

The life history of a gall-inducing mite: summer phenology, predation and influence of gall morphology in a sugar maple canopy

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- Abstract**
- 1 Eriophyoid mites are among the most ubiquitous gall-inducing arthropods, and are adapted species-specifically to a broad diversity of plants, although their life histories remain poorly studied outside agricultural systems.
 - 2 We examined the seasonal phenology of a leaf-galling eriophyid mite, the maple spindle gall mite *Vasates aceriscrumena* (MSGM), in naturally occurring stands of sugar maple *Acer saccharum* in south-central Ontario in 2007 and 2008.
 - 3 Galls were first induced in spring (mid-May) and were devoid of mites by late August. In the study region, MSGM appears to have at least two generations, with overwintering, deutogyne females that initiate galls in spring (mid-May) after leaf flush, giving rise to a generation of protogyne (primary) females and a few morphologically similar males (<1 for every 10 females) and, subsequently, to a new generation of deutogyne females in mid-July to early August. In July, some galls can be highly crowded, with 50–200 individuals per gall.
 - 4 In addition, a tarsonemid mite, *Tarsonemus acerbilis*, was found in approximately 40% of MSGM galls examined. As much as 95.4% of galls in 2007 and 97.4% in 2008 that contained tarsonemid larvae did not contain MSGM eggs (by contrast, only 2.3% of tarsonemid-free galls contained no MSGM eggs), suggesting that these juveniles feed, at least opportunistically, on MSGM eggs.
 - 5 Gall ostiole morphology appeared to influence both MSGM and *Tarsonemus* densities within galls, with ‘open’ ostioles (versus ‘closed’) being much more susceptible to invasion by the tarsonemid. The latter is likely to be an important regulator of MSGM populations. We hypothesize that the two ostiole types are the result of selection pressures on the gall inducer, favouring closed gall entrances for increased protection, and possibly also on the host tree, favouring open galls to increase predator access.

Keywords *Acer saccharum*, egg predation, Eriophyidae, maple spindle gall mite, natural enemies, phenology, Tarsonemidae, *Tarsonemus acerbilis*, *Vasates aceriscrumena*.

Introduction

Gall-inducing arthropods are specialized plant parasites that have evolved intimate associations with their hosts. Besides exceptional tenuipalpid and tarsonemid mites capable of inducing galls on their hosts (Walter *et al.*, 2009), the large majority

of gall-inducing mites belong to the Eriophyoidea (Lindquist, 1998). Eriophyoids are highly host-specific (Skoracka *et al.*, 2010) and some may have dramatic impacts on their host plants. Among the approximately 4000 described species of eriophyid mites that occur worldwide (de Lillo & Skoracka, 2010), over one quarter of them induce galls (including erineae) on the leaves, buds, twigs or flowers of their host plant (Jeppson *et al.*, 1975; Oldfield, 2005). The roles of eriophyid mites as disruptors of plant growth and physiology (Larson, 1998; de Lillo

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& Monfreda, 2004; Petanovic & Kielkiewicz, 2010), as well as plant virus vectors (Oldfield & Proeseler, 1996), have been documented to some extent, although their life histories remain poorly studied outside agricultural and horticultural systems (de Lillo & Skoracka, 2010). Recently, eriophyid life histories have received more attention for their role as biocontrol agents of weeds (Ozman & Goolsby, 2005; Smith *et al.*, 2010). Eriophyids induce galls on a variety of trees in tropical and temperate forests, although their minute size and the remote locations of galls in forest canopies are probable reasons for the dearth of studies in natural settings.

Acer saccharum is inhabited by eight species of eriophyoids, which are mostly confined to that host (Hodgkiss, 1930; Amrine & Stasny, 1994; Baker *et al.*, 1996). Among those, the maple spindle gall mite *Vasates aceriscrumena* (Riley & Vasey, 1870) (hereafter MSGM) is a conspicuously prevalent species on sugar maple that induces spindle or finger-shaped galls on the leaves of its host (Jeppson *et al.*, 1975). In the eastern province of New Brunswick, Canada, approximately 75% of maples were reported with 22% of their leaves infested on average (Hall, 1994). In stands of south-central Ontario, Canada, a typical (mature) maple contains approximately 10^4 to 10^5 MSGM galls, with several dozens of mites per gall; calculations based on Thomas *et al.* (2010). MSGM was also reported from *Acer rubrum* and *Acer leucoderme* (Herrick, 1935), although mites on these hosts are most likely distinct species (J. Amrine, personal communication). MSGM shows a preference for the upper canopy (>20 m) leaves of older trees (>30 cm diameter) and causes pronounced declines (up to 50%) in photosynthetic capacity in infested leaves, which likely lead to reductions in radial tree growth (Patanekar *et al.*, 2011). However, despite its ubiquity and profound impact on the performance of sugar maple, which is a regionally dominant tree and a valuable source of timber and unique food commodity, the biology of this mite and of its natural enemies is poorly known. Details on the life history of MSGM are almost absent in the literature (Hodgkiss, 1930; Martineau, 1984).

A better understanding of the life history of MSGM would help clarify the relationship between MSGM and sugar maple's growth, yield and physiological response to variations in mite galling intensity through time or space. In the present study, we investigated (i) some details of the life history of *V. aceriscrumena* through the season, including gall formation and the densities of different life stages and female forms, and (ii) the relationship between MSGM, gall ostiole morphology and a gall-invader.

Materials and methods

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 *A. saccharum* accounts for approximately 55% of tree basal area on upland sites. We accessed tree canopies using a mobile elevating work platform (Scanlift 240; Kesla Oyj, Finland) that enabled us to sample up to 24 m in height (i.e. 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 (approximately mid-May) until galls were completely devoid of mites (mid-August).

MSGM gall morphology and development

To determine temporal development of galls, we measured gall dimensions in a subset of galls ($n = 298$) collected during summer (18 June to 17 August) in 2007. We noted variation in gall entrances (ostioles) and hence scored ostiole condition to test any correlation 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 largely covered by gall/leaf tissue were scored as 'closed' (see Supporting information, Fig. S1). To test whether new galls were initiated later in the summer, a total of 39 galled and 25 ungalled canopy leaves (at a height of approximately 20 m) from four mature trees were randomly selected and tagged on 19 May 2007 and gall development was monitored until galls were devoid of mites (18 August 2007).

MSGM phenology

Leaves were sampled from the canopy and galls excised from sampled leaves and dissected on the same day or stored at approximately 4 °C until dissection within 2 days. For counting mites, entire galls, along with the surrounding leaf tissue, were extracted and adhered horizontally onto double-sided foam tape. This enabled a single longitudinal incision, thereby causing minimal damage to the contents. For each sampling date, galls were scored for founding female adults, eggs, immatures (larvae and nymphs combined), protogyne females and new deutogynes, as well as for the presence of other arthropods, under a dissecting stereoscope (Wild, Switzerland) with additional magnification using a $\times 1.5$ objective lens (total magnification: $\times 60$). First-instar (larvae) and second-instar (nymphs) immatures were not reliably distinguished from each other under a dissecting scope, and so these were scored together as 'immatures'. Founding female adults (deutogynes inducing galls in spring), however, were easily distinguished from other stages and female forms: they were larger than immatures, distinctly orange–brown in colour and were usually found at the apical tip of the gall chamber. Protogyne females were also larger, although they had a similar colour to the immature stages (pale white). New deutogynes were similar to founding deutogynes in colour and size. Although smaller, males were otherwise similar to protogynes, and so could only be differentiated reliably under a compound microscope. Because of time constraints and the relative rarity of males, we combined both sexes. Over 150 specimens were slide-mounted and examined under a compound scope to confirm our determination of life stages made under a dissecting stereoscope. Voucher specimens are stored in the Canadian National Collection of Insects, Arachnids, and Nematodes (Ottawa, Canada).

In 2007, leaves were collected from 19 May to 18 August (≥ 1 per week) from two mature sugar maple trees approximately 15 m apart. To test for variation between years

and across tree age, 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, the mean number of founding adults, eggs, immatures and new deutogyne adults per gall was calculated. During 5–8 May 2008, a total of 20 terminal twigs were collected from five haphazardly selected saplings to test for the presence of MSGM (deutogynes).

Occurrence and phenology of a tarsonemid mite

In 2007, a frequently occurring mite was discovered within MSGM galls and identified as *Tarsonemus acerbilis* Delfinado, 1978 (Tarsonemidae). Its phenology was studied in 2007 and densities were estimated using a method similar to that for MSGM (see below). Approximately 60 tarsonemid specimens collected from 14 dates across the summer were slide-mounted to confirm the species under a compound microscope. Phenology was excluded from 2008 as a result of the haphazard nature of sampling during that year (i.e. different maple individuals were sampled on different dates as a result of logistical constraints) and, often, no tarsonemids were found in galls. However, the total numbers of tarsonemid eggs, larvae and adults were scored from both years.

Statistical analysis of MSGM and tarsonemid phenology

Peak densities in eggs, immatures, adults and progeny were estimated by fitting a smoothing spline function with confidence intervals (Bonferroni method) to the MSGM (2007 and 2008) and *T. acerbilis* (2008) phenology data ('fields' package in R, R Development Core Team, 2011). Cubic smoothing splines provide a locally weighted mean of regression based on a flexible smoothing parameter λ that controls the 'smoothness' of the resulting curve (Eubank, 1999); λ was chosen by the default procedure of the R function `sreg` (). Nonparametric analyses (Kruskal–Wallis test) and Dunn's multiple comparison tests were performed to check for differences in median numbers of SGM life-stages between years and locations.

Results

MSGM gall morphology and development

Galls 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) but approximately 65% (252/391) of early galls in 2007 and approximately 27% in 2008 (20/74 galls) were empty and appeared to have been abandoned after initiation. The height of fully developed galls measured 2–5 mm (2.8 mm \pm 1.0), and gall height was correlated with gall content: larger galls contained more eggs and immatures (Spearman's $\rho = 0.487$,

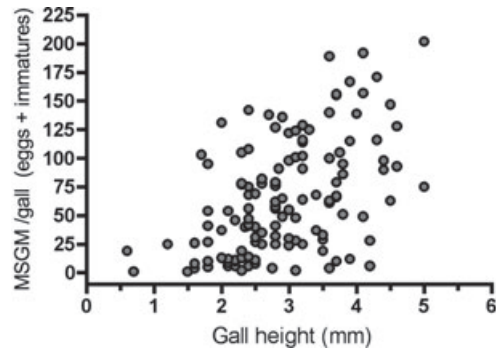


Figure 1 Maple spindle gall mite (MSGM) gall morphology in relation to MSGM populations from sugar maple canopy leaves in Haliburton Forest, Ontario, in 2007. Gall height was positively correlated with the number of eggs + immatures per gall (a) (Spearman's $\rho = 0.4867$, $n = 124$, $P < 0.001$).

$n = 124$, $P < 0.001$; Fig. 1). Fully developed galls exhibited a typical 'nail' or 'spindle' shape, with the inner chamber and gall entrance devoid of any hair-like trichomes (see Supporting information, Fig. S1). Galls remained red through the season or, on some leaves, turned yellow–green late in the season. Gall ostiole type had an effect on MSGM populations, with mean MSGM eggs and immatures significantly lower in galls with 'open' ostioles than 'closed' ostioles (Mann–Whitney: $U_{\text{eggs}} = 5030$, $P < 0.001$; $U_{\text{immatures}} = 4471$, $P < 0.001$; Fig. 2a).

MSGM phenology

A single, founding deutogyne was found from mid-May (days 139 and 132; 2007 and 2008, respectively) until early July (days 191 and 193; 2007 and 2008; Fig. 3a, f) in 40.3% (2007) and 78.3% (2008) of galls. Two deutogynes were found instead of one in 1.1% (9/812) of galls in 2007; 58.6% (2007) and 21.7% (2008) of galls inspected were found to be empty. By mid-summer (11 July 2007 and 8 July 2008), all founding females in galls were dead and shriveled at the apex of galls (Fig. 3a, f). MSGM eggs were first observed on 19 May 2007 and 22 May 2008 and, based on the fitted splines, egg densities peaked by approximately 21 June 2007 and 7 July 2008 (Fig. 3b, g). However, because these represent the eggs of more than one overlapping generation, the shown curves and peaks are somewhat artificial. No eggs were seen after 12 July in 2007 and 22 July in 2008. Immatures were first observed 19 days after the eggs first appeared (7 June 2007 and 10 June 2008) and peak immature density (of overlapping protogyne and new deutogyne generations) was reached at approximately mid-July in both years (Fig. 3c, h). Protogynes were first observed on 14 June 2007 and peaked in mid-July (Fig. 3d). Unfortunately, protogynes were not rigorously distinguished from immatures in 2008, and so immatures and protogynes are represented in the same curve (Fig. 3h). However, because protogynes appear to have much lower abundances than immatures (Fig. 3c, d), Fig. 3(h) may largely represent the numbers of 2007 immatures. New deutogynes appeared in July and peaked at approximately 10 August 2007 and 5 August 2008 (Fig. 3e, i). From mid-July to mid-August, most deutogynes were found aggregated

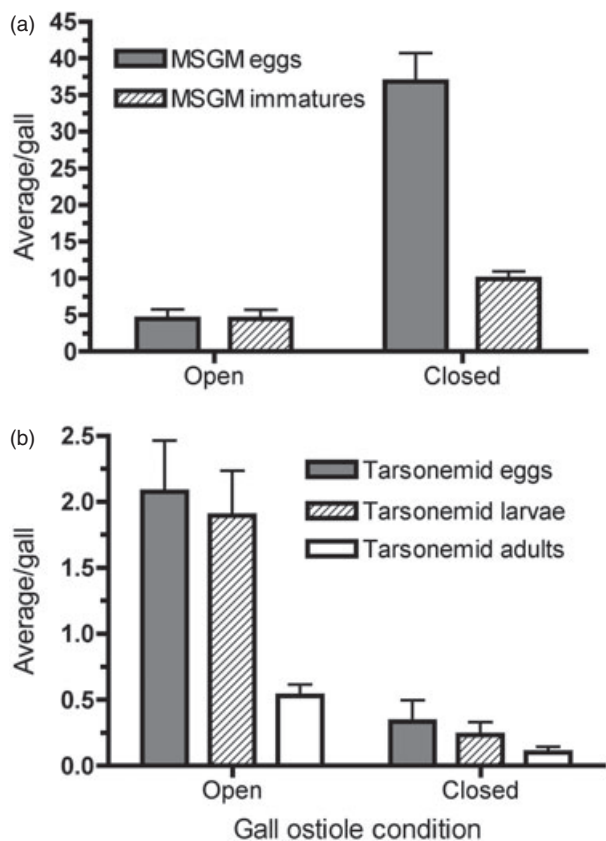


Figure 2 Maple spindle gall mite (MSGM) gall ostiole condition ('open' versus 'closed') in relation to (a) MSGM eggs and immatures (larvae + nymphs) and (b) *Tarsonemus* eggs, larvae and adults. Open galls had significantly fewer MSGM and significantly more *Tarsonemus* than closed galls. Differences between 'open' and 'closed' galls are significant at $P < 0.001$ for all groups except for tarsonemid adults, significant at $P = 0.0023$.

in a small proportion of galls: in 2007, approximately 95% (561/589) of them occurred in two of 11 galls and, in 2008, approximately 61% (450/733) occurred in three of 76 galls.

The mean numbers per gall of the various life stages differed mainly between years, although not significantly between trees versus saplings. MSGM eggs (Kruskal–Wallis: $K = 51.9$), immatures (Kruskal–Wallis: $K = 51.9$) and new deutogynes (Kruskal–Wallis: $K = 179.6$) were all fewer in galls of 2007 canopy leaves versus 2008 (Table 1).

In both years, new deutogyne adults were observed exiting galls on several occasions in the fall and crawling in two distinct directions, either (i) towards the leaf edge where they aggregated in groups (20–50 mites) and engaged in 'waving' behaviour in an apparent effort to be dispersed by local winds or (ii) moving along the midrib and secondary leaf veins towards the branch via the petiole (Sabelis & Bruin, 1996; Michalska *et al.*, 2010).

From the terminal twigs collected in mid-May 2008, 279 deutogynes were found from a total of 46 leaf buds, including 24 open buds (i.e. scales enclosing the embryonic leaves were fallen or open) and 20 unopened buds. Almost all MSGM individuals (97.1%) occurred in open buds. Most (75%)

open buds had MSGM, with a mean \pm SD of 10.5 ± 12.7 deutogynes per bud, whereas only 25% of unopened buds had MSGM, with 0.4 ± 1.0 per bud.

Occurrence, phenology and role of *T. acerbilis*

A few other organisms were seen in MSGM galls: cecidomyiid larvae (in one gall), coccid scales (one gall), six nymphs of tydeid mites (four galls) and three females of *Dendroptus cf. edwardi* (Delfinado, 1978) (Tarsonemidae) (one gall). Only one species was common throughout the summer, the tarsonemid mite *T. acerbilis*. This species occurred in 40% (351/887) of galls in 2007 and 42.5% (133/313) in 2008. It was not uncommon to find multiple females in a single gall early in the season. Mites (presumably) entered the galls through ostioles during gall formation, and laid opaque, ovoid eggs. Spline analysis revealed that there were three marked peaks in egg densities and larvae in 2007, suggesting multiple, short generations (Fig. 4a, b). Larvae were first observed on 31 May, 12 days after the eggs were first detected in galls. Eggs developed rapidly, and larvae emerged in galls 1 week before the emergence of MSGM larvae. No obvious peaks were seen in adults until later in the season (third week of July onwards) when adults increased substantially to a mean maximum of approximately 25 adults per gall. No adults were found in galls when last sampled on 17 August 2007 but, in 2008, adults were found until 29 August, which was the last day of sampling. Mean densities of *T. acerbilis* life stages are summarized in Table 1.

In both years, we found a strong relationship between the presence of *T. acerbilis* larvae in galls and the absence of host eggs (2007: Mann–Whitney: $U = 1394$, $P < 0.001$; 2008: $U = 25$, $P < 0.001$; Fig. 5). MSGM eggs were present in only 6.0% of galls in 2007 (8/130) and 2.6% in 2008 (1/38) that contained *T. acerbilis* larvae versus in 95.5% (273/286 in 2007) and 100% (115 galls in 2008) of early season galls without tarsonemid larvae. No correlation was observed with tarsonemid adults, such that MSGM eggs were present in 20.0% (21/110) of early-season (i.e. before *T. acerbilis* larvae appeared, by approximately mid-June) galls with *T. acerbilis* adults, as opposed to 30.5% (51/167) of galls without *T. acerbilis* adults in 2008.

MSGM gall ostiole condition was related the number and frequency of tarsonemids in galls. The number of *T. acerbilis* adults, eggs and larvae was higher in galls with open ostioles versus closed ostioles (Mann–Whitney: $U_{adults} = 8717$, $P = 0.0023$; $U_{eggs} = 8391$, $P < 0.001$; $U_{larvae} = 8430$, $P < 0.001$; Fig. 2b). Also, 25.3% (43/170) of galls with open ostioles contained adults compared with only 5.5% (7/128) of galls with closed ostioles; 27.6% (47/170) of 'open' galls contained *T. acerbilis* larvae compared with 5.5% (7/128) of 'closed' galls.

Discussion

The maple spindle gall mite follows a life cycle typical of galling eriophyoids on temperate deciduous hosts, characterized by at least one generation of primary, protogyne females during

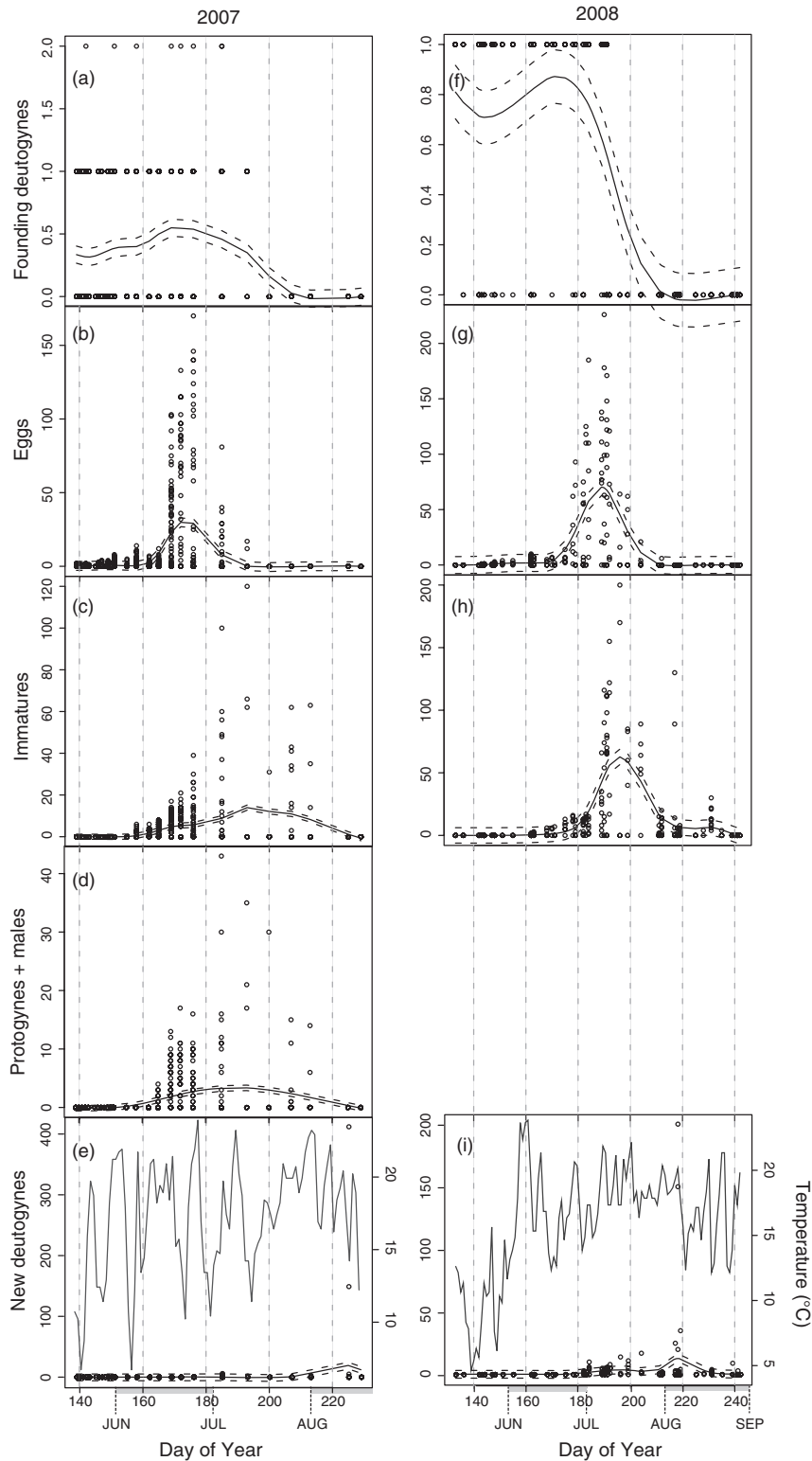


Figure 3 Phenology of maple spindle gall mite life stages from galls on sugar maple leaves during the growing season of the host in Haliburton Forest, Ontario. Data collected from May to August in 2007 (left) and 2008 (right). Phenology was scored for founding (deutogyne) females (a, f); eggs (b, g); immatures (larvae + nymphs) (c, h); protogyne adults (+ males) (2007 only; d) and new deutogyne adults (e, i). Cubic smoothing splines (\pm 95% confidence limits) were fit to the data to detect peaks in these life stages. Note that Figs (b) and (g), and (c) and (h), represent the eggs and the immatures, respectively, of protogyne and deutogyne generations combined; 2008 data: (h) represents both immatures and protogyne adults. Mean daily temperatures ($^{\circ}$ C) for 2007 and 2008 are shown in (e) and (h), respectively.

Table 1 Summary of *Vasates aceriscrumena* and *Tarsonemus acerbilis* life stages from galls on canopy leaves in two mature sugar maple trees (2007) and canopy and understorey sapling leaves (2008) in Haliburton Forest, Ontario

		2007 Canopy	2008 Canopy	2008 Sapling
Number of galls dissected		887	71	242
<i>V. aceriscrumena</i>	Number of mites (all stages)	10 091	1842	3682
	Mean/gall			
	Founding adults	0.39 ± 0.51	0.35 ± 0.48	0.51 ± 0.50
	Eggs	6.9 ± 22.0 ^a	11.8 ± 29.0 ^b	14.7 ± 37.6 ^b
	Immatures (larvae + nymphs)	3.6 ± 12.0 ^a	6.6 ± 16.3 ^b	6.8 ± 32.3 ^b
	Protogynes	0.97 ± 3.46	NA	NA
New adults (deutogynes + males)	0.7 ± 14.7 ^a	7.2 ± 31.5 ^b	1.1 ± 3.4 ^b	
<i>T. acerbilis</i>	Number of mites (all stages)	3082	482	627
	Mean/gall			
	Adults	1.18 ± 6.74	4.95 ± 11.98	0.94 ± 3.82
	Eggs	1.54 ± 3.75	1.23 ± 4.09	0.60 ± 2.44
Larvae	0.75 ± 2.62	0.61 ± 1.80	1.05 ± 4.54	

Data are shown as the mean ± SD. For *V. aceriscrumena*, different superscript letters represent significant differences in median values ($P = 0.05$) based on nonparametric Kruskal–Wallis tests with Dunn's post-hoc comparisons. Note that in 2008 protogynes were not distinguished from immatures.

the summer, and a subsequent generation of overwintering, deutogyne females, which induce galls during the next spring. It appears that gall ostiole condition influences MSGM populations, with 'open' galls having lower numbers of MSGM eggs and immatures compared with almost closed galls. Open galls harbour substantial populations of another mite, *T. acerbilis*, which appears to be preying on maple spindle gall mite eggs. Hence, the shape of gall ostioles may indirectly determine mortality rates in the sugar maple spindle gall mite.

Life cycle and gall development

Where MSGM spends the winter is unclear. Deutogynes were presumed to overwinter in bud clusters (Baker *et al.*, 1996), although they may be more likely do so in bark crevices or beneath overlapping bark layers of branches and tree trunks (Hodgkiss, 1930; J. Amrine, personal communication). As indicated by the present study, deutogynes gather in leaf buds by early May and induce a gall on the upper side of young primordial leaves by feeding on the underside. During gall initiation, some deutogynes may either abandon the feeding site or may be preyed upon when they are vulnerable on the exposed leaf surface (given the absence of mites in some galls, and also observations that galls may keep growing after interrupted feeding; Royalty & Perring, 1996). Furthermore, more than one deutogyne may enter a gall, either because one female had abandoned its own gall or because two or more females may have fed close enough to produce a single gall (Oldfield, 1969). After the gall starts to expand, the deutogyne(s) lay eggs inside. Eggs hatch into larvae, develop into nymphs and later emerge as adult female protogynes that are morphologically distinct from deutogynes (originating from fertilized, diploid eggs), along with some males (from unfertilized, haploid eggs). Males appear to be rare, resulting in a sex ratio (>90% female) similar to the few other eriophyid species that have been studied (84–95% females; Sabelis &

Bruin, 1996). Protogynes may represent a single generation between the founding deutogynes and the production of new deutogyne females; however, we cannot exclude the possibility of an additional protogyne generation. The new deutogynes leave galls for hibernation sites off the leaves, presumably after insemination with a spermatophore left by males in the galls of the same or the previous generation. If any overwintering females remain unfertilized before winter, then they would presumably produce males parthenogenetically the next year before mating with them subsequently (Oldfield & Newell, 1973a, b; Oldfield & Michalska, 1996).

The high variation in deutogyne density during later summer is puzzling. Although immatures were relatively numerous in galls towards the end of July 2007 and mid-August 2008, the new deutogynes were few and often absent, except in a small proportion of galls that harboured over a hundred deutogynes in mid-August, surpassing the number of immatures seen. The near absence of deutogynes in most galls suggests that they leave their gall early after their emergence for overwintering quarters or, more strategically, to first locate a male spermatophore inside another gall to minimize inbreeding (if no males were present in their original gall). Oldfield and Newell (1973b) suggest that aggregation by deutogynes and males within a few galls in later summer is a means of facilitating outbreeding. Mortality as a result of fungal pathogens may be another factor contributing to high variation in densities of deutogynes and other life stages (McCoy, 1996). Fungal hyphae and spores were observed in many galls. Most of these galls contained one or more dead MSGM individuals, in contrast to galls lacking fungi where carcasses were rare (R. Patankar, personal observation).

There was significant variation in height among fully developed galls, which correlated with the number of MSGM eggs and immatures. A similar correlation has been observed in galls induced by *Aceria lantanae* Cook on *Lantana camara* L. leaves (R. M. d. S. Isaias, personal communication). Because

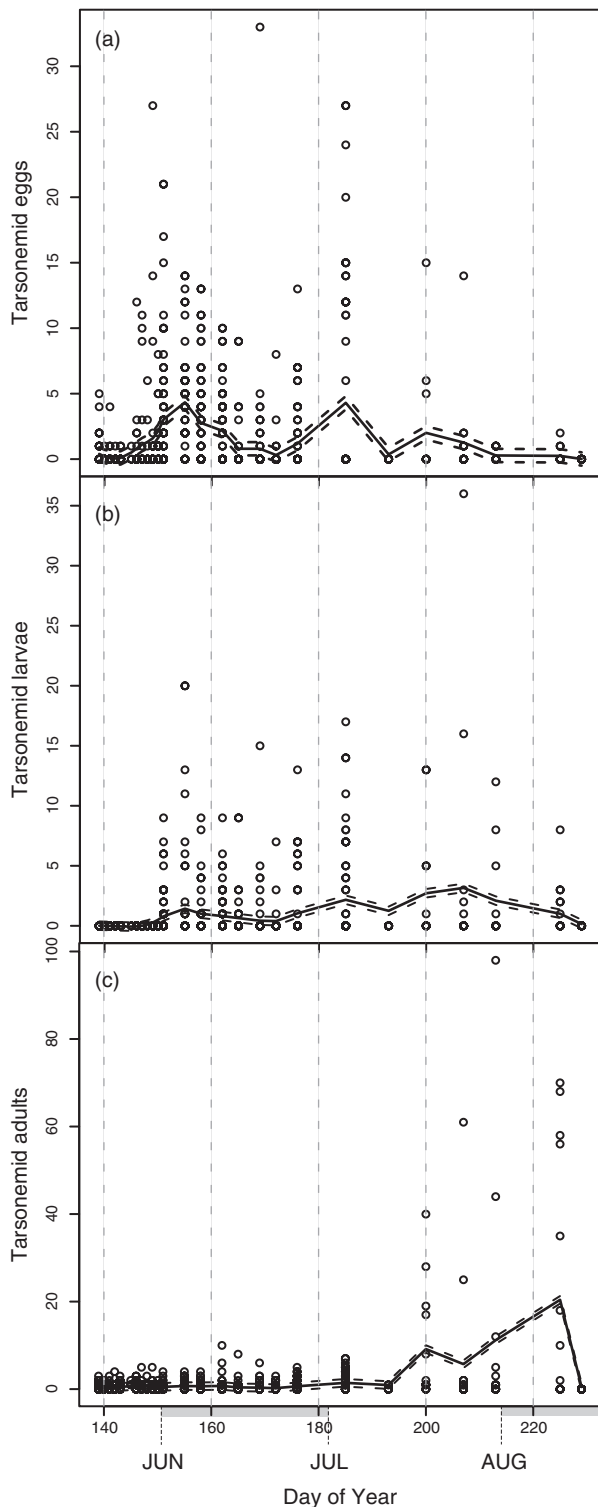


Figure 4 Phenology of *Tarsonemus acerbilis* found in maple spindle gall mite galls during the growing season of sugar maple in Haliburton Forest, Ontario. Data were collected from May to August in 2007 for the life-history stages: (a) eggs (b) larvae and (c) adults. Cubic smoothing splines (\pm 95% confidence limits) were fit to the data to detect peaks in numbers.

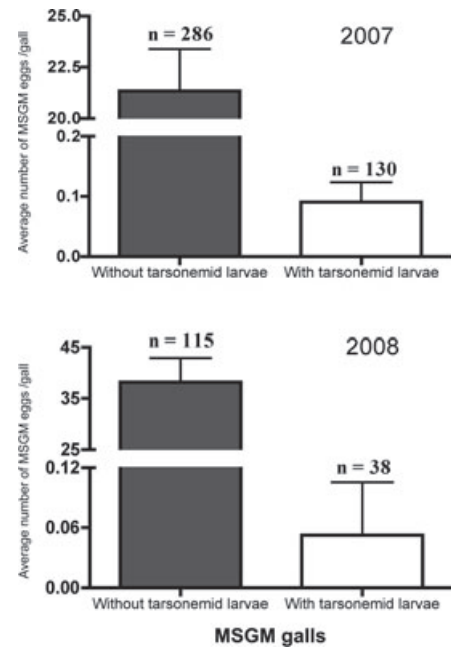


Figure 5 Mean number of maple spindle gall mite (MSGM) eggs in galls with (clear bars) and without (shaded bars) tarsonemid larvae from sugar maple canopy leaves in Haliburton Forest, Ontario, from 2007 and 2008. Almost all galls that contained tarsonemid larvae were devoid of MSGM eggs (2007: Mann-Whitney: $U = 1394$, $P < 0.001$; 2008: Mann-Whitney $U = 25$, $P < 0.001$).

feeding stages of MSGM other than the founding deutogyne have not yet appeared by the time galls attained their full height, only the feeding activity of that female could influence gall size. Females capable of inducing larger galls may have higher reproductive potential. Alternatively, higher mortality could occur in smaller galls, resulting from resource or interference competition, or from differential establishment of pathogenic fungi. Another, non-exclusive explanation is that larger galls have more nutritious cells, allowing for higher fecundity and population increase.

As previously reported (Hodgkiss, 1930; Keifer *et al.*, 1982), MSGM galls strongly vary in colour. It is unclear whether this variation is a result of the differential responses of host leaves to galling or a result of MSGM itself. Non-green pigments could serve a protective role by preventing photo-inhibition and photo-oxidation of gall tissue, thereby preserving the food source of the mites, especially in the canopy where these processes are more intense (Inbar *et al.*, 2010). Alternatively, yellow or red pigmentation could reflect elevated levels of host immune defenses (Schaefer & Rolshausen, 2006).

Tarsonemids in galls

With approximately 40% of galls harbouring *T. acerbilis*, MSGM galls appear to be a major habitat for this mite. Galls with broadly open ostioles were particularly susceptible to invasion, with approximately 25% inhabited by *T. acerbilis* adults, as opposed to 5.5% of galls with almost closed ostioles (see Supporting information, Fig. S1). In addition, 'open' galls had

approximately seven- and two-fold fewer MSGM eggs and immatures, respectively, than 'closed' galls. Although adults may also prey on eriophyids, the larvae of *T. acerbilis* appear to be the most avid predators of MSGM eggs, as suggested by the near absence of MSGM eggs in the presence of *T. acerbilis* larvae. When MSGM and tarsonemid eggs appear in galls at the same time, the earlier hatching of *T. acerbilis* eggs would enable them to feed on MSGM eggs, with many yet unhatched. Although phytoseiid mites and other itinerant predators may inflict mortality on MSGM populations during migration between galls or to overwintering sites (Sabelis, 1996), *T. acerbilis* may be the main mortality factor within galls and a major regulator of MSGM populations. Because MSGM eggs are largely absent from galls after mid-July, *T. acerbilis*, still common in galls until mid-August as larvae and adults, might feed on fungi inside galls. *Tarsonemus acerbilis* was described based on specimens collected in eriophyid galls on the leaves of silver maple *Acer saccharinum* L. (Delfinado, 1978), and it has also been found on red maple associated with another eriophyid (E. Lindquist, personal communication). Other tarsonemids have been found in eriophyid galls (Delfinado, 1978; Lindquist & Smiley, 1978; Kim *et al.*, 1998) and species from at least three genera were observed (or strongly suspected) preying on the eggs of tydeid, tetranychoid and eriophyid mites (Smiley & Landwehr, 1976; Mitrofanov *et al.*, 1986; Lin *et al.*, 2002; Ozman & Goolsby, 2005) or even the post-egg stages of eriophyids (Villanueva *et al.*, 1996). Hence, the available evidence suggests that *T. acerbilis* is an opportunistic, facultative predator of eriophyid eggs on maple trees in general, including vagrant eriophyids, that commonly use galls (e.g. pouch galls and erineae) as shelters and as a food source of mite eggs.

Evolution of gall morphology

Enclosed galls with narrow openings such as those induced by *V. aceriscrumena* provide protection against desiccation, and against most predators (Sabelis & Bruin, 1996); however, such galls are still vulnerable to small mite intruders such as tarsonemids and tydeids that facultatively feed on eriophyids (Perring & McMurtry, 1996). Small species or immatures of more voracious predatory mites, such as phytoseiids and stigmatids, and even primarily fungivorous oribatids, could also penetrate galls and prey on eriophyids (N *et al.*, 1986; Thistlewood *et al.*, 1996; Kawashima & Amano, 2004). The more protected the gall is (e.g. enclosed ostiole, dense trichomes in gall chamber), the less likely predators and competitors can enter. We speculate that selection pressures may be acting on eriophyids to induce galls with closed or guarded entrances allowing mainly or only the passing of eriophyids. It is also possible, however, that more tightly closed galls have higher humidity and are thus subjected to stronger fungal infestations, and this could explain the apparent severe mortality rate of MSGM immatures in closed galls (compared with egg numbers; Fig. 2a). A selective trade-off may therefore be acting on MSGM, between open galls with low fungal attacks, and almost predator-free, 'closed' galls. On the other hand, the host tree might also be selected upon, to keep the galls as open as possible, allowing predators (i.e. the plant bodyguards) access into galls to eliminate the plant 'parasites'. Given the remarkably large effects of MSGM

galls on performance of host trees (Pankar *et al.*, 2011), this evolutionary dynamic could have important implications at the scale of the forest community.

Supporting information

Additional Supporting information may be found in the online version of this article under the DOI reference:

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Fig. S1. Scanning electron microscopy image of a dissected maple spindle gall mite *Vasates aceriscrumena* (MSGM) gall showing eggs and immatures within the inner chamber (A) and variation in ostiole morphology (B, C) among early-season MSGM galls on *Acer saccharum* leaves: (B) an 'open' entrance and (C) a 'closed' gall.

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