height within approximately 15-17 days. In 2007, minute galls were already present when the canopy was first sampled on 19 May. In 2008, galls were first observed on flushing leaves from understory saplings on 12 May. No additional galls developed on the 39 galled and 25 ungalled leaves during the summer of 2007. Incipient galls contained a single adult female (deutogyne) (Figure 2.2a) but a few galls were empty and appeared to have been abandoned after initiation. Height of fully developed galls measured 2–5 mm (average =2.81 mm ± 0.98), and gall height was positively correlated with gall content: larger galls contained more eggs and immatures (Spearman’s $\rho = 0.487$, $n = 124$, $P < 0.001$, Figure 2.3). Fully developed galls either remained red through the season or, on some leaves, turned yellowish-green. Gall ostiole type had an effect on SGM populations with mean SGM eggs and immatures significantly lower in galls with ‘open’ ostioles than ‘closed’ ostioles. (Mann-Whitney $U_{\text{eggs}} = 5030$, $P < 0.001$; $U_{\text{immatures}} = 4471$, $P < 0.001$, Figure 2.4a).

2.4.2 SGM Phenology

SGM eggs (circular, translucent; Figure 2.2c) were first observed on 19 May 2007 and 22 May 2008, and based on the fitted splines, egg densities peaked on 21 June 2007 and 7 July 2008 (Figure 2.5b, f). Immatures (Figure 2.2d) were first observed 19 days after eggs first appeared (7 June 2007 and 10 June 2008) and peak immature (larval + nymphal) density was reached on 12 July 2007 and 14 July 2008 (Figure 2.5c, g). New
overwintering deutogynes arrived in July and peaked on 10 August 2007 and 5 August 2008 (Figure 2.5d, h). These mites resembled the founding females in appearance and colour (orange-brown). However, by the time these new adults emerged, most founding females were found dead and shivered up at the apex of galls. Hence F2 deutogynes were easily distinguishable from founding females in the same galls. Founding female adults were present in galls at relatively low levels (i.e. not all galls had females) throughout the season but by mid summer (11 and 8 July 2007 and 2008 respectively), all founding females in galls were dead (Figure 2.5a, e). We detected the presence of ‘protogynes'(primary female form), initially scored as ‘big’ larvae (Figure 2.2b), in galls in mid June using scanning electron microscopy (additional SEM photographs of protogynes and deutogynes in Appendix A). The presence of protogynes points to the likelihood of males in this species and we suspect their presence among the protogynes, but we did not actively look for them. Interestingly, during the period when F2 adults were found (mid July to mid August), large aggregations were found in a small proportion of galls: in 2007, ~ 95% (561 / 589) of them occurred in 2 of 11 galls, and in 2008, ~ 61% (450 / 733) occurred in 3 of 76 galls.

The average numbers (per gall) of the various life stages differed between years and between locations (trees versus saplings in 2008). Post-hoc Dunn’s multiple comparison tests revealed that the number of SGM eggs was significantly lower in 2007 when compared to 2008 saplings but not in comparison to 2008 trees; there was no significant difference between 2008 saplings and trees (Kruskal-Wallis K = 6.69, P = 0.0353). Larvae were significantly lower in 2007 when compared to trees and saplings from 2008; as with eggs, there was no significant difference between 2008 saplings and
trees (Kruskal-Wallis K = 51.88, P < 0.001). SGM larvae were similarly lower in 2007 compared to 2008 (regardless of location) and there was no significant difference between 2008 trees and saplings (Kruskal-Wallis K = 179.60, P < 0.001). Average densities of SGM in 2007 are summarized in Table 2.1.

In both years emerging F2 deutogyne adults were observed exiting galls on several occasions in the Fall and crawling in two distinct directions, either: a) towards the edge of a leaf surface where they aggregated in groups (20–50 mites) and engaged in ‘waving’ behavior in an apparent effort to be dispersed by local wind events or b) moving along the midrib and secondary leaf veins towards the branch via the petiole (Michalska et al. 2010, Sabelis and Bruin 1996).

2.4.3 Occurrence, phenology and role of *Tarsonemus* sp.

The tarsenemid mite (Figure 2.6) occurred in 40% (351/887) of galls scored in 2007 and 42.5% (133 / 313) in 2008. It was common to find multiple female adults in a single gall early in the season. Mites entered the galls through ostioles during gall formation and laid opaque, ovoid eggs. Smoothing splines revealed that there are three marked peaks in egg densities and larvae in 2007 suggesting multiple, rapid generations (Figure 2.7a,b). Larvae were first observed on 31 May, 12 days after eggs were first detected in galls. Eggs developed rapidly, and larvae emerged in galls one week before the emergence of SGM larvae. The time intervals between tarsenemid egg peaks ranged between 15-29 days and ranged from 20-25 days between larval peaks. No apparent peaks were seen in adults until later in the season (19 July onwards) when adults increased to an average maximum of ~25 adults per gall. No adults were found in galls when we last sampled for SGM on 17
August in 2007, but in 2008 adults were found until 29 August, the last day of SGM sampling. Average densities of the various tarsonemid life stages are summarized in Table 2.2. In both years, we found a strong relationship between the presence of tarsonemid larvae in galls and the absence of host eggs in both years (2007: Mann-Whitney U = 1394, \( P < 0.001 \); 2008: \( U = 25, P < 0.001 \); Figure 2.8).

SGM eggs were present in only 6.0% of galls in 2007 (8/130) and 2.6% in 2008 (1/38) that contained tarsonemid larvae vs. in 95.5% (273/286 in 2007) and 100% (115 galls in 2008) of early season galls without tarsonemid larvae. In contrast, SGM eggs were present in 20.0% (21/110) of early-season galls (i.e. before tarsonemid larvae appeared) with tarsonemid adults vs. 30.5% (51/167) of galls without.

SGM gall ostiole condition influenced the number and frequency of tarsonemids in galls. The number of tarsonemid adults, eggs and larvae was significantly higher in galls with open ostioles vs. closed ostioles (Mann-Whitney \( U_{\text{adults}} = 8717, P = 0.0023 \); \( U_{\text{eggs}} = 8391, P < 0.001 \); \( U_{\text{larvae}} = 8430, P < 0.001 \); Figure 2.3b); 25.3% (43/170) of galls with open ostioles contained adults compared to only 5.5% (7/128) of galls with closed ostioles. Additionally, 27.6% (47/170) of ‘open’ galls contained tarsonemid larvae compared to 5.5% (7/128) of ‘closed’ galls.

2.5 DISCUSSION

In temperate deciduous systems, eriophyoid mite galls are initiated when overwintering females (known as deutogynes) appear from under hibernaria (bud scales, bark etc.) to feed on new leaves as they begin to flush in spring. SGM galls are similarly initiated by
deutogynes on emerging sugar maple leaves. In a review of various known forms of eriophyid life cycles, Manson and Oldfield (1996) note that deuterogeny (i.e. the presence of secondary, overwintering females) occurs in deciduous hosts as a survival strategy in order to counteract leaf loss; ‘impregnated’ deutogynes undergo diapause in buds (or similar refugia) during winter before emerging in the spring to feed and oviposit in galls. Deutogynes are usually found in addition to primary female forms (protogynes) and males, however, exceptions have been found where only deutogynes and males exist (Oldfield 1969). In the case of SGM, we detected deutogynes in early season galls and protogynes later in the season (late June; Figures 2.1a and 2.1b) confirming the presence of two female morphs. The few studies on galling mites that have looked at sex ratios in the field have found significantly higher numbers of females (84-95 %) than males (Sabelis and Bruin 1996); however, we have yet to detect males in SGM galls. The presence of the primary protogyne suggests that males are also present, but probably exist in very small numbers compared to females.

2.5.1 SGM gall morphology
Galls attained their maximum height within 15-17 days after initiation and there was significant variation in height among fully developed galls, which correlated positively with gall content (SGM eggs and immatures). Similar correlations were observed in galls induced by Aceria lantanae Cook on Lantana camara L. leaves. (Rosy Mary dos Santos Isaias, personal communication). As previously noted by Keifer (1982), SGM galls strongly vary in colour. It is unclear whether this variation is due to differential responses of host leaves to galling or due to SGM itself. There are several possible reasons for the expression of non-green pigments (e.g. red, yellow) in galls. First, such pigments could
serve a protective role by preventing photo-inhibition and photo-oxidation of gall tissue at high light levels in the canopy (Inbar et al. 2010). Alternatively, yellow or red pigmentation could correspond with elevated levels of host chemical defenses (Schaefer and Rolshausen 2006).

The variation in ostiole morphology had highly significant consequences for SGM populations. Galls with ‘open’ entrances had significantly fewer SGM eggs and immatures compared to galls with closed entrances. This is likely due to the susceptibility of ‘open’ galls to tarsonemid mite invasion (see below). However, as with gall colouration, it is unclear as to what factors are responsible for differences in ostiole morphology. Nevertheless, this variation in morphology represents a fascinating aspect of gall-inducer biology and warrants further examination to see how strong an effect gall ostioles might have on gall-inducing populations, either directly or indirectly.

2.5.2 Phenology of SGM

The appearance of protogynes early in the season (mid June) indicates that SGM follows a bivoltine life cycle. While peaks in numbers of immatures and F2 progeny are consistent between years (irrespective of whether phenology was scored from the same locations or from haphazard sampling of trees and understory saplings), there is a difference in peak egg emergence between years. In 2008, egg peak (7 July) was much closer to peak in the number of immatures (14 July) than in 2007 (21 June and 12 July for eggs and immatures respectively) resulting in a gap of only 7 days between egg and immature peaks in 2008. The reason for this observed difference is unclear, but it is possible that actual egg peaks might have been missed during either sampling year. The bivoltine life cycle observed indicates a slow development compared to other multivoltine species on evergreen hosts,
and is similar to that of several other eriophyoids inducing galls on deciduous hosts (Oldfield 1969, Vaneckova-Skuhrava 1996). We suspect that protogynes (F1) that appear in summer are either fertilized by males appearing with them or produce males parthenogenetically (Helle and Wysoki 1983) and then mate with male offspring to produce overwintering deutogynes (F2) (Manson and Oldfield 1996). F2 deutogynes appearing in late summer (mid July - August) might then either be fertilized by male spermatophores (to produce protogynes via stored sperm) or remain unfertilized (to produce males parthenogenetically) the following year, similar to other eriophyoids with deuterogeny (Oldfield and Michalska 1996, Oldfield and Newell 1973a,b).

Several aspects relating to phenology remain unanswered of which we highlight ones that warrant immediate investigation. First, the presence of ‘primary’ protogynes implies that males are present in this species as well, as the role of protogynes is hard to define in the absence of males. This needs to be ascertained by specifically searching for males. Leading from this, it would also be interesting to know what proportion of deutogynes arriving at the end of summer are fertilized by males or overwinter as unfertilized adults. Also unclear is the proportion of protogynes in galls. However, identifying and confirming the presence of protogynes in galls (using SEM) proved to be fairly challenging and time-consuming, and therefore determining such ratios proved beyond the scope of the present study. This will have to be examined in more detail. Nevertheless, we feel this paper provides a starting point for such life history work.

Our results indicate that, on average, the numbers of eggs, immatures and F2 deutogynes per gall were higher in 2008 than in 2007. Because sampling from various locations (i.e. different trees and saplings in 2008) can lead to skewed estimates of
fecundity, we avoided calculating fecundity for 2008; estimates derived from the 2007 sampling method are probably more reliable (as galls were collected from the same locations over the study period), at least for eggs and immatures. Field estimates of F2 deutogynes are less likely to be reliable, as deutogynes tend to disperse soon after emerging. As such, several possible interacting factors may contribute to variations in deutogyne survivorship, including dispersal, mating behaviour, and mortality due to pathogens and predators. It is possible that the swift dispersal of deutogynes out of galls to seek shelter has masked actual survivorship. However, our data suggest that other factors are also involved, because galls tended to have either several or no new adults in August. It is possible that F2 deutogynes and males aggregate within a few selected galls where the females pick up spermatophores deposited by males before dispersing to hibernaria (Oldfield and Newell 1973b). This insemination prior to overwintering is common in species with overwintering deutogynes (Manson and Oldfield 1996). Finally, in addition to dispersal and aggregation, mortality due to pathogens might be an equally important factor that contributes to low F2 survival. We observed the presence of fungi in many galls. Most of these galls contained one or more dead SGM individuals, in contrast with galls lacking fungi where carcasses were rare (Patankar, pers. observation). Pathogenic fungi have been observed to cause serious mortality in eriophyids species (Leatherdale 1965), and may have contributed to losses of entire broods of SGM, producing strong contrast in populations among galls.

While we provide some field-based estimates of life history parameters for SGM, further rearing experiments in controlled environments are required to accurately estimate intrinsic growth rates, mortality, survivorship and other relevant population parameters.
Based on the observed phenology and confirmed life stages of SGM, the hypothesized life cycle of this mite is described in Figure 2.10.

2.5.3 Predation by tarsonemid mites

SGM galls appear to be one of, if not the main habitat, of an undescribed species of *Tarsonemus*. It appears that the ability to occupy galls is, at least in part, dependent on the type of SGM gall entrance; galls with open entrances are, in particular, susceptible to invasion by this species. About 25% (43/170) of galls with ‘open’ ostioles were inhabited by tarsonemid adults versus 5.5% (7/128) of galls with ‘closed’ ostioles. Based on our observations, it is reasonable to conjecture that other small mites such as tarsonemids take advantage of open gall entrances early in the season. Alternatively, galls could be prised open by tarsonemids in order to gain access. However, ‘open’ galls do not exhibit visible chewing damage (see Figure 2.9). This mite inhabited ~ 40% of SGM galls that we dissected. While it is unclear what the adult stage is feeding on (SGM eggs appear to survive, albeit in low numbers, in the presence of tarsonemid adults), the larvae of *Tarsonemus* sp. are seemingly predators of SGM eggs, as suggested by the near absence of SGM eggs in the presence of *Tarsonemus* larvae. Additionally, while SGM and tarsonemid eggs appear in galls at the same time, hatching of *Tarsonemus* eggs earlier than SGM eggs might enable them to feed on (as yet un-hatched) SGM eggs. So, while phytoseiid mites and other predators wandering on the foliage may inflict serious mortality to SGM populations during migration between galls or to overwintering sites (Sabelis 1996), this tarsonemid species may be the main mortality factor within galls. However, one question remains: what happens when SGM eggs run out? If *Tarsonemus* larvae feed primarily on eriophyid eggs as we suspect, then the newly formed adults must
leave depleted galls in search of new, unexploited SGM galls, or other microhabitats
sheltering eriophyids, e.g. erinea, induced on sugar maple by two *Aceria* species (Baker et
al. 1996). Hence it is likely that this species is simply opportunistic (i.e. facultative) in
utilizing SGM galls as a source of food and shelter, but is not entirely dependent on them.

Tightly enclosed galls with narrow openings like those induced by *Vasates aceriscrumena*
provide protection against desiccation, and more importantly against the
majority of predators (Sabelis and Bruin 1996), but may be vulnerable to the smallest mite
intruders. Although it has not been observed, the adults of some tarsonemids, tydeids,
stigmaeids, as well as immatures of phytoseiids could possibly penetrate eriophyid galls
and prey on their eggs (Perring et al. 1996, Sabelis 1996, Thistlewood et al. 1996, Oldfield
2002). A recent field study by Kawashima and Amano (2004) found that galls induced by
an *Acalitus* sp. (Acari: Eriophyoidae) in Japan were frequently occupied by the phytoseiid
mite *Typhlodromus vulgaris* (Ehara) which appeared to prey on the host species within
galls. Among tarsonemids, at least three species of *Dendroptus* species are known to
invade eriophyid galls (Beer 1963). While Beer thought *Dendroptus* inquilines were driving
their eriophyid hosts out of their own galls via disturbance in the galls, Lindquist and
Smiley (1978) suspected that they were preying, at least occasionally, on eriophyid eggs.
Other tarsonemids are free-living predators of arthropod eggs. The larvae and adults of
*Acaronemus destructor* (Smiley and Landwehr 1976) feed on tenuipalpid and tetranychid
mite eggs on pine trees in California, and at least another species of *Acaronemus* appear to
do the same in Italy (Smiley and Landwehr 1976; Lindquist 1986). *Tarsonemus praedatorius*
(Lin) prey on tetranychid eggs on bamboo (Lin et al. 2002) and *Dendroptus suskii*
Sharonov & Livshits feed on tydeid eggs (Mitrofanov et al. 1986). Other
tarsonemids (*Tarsonemus* and *Dendroptus* spp.) have also been reported as predators of post-egg stages of eriophyid mites and phylloxerids (Forneck et al. 1998, Villanueva et al. 1996). While some predation by adult *Tarsonemus* may occur, we suspect that it is mainly the larval tarsonemids that take advantage of SGM eggs in galls. The unknown *Tarsonemus* sp. found in SGM galls appears to be a major source of mortality of SGM and thus may play an important role in regulating populations of SGM.

2.6 CONCLUSIONS

Gall-inducing eriophyid mites are important host-specific plant parasites in forest communities. Yet, in spite of their known impacts on host trees, rarely has their basic life-history been examined in such ecosystems. The present study is novel in that it examines several aspects relating to the seasonal life history of naturally occurring forest populations of a gall-inducing mite. The phenology of various stages, along with gall formation and development was observed from remote canopy locations, providing important information on the natural rates of development in a ubiquitous forest pest species. Further, we quantitatively examined the role of a frequently occurring (undescribed) tarsonemid mite, and propose that it is likely an opportunistic predator of gall mite eggs. Despite its observational approach, the present paper enhances what is presently known about eriophyid mites and their interactions with natural enemies in forest ecosystems.
2.7 TABLES

Table 2.1 Summary of *Vasates aceriscrumena* life stages from galls dissected from (a) canopy leaves from two mature sugar maple trees (2007) and (b) canopy and understory sapling leaves (2008) in Haliburton Forest, Ontario. Numbers in parentheses = ±1 standard deviation. The different letters represent significant differences in median values (at P = 0.05) based on non-parametric Kruskal-Wallis tests with Dunn’s post-hoc comparisons.

<table>
<thead>
<tr>
<th></th>
<th>2007 canopy</th>
<th>2008 canopy</th>
<th>2008 sapling</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. galls dissected</strong></td>
<td>887</td>
<td>71</td>
<td>242</td>
</tr>
<tr>
<td><strong>Total no. individuals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adults</td>
<td>350</td>
<td>25</td>
<td>123</td>
</tr>
<tr>
<td>eggs</td>
<td>6117</td>
<td>835</td>
<td>3559</td>
</tr>
<tr>
<td>immatures (larvae+nymphs)</td>
<td>3035</td>
<td>468</td>
<td>3314</td>
</tr>
<tr>
<td>new adults (F2)</td>
<td>589</td>
<td>514</td>
<td>259</td>
</tr>
<tr>
<td><strong>Average per gall</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adults</td>
<td>0.4 (0.51)</td>
<td>0.4 (0.48)</td>
<td>0.5 (0.50)</td>
</tr>
<tr>
<td>eggs</td>
<td>6.9 (22.0)a</td>
<td>11.8 (29.0)b</td>
<td>14.7 (37.6)b</td>
</tr>
<tr>
<td>Immatures (larvae+nymphs)</td>
<td>3.6 (12.0)a</td>
<td>6.6 (16.3)b</td>
<td>6.8 (32.3)b</td>
</tr>
<tr>
<td>new adults (F2)</td>
<td>0.7 (14.7)a</td>
<td>7.2 (31.5)b</td>
<td>1.1 (3.4)b</td>
</tr>
</tbody>
</table>
Table 2.2  Summary of life stages of a tarsonemid mite (*Tarsonemus* sp.) found in SGM galls dissected from (a) canopy leaves from two mature sugar maple trees (2007) and (b) canopy and understory sapling leaves (2008) in Haliburton Forest, Ontario. Numbers in parentheses = ±1 standard deviation.

<table>
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<tr>
<th></th>
<th>2007</th>
<th>2008</th>
<th>2008</th>
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</thead>
<tbody>
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<td></td>
<td>canopy</td>
<td>canopy</td>
<td>saplings</td>
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<td>71</td>
<td>242</td>
</tr>
<tr>
<td><strong>Total no. individuals</strong></td>
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<td></td>
</tr>
<tr>
<td>adults</td>
<td>408</td>
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<tr>
<td>eggs</td>
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<td>144</td>
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<tr>
<td>larvae</td>
<td>662</td>
<td>43</td>
<td>255</td>
</tr>
<tr>
<td>new adults (F1)</td>
<td>639</td>
<td>345</td>
<td>215</td>
</tr>
<tr>
<td><strong>Average per gall</strong></td>
<td></td>
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<td></td>
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<tr>
<td>adults</td>
<td>0.46 (0.86)</td>
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<td>0.05 (0.37)</td>
</tr>
<tr>
<td>eggs</td>
<td>1.54 (3.75)</td>
<td>1.2 (4.09)</td>
<td>0.6 (2.44)</td>
</tr>
<tr>
<td>larvae</td>
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<td>0.6 (1.8)</td>
<td>1.05 (4.54)</td>
</tr>
<tr>
<td>new adults (F1)</td>
<td>0.86 (6.74)</td>
<td>4.86 (11.98)</td>
<td>0.88 (3.82)</td>
</tr>
</tbody>
</table>
Figure 2.1  A) Using the mobile elevating platform to access sugar maple leaves galled by *Vasates aceriscrumena* (maple spindle gall mite, SGM) in Haliburton Forest, Ontario. B) Early season (mid-May) SGM galls on flushing sugar maple leaves in the forest understory. C) SGM galls on fully expanded sugar maple canopy leaves later in the summer (also notice damaged, darkened galls).
Figure 2.2  Scanning electron micrographs of some of the life stages of SGM obtained from sugar maple stands from Haliburton Forest, Ontario.  A) the overwintering deutogyne female, B) the primary protogyne female, C) a single translucent, ovoid SGM egg and D) SGM larvae and eggs within a gall.
Figure 2.3 SGM gall morphology in relation to SGM populations from sugar maple canopy leaves in Haliburton Forest, Ontario in 2007. Gall height was positively correlated with the number of eggs+immatures per gall (a) (Spearman’s $\rho = 0.4867$, $n = 124$, $P < 0.001$).
Figure 2.4  SGM gall osiotole condition (‘open’ versus ‘closed’) in relation to a) SGM eggs and immatures (larvae+nymphs) and b) *Tarsonemus* eggs, larvae and adults. Open galls had significantly fewer SGM and significantly more *Tarsonemus* than closed galls. Differences between ‘open’ and ‘closed’ galls are significant at P < 0.001 for all groups except for tarsonemid adults, significant at P = 0.0023.
Figure 2.5  Phenology of SGM from galls on sugar maple during the growing season of the host in Haliburton Forest, Ontario. Data were collected from May to August in 2007 (left panel) and 2008 (right panel). Phenology was scored for the following life-history stages: 1) adult founding females 2) eggs 3) immatures (larvae + nymphs) and 4) new adults (F2). Cubic smoothing splines were fit to the data to detect peaks in these life stages.

(Continued from previous page)
Figure 2.6  Scanning electron micrographs of A) a female and B) male of the *Tarsonemus* sp. obtained from SGM galls in sugar maple stands from Haliburton Forest, Ontario.
Figure 2.7  Phenology of the undescribed *Tarsonemus* sp. found in SGM galls during the growing season of sugar maple in Haliburton Forest, Ontario. Data were collected from May to August in 2007 for the following life history stages: a) eggs b) larvae and c) adults. Cubic smoothing splines were fit to the data to detect peaks in numbers of eggs, larvae and adults.
Figure 2.8  Average number of SGM eggs in galls with (clear bars) and without (shaded bars) tarsonemid larvae from sugar maple canopy leaves in Haliburton Forest, Ontario from 2007 and 2008. Virtually all galls that contained tarsonemid larvae were devoid of SGM eggs (2007: Mann-Whitney U = 1394, P < 0.001; 2008: Mann-Whitney U = 25, P < 0.001).
Figure 2.9  Variation in ostiole morphology among early-season SGM galls on *A. saccharum* leaves: A) a gall with an open entrance and B) a ‘closed’ gall.
Figure 2.10  SGM life cycle with hypothesized alternative pathways based on the observed phenology and (confirmed) life stages of SGM from sugar maple canopies in Haliburton Forest. The presence of the primary ‘protogyne’ indicates the presence of males in the system. Galls are initiated in spring when overwintering deutogynes emerge from hibernaria to induce galls on immature leaves. Primaries (protogynes and males) appear in the early part of summer (mid June) and reproduce to give rise to a new generation of overwintering deutogynes that emerge in mid summer (mid-late July). These new deutogynne adults are either inseminated by spermatophores deposited in galls by males earlier in the summer (to produce diploid females) or overwinter unfertilized to produce haploid males the following year. Overwintering deutogynes exit galls before leaf fall (late August) and either a) migrate back into the main branch via the petiole or b) migrate to the leaf edge where they are then dispersed passively by local wind currents onto other branches/trees. It appears that this species overwinters primarily in the buds of its host.
Figure 2.11 Scanning electron micrograph of a SGM gall dissected in half (longitudinally) showing eggs and immatures inside. Gall ostiole (entrance) is towards the lower right side of the image.
2.9 REFERENCES


CHAPTER 3: GALLING BY *Vasates aceriscrumena* DRIVES DECLINES IN *Acer saccharum* CANOPY PHOTOSYNTHESIS
3.1 **ABSTRACT**

Mature forest canopies sustain an enormous diversity of herbivorous arthropods; however, with the exception of species that exhibit massive outbreaks, canopy arthropods are thought to have relatively little influence on overall forest productivity. Diminutive gall-inducing mites (Acari; Eriophyoidae) are ubiquitous in forest canopies and are almost always highly host specific, but in spite of their pervasive occurrence, the impacts of these obligate parasites on canopy physiology have not been examined. Here we document large declines in photosynthetic capacity (~ 60%), stomatal conductance (~50%) and water use efficiency (~ 30%) in canopy leaves of mature sugar maple (*Acer saccharum*) trees frequently infected by galls of the maple spindle gall mite *Vasates aceriscrumena*. Remarkably, such large impacts occurred at very low levels of galling, with the presence of only a few galls (occupying < 1% of leaf area) compromising gas-exchange across the entire leaf. In contrast to these extreme impacts on leaves of adult trees, galls had no detectible effect on gas-exchange of maple saplings, implying large ontogenetic differences in host tolerance to mite galling. Further, we found a significant negative correlation between canopy tree radial increment growth and levels of mite galling. Increased galling levels and higher physiological susceptibility in older canopy trees suggest that gall-inducing mites may be major drivers of “age-dependent” reductions in physiological performance and growth of older trees.
3.2 INTRODUCTION

Gall-inducing arthropods are intimately associated herbivores that influence host plants in a number of ways. The initiation and maintenance of galls on plant organs (i.e. leaves, stems, fruits, and buds) have been shown to alter host traits such as plant architecture (Larson and Whitham 1997), shoot growth (Vuorisalo et al. 1990) and nutrient allocation (McCrea et al. 1985), in addition to impacting whole-plant growth and survival (Hakkarainen et al. 2005). Similarly, foliar gas-exchange processes (i.e. photosynthesis, stomatal water conductance and water use efficiency) have shown significant modifications, ranging from compensatory increases (Fay et al. 1993, Dorchin et al. 2006) to decreases (Larson 1998, Florentine et al. 2005). Thus the limited information available suggests that impacts of gall formation on gas-exchange processes are inconsistent and likely determined by the type of gall inducer attacking the host plant (Welter 1989).

However, fundamental questions with respect to the influence of gall inducers on their hosts remain unaddressed: Are galling impacts (either positive or negative) on gas-exchange processes restricted to the vicinity of galls or extended across the entire leaf? Assuming that leaves respond to galling, at what level of galling (on a per leaf or per area basis) do gas-exchange processes begin to respond? Questions such as these are important not only for understanding the nature of impacts, but also when attempting to ‘scale up’ effects to the whole tree or plant-level.

The role of gall inducers as drivers of host physiological functioning in forest ecosystems has been the subject of little previous attention. Prior studies on galling arthropod-plant interactions have largely examined impacts of galling on crops, herbaceous plants, shrubs or common species from manicured or agricultural systems;
studies on galling impacts in forests have been limited to understory saplings (Aldea et al. 2006). Although common to ubiquitous in forest canopies (Ribeiro and Basset 2007), little is known about the influence of leaf-galling arthropods on the health and physiology of mature forest trees. Mature trees differ from seedlings and saplings in physiology (Thomas and Winner 2002), growth requirements and defense strategies, and hence can differ in their response to herbivory (Boege and Marquis 2005), but the remote position of gall inducers in the canopy has discouraged detailed examinations into their interactions with host trees.

We addressed the following questions about the interaction between a gall-inducing mite and its host sugar maple (Acer saccharum Marsh) in a temperate mixed deciduous forest stand: 1) What is the impact of mite-galling on gas-exchange processes in canopy leaves? 2) Are the impacts of gall formation restricted to the galled area or do they extend to ungalled portions of infected leaves? 3) Does the response of gas-exchange processes to galling differ through the ontogeny of the host? 4) What is the nature of the galling-photosynthesis relationship? 5) What is the distribution of galls across tree size classes in a given forest stand? 6) Are there any detectable galling impacts beyond the leaf level, i.e. on shoot and whole-tree growth?

3.3 Methods

3.3.1 Study site and canopy access

The study site was located within the Haliburton Forest and Wildlife Reserve in Ontario, Canada (45°13’ N 78°35’ W). Acer saccharum is the dominant species in northern
hardwood forests of eastern North America and accounts for ~60% of tree basal area in the study area. Leaves of sugar maple are galled by the highly host-specific eriophyoid gall mite, *Vasates aceriscrumena* (Riley) (maple spindle gall mite, hereafter SGM) (Jeppson et al. 1975). This mite is likely the single most common macroscopic animal present in sugar maple canopies in the study area: one typical canopy tree contains $10^4$-$10^5$ SGM galls, each of which occupies less than 0.1% of the area of an individual leaf (see below) and houses 10-100 mites. Tree canopies were accessed using a mobile elevating work platform (Scanlift 240, Kesla Oyj, Kesälahti, Finland).

### 3.3.2 Foliar gas exchange

Gas-exchange parameters were measured *in situ* using an infrared gas analyzer (Li-6400 portable photosynthesis system, LI-COR, Lincoln Nebraska, USA) in 10 mature sugar maple trees in 2007, and in 5 additional trees in 2008 (to determine the nature of the photosynthesis-galling intensity relationship). To test for ontogenetic differences in response to galling, we measured similar gas-exchange processes on galled leaves (with comparable gall intensities of 20-50 galls per leaf) in 6 saplings located in treefall gaps. There is evidence – at least from tropical forests – that photosynthetic capacity ($A_{\text{max}}$) is closely related to integrated diurnal carbon gain at the leaf level (Zotz and Winter 1993). Hence we measured photosynthetic capacity ($A_{\text{max}}$), stomatal water conductance ($g_s$), transpiration (E) and instantaneous water use efficiency (WUE, ratio of $A_{\text{max}}$ to E) in galled areas of affected leaves, in gall-free areas of the same leaves (>3 cm from the nearest gall), and in nearby gall-free (control) leaves. We included gall-free areas of affected leaves to observe if galling-impacts extended to the entire leaf or were confined locally to areas surrounding galls. We chose gall-free leaves from the same branches (but
different shoots) as controls, thereby ensuring a similar canopy position and environmental conditions. Gas-exchange measurements and leaf surveys were made at the upper (16-25 m height) and lower (6-12 m) thirds of tree canopies; saplings were located in large gaps in the same area (within 150 m of measured trees). Measurements were made during the months of June to August in 2007 and 2008, on fully expanded leaves, with temperatures of 20-25°C and 50-80% relative humidity (0.5-1.6 KPa vapor pressure difference), between 8:30 - 11:00, to avoid possible midday photosynthetic depression effects. For both galled and gall-free groups, only leaves that were free from other forms of herbivory or pathogenic damage were selected for measurement. We used a one-way ANOVA and two-tailed Student’s t-test respectively to examine differences between control and infected leaves in mature trees and saplings.

3.3.3 Leaf nutrient analyses
A subset of control and galled leaves were collected and dried for carbon and nitrogen analyses. For galled leaves, galls were excised and galls vs. ungalled parts of leaves processed separately. Tissue samples were then crushed in liquid N, and leaf tissue samples were analyzed for differences in carbon and nitrogen content using an ECS 4010 analyzer (Costech Analytical Technologies, Valencia, CA.).

3.3.4 Estimation of galling – photosynthesis relationship
To quantify the relationship between SGM infection and photosynthetic performance, we measured photosynthesis ($A_{max}$) across a range of galling intensities in the canopy. We then log-transformed predictor (number of galls) and response ($A_{max}$) variables, and back-transformed slope and intercept parameters to estimate the non-linear (allometric)
relationship. A model 1 linear regression was employed to examine the relationship between tree size (diameter at breast height; hereafter dbh) and photosynthesis in control and galled leaves.

3.3.5 Galling frequency in relation to host ontogeny and tree growth
To quantify size-dependent patterns of galling, leaves from 45 trees and 14 saplings (located in the understory and tree-fall gaps) were collected randomly using the canopy lift. We collected 200 leaves per tree (100 each from the upper and lower canopy) and 100 leaves per sapling, in addition to size (dbh) and height. We pooled the data from upper and lower canopy leaves, as there was no significant difference in mean galling intensities between the two canopy positions.

To determine if whole tree growth correlated with galling frequency (percentage of galled leaves/tree), partial increment cores including at least 10 of the most recent annual rings were obtained from 30 of the 45 trees. Mounted cores were finely sanded and radial increment measured with a table mounted tree-ring increment measurement system (T.R.I.M # 8505, OMNR, Toronto, Canada) and dissecting scope (Wild M3 Heerbrugg, Switzerland). A linear regression was employed to examine the relationship between galling frequency (percentage of galled leaves/tree) and radial tree growth (mm), averaged over the 2 most recent 5-year periods.

3.3.6 Percentage of leaf area occupied by SGM galls
We estimated the total area occupied by a single gall (assuming that galls approximate a cylinder in geometry) using average height and width values measured from 294 galls (area of 1 gall  = 4.48 mm$^2$, ±1 s.d. = 2.38). Average leaf area from ~ 80 canopy leaves
was 43.52 cm² (±1 s.d. = 8.81). Hence, the percentage of leaf area occupied by a single
gall was 0.011%.

3.3.7 Galling intensity in relation to leaf size and shoot extension

To examine the relationship between galling and leaf size, galling intensity (number of
galls per leaf), leaf area and petiole length were measured from 535 leaves sampled from
seven trees in 2008. Leaf area (cm²) was measured using a leaf area meter (LI-COR LI
3100, Lincoln, Nebraska). Leaf canopy position (upper, middle or lower canopy) was
noted to examine differences in leaf traits across the vertical distribution of leaves.
Nonparametric Spearman rank correlation test was used to test for the relationship
between leaf area and galling intensity as leaf areas of galled and gall-free leaves exhibited
strong departures from normal distributions.

Shoot extension (length in mm) was measured from 46 terminal shoots collected
from eight trees in 2008. Growth (extension) was measured for the last four years using
callipers. To examine effects of galling on shoot extension, we first calculated the
percentage of leaves that were galled and the total number of galls per shoot. Based on the
percentage of galled leaves, shoots were placed into the flowing infection classes: no
infection (0 galled leaves), low (1-25 % galled leaves), medium (26-75% galled leaves),
high (76-100% galled leaves). Shoots were similarly grouped arbitrarily into classes based
on the total number of galls per shoot as follows: control (0 galls), low (1-14 galls), high
(>15 galls). Comparison between galled shoots revealed no differences in shoot extension
using either of the above two parameters and hence data was pooled from all galled
shoots. Two-tailed unpaired Student *t*-tests were used to test for differences in shoot extension between galled and gall-free shoots.

3.4 RESULTS

3.4.1 Foliar gas-exchange

Galled leaves in mature canopy trees (height > 18 m) showed dramatic reductions in photosynthetic capacity ($A_{\text{max}}$; $F_{2,154} = 63.5$, $P < 0.001$, Figure 3.1a), stomatal conductance ($g_s$; $F_{2,154} = 42.16$, $P < 0.001$, Figure 3.1b) and instantaneous water use efficiency (WUE, ratio of $A_{\text{max}}$ to transpiration ($E$); $F_{2,154} = 3.91$, $P < 0.001$, Figure 3.1c), compared to ungalled controls. Un-galled portions of galled leaves of mature canopy trees likewise showed large reductions in $A_{\text{max}}$ and $g_s$ compared to controls, with values similar to those of galled parts of leaves (Figure 3.1). WUE in ungalled portions of galled leaves was lower than in control leaves, but this difference was not significant. Hence, the impact of SGM galling on gas-exchange processes was not restricted just to the vicinity of galls but extended to ungalled portions as well. In contrast, there were no detectable effects of galling on photosynthetic gas-exchange in saplings ($A_{\text{max}}$: $t = 0.143$, d.f. = 40, $P=0.89$; $g_s$: $t = 0.3941$, d.f. = 40, $P=0.6956$; WUE: $t=0.881$, d.f.=40, $P = 0.38$, Figure 3.1), indicating clear ontogenetic differences in response.

3.4.2 Leaf nutrients

Carbon (Trees: $F_{2,78} = 2.52$, $P = 0.0872$; Saplings: $F_{2,76} = 1.7014$, $P = 0.1895$) and C:N ratio (Trees: $F_{2,78} = 2.26$, $P = 0.1109$; Saplings: $F_{2,76} = 0.9287$, $P = 0.3996$) did not differ significantly between galls, surrounding ungalled tissue or control leaves among either
trees or saplings, indicating that SGM galls do not act as strong sinks for photosynthetic assimilates. Nitrogen level (% dry weight) was marginally lower in galls (mean = 1.75%, s.e.m = ± 0.036) than in surrounding ungalled tissue (mean = 1.88%, s.e.m = ± 0.038) or control leaves (mean = 1.89%, s.e.m = ± 0.034) within trees (F$_{2,78}$ = 3.4364, P = 0.0573) but no such difference in nitrogen was detected in saplings (F$_{2,76}$ = 1.2644, P = 0.2884).

3.4.3 Galling – photosynthesis relationship
Leaves showed significant reductions in photosynthesis (>30%) even at low levels of mite galling (< 10 galls per leaf) but continued to photosynthesize at high levels of galling (>100 galls per leaf, Figure 3.2), indicating that SGM-induced galls have considerable impacts even at very low intensities, but also do not completely eliminate net carbon uptake at high galling levels.

3.4.4 Galling frequency in relation to host ontogeny and whole tree growth
Of the 45 mature trees sampled, 41 (91%) were infected with SGM galls, with an average of 40.3% of leaves (across all tree sizes of trees > 20 cm dbh) infected. In contrast, among saplings galling was found on an average of only 4.7% of leaves sampled. Canopy sugar maple trees carried appreciably higher mite loads than saplings with both the percentage of leaves galled (Figure 3.3a) and number of galls per (galled) leaf (Figure 3.3b) being significantly greater.

The increase in galling frequency was accompanied by significant declines in incremental tree growth when the last 5-year growth patterns were examined ($r^2 = 0.22$, F = 8.025, P = 0.008; Figure 3.4a). However, no such significant responses were detected
when the previous 5-year (i.e. 6-10 years back) growth patterns were examined ($r^2 = 0.12$, $F = 3.776, P = 0.063$; Figure 3.4b). Radial increment did not correlate with tree size (range = 16.5 – 44.5 cm dbh) for the 30 trees that were examined ($r^2 = 0.042, F = 1.212, P = 0.28$).

3.4.5 Galling intensity in relation to leaf size and shoot extension

Galling intensity was positively correlated with leaf area (Spearman rho = 0.199, $p < 0.001$, Figure 3.5) when all leaves were analyzed together, suggesting that dispersing mites are more likely to land on larger leaves. Shoot extension (length in mm) did not differ between galled and ungalled shoots when averaged over the last four years ($t = 0.3403$, d.f. = 44, $P = 0.7353$) or when measured for the present year’s growth ($t = 1.099$, d.f. = 44, $P = 0.2776$; Figure 3.6).

3.4.6 Quantifying galling effects on age-related changes and canopy-averaged photosynthesis

The observed increases in galling with tree age (Figure 3.3), and greater effects of galling on photosynthesis observed in mature trees than saplings (Figure 3.1), suggest that galling may in part drive “age-related” changes in leaf gas-exchange. To estimate the magnitude of this effect, we calculated gall-related impacts through ontogeny by estimating the difference in average $A_{max}$ values when an ungalled 20 cm dbh tree either a) remains ungalled or b) becomes galled at 50 cm dbh at average rates of galling. $A_{max}$ in the upper canopy of a 20 cm ungalled tree = 8.26 µmol m$^{-2}$ s$^{-1}$ (from Figure 3.7). Assuming the tree becomes galled by 50 cm dbh, given average incidences of galling (Figure 3.3, ~ 50% galled leaves), the resulting $A_{max}$ will be 4.67 (i.e. average $A_{max}$ of galled (~50%) and ungalled leaves). However, if a tree remains ungalled at 50 cm, the corresponding average
A_{\text{max}} is 5.61 (based on observed values for ungalled leaves from Figure 3.7). The direct galling effects thus account for ~26% of the overall “age-related” decline in A_{\text{max}} across this size range. To estimate the effects of galling at the canopy-level of integration, we estimated a canopy-averaged A_{\text{max}} using leaf data from 33 canopy trees (size range = 14.9 – 67.4 cm dbh; 100 – 200 leaves/tree) that were surveyed for galls in 2007. For each tree, we first calculated mean A_{\text{max}} assuming the tree was completely ungalled based on the A_{\text{max}}-dbh relationship from Figure 3.7 (A_{\text{max}} = 9.988 – 0.8759*dbh, r^2 = 0.6385, P = 0.0174). Using this value, we next calculated A_{\text{max}} for every leaf (galled and ungalled) within each tree using the A_{\text{max}}-galling relationship from Fig. 2 (log A_{\text{max}} = a – 0.263*log_{10}(1 + \text{Galls}), where a = y-intercept at x = 0 galls for a given dbh from Figure 3.7). We then calculated mean A_{\text{max}} per tree based on A_{\text{max}} values from all leaves (galled and ungalled). The average reduction in A_{\text{max}} across the entire stand of trees was ~15.6% (range: 0.5 – 34%, ±1 s.d. = 8.74).

### 3.5 Discussion

Gall induction by maple spindle gall mites caused large reductions in gas-exchange in mature canopy leaves, but had no such impact on saplings. Among leaves of mature trees, A_{\text{max}} decreased by 57% in galled portions and 47% in ungalled portions of gall-infected leaves relative to gall-free leaves. Correspondingly, stomatal conductance (g_s) decreased by 48 % and 47% in the same regions. Instantaneous WUE also decreased with galling but the difference was only significant between gall-free leaves and galled portions of infected leaves. Remarkably, the presence of even a few galls representing <0.5% of leaf
area was enough to significantly reduce $A_{\text{max}}$, $g_s$ and WUE (Figure 3.2). Although there were no negative effects of galling on shoot extension growth, there was a highly significant negative correlation between recent tree radial increment and galling intensity (Figure 3.4a) suggesting that gas-exchange effects strongly impact whole-tree growth.

Galls had comparable levels of carbon to that of surrounding tissue and control leaves and therefore did not appear to act as strong sinks for photoassimilates, unlike certain insect galls (McCrea et al. 1985, Larson and Whitham 1991). This could be due to the fact that eriophyoid mites do not intercept vascular tissues directly during gall induction and the region of mechanical gall initiation is restricted to cell walls of host epidermal cells (Westphal and Manson 1996, Kane et al. 1997). Leaf nitrogen was lower in galls of canopy leaves when compared to surrounding ungalled tissue and control leaves. Although this difference was marginal, this pattern is consistent with results from other plants galled by eriophyoid galling mites (Hartley 1998). Hence, unlike in the case of certain gall-inducing insects, mite galls do not appear to be significant sinks of nitrogen, at least at this scale of examination.

SGM galling had no negative impact on leaf size. Rather, number of galls showed a positive correlation with leaf size. There are two possible explanations for higher gall densities found on large leaves. One is that vigorously growing leaves that attain larger areas in comparison to slow growing ones represent better resources for herbivores and hence are exploited more (plant vigor hypothesis: (Price 1991). However, galling mites are passively dispersed by local wind patterns (Sabelis and Bruin 1996) and hence are unlikely to be able to ‘choose’ more vigorous leaves to settle on. A more likely explanation for higher numbers of galls on larger leaves is simply that within an
individual canopy, mites are more likely to encounter leaves with larger surface areas. Earlier studies examining the relationship between gall densities and shoot growth found positive (Fritz et al. 1987, Fritz et al. 2000) and negative (Willson and O’Dowd 1990) associations. However, galling by SGM appears to have no impact on shoot extension in maple shoots. Similarly, galling by the eriophyoid mite *Aculus tetanothrix* had no impact on shoot lengths in several willow host species (Kuczynski and Skoracka 2005).

Galling levels (percentage of galled leaves and galls per galled leaf) were significantly higher in mature trees than understory saplings. Apart from a handful of studies, little is known about the distribution of gall-inducing arthropods in forests. Factors associated with the upper canopies of large trees, such as leaf sclerophylly (Ribiero and Basset 2007), increased tannin levels, and decreased water and nutrient levels, often represent harsh environments for natural enemies such as predators, parasitoids and fungi, but may be favorable habitats for galling insects (Fernandes and Price 1992, Price et al. 1998). The patterns observed here are similar to prior observations that arthropod gall abundances generally show strong vertical gradients in forest ecosystems (Fonseca et al. 2006), with taller and drier canopies usually carrying higher gall loads compared to moister sub-canopies and understory environments. However, patterns of arthropod gall distribution appear highly varied (Ribiero and Basset 2007), and an often-neglected stage along host ontogeny in these studies is old, senescent trees. We recently examined general patterns of herbivory in *A. saccharum* across a range of tree size classes at Haliburton, Ontario: consistent with the data reported here, spindle gall mites were most abundant in the canopies of old trees (i.e. > 55 cm dbh) and least abundant in
the smallest trees; however the abundance of other galling arthropods did not vary significantly across tree size classes (Thomas et al. 2010).

In addition to leaf-level effects, we detected a significant negative relationship between galling frequency (expressed as percentage of leaves that were galled) and radial growth (Figure 3.4a). Several alternate explanations exist for this observed pattern: a) mites 'choose' to form galls on slow-growing trees; b) slow-growing trees are more susceptible to mite infection and therefore show an accumulation of mite populations and galling levels; c) high galling prevalence is responsible for declines in tree growth. Given the dispersal mechanisms of eriophyoid mites, it is unlikely that mites are able to select hosts. First, mites are poor dispersers that rely on local wind events to carry them to suitable habitats, and thus host-choice would be inhibited by passive dispersal (Sabelis and Bruin 1996). Second, gall mites in temperate systems are able to reestablish and maintain populations on hosts via the presence of a secondary female morph (known as the ‘deutogyne’). Deutogynes exit galls in late summer and return (via the petiole) to branches where they overwinter; they re-appear the following spring to induce galls on newly flushing leaves (Manson and Oldfield 1996). This ensures continued survival of mites on individual hosts. It seems likely that once a suitable tree is discovered and colonized, populations will continue to reestablish and grow in the same tree over time thus resulting in ‘islands’ of high gall density trees surrounded by poorly-galled or gall-free trees (Egan and Ott 2007). Long-term manipulative experiments would be necessary to rigorously distinguish the directionality of causation between slow growth and SGM galling levels. However, the inference that galling causes reduced growth is consistent with: (1) the large observed photosynthetic impacts; and (2) a higher correlation with
recent growth than with earlier growth (Figure 3.4), suggesting cumulative impacts on growth with time.

Peak $A_{\text{max}}$ in $A. \text{saccharum}$ occurs when trees reach a size of $\sim 13$ cm DBH, close to the size at onset of reproduction, and gradually declines as trees increase in size and age (Thomas 2010). We found similar patterns in the gall-free leaves on eight trees from which we collected gas-exchange data; however, $A_{\text{max}}$ on galled leaves was drastically reduced and fell to a uniformly low level across all tree sizes (Figure 3.7). Putting together the data on the increase in galling frequency and number of galls per galled leaf (Figure 3.3) with the impacts on photosynthesis (Figure 3.2), we estimate that the direct effects of galling account for $\sim 26\%$ of the total “age-related” photosynthetic decline (see results above). If we consider the sampled mature trees in this study as a representative population of within a mixed-age forest, galling is estimated to result in a $\sim 16\%$ decline in the average $A_{\text{max}}$ value through the canopy (see results above).

Declines in photosynthetic capacity and growth rates as trees age have largely been attributed to biophysical limitations on physiological processes of large trees. A commonly cited explanation is that large trees experience increased hydraulic limitation with increasing hydraulic pathlength leading to stomatal closure (Ryan et al. 2006) and/or reduced leaf expansion (Woodruff et al. 2004) in upper canopies, resulting in decreased carbon uptake. The present study provides a specific example of a biotic mechanism that leads to a large age-related physiological decline in trees, analogous to biotic mechanisms of ageing common in other systems (Moller and De Lope 1999). Spindle gall mite parasite loads also prompt differential physiological functioning between sapling and mature sugar maple canopies, as predicted by the theory of
“antagonistic pleiotropy” (Williams 1957).

Previous studies measuring indirect impacts of galling on photosynthesis found decreases in photosynthetic efficiency away from the site of gall damage (up to 4 mm), but these differences were marginal (Aldea et al. 2006). One potential mechanism that might explain why infection by low numbers of minute SGM galls could alter leaf-level photosynthesis is stomatal closure to compensate for non-stomatal water loss in and around elongated spindle-shaped galls that visibly disrupt the plant cuticle. Reductions in stomatal conductance do indicate that canopy leaves respond to galling by closing their stomata; WUE also decreased in response to galling, indicating either increased non-stomatal water loss in galled leaves and/or a shift in stomatal regulation. Leaves in the canopies of large forest trees are often under physiological water stress due to hydraulic limitation of water transport (Woodruff et al. 2004, Ryan et al. 2006), and must also cope with high radiant heat loads in the upper canopy. Reduced stomatal conductance in galled leaves will result in decreased latent heat loss, which could further compromise photosynthesis of galled leaves in the upper canopy. In addition to effects mediated by leaf water and energy balance, eriophyoid mites have been found in some cases to transmit viruses (Jeppson et al. 1975), and we cannot exclude disease transmission as a possible mechanism for the observed patterns. Finally, it is also possible that the physiological costs of induced defenses could play an important role (Zangerl et al. 2002). Further studies are required to distinguish among these mechanisms.

Current estimations of global carbon cycles point to middle-aged and old-growth forests as important carbon dioxide sinks due to positive net primary productivity (Luyssaert et al. 2008, Lewis et al. 2009). In temperate forests, productivity periodically
declines due to large-scale insect defoliations (Mattson and Addy 1975a), and recent evidence indicates a shift from carbon sink to carbon source in these systems during outbreaks (Kurz et al. 2008a). However, impacts of “chronically associated” arthropod parasites, such as eriophyoid gall mites, have largely been neglected despite their potentially large influences on forest carbon cycles. The present study shows that gall formation by eriophyoid mites can have large impacts on physiological performance, and that galling incidence is also associated with declines in whole-tree growth in mature temperate forest canopies. Gall formation is thus plausibly a major driver of large-scale forest processes including both age-related declines in tree performance, and patterns of forest growth and net primary productivity.
Figure 3.1  Net photosynthetic capacity $A_{\text{max}}$ (a), net stomatal conductance $g_s$ (b) and instantaneous water-use efficiency WUE, measured as the ratio of $A_{\text{max}}$ to $E$ (transpiration), (c) of leaves or leaf portions in the crowns of sugar maple saplings and trees from Haliburton Forest, Ontario measured for: 1) gall-free (control) leaves, 2) galled portions of leaves and 3) un-galled portions of galled leaves (canopy trees only) affected by the maple spindle gall mite. Different letters indicate significant difference between groups within each category at $P < 0.001$. 
Figure 3.2 Relationship between net photosynthetic capacity \( (A_{\text{max}}) \) and number of galls per leaf from a mature sugar maple canopy in Ontario. \( A_{\text{max}} \) decreased dramatically (from 6.7 to 3.9 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)) in the presence of just a few (<10) galls, but this effect saturated at higher galling intensities. Filled circles represent \( A_{\text{max}} \) averages (±1 s.d.) of binned gall numbers (equal sized bins of N=8 replicates) expressed on a per leaf basis. (Non-linear regression of unbinned data: \( \log_{10} A_{\text{max}} = 0.779 - 0.263^* \log_{10} (1 + \text{Galls}) \), \( r^2 = 0.283, F = 37.25, P < 0.001 \)). Percentage of leaf area occupied by galls is estimated using average height and diameter of galls, and assuming cylindrical geometry. * = Control (gall-free) leaves.
Figure 3.3  Percentage of leaves galled (a) and number of galls per galled leaf (b) in four size categories: 1) saplings (dbh < 10 cm), 2) 10-20 cm dbh 3) 21-30 cm dbh, and 4) 31-55 cm dbh, from a survey of sugar maples in Haliburton Forest, Ontario. Error bars represent +/- 1.00 s.e.m.
Figure 3.4  Relationship between percent galled leaves per tree (expressed as arc-sine transformed proportions) and a) last 5-year and b) previous 5 (6-10) year incremental tree growth from 30 sugar maples in Haliburton Forest, Ontario. Tree growth values are log-transformed incremental growth measurements (mm).
Figure 3.5  The relationship between SGM gall intensity (galls/leaf) and leaf size (area in cm$^2$) from 535 leaves collected from the *A. saccharum* canopy in Haliburton forest. Leaf size and gall intensity were positively correlated (Spearman’s rho = 0.199, p < 0.001).
Figure 3.6  Comparison of growth (length and width in cm) between 46 galled and ungalled *A. saccharum* canopy branchlets from Haliburton Forest, Ontario. Un-paired two-sample t-tests revealed that there was no difference in growth parameters between galled and ungalled shoots when averaged over the last four years ($t = 0.3403$, d.f. = 44, $P = 0.7353$) or when measured for the present year's growth ($t = 1.099$, d.f. = 44, $P = 0.2776$).
Figure 3.7  Photosynthetic capacity ($A_{\text{max}}$) in leaves from eight sugar maple trees in a mature forest canopy in Haliburton Forest, Ontario. While $A_{\text{max}}$ in control leaves (open circles) decreased sharply (from 8 to 6 µmol m$^{-2}$ s$^{-1}$; $r^2 = 0.6385$, $p = 0.008$), $A_{\text{max}}$ in galled leaves (filled diamonds) remained unaltered at significantly lower levels (< 3.0 µmol m$^{-2}$ s$^{-1}$, $r^2 = 0.3355$, $p = 0.1325$) with increasing tree size (measured in cm as diameter at breast height DBH). Error bars represent ± 1 s.d.
3.7 REFERENCES


CHAPTER 4: THE SPATIAL DISTRIBUTION OF *Vasates aceriscrumena* IN A TEMPERATE FOREST PLOT AND ITS ASSOCIATION WITH LOCAL SPECIES DIVERSITY
4.1 ABSTRACT

Gall-inducing arthropods are host-specific plant parasites that characteristically have highly non-random distributions, being strongly aggregated with and among their host plants and trees. However, studies on galling arthropod distributions have so far excluded the influence of inherent spatial structure in relation to host distributions. We examined the spatial distribution of a commonly occurring arthropod, the maple spindle gall mite *Vasates aceriscrumena* (Acari; Eriophyoidae; hereafter SGM) that induces galls on its host sugar maple *Acer saccharum* (a dominant broadleaf species in the region) in an 8.8 ha forest plot. All trees in the plot above 10cm diameter at breast height (dbh) were identified and geo-referenced. Mantel and partial Mantel tests were performed to determine correlations between mite gall densities and several biotic and abiotic descriptors including a) host (maple) stem densities and basal areas, b) overall tree densities c) tree species diversity d) densities of other associated tree species and e) topography (slope, elevation and aspect). We found strong positive associations between gall densities and overall species diversity and overall stem density. This was the case even when controlling for the potential influence of topography within the plot. Host (sugar maple) density and basal area were not strongly correlated with gall density, contrary to expectations. SGM density was positively correlated with plot slopes but had no significant association with either elevations or aspects. Based on its strong association with species diversity, we suggest that SGM, a host-specific, dispersal-limited canopy parasite is, in part, linked with local community dynamics.
4.2 Introduction

Forest canopies worldwide host a variety of natural enemies including parasites, pathogens and herbivores. A large number of these are host-specific and are thought to be responsible, in part, for the maintenance of tree species diversity in both tropical (Janzen 1970, Connell 1971, Wright 2002) and temperate forests (Packer and Clay 2000) through density- and distance-dependent mechanisms (i.e. the Janzen-Connell effect). In temperate forests, familiar examples of host-specific natural enemies include fungal pathogens (Packer and Clay 2000) and parasitic plants such as dwarf mistletoes (Hawksworth and Weins 1996). Dispersal-limited canopy parasites such as dwarf mistletoes are known to have significant negative impacts on hosts, including reductions in growth (Filip et al. 1993) and physiological function such as photosynthetic capacity and water use efficiency (Meinzer et al. 2004).

One group of host-specific canopy parasites that have received relatively little attention, especially within the context of natural forest communities, are gall-inducing arthropods (hereafter GIA). Along with leaf miners, they comprise the most specialized group of phytophagous arthropods due to their extreme host specificity (Gaston et al. 1992, Goncalves-Alvim and Fernandes 2001). Given this high host-specificity, one might predict strong positive relationships between host density and GIA abundance. Several studies examining both species richness and the association of individual species with host and environmental correlates have provided some insight into the mechanisms and patterns of GIA distributions. GIA species richness, for example, has been found to be positively correlated with sclerophyllus vegetation (Price et al. 1998), xeric habitats (Fernandes and Price 1988), plant structural complexity and plant species richness (Cuevas-Reyes et al. 2004a) and negatively correlated with soil nutrient richness (Cuevas-Reyes et al. 2004b). In autecological studies on gall inducers, a range of host and environmental factors that influence gall distributions have been uncovered, though patterns vary depending on the study species. For example, general spatial distribution patterns of galls across the ontogeny of hosts remain unclear, with increasing gall density in some species (Patankar et al. unpubl.) and decreasing gall density among others, with
host tree age (Fonseca et al. 2006, Ribeiro and Basset 2007). Other factors that influence the spatial distribution of GIA within a host species include host size (Thomas et al. 2009), light requirements (Basset 2001, Castellanos et al. 2006), canopy position (Kampichler and Teschner 2002), Thomas et al. 2010), reproductive status (Ishihara et al. 2007) and the susceptibility (and palatability) of individual hosts (Egan and Ott 2007).

To date, most prior spatial studies on GIA distributions have focused primarily on within-plant canopy distributions (Kampichler and Teschner 2002), patterns across ontogenetic stages (Fonseca et al. 2006, Patankar et al. in review) or their position within the context of individual plants (Kuczynski and Skoracka 2005) with an aim to tease out microhabitat preferences. Only a few studies have examined spatial distributions at local spatial scales (Biedermann 2007). GIA are known to shown high host fidelity even to individual plants, in part due to the isolation of gall populations on individuals and the relative rarity of palatable hosts, which can eventually lead to the formation of ‘demes’ or genetically distinct gall-inducer populations in some species (see Egan and Ott 2007). Aggregated, non-random distributions imply spatial structure (autocorrelation) in GIA distributions, analogous to distributions of other types of host-specific canopy parasites such as dwarf mistletoes (Shaw et al. 2005). However, no prior studies that have examined the distribution of GIA in relation to hosts (and other habitat variables) have specifically accounted for spatial structure, which is certain to exist where non-random aggregations are found (see Fortin and Dale 2005); most correlations (positive or negative) have simply relied on simplistic linear models while leaving out important (but often neglected) effects of spatial autocorrelation. Any positive or negative correlative patterns based on such models might thus suffer from a lack of independence from the underlying influence of geographic space (or related factors). Moreover, these studies have generally relied on sampling a few individual hosts; no large-scale GIA-host study accounting for spatial autocorrelation has yet been attempted.

Here we use spatially explicit methods to examine the distribution of a commonly occurring GIA on a dominant forest tree species in a northern temperate mixed forest. The maple spindle gall mite Vasates aceriscrumena (Riley; hereafter SGM) induces galls on leaves of its host sugar maple Acer saccharum (Marsh.), a widely distributed and
economically important broadleaf species in forests of eastern North America. It is by far the most dominant tree species in the study area with large stems (≥ 10 cm diameter at breast height; hereafter dbh) alone comprising ~ 41% of the total basal area. We have shown previously that galling by this mite causes significant declines in leaf-level gas-exchange processes in canopies of mature maple trees; high galling intensity also appears to have a negative effect on radial increment growth of trees (Patankar et al. in review). Further, sampling individual trees across a range of tree size classes revealed that the distribution of this mite is highly skewed, with the oldest trees carrying the heaviest mite loads while understory saplings have negligible loads (Patankar et al. in review). Given its extreme host fidelity (mites are able to re-establish on susceptible hosts repeatedly), putative passive (wind-borne) dispersal mode and affinity for older trees, we hypothesized that such patterns of distribution (i.e. higher densities on older trees) would persist at a larger spatial scale, i.e. at a stand level. At this scale, host trees and patches would represent ‘islands’ in a heterogeneous environment. It is possible that SGM densities might increase as a function of increasing host density, as proposed by the resource concentration hypothesis (Root 1973) (i.e. locations in the site that have more A. saccharum stems/unit area might also have higher mite densities). Alternatively, heavily galled trees might in fact be rare, and gall densities might instead be influenced by other biotic factors such as overall plant/tree densities and local species composition or diversity. These factors might in turn be strongly influenced by (unknown) underlying environmental or topographical features such as soil moisture, slopes or elevation gradients at this scale.

The aim of this paper was to determine the influence of biotic and abiotic factors on the spatial distribution of SGM galls in a temperate forest plot. Specifically, we asked the following questions: (1) Is SGM gall density spatially clumped and at what scale? (2) How is SGM density correlated with underlying environmental features such as slope, aspect and elevation within the forest plot? (3) Is gall density correlated with sugar maple stem density and basal area, and in particular with older maple trees? (4) Is SGM gall density correlated with other commonly occurring tree species? and (5) Is SGM gall density correlated to overall stem density or species diversity in the study plot? We
hypothesized that SGM density would correlate strongest with host tree density (as proposed by the Resource Concentration Hypothesis) and, when examined across a range of host size classes (diameter at breast height 10 cm and above), that the oldest trees would carry the highest mite densities (based on prior observations). We also predicted that areas with steeper slopes would be positively correlated with SGM gall density; these areas with shallow soils and rocky outcrops are expected to represent ‘stressed’ habitats and there is prior evidence of GIA preferences towards environmentally stressed areas (Fernandes and Price 1998).

4.3 METHODS

4.3.1 Study site description

Sampling was conducted within a recently established forest mega-plot (13.5 ha) in Haliburton Forest and Wildlife Reserve, Ontario (Latitude: 45.2901, Longitude: -78.6377, Figure 4.1). The plot is situated near the west-central shoreline of a lake (Figure 4.1b) within Haliburton Forest, and is part of the plot system established by the Center for Tropical Forest Science (CTFS). The site supports mixed hardwood forest, of the Great Lakes - St. Lawrence forest type. Tree species include sugar maple (*Acer saccharum* Marsh), yellow birch (*Betula alleghaniensis* Britton), American beech (*Fagus grandifolia* Ehrh.), white ash (*Fraxinus americana* L.), red maple (*Acer rubrum* L.), red oak (*Quercus rubra* L.), black cherry (*Prunus serotina* Ehrh.), Eastern white pine (*Pinus strobus* L.), and Eastern hemlock (*Tsuga canadensis* (L.) Carr.). All trees >10 cm dbh (diameter at breast height) were identified, geo-referenced and assigned a permanent id as part of the first CTFS census, conducted prior to this study. In total, there are 7211 stems ≥ 10 cm dbh in the entire plot. Sugar maple is the dominant species here with 2545 stems comprising 35.3% of stems ≥ 10 cm dbh. The plot is divided into 367 20x20 m grids with permanent grid-posts marking the Northwest corner of each 20-m2 quadrant (Figure 4.1d).
4.3.2 Data collection

In 2008 leaves were collected from 231 grid-posts encompassing a rectangular area of 8.8 ha within the mega-plot (Figure 4.1d). Leaf collection took place in early October, immediately after leaf fall. At each grid-post, newly fallen leaves (n≥50) within a 1 m radius were randomly collected from the canopy floor and stored in brown bags. In addition to the grid-posts, leaves were collected from a total of 20 ‘offset’ points at 2, 5 or 10 m from posts in one of the four cardinal directions. The location (grid-post id), distance (2, 5, or 10m) and direction (north, south, east, west) of these points were randomly generated with the purpose of gathering supplementary gall density data at known points in addition to data collected at grid-posts. As the plot is bordered by lake margin on two sides, we restricted sampling to a minimum distance of 20 m from lake margins to minimize potential edge effects. Leaves were stored in marked brown paper bags (1 bag per grid-post) and transported to the lab wherein they were dried before counting. Sugar maple leaves were then scored for number of galls. For each grid-post, a minimum of 50 leaves (where available) were scored, but in bags with fewer leaves, all sugar maple leaves were scored. For each grid-post, we calculated mean SGM gall density by dividing the total number of galls by the number of leaves counted. Slopes and aspects were calculated from elevations that were previously measured at individual grid-posts.

4.3.3 Spatial analyses

We examined the relationship of SGM gall densities with four biotic variables (overall stem density, species diversity, sugar maple basal area and stem density) and three abiotic site features (elevation, slope and aspect of the plot). In addition, correlations between SGM gall density and stem density of seven commonly occurring species were examined. As the strength of relationships between SGM gall density and descriptors can vary across scales, statistical tests were performed on a series of circular samples that were centred on the posts and differed in radius (scale) from 5 to 35m. We used Mantel and partial Mantel tests to examine relationships between the various variables. Mantel tests examine the correlation between distance (or similarity) matrices of any two given variables (e.g. SGM density and overall species diversity). Entries in a given matrix do not represent the original raw values but instead are based on some measure of distance (or similarity).
between individual ‘points’ of that particular variable (e.g. SGM densities, maple densities, geographic distance etc.; (Fortin and Gurevitch 1993). The normalized Mantel statistic $r$ is calculated similar to Pearson’s product-moment coefficient (by first normalizing each matrix) and ranges from a value of -1 to 1.

Partial Mantel tests were used to examine the relationship between two variables while controlling for a third (e.g. the correlation between SGM density and species diversity controlled for overall stem density(Legendre and Fortin 1989). The significance of the resulting Mantel $Z$ or $r$ statistic was assessed by comparing it with a reference distribution obtained by permuting the arrangement of the elements of any one of the distance matrices a number of times, each time calculating the Mantel statistic (Fortin and Gurevitch 1993)the R software package ‘vegan’ uses a default 999 permutations). We did not go beyond 35m for all our analyses as the eastern and southern boundaries of the study plot were close to lake margins (< 40m) and hence this might have lead to erroneous correlation values due to potential edge effects. Partial Mantel tests were then used based on the appropriate scale to examine SGM gall density in relation to the above variables conditioned on space.

A contour map was produced for data visualization to detect areas with high gall densities within the sample plot (Figure 4.2a). The contour map was produced using bilinear interpolations (linear interpolations in two directions) of gall densities at individual posts. Additionally, contour maps of species diversity, overall stem density, sugar maple density and sugar maple basal area were based upon 30m circular sample plots, centred on the grid-posts. Further, slopes and elevations within the study plot were plotted as contour maps. All maps were created with the R software package; statistical analyses (Mantel and partial Mantel tests) were computed using the vegan package in R.

4.4 RESULTS

The Mantel tests revealed that associations between SGM gall density and a) sugar maple density b) sugar maple basal area c) overall stem density and d) tree species diversity were
strongest at 30m (Figure 4.2). At this scale, species diversity ($r = 0.3217; P = 0.001$) and overall stem density ($r = 0.3357, P = 0.001$) were most strongly correlated with SGM gall density among the included descriptors. Sugar maple basal area (across all tree sizes) did not correlate as strongly with gall density, although this relation was positive ($r = 0.2232, P = 0.001$). Sugar maple density (across all size classes) showed weaker correlations than basal area ($r = 0.1868, P = 0.001$). When partitioned into size classes, correlations between gall densities and basal area were strongest at the smallest size class ($r_{10-20} = 0.2339, P = 0.001$); gall densities were negatively correlated with basal areas of the largest maple stems but this was not significant ($r_{50-80} = -0.0622, P = 0.952$; Figure 4.4). The correlation between gall density and sugar maple density partitioned into size classes was bimodal, with 10-20 cm dbh and 30-40 cm dbh trees showing the strongest correlations amongst the size classes ($r_{10-20} = 0.1965, P = 0.001$; $r_{30-40} = 0.1777, P = 0.001$, Figure 4.5).

Mantel tests with seven commonly occurring tree species revealed that among these species, SGM gall densities were positively and significantly correlated with only three species (marginally with *Tsuga canadensis*), of which red maple (*Acer rubrum*) had the strongest association (Table 4.2). The partial Mantel tests at 30m conditioned on ‘space’ as the third factor produced slightly lower correlation coefficients for the various descriptors but the general pattern was the same as with the Mantel tests (Table 4.2). Because tree species diversity was strongly correlated with overall stem density, we repeated the partial Mantel test between SGM gall density and species diversity, this time accounting for the effect overall stem density. The results revealed that this correlation was reduced when conditioned on overall stem density (Table 4.1). To test for the diversity effect on SGM gall density independent of species composition, the partial Mantels were repeated, this time controlling for the potential influence of *Acer rubrum*, which had the strongest association with galls among the seven species. Species diversity continued to have strong correlations with SGM gall density when controlled for *A. rubrum* densities ($r = 0.3105, P = 0.001$; Table 4.1). As expected, the strongest association between descriptors was between sugar maple density and sugar maple basal area ($r_{\text{Mantel}} = 0.7216, P = 0.001$; Table 4.1). We examined the relationship between SGM gall density and three topographical variables at individual grid-posts in the study area: a) elevation b)
slope and c) aspect. Elevations and aspects were not significantly correlated with corresponding gall density at posts \(r_{\text{Elevation}} = 0.0491, P = 0.087; r_{\text{Aspect}} = -0.0574, P = 0.962\) while slopes were positively correlated with SGM density \(r = 0.1210, P = 0.03\), Table 4.3).

The contour map centred on grid-posts revealed that SGM gall densities were highest in the lower (southern) end of the sample plot (Figure 4.2a). Overall stem density and species diversity (at 30 m radius) were similarly higher in these areas than in the rest of the plot (Figure 4.2b, c); sugar maple density and basal area however, did not correspond with high gall densities, as revealed by the spatial analyses above (Figure 4.2d,e). Slopes were similarly the steepest towards in the southern half of the plot (Figure 4.2f) but elevations or aspects did not show any patterns in the study area.

4.5 DISCUSSION

As expected, the occurrence of galls in the plot followed an aggregated, non-random distribution within the study area with patches of high gall densities (Figure 4.2a). Although from the contour map there appears to be some spatial structure, when tested as a separate descriptor, space had negligible influence on distribution of SGM densities. This implies that other factors are likely responsible for the distribution of galls, as described below.

Mantel tests reveal that among the above four biotic descriptors, species diversity and overall stem density had the strongest positive associations with mite gall densities; this result persisted even when controlling for the influence of geographic distance (‘space’). Although the relationship between gall density and species diversity was lowered when controlled for overall stem density, the positive association remained significant and was similar in magnitude to the association between SGM galls and sugar maple density (controlled for space). Similarly, this association remained strong even when controlling for the potential influence of the three site features namely, elevation, slope and aspect. Because species composition can potentially influence the above
relationship, we tested this correlation while controlling for the density of one tree species, red maple (*A. rubrum*), as this had the strongest correlation with SGM gall density among the associated tree species in the plot. The relationship between species diversity and SGM gall density remained strong even when controlling for red maple, implying that red maple did not influence this relationship.

The strong positive correlation of gall density with species diversity was surprising, and was in contrast to our initial hypothesis. We had predicted that with increasing (local) species diversity in the plot, there would be fewer available sugar maple individuals for SGM to colonize and establish on, and that this might lead to low numbers of SGM galls. Several possible explanations exist for the observed correlation, none of which are likely to be mutually exclusive. First, isolation of susceptible maples in species-rich areas could result in a build-up of gall densities in these trees over time. Isolated trees might be harder to colonize at first, but it is possible that established populations of SGM on isolated trees might in fact be released from selective pressures enforced by natural enemies that fail to migrate to and establish on such isolated trees (Faeth and Simberloff 1981). Faeth and Simberloff (1981) observed that leaf miner survival in three species of oak was significantly higher in isolated compared to non-isolated trees, most likely due to decreased parasitism rates in these trees (however, this was not followed by subsequent increases in leaf miner abundances). Similarly, (Ozanne et al. 2000) observed that the composition of arthropods on isolated Scott's pine (*Pinus sylvestris* L.) trees was significantly different from that in *P. sylvestris* stands, with certain herbivores attaining higher densities in isolated trees, probably due to lower establishment by predators (cf. Faeth and Simberloff 1981). Long-term monitoring of individual trees in these areas might expose such a relationship between isolation, galling density and the presence of natural enemies, especially on older trees. Another possible explanation for high gall densities in species-rich areas is an increase in the abundance and richness of predatory arthropods as a function of tree species diversity/richness that might select heavily against the primary natural enemy of SGM, a gall-invading tarsonemid mite (Acari: Tarsonemidae). In a separate study examining SGM life-history, we found that > 40% of SGM galls (sampled across two years) contained this opportunistic gall-invader
making it by and large the most dominant arthropod species found in SGM galls. This mite is more active (and is often seen roaming on the leaf phylloplane) thereby making it vulnerable to bigger arthropod predators such as Phytoseiid mites and small insects such as coccilids and thrips (for further examples see (Lindquist 1986). Such an indirect ‘release’ from the gall-invader by larger predatory arthropods might result in higher populations of SGM in areas of high species richness. Future studies on arthropod assemblages across a gradient of host densities might uncover strong multi-trophic patterns with respect to SGM. A third alternative explanation could be that higher gall densities on maples could, in part, lead to greater species diversity. This would be possible if the influence of galling somehow lead to reduced performance in individual maple trees, thereby leading to higher local species diversity. Indeed, in a prior study examining the impacts of SGM galling on sugar maple, we found that gall formation lead to significant declines in leaf gas exchange (> 50% reduction in carbon uptake in infected leaves) and declines in radial incremental growth in SGM-infected trees (Patankar et al. in review). Hence it is possible that heavily impacted maples might have significantly lower competitive effects as a consequence of impeded growth and performance; this could possibly lead to the establishment of other species as a result. Finally, high diversity patches could result in greater use of resources by competing tree species through niche complementarity, and this could result in higher ‘stress’ and therefore higher susceptibility to galling in sugar maple. Increases in gall density in stressed versus unstressed hosts have been observed in other systems (e.g. De Bruyn 1995, Cuevas-Reyes et al. 2004b).

Interestingly, gall densities did not correlate as strongly with sugar maple densities, contrary to our hypothesis based on the resource concentration hypothesis (Root 1973). We predicted that, given its extreme host specificity, SGM densities would strongly correlate with host densities, but sugar maple densities only explained ~ 19% of the variation in mite gall densities. This pattern however, appears to be far from abnormal with respect to gall inducers. In a study examining the relationship between galling insect species richness and several biotic factors including host plant density in a Mexican tropical dry forest, it was found that in only 46% of galling species did gall densities strongly correlate with host plant densities (Cuevas-Reyes et al. 2004a). Similarly,
Abrahamson et al. (1983) found that while there was a positive correlation between gall insect and host plant occurrence, gall densities did not strongly increase with an increase in host plant densities. The association with sugar maple basal area was slightly stronger, but even this was not as strong as either overall stem density or species diversity. Among the different maple size classes, basal area of 10-20cm dbh trees had the strongest correlation with mite densities, similar to stem densities of 10-20m sugar maples. Although gall densities appear to show a bimodal distribution with respect to maple density, it is more likely that areas with high densities of 10-20 cm trees show increased galling intensity on larger trees. It must be noted that unlike in the present study, where gall densities are estimated based on freshly fallen leaves at grid-posts, two prior independent surveys of galling frequencies involved exhaustive sampling of individual trees across a range of size classes, and as such are likely more representative of actual galling patterns. First, in a prior study examining general patterns of herbivory on sugar maple in Haliburton, it was found that the oldest maples trees harboured the highest SGM abundances, opposite to that of other maple herbivores (Thomas et al. 2010). Further, a second survey of SGM densities on individual maples confirmed this pattern (Patankar et al. in review).

The positive association between SGM gall densities and slopes in the plot indicates that SGM might prefer to form galls on trees in areas within the plot that experience more stressful conditions such as lower soil nutrient levels or moisture stress. Areas with such environmental conditions are thought to harbour higher GIA species richness (see Fernandes and Price 1988, Price et al. 1998).

The maple spindle gall mite on sugar maples in Haliburton Forest appears to pose a unique and interesting phenomenon with respect the role of specialist natural enemies in forest canopies. This is because SGM (a host-specific, dispersal-limited canopy parasite) has the strongest association with species diversity, and not with any of the host-related factors such as host density or basal area. This strong association remains even when accounting for the underlying effects of potentially influential abiotic features (such as slope and elevation). Higher densities of natural enemies in larger patches of hosts would definitely be expected from the standpoint of the resource concentration.
hypothesis, and probably from Janzen-Connell processes, where one could expect greater numbers of host-specific natural enemies in areas of high host densities (Clark and Clark 1984). However, SGM appears to show an alternative pattern, with a preference for higher local species diversity, at least at this scale of examination. This pattern is all the more extraordinary given that SGM is the single most common aboveground arthropod on sugar maple, itself the dominant species in Haliburton Forest (Thomas et al. 2010).

But what are the long-term implications of such a pattern on community dynamics? Based on our findings here, there are ‘interesting dynamics’ that could possibly emerge from the patterns described. If patches of pure sugar maple show low galling impacts, this should enhance the capacity of sugar maple to persist in these patches. Conversely, if patches with high species diversity negatively impact sugar maple through increased galling, this should increase the capacity of these high diversity patches to persist - particularly by reducing the competitive effects of sugar maple on more light-demanding tree species. In a theoretical context this dynamic could act to maintain diversity transiently, but is not dynamically stable (as it involves a positive feedback mechanism). Further long-term monitoring of infected and non-infected maples in patches and isolation might shed light on the responses of growth, reproductive success, and establishment of con-specifics (and competitors) to mite galling, and thus provide further insight on important natural enemy–regulated processes that govern tree species diversity in temperate forests.

The present study is the first to explicitly examine the spatial characteristics of a commonly occurring GIA (thought to be the most abundant aboveground herbivore on its host, sugar maple) and several associated biotic and abiotic factors. Although present in diverse natural communities across the globe, the role of GIA remains poorly studied. Based on our findings here we suggest that host-specific natural enemies such as gall inducing arthropods in temperate forest communities might play an important role with respect to forest species dynamics and should thus be incorporated into studies that examine the role of natural enemies in forests.
4.6 Tables

Table 4.1 Results ($r$ and corresponding $P$ in parenthesis) of Mantel (above the diagonal) and partial Mantel (below the diagonal) tests between gall density and four descriptor variables in the Haliburton Forest mega-plot. Partial Mantel statistics between gall density and space differed when conditioned on the remaining three descriptors. Hence corresponding $r$ and $P$ values here are as follows: $^a$ = conditioned on stem density, $^b$ = species diversity, $^c$ = sugar maple density. Partial Mantel statistics between descriptors are conditioned on space. $^*$, $^†$, $^¢$, $^\$ = Partial Mantel statistic between SGM gall density and species diversity conditioned on slope $^*$, aspect $^¢$, overall stem density $^†$ and $A. \ rubrum$ density $^\$; n.a. = tests not applicable.

<table>
<thead>
<tr>
<th></th>
<th>Gall density</th>
<th>Stem density</th>
<th>Species diversity</th>
<th>Sugar maple density</th>
<th>Sugar maple basal area</th>
<th>Space</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gall density</td>
<td></td>
<td>0.3357</td>
<td>0.3217</td>
<td>0.1868</td>
<td>0.2231</td>
<td>0.0944</td>
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<td>Stem density</td>
<td>0.3252</td>
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<td>0.4271</td>
<td>0.2367</td>
<td>0.3135</td>
<td>0.1883</td>
</tr>
<tr>
<td>Species diversity</td>
<td>0.3184, 0.3072$^a$, 0.2094$^†$, 0.3105$^¢$, 0.3157$^$</td>
<td>0.4249</td>
<td></td>
<td>0.1718</td>
<td>0.3056</td>
<td>0.0555</td>
</tr>
<tr>
<td>Sugar maple density</td>
<td>0.1742</td>
<td>0.2118</td>
<td>0.1651</td>
<td>-</td>
<td>0.7216</td>
<td>0.1680</td>
</tr>
<tr>
<td>Sugar maple basal area</td>
<td>0.2066</td>
<td>0.2789</td>
<td>0.302</td>
<td>0.7121</td>
<td>-</td>
<td>0.2599</td>
</tr>
<tr>
<td>Space</td>
<td>0.0338$^a$, 0.065$^b$, 0.081$^c$</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 4.2  Mantel and partial Mantel statistics ($r =$ Pearson correlation coefficient, $P =$ significance value) between SGM gall densities at grid-posts and seven tree species from the Haliburton Forest mega-plot. Tests were based on individual tree densities calculated within a 30m radius from grid-posts. The partial Mantel test takes into account the effect of 'space' (geographic distance) when testing for associations between tree densities and mite gall densities.

<table>
<thead>
<tr>
<th>Tree Species</th>
<th>Mantel Statistic ($r$)</th>
<th>P-value</th>
<th>Partial Mantel Statistic ($r$)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acer rubrum</em></td>
<td>0.1460</td>
<td>0.01</td>
<td>0.147</td>
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<td><em>Tsuga canadensis</em></td>
<td>0.1176</td>
<td>0.058</td>
<td>0.1178</td>
<td>0.045</td>
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<tr>
<td><em>Quercus rubra</em></td>
<td>0.1053</td>
<td>0.045</td>
<td>0.1077</td>
<td>0.047</td>
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<tr>
<td><em>Fagus grandifolia</em></td>
<td>0.0551</td>
<td>0.13</td>
<td>0.0498</td>
<td>0.163</td>
</tr>
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<td><em>Betula alleghaniensis</em></td>
<td>-0.0285</td>
<td>0.682</td>
<td>-0.0335</td>
<td>0.724</td>
</tr>
<tr>
<td><em>Fraxinus americana</em></td>
<td>-0.0621</td>
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</tr>
<tr>
<td><em>Pinus strobus</em></td>
<td>-0.0200</td>
<td>0.497</td>
<td>-0.0171</td>
<td>0.448</td>
</tr>
</tbody>
</table>
Table 4.3  Mantel and partial Mantel statistics ($r =$ Pearson correlation coefficient, $P =$ significance value) between SGM gall densities at grid-posts and two site features from the Haliburton Forest mega-plot. Tests were based on individual tree densities calculated within a 30m radius from grid-posts. The partial Mantel test takes into account the effect of ‘space’ (geographic distance) when testing for associations between site features and mite gall densities. $n.a.$ = tests not applied.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Mantel Statistic ($r$)</th>
<th>P- value</th>
<th>Partial Mantel Statistic ($r$)</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
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<td>0.03</td>
<td>0.1042</td>
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</tr>
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<td>Elevation</td>
<td>0.0491</td>
<td>0.087</td>
<td>0.0490</td>
<td>0.085</td>
</tr>
<tr>
<td>Aspect</td>
<td>- 0.0574</td>
<td>0.962</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>
4.7 Figures

Figure 4.1 Haliburton Forest mega-plot, Ontario, Canada. A) regional location in relation to the Great Lakes, B) the mega-plot in relation to Havelock Lake C) the SGM gall density study plot highlighted in green.
Figure 4.2 Correlation coefficients between gall density and three descriptors (squares = species diversity; circles = overall stem density; triangles = *A. saccharum* density) from Mantel tests at six different scales (5m-30m) of analyses. Filled symbols represent values that are significant at $P = 0.05$. 
Figure 4.3  Contour maps of a) SGM gall density (galls/leaf) b) overall stem density (stems/ m²) c) species diversity (number of species) d) sugar maple stem density (stems/m²) e) sugar maple basal area (cm²) and f) slopes (% slope) from the 8.8 ha study area of the Haliburton Forest mega-plot, Ontario. Sugar maple density, basal areas, overall stem density and species diversity were calculated within a 30m radius from 231 individual gridposts spaced 20m apart. Gall densities, elevations and slopes were calculated at the gridposts.
Correlation coefficients of partial Mantel tests between SGM gall density and sugar maple stem densities (conditioned on space) partitioned into 4 size classes (10-20 cm, 20-30 cm, 30-40 cm, 40-50 cm dbh) from the Haliburton forest mega-plot. The y-axis represents the standardized partial Mantel statistic (Pearson’s r). Densities from all maple size classes were significantly correlated with SGM gall density (P< 0.05).
Figure 4.5  Correlation coefficients of partial Mantel tests between SGM gall density and sugar maple basal areas (conditioned on space) partitioned into 5 size classes (10-20 cm, 20-30 cm, 30-40 cm, 40-50 cm, 50-80 cm dbh) from the Haliburton forest mega-plot. The y-axis represents the standardized partial Mantel statistic (Pearson's r). Basal areas from 10-50 cm were significantly correlated with SGM gall density (P< 0.05); there was no significant correlation between SGM and 50-80 cm trees.
4.8 References


CHAPTER 5: CONCLUSIONS
5.1 Thesis Summary

The impact of gall-inducing arthropods on the growth and physiology of large canopy trees has remained largely unexplored. My thesis examined interactions between one such canopy arthropod, the leaf-galling eriophyid mite *Vasates aceriscrumena*, and its host tree species sugar maple (*Acer saccharum*) in a mixed temperate forest in central Ontario, Canada. Specifically, my work examined: 1) the previously unknown life cycle of the mite; 2) the influence of gall formation on leaf physiology across two distinct ontogenetic stages, saplings and mature trees; 3) the relationship between galling and tree age and growth; and 4) the spatial distribution of this mite across the ontogeny of the host and at the forest stand level. I examined this host-parasite relationship as it scaled up from the leaf to forest-stand level. As a result, chapters within the thesis address a range of questions from diverse fields including arthropod life history strategy and interactions with natural enemies, host-herbivore interactions (specifically, foliar parasite impacts on tree ecophysiology and growth) and the spatial distribution of a dispersal-limited parasite in relation to forest species diversity.

5.2 Major Findings

5.2.1 Phenology of *Vasates aceriscrumena* (SGM)

My work showed that *V. aceriscrumena* follows a life cycle similar to other gall-inducing mites on deciduous hosts, with the presence of two females morphs, namely the gall-inducing deutogyne and the primary protogyne. Galls of *V. aceriscrumena* were exploited by an unknown species of gall-invading tarsonemid mite (Acari: Tarsonemidae). Although tarsonemid mites have been observed in eriophyid mite galls before (Beer 1963, Lindquist...
and Smiley 1978), their exact role within these habitats has been unclear. Here, I show that an unknown *Tarsonemus* species has a significant influence on SGM populations within galls, drastically reducing the number of SGM eggs and larvae, and hence appears to be a major source of mortality. This mite may thus play an important role in regulating populations of SGM.

5.2.2 Impacts of *V. aceriscrumena* on leaf-level physiology and whole-tree growth

Mature trees differ from seedlings and saplings in physiology (Thomas and Winner 2002), growth requirements and defence strategies, and hence can differ in their response to herbivory (Boege and Marquis 2005). However, there has been little prior research on the influence of ‘chronically-associated’ arthropod parasites such as eriophyid gall mites on host trees, despite their potentially large influences on tree health and forest carbon cycles. I found that galling by SGM drastically reduces gas-exchange processes (photosynthetic capacity, stomatal conductance and water use efficiency) in infected leaves of canopy trees but no such impacts are seen in infected understory sapling leaves. Interestingly, no detectible differences are found in either leaf size or shoot growth between galled and ungalled leaves but there is an association between galling incidence and declines in whole-tree growth in mature sugar maple canopies. Gall formation may thus contribute, at least in part, to changes in large-scale forest processes including both age-related declines in tree performance, and patterns of forest growth and net primary productivity.

5.2.3 The spatial distribution of *V. aceriscrumena* in a mixed forest stand

Gall-inducing insects (GIA) are known to shown high host fidelity even to individual plants (Egan and Ott 2007). However, no prior studies examining the distribution of GIA in relation to hosts (and other habitat variables) have specifically accounted for spatial
structure, which is certain to exist where non-random aggregations are found (Fortin and Dale 2005). Moreover, these studies have generally relied on sampling a few individual hosts; no large-scale GIA-host study accounting for spatial autocorrelation has yet been attempted. In Chapter 4, I show that gall densities of SGM has strong correlations with local species diversity, even when accounting for potential influence of geographic space and environmental and biotic covariates such as topography, overall stem density and densities of associated species such as red maple (Acer rubrum). Further, the above correlation is consistently stronger than that between SGM gall density and its host, sugar maple. Thus SGM, a host-specific dispersal-limited canopy parasite, is plausibly linked with local community dynamics in Haliburton Forest.

5.3 ECOLOGICAL SIGNIFICANCE

My work provides new insights into a little known, but potentially important, interaction between a dominant forest species and its most abundant foliar herbivore, a gall-inducing mite. SGM, a dispersal-limited canopy parasite, has drastic impacts on mature sugar maple trees, but no apparent impacts on saplings. The results of this research provide insights into the broader phenomenon of senescence and ageing in old forest canopies, and biotic mechanisms that might contribute to them. The long-term implications of such impacts on hosts suggest that sustained high levels of galling on trees could lead to declines in tree health and productivity. Crucially, such parasite-induced declines might in part be responsible for increases in local species diversity. Finally, there is the possibility that gall-inducing herbivores such as SGM could have an influence on carbon cycles in forests, and based on the findings presented in this thesis, which warrants further examination.
5.4 **APPLIED SIGNIFICANCE**

*Acer saccharum* is a commercially important species in the silviculture industry, not just regionally, but also in major parts of eastern North America. However, the role of ‘chronic pests’ such as SGM has seldom been studied. Here, I show that galling by SGM could have potential long-term impacts on whole tree growth in sugar maple. Specifically, my research sheds light on particular size classes of maple that are susceptible to SGM galling. Such information could be especially useful within the context of uneven-aged forest management systems such as selection silviculture, where individual infected trees could be identified for appropriate remedial measures (such as miticide treatment) to arrest growth declines. A recent silviculture example of where this has been attempted is in the treatment of Eastern cottonwood (*Populus deltoides* Bartr.) clones infected by the cottonwood leafcurl mite *Aculops lobuliferus* (Keifer) (Coyle 2002).

Overall, the results brought about by this thesis will enable concerned foresters to answer/address specific questions pertaining to maple health/management and mite infection such as ‘Where are gall infection rates likely to be higher, in maple-specific patches or heterogeneous ones?’ and ‘Which size classes appear most susceptible to galling by SGM?’ Alternately, if high rates of SGM infections on isolated maples do indeed serve to promote local species richness, such maples may be identified and retained due to their potential role in contributing towards local biodiversity. Hence this work will contribute towards more informed management decisions in silviculture, especially in the context of selective system silviculture.
5.5 CONCLUDING REMARKS

There has been no prior research examining the role of galling arthropods on mature canopy trees, especially at multiple scales. My research is the first to examine the effects of galling on canopy-level gas-exchange, in addition to the potential effects of galling on whole tree growth. Additionally, important differences in host response to galling along ontogeny are determined. The high relative importance of water limitation in canopy trees may exacerbate negative effects of gall formation related to water loss. Given the high dominance of sugar maple, and the prevalence of SGM in the region, such effects may have large implications to forest carbon processes and forest hydrology. Furthermore, the discovery of the SGM-gall invader interaction will provide new insight into probable mechanisms that regulate gall mite populations on mature tree canopies. Gall mites are an important prey source for a number of predaceous mite species in the canopy, the main ones of which are mites belonging to the family Phytoseiidae. Indeed, some of them have been shown to exploit eriophyid galls for shelter (Kawashima and Amano 2004). However, their interactions with fungivorous and phytophagous Tarsonemid mites are not well explored (Beer 1963, Lindquist and Smiley 1978). This work represents the first study to quantify the occurrence of a gall-invading predator, and also show that gall intrusion by this unknown mite leads to declines in the various life stages of the host SGM. Lastly, there are strong indications that SGM is related to local tree species diversity. This is a novel phenomenon where a host-specific, dispersal-limited canopy parasite might, at least in part, be influential in the maintenance of local species diversity.

The ability to induce galls is in itself an intriguing phenomenon, and this arguably makes gall-inducing arthropods amongst the most fascinating group of host-specific herbivores. There are potentially thousands of GIA species in forest canopies worldwide,
and yet they remain disproportionately underrepresented in ecological literature. This is especially true for diminutive gall-inducing mites (adults average no more than ~ 400 µm) that remain among the most, if not the most, challenging group of arthropods to work on. Their role as plant pests in agricultural and horticultural systems (Lindquist et al. 1996) are well documented, and their function as bio-control agents in invasive plant species management remains all the more relevant in a globalized world (Skoracka et al. 2010). However, as the main findings of my thesis would argue, ‘chronically-associated’ pests such as gall-inducing mites are a crucial part of global forest canopies, and could play critical roles both as natural enemies of host tree species and as agents of change in forest canopies worldwide.
5.6 REFERENCES


Scanning electron micrographs of SGM overwintering deutogynes (a, b) and primary ‘protogynes’ (c, d). Notice the increased ornamentation on the prodorsal shield (d) and presence of spinules (c, white arrow) on the protogyne compared to the deutogyne (a, b respectively).
Appendix B

Scanning electron micrographs of a female *Tarsonemus* sp. adult found in SGM galls in the sugar maple of Haliburton Forest, Ontario.
Appendix C  Attempted SGM inoculation experiments.

Two separate manipulative experiments were attempted to inoculate ungalled sugar maples with SGM galls. Both experiments involved inoculating saplings with infected mature canopy branches. Both attempts did not result in successful inoculations although there were indications that a few sapling leaves might have ‘taken’ 1-2 galls. Based on the results here, it is recommended that future manipulative attempts involve the experimental transfer of individual overwintering females just prior to or at the onset of sugar maple bud break in the spring. However it must be noted that saplings appear to have a higher level of resistance to SGM galling compared to canopy trees, and this could have lead to the failure of saplings to take up SGM in the first place. Hence even experimental transfers of individual females may not necessarily result in successful galling in sugar maple saplings.

A) Inoculation of sugar maple saplings in forest gaps with SGM-infected canopy branches.

On 30 August 2007, forty sugar maple saplings (< 3 cm diameter at breast height) in the Haliburton forest understory were searched exhaustively for galls and then tagged as either galled or ungalled saplings. Individual gall-infected canopy branchlets from neighbouring mature trees were tagged during the same period. On 5 May 2008, previously marked branchlets were cut from the canopies of matures trees and tied onto gall-free saplings (tagged in 2007) just before bud burst. Individual branches were then observed during their development in the early growing season to determine if saplings leaves became ‘infected’ by overwintering by SGM emerging from buds of previous-galled canopy branchlets. Only three leaves in total appeared to ‘take up’ galls (1-2 galls/leaf).

B) SGM inoculation of maple saplings by grafting with mature branches.

In 2008, 200 sugar maple saplings were collected from the forest understory and repotted at the Faculty of Forestry greenhouse. Half of these were selected for grafting with previously tagged infected and gall-free canopy branchlets. Canopy branchlets were grafted onto the main stem of saplings using conventional cleft grafting techniques. Almost all the grafted saplings failed to take up the canopy ‘scions’ (grafts) resulting in a failure to inoculate saplings with galls. A few saplings that were not grafted had low levels of galls, most likely by SGM that overwintered in their buds.