Title: Contribution of hardwood trees to budworm – parasitoid food web dynamics

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Abstract

A major pest of Atlantic forests is the spruce budworm caterpillar which outbreaks every 35 years and causes large scale tree mortality. Historically, budworm management has largely ignored other species in the food web. Broadening the focus could reduce budworm outbreaks while balancing the multiple demands on our forests. However, the food web surrounding budworm including other caterpillar species that are attacked by budworm parasitoids has been relatively undersampled and under-researched. Therefore, we tested two hypotheses: the **alternating hardwood-softwood parasitoids hypothesis** where parasitoids attack other caterpillars on hardwoods when budworm are rare and attack budworm on balsam fir or other softwoods when budworm are plentiful, and the **mixed stands natural enemies hypothesis** where stands with a mixture of softwood and hardwood trees harbour greater abundances and diversity of budworm parasitoids. We tested these hypotheses using stable isotope analysis of budworm parasitoids and through community analyses of parasitoids sampled along a hardwood gradient. We found indications that parasitoids do attack caterpillars on hardwoods and budworm on balsam fir, but found mixed results for the natural enemies hypothesis. Our study highlights the importance for budworm management of understanding the dynamics of the food web surrounding budworm.

Keywords

Choristonuera fumiferana, *Abies balsamea*, hardwood, parasitoids, trophic relationships, natural enemies hypothesis

Introduction

Spruce budworm (*Choristonuera fumiferana*) in eastern North American forests have massive outbreaks every 35 years (Royama et al. 2017). These outbreaks last about 5-15 years, severely defoliating balsam fir trees and causing high growth loss and tree mortality (Hennigar et al. 2008). Budworm outbreaks have been known to damage 50 million hectares of eastern Canadian forests and have large impacts on the forestry sector (Chang et al. 2012). Consequently, finding methods to reduce budworm outbreaks is important to maximize forestry economic activity while minimizing losses of balsam fir and species of spruce.

Many methods to reduce budworm outbreaks focus on budworm and neglect other species and downstream consequences. Chemical insecticides, including tebufenozide, are regularly used to control budworm outbreaks with mixed results (Holmes and MacQuarrie 2016). These chemical insecticides often kill or impair other caterpillars, natural enemies, and local wildlife (McCravy et al. 2001; Holmes and MacQuarrie 2016). Specifically, when chemical insecticides kill caterpillars, including budworm, any internally feeding parasitoids are also killed affecting subsequent generations of budworm's natural enemies (Brown 1994). Similarly, spraying forests with *Bacillus thuringiensis* (B.t.), a bacterium that produces a spore toxic to lepidoptera (butterflies and moths, including budworm), can have detrimental effects on the natural enemies of budworm by killing any internally feeding parasitoids (Nealis and van Frankenhuyzen 1990). Furthermore, B.t. kills other lepidoptera which can have downstream consequences on local populations of birds that consume lepidoptera (Sopuck et al. 2002). Another method is to pre-emptively cut down spruce and balsam fir trees, thus reducing the food source of budworm (Crook et al. 1979; Bauce 1996). Thinning has variable effectiveness depending on the stage of the budworm cycle and on the hardwood content (Crook et al. 1979; Bauce 1996). All of the above methods have been incorporated into the more holistic management approaches of integrated pest management (IPM) and ecosystem management (EM), where the focus of management has been expanded to the whole forest ecosystem and multiple techniques are used that balance pest management, economic outcomes, and human and environmental health (Alfaro and Langor 2016). These two approaches highlight the importance of understanding the whole food web surrounding the pest species to augment natural mechanisms that can reduce the severity of pest outbreaks and sustainably manage the multiple demands on eastern North American forests.

When considering the whole food web, a promising alternative to reduce the severity of budworm outbreaks is to use insects that parasitize and then kill a caterpillar host (parasitoids). The budworm population fluctuations are generally considered to be a predator - prey cycle, but in this cycle, the predator is actually a collection of natural enemies including invertebrate predators, birds and parasitoids (Royama et al. 2017).

Within this collection of natural enemies, parasitoids have arguably the strongest impact on budworm mortality causing between 30-90% mortality depending on the surrounding forest composition and the point in the budworm cycle (Cappuccino et al. 1998; Royama et al. 2017). Furthermore, the parasitoid community responds strongly to budworm density with increases in diversity cascading up parasitoid trophic levels (the bird feeder effect) (Eveleigh et al. 2007). With such strong mortality effects and community responses, parasitoids are a promising natural mechanism for reducing the severity of budworm outbreaks.

Hardwood trees may support larger populations of budworm parasitoids, and thus have the potential to mute budworm outbreaks. Mixed forest stands containing hardwood trees have been found to have lower balsam fir defoliation compared to balsam fir dominated stands during budworm outbreaks (Su et al. 1996; Zhang et al. 2018, 2020). Furthermore, Eveleigh et al. (2007) found lower peak budworm densities in heterogeneous plots compared to homogeneous plots. One reason for defoliation and density differences is greater dispersal loss of early instar budworm larvae in hardwood dominated plots (Zhang et al. 2020). Another mechanism suggested for these patterns is that hardwood trees maintain a steady variety of other caterpillars which harbour a greater diversity and abundance of parasitoids overall (Eveleigh et al. 2007). Indeed in an initial survey, Eveleigh et al. (2007) did find increased diversity and abundance of parasitoids in plots with greater proportions of hardwood trees. Essentially this is the natural enemies hypothesis which predicts that in agroecosystems, there should be increased abundances of natural enemies in species rich plant assemblages compared to species poor assemblages (Letourneau 1987). Even with some important differences between agroecosystems and forest plantations (see Koricheva et al. (2006)), forest plantations could be argued as similar in the sense that monocultures of plants or crops are planted and harvested. Consequently, testing the natural enemies hypothesis in the budworm food web is a worthwhile pursuit because supporting natural enemy populations has the potential to reduce the severity of budworm outbreaks.

To date, the quantification of the feeding relationships of parasitoids on balsam fir and hardwoods has been sparsely researched, nor has the natural enemies hypothesis been explicitly tested for budworm. First, we need to establish whether parasitoid communities actually attack caterpillars on hardwoods and balsam fir. This has been anecdotally done (Krombein et al. 1979) and there has been quantification of parasitoids attacking non-budworm caterpillars on balsam fir (Eveleigh et al. 2007; Smith et al. 2011; Greyson-Gaito et al. 2021) . These non-budworm caterpillars are regularly found on hardwood trees. Yet, due to the understandable focus on pest species by the forestry industry, eastern North American hardwood non-pest caterpillar populations have rarely been sampled, limiting our understanding of the feeding relationships of budworm parasitoids. Second, if parasitoids do attack caterpillars on hardwoods, we need to establish if the natural enemies hypothesis can be applied to balsam fir - hardwood forest stands. Although the importance of tree diversity to budworm control has been periodically brought up since the 1920s (Miller and Rusnock 1993), tests of tree diversity have rarely examined budworm parasitoid diversity and abundance, and instead have mostly examined budworm defoliation (Su et al. 1996; Zhang et al. 2018) or quantified parasitism rates (Legault and James 2018; Zhang et al. 2020). Overall, a comprehensive examination of the whole budworm food web including caterpillars and parasitoids on hardwoods is required.

We endeavoured to facilitate this whole budworm food web examination using a variety of methods spanning multiple years and stands with varying hardwood content. We hypothesized that parasitoids attack other caterpillars on hardwoods when budworm are rare and attack budworm on balsam fir or other softwoods when budworm are plentiful (**alternating hardwood-softwood parasitoids hypothesis**). Using stable isotope analysis, a common tool for examining feeding relationships, we predicted that parasitoids collected in years with low budworm density would have similar stable isotope signatures to hardwood signatures, and parasitoids collected in years with high budworm density would have similar signatures to balsam fir signatures. We also hypothesized that the stands with a mixture of softwood and hardwood trees would harbour greater abundances and diversity of budworm parasitoids (**mixed stands natural enemies hypothesis**). Using a variety of budworm reared and Malaise caught parasitoids, we tested whether parasitoid abundances, richness, and phylogenetic community structure differed along a hardwood gradient. Importantly, we found indications that parasitoids do attack caterpillars on hardwoods and budworm on balsam fir, but found mixed results for the natural enemies hypothesis, with trends for lowered parasitoid abundances and consistent phylogenetic clustering in balsam fir dominated stands.

Methods

Study sites

All sampling was done in the Acadia Research Forest (ARF) near Fredericton (66°25'W, 46°00'N). The ARF is a 9,000 ha (22,230 ac) experimental forest with a mixture of softwood, hardwood, and mixed wood stands. Spruce (*Picea* spp.) and balsam fir (*Abies balsamea* (L.) Mill.) are the most abundant trees (Swift et al. 2006). All plots were outside areas of aerial application of insecticides for budworm control.

Alternating hardwood-softwood parasitoids hypothesis

Sampling

All sampling was performed in the same balsam fir dominated plot in ARF for the years of 1982, 1984, 1986, and 1987. Parasitoids were collected using modified 1 m^3 Malaise traps (Nyrop and Simmons 1982). A Malaise trap was placed with the open sides perpendicular to the tree trunk at the top, middle, and lower crown levels of three balsam fir trees separated by approximately 100 metres (i.e. 3 traps at each crown level, 9 traps in total). The Malaise traps were placed in the same trees every year beginning in May and ending in September. Flying insects were collected daily, immediately stored in 70% ethanol, and frozen at -7°C until preparation for stable isotope analysis in 2017 (except insects collected in 1982 which were stored without ethanol but still in the freezer). In 2017, we separated fourteen Dipteran species and three species of Ichnuemonids and Braconids that were sampled in the Malaise traps into three functional groups (see Table S1): Group 1, parasitoids that attack budworm larvae before budworm diapause, and parasitoids that attack post-diapause budworm but do not require alternate caterpillars to overwinter; Group 2, Diptera that attack post-diapause budworm but where it is unknown whether the Diptera need an alternate caterpillar to overwinter; Group 3, any parasitoid species that attacks post-diapause budworm and require an alternate caterpillar in which to overwinter. These three groups were then further split into three periods to capture the phenology of the parasitoid emergences from budworm and other caterpillars: May/June, July, and August/September. When there were fewer than 50 total individuals in a group and time period, all individuals were used for stable isotope analysis. When there were more than 50 total individuals in a group and time period, we randomly selected 50 individuals and ensured the proportions of selected individuals of each species matched the proportions of total number of individuals for each species (within the group and time period). We removed legs and wings from all individuals, keeping the mass of legs and wings approximately constant between individuals and species. Legs and wings were combined for each group and time period and were dried at 60°C for at least 48 hours. We used legs and wings because many parasitoids as adults consume non-host nutrient sources, and legs and wings have a slower turnover rate compared to other body parts (Gratton and Forbes 2006; Benelli et al. 2017)

Baselines for the stable isotope analysis consisted of balsam fir and hardwood foliage, and caterpillars from these sampled foliage in 2017. Beginning on May 30th and ending on June 27th, we sampled a metre long branch from the mid canopies of 45 balsam fir trees from 9 plots in ARF once a week (see **Mixed stands natural enemies hypothesis** for the selection of these balsam fir trees). Each week, we also sampled a metre long branch from the most abundant hardwood tree species in each plot. On the 17th July and on the 4th August, we randomly sampled a single balsam fir branch from each plot, and we sampled branches from the same hardwood species as we sampled in June (a branch per species in each plot). We sampled foliage without any noticeable herbivory damage from all branches. This foliage was rinsed with distilled water and dried at 60°C for at least 48 hours. We ground the foliage and ensured that the combination of different hardwood species in each plot's ground sample matched the proportions of hardwood trees found in each plot. This was repeated for June, July and August. From the balsam fir branches and the hardwood branches, we collected all caterpillar individuals and separated them into caterpillars from balsam fir or hardwoods and by plot and by time period. The caterpillar samples were dried at 60°C for at least 48 hours. All parasitoid, caterpillar and foliage samples were analysed for carbon and nitrogen isotope ratios at the University of Windsor GLIER (Windsor, ON, Canada) laboratories.

Statistical Analyses

The δ13C of the parasitoid samples were enriched by 16% compared to the baselines probably because the parasitoid samples were stored in ethanol for about 30 years (Jesus et al. 2015). Therefore, we were not able to use any standard mixing model analyses. Instead, we decided to compare the δ13C between years, time periods (budworm larvae present or absent), and functional groups because we knew that there were consistent differences in δ13C between hardwood and softwoods which were transferred to the caterpillars (Balsam fir and hardwood Welch t-test: $t = 2.813$, $df = 40.219$, $P = 0.00756$. Balsam fir caterpillars and hardwood caterpillars Welch t-test: $t = 3.161$, $df = 39.161$, $P = 0.00303$). Note, from the three sampling periods above (May/June, July, August/September), we simplified the periods into two time periods of budworm larvae present (May/June) and budworm larvae absent (July and August/September) by averaging the δ13C values of the July and August/September periods. We ran a generalized least squares regression to test the effects of year, time period (budworm larvae present or absent), parasitoid group, and all interactions on the δ13C of sampled parasitoid legs and wings (function gls, R package nlme, version 3.1-137, (Pinheiro et al. 2018)). We added a varIdent structure to account for heterogeneity in variation between the time periods. We fitted the full model using maximum likelihood estimation and then used backwards selection with log likelihood ratio tests to select the final fixed effects. We refitted the final model using restricted maximum likelihood estimation to give unbiased maximum likelihood predictors (Zuur et al. 2009) .

Mixed stands natural enemies hypothesis

Sampling – reared parasitoids

To understand the response of parasitoid communities to seeding of budworm caterpillars along a hardwood gradient, in 2014, nine 150 metre by 120 metre plots were selected, where three were balsam fir dominated (70% balsam fir – BFBF), three were hardwood tree dominated (75% hardwood – HWBF), and three had an even mixture of balsam fir and hardwood trees (40-60% balsam fir – BFMX). The nine plots were chosen using a forest cover map provided by the ARF, lidar maps, and ground truthing. In 2015, 2016, and 2017 five balsam fir trees, at least 20 metres apart and with healthy crowns, were chosen within each plot in the ARF (45 total). Different trees were selected in each year. In April of 2015, 2016, and 2017, 2,000 2nd instar budworm individuals were placed onto each of the 45 trees. Budworm individuals were reared by Insect Production Services (IPS) at the Great Lakes Forestry Centre in Sault St Marie, Ontario on a bed of gauze, which were cut up into squares of about 250 caterpillars. We placed a total of eight squares on each of the 45 trees, with each square being pinned to the underside of single branch in the mid-crown layer that had new growth. While budworm were active in the summers of 2015-2017, one foliated balsam fir branch from each of the 45 trees was sampled at least once a week. We sampled those branches that had a guaze pinned to them to maximise the collection of budworm larvae. Budworm were collected from these branches and placed in vials with artificial diet made at IPS (McMorran 1965). From these sampled budworm, we counted the number of budworm and the number of parasitoids that emerged.

Sampling – Malaise caught parasitoids

To passively examine the abundance and diversity of budworm parasitoids along a hardwood gradient, we placed a Malaise trap in every plot chosen above, close to one of the trees where budworm individuals were seeded, between May 19th 2016 and August 11th 2016. The flying insects from the Malaise traps were sampled once a week when budworm larvae were present, and once a month when budworm larvae were absent. We separated out individuals belonging to insect families that we knew contained species that attack budworm. These families included Tachinidae, Sarcophagidae, and Ichnuemonidae. We stored the collected parasitoids in 70% ethanol and in a refrigerator at 4°C, until they were barcoded.

DNA Barcoding

To quantify parasitoid diversity and phylogenetic structure, we used DNA barcodes. Tissue samples were taken using 1-6 legs and placed in 30 µL of 95% ethanol and stored at -20°C. Mitochondrial DNA from the cytochrome *c* oxidase I (COI) region (the standard animal DNA barcode locus) was amplified and sequenced at the Biodiversity Institute of Ontario (BIO; University of Guelph, Ontario). High resolution photographs were taken of wet specimens under a dissecting microscope using Leica Application Software V4.9. Sequences and photographs were uploaded to the Barcode of Life Data System (Ratnasingham and Hebert 2007). For diversity measurements, we used Barcode Index Numbers (BINs), a DNA-based delineation of species based on patterns of intra and interspecies variations outlined by Ratnasingham & Hebert (2013). We constructed a single-representative maximum likelihood tree in MEGA6 based on estimation of the best substitution models in MEGA6 (Nei and Kumar 2000; Tamura et al. 2013).

Statistical Analyses

Parasitoid abundances

For the reared parasitoids, we performed an ANOVA with per capita emergences (reared parasitoid abundances divided by number of budworm reared in each plot) as the response variable and forest type, year and their interaction as the explanatory variables. We used per capita emergences instead of abundances to account for potentially different abundances of budworm sampled between forest types and years. For the Malaise caught parasitoids, we performed an ANOVA with abundances as the response variable and forest type as the explanatory variable.

Parasitoid richness

We ran an ANOVA on the log10 of the Chao1 estimation of species richness in each plot (function ChaoSpecies, R package SpadeR, version 0.1.1, (Chao et al. 2016)) with forest type as the explanatory variable for the Malaise caught parasitoids.

Phylogenetic community structure

To examine how hardwood content affects phylogenetic community structure of budworm parasitoids, we calculated the mean nearest taxon distance (MNTD) using maximum likelihood trees between the three forest types for the Malaise caught parasitoids. Maximum likelihood trees used a general time reversible model with discrete gamma distribution and under the assumption that sites are evolutionarily invariable (Nei and Kumar 2000; Tamura et al. 2013). The standard effect size of the MNTD was then calculated and phylogenetic clustering and dispersion assessed by performing 1000 random permutations of hardwood content associations to simulate a distribution of MNTD for each community. The significance of the observed MNTD values for each community was examined with a two-tailed test of significance ($p = 0.05$) (function ses.mntd, R package Picante, version 1.7, (Kembel et al. 2010)).

As a further comparison of phylogenetic clustering in plots differing in hardwood content, we calculated the mean nearest taxon distance (MNTD) and assessed phylogenetic clustering and dispersion (function ses.mntd, R package Picante, version 1.7, (Kembel et al. 2010)) of reared parasitoids collected from the three plots in Eveleigh et al. (2007). Note, these parasitoids were reared from both budworm and other caterpillars found on the study's balsam fir trees. Eveleigh et al. (2007) compared the richness of reared parasitoids between three plots with differing tree compositions (tree basal area, Plot 1: balsam fir 98%, Spruce 1%, Hardwood 1%. Plot 2: balsam fir 77%, spruce 8%, hardwood 14%. Plot 3: balsam fir 50%, spruce 36%, hardwood 14%). A subset of these parasitoid species were preserved at -20ºC then DNA barcoded to explore how genetic estimates of isolation and species identification changed the estimates of food web connectance (connectance was reduced as the number of nodes increased) (Smith et al. 2011). However, Smith et al. (2011) did not report estimates of phylogenetic community structure for the parasitoids of these three plots, and so in this study we add an examination of phylogenetic community structure of parasitoids sampled in the 1980s and compare with phylogenetic clustering of parasitoids sampled along a hardwood gradient in 2016. For further details of the three plots and all sampling and rearing procedures, see Lucarotti et al. (2004), Eveleigh et al. (2007) (SI Materials and Methods) and Royama et al. (2017).

Results

Alternating hardwood-softwood parasitoids hypothesis

The final model explaining δ13C included year, group, time period (budworm larvae present or absent), and the interactions of year with time period (year:time period interaction, L = 13.230, P = 0.0013, df = 1, log likelihood ratio test, Figure 1) and group with time period (group:time period interaction, L = 28.900, P < 0.0001, df = 1, log likelihood ratio test, Figure 1). Group one parasitoids became slightly more negative by approximately 0.5% each year, and group one parasitoids caught when budworm were absent had more negative δ13C values by 2.4% compared to group one parasitoids caught when budworm were present. δ13C values for group two parasitoids became less negative overtime by approximately 1.6% each year. Group three parasitoids showed a difference of 12.2% in δ13C between when budworm larvae were present and absent. When budworm larvae were present, group three parasitoids had more negative δ13C values similar to hardwood trees (average δ13C of hardwood trees in 2017 = -30.222). When budworm larvae were absent, group three parasitoids had less negative δ13C values similar to balsam fir trees (average δ13C of balsam fir trees in 2017 = -29.521). In comparison to the difference in δ13C between when budworm were present or absent, δ13C for group three parasitoids changed little with no noticeable trend between years.

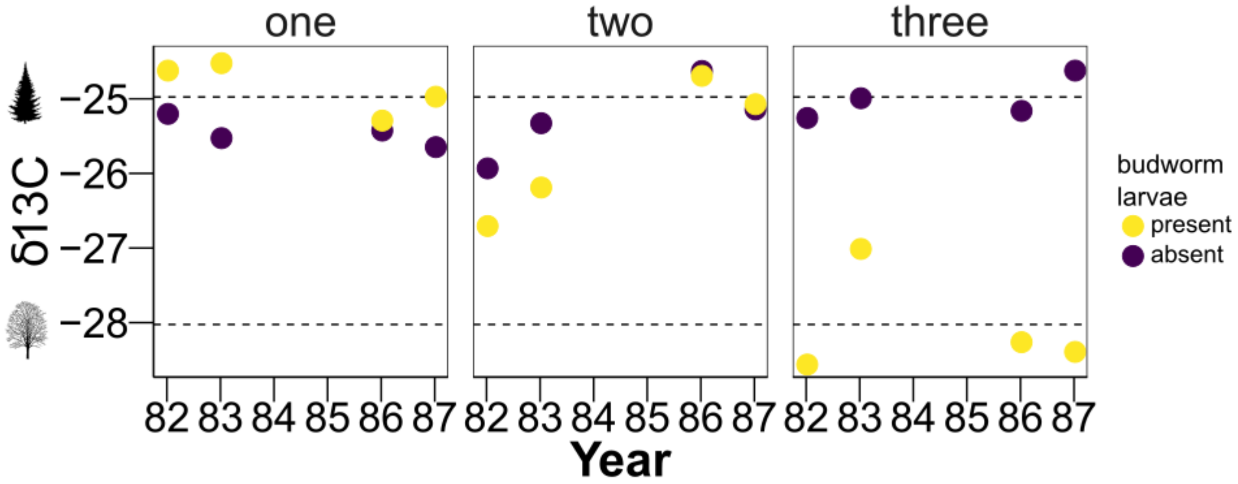

Figure 1 Several parasitoid species attacked budworm when budworm larvae were

plentiful and other caterpillars on hardwoods when budworm larvae were rare. δ13C for three groups of parasitoid species: group one attack only budworm within a year (left plot), group two attack budworm and likely caterpillars on hardwoods within a year (centre plot), and group three alternate between attacking budworm and caterpillars on hardwoods within a single year (right plot). Budworm populations peaked in 1985. δ13C was measured on parasitoids captured in May and June when budworm larvae were present and in July, August, and September when budworm larvae were absent. Dashed lines depict the average δ13C value for the group three parasitoids when budworm were present and absent (used as estimates for the balsam fir and hardwood foliage δ13C values). See Figures S1, S2, S3 for time series of the abundances of the parasitoids in each group. Balsam fir and red maple images shown on the y-axis are publicly available from Natural Resources Canada, Canadian Forest Service.

Mixed stands natural enemies hypothesis

Parasitoid abundances

Generally, hardwood content did not appear to affect parasitoid per capita emergence and abundance, but the year of 2016 exhibited correspondence of lowered per capita emergence and abundance in balsam fir dominated plots. The per capita emergences of reared parasitoids were significantly different between years but not between forest types (two-way ANOVA, Year: F = 27.254, df = 1, P < 0.001, Cohen's f = 1.14, Power = 1. Forest type: F = 0.247, df = 2, P = 0.784, Cohen's f = 0.15, Power = 0.202, Figure 2a). Although the mean abundance of Malaise-caught parasitoids in mixed wood plots (BFMX) and hardwood dominated plots (HWBF) forest types was 77% larger than the mean abundance of Malaise-caught parasitoids in balsam fir dominated plots (BFBF), the abundance of Malaise-caught parasitoids did not significantly differ between forest types (ANOVA, $F_{2,6}$ = 1.857, P = 0.236, Cohen's f = 0.787, Power = 0.360, Figure 2b).

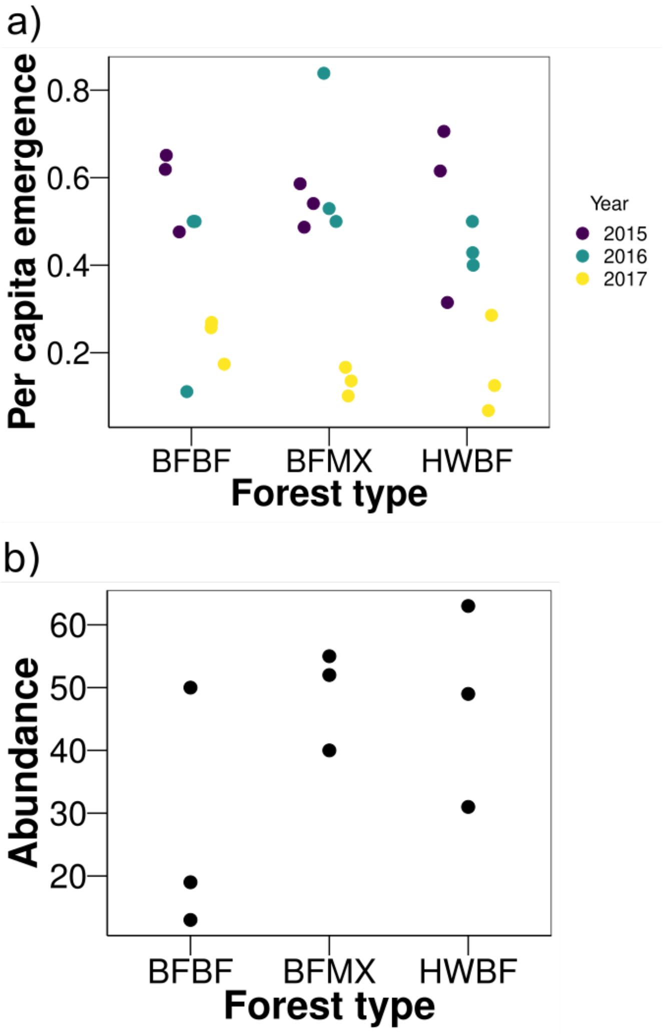

Figure 2 Generally, hardwood content did not appear to affect per capita emergence and abundance, but the year of 2016 exhibited correspondence of lowered per capita emergence and abundance in balsam fir dominated plots. a) Per capita emergences of reared parasitoids from budworm in 2015, 2016 and 2017 and b) abundance of Malaise-caught parasitoids in May through to September of 2016 from three plots each of three forest types in the Acadia Research Forest. BFBF is balsam fir dominated, BFMX is a mixture of balsam fir and hardwood trees, and HWBF is hardwood dominated.

Parasitoid richness

The Chao1 number of species of Malaise caught ($F_{2,6}$ = 0.546, P = 0.605, Cohen's f = 0.43, Power = 0.136) parasitoids did not differ between forest types.

Phylogenetic clustering

Plots dominated by balsam fir were consistently phylogenetically clustered. Phylogenetic clustering was found in the balsam fir dominated plots with Malaise caught parasitoids from 2016 (BFBF MNTD z =-2.375, P = 0.005. Figure 3a). Neither phylogenetic clustering nor dispersion were found in the mixed forest plots and the hardwood dominated plots with Malaise caught parasitoids from 2016 (BFMX: MNTD z = 1.191, P = 0.888. HWBF: MNTD z = -1.303, P = 0.096. Figure 3a). Phylogenetic clustering was tentatively found in Plot 1 from the 1980s (Balsam fir dominated MNTD z = -1.639, p = 0.053, Figure 3b). Neither phylogenetic clustering nor dispersion were found in the two other plots from the 1980s (Plot 2: MNTD z = -1.509, p = 0.066. Plot 3: MNTD z = -0.546, p = 0.0.296. Figure 3b).

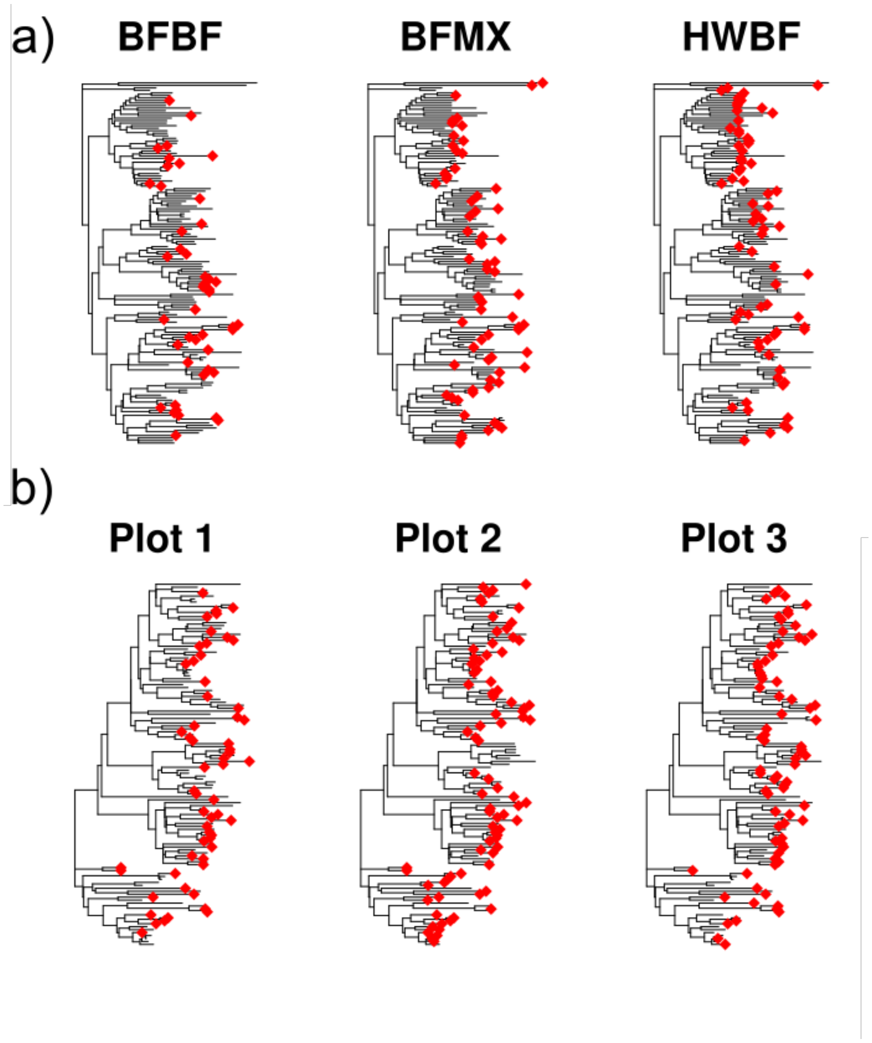

Figure 3: Phylogenetic clustering was consistently found in balsam fir dominated plots. a) Phylogenies of Malaise caught parasitoid communities with presence denoted by diamonds in three balsam fir dominated plots (BFBF), three mixed wood plots (BFMX), and three hardwood dominated plots (HWBF) in Acadia Research Forest in 2016. b) Phylogenies of parasitoid communities with presence denoted by diamonds for Plots 1, 2 (Acadia Research Forest), and 3 (Saint-Quentin) for all years sampled (1983-1995).

Tree basal area, Plot 1: balsam fir 98%, Spruce 1%, Hardwood 1%. Plot 2: balsam fir 77%, spruce 8%, hardwood 14%. Plot 3: balsam fir 50%, spruce 36%, hardwood 14%.

Discussion

Our study has shown that the budworm parasitoid community was trophically linked to hardwood trees, and hardwood content likely affects budworm community structure. From comparing the stable isotopes of parasitoids during a budworm outbreak, we found that several parasitoids attacked budworm when budworm were plentiful and attacked other caterpillars on hardwood trees when budworm were rare. We also sampled parasitoids along a hardwood gradient by two methods: budworm rearing and Malaise trapping. Although individual analyses of parasitoid abundance and richness did not find differences along the hardwood gradient, phylogenetic community structure was consistently clustered in balsam fir dominated plots. Taken together, our study highlights the need to include hardwood trees when examining budworm dynamics and the need to carefully consider the scale of hardwood tree placement when attempting to reduce budworm outbreaks.

Considering the small sample sizes, we still found support for the alternating hardwood-softwood parasitoid hypothesis. Group three parasitoids provide us with the clearest comparison of balsam fir and hardwood usage because they attack budworm in the summer every year but then must overwinter in other caterpillar species that are often on hardwoods (Maltais et al. 1989; Cusson et al. 1998; O'Hara 2005). The difference in group three parasitoid δ13C between when budworm were present (group three parasitoids emerging from other caterpillar species to attack budworm) and absent (group three parasitoids emerging from budworm to attack other caterpillars) matches what we know of the life history of group three parasitoids (Figure 1). Therefore, we can be confident that any comparable changes in δ13C for the other groups should be due to the parasitoids changing their attack rates on budworm on balsam fir and other caterpillar species on hardwoods. Group one parasitoids seemingly did not attack other caterpillars on hardwoods as budworm densities declined (Figure 1), consistent with other studies that concluded that these parasitoids attack budworm more than other caterpillar species (O'Hara 2005; Cossentine et al. 2007). Another possibility is that the populations of these parasitoids are supported by other caterpillar species that feed on balsam fir as suggested by *Apanteles fumiferana* and *Glypta fumiferana* attacking other caterpillar species on balsam fir (Greyson-Gaito et al. 2021) . Consequently, sampling and rearing of caterpillars on balsam fir and hardwoods when budworm are rare is required to establish how group one parasitoid populations are maintained. In contrast to group one parasitoids, group two parasitoids exhibited greater change in δ13C over time suggesting that these parasitoids likely attacked other caterpillars on hardwoods and then attacked budworm on balsam fir (Figure 1). Again sampling and rearing of caterpillars on balsam fir and hardwoods when budworm are rare is crucial to understanding the contributions of caterpillars on hardwoods to the population dynamics of group two parasitoids.

Interestingly, the pattern that groups two and three parasitoids exhibited could be classed as coupling, an important stabilizing ecological mechanism (McCann et al. 2005). Coupling usually occurs when a generalist consumer attacks prey from two or more spatially separate subgroups of a larger food web (resource compartments) (McCann et al. 2005). In the budworm food web, the parasitoids may be coupling the balsam fir and hardwood resource compartments over time which, if fostered, could mute the budworm oscillations, leading to less severe outbreaks. Overall, there is a strong suggestion that several parasitoids do attack other caterpillars on hardwoods when budworm are rare and attack budworm on balsam fir when budworm are plentiful, thus coupling the softwood and hardwood food webs. A promising method for evaluating this softwood/hardwood coupling is by using qPCR approach to determine whether and by what a budworm larvae has been parasitized (Nisole et al. 2020). This method promises to significantly reduce sorting time and costs. However, this method requires *a priori* knowledge of what species to include in the qPCR assay. To ensure that the coupled effects we suggest here are indeed measurable in future qPCR assays, we suggest that DNA libraries of budworm parasitoids and parasites be expanded to include representation from hardwood forest parasitoid communities. Overall, sampling of parasitoids and caterpillars on softwoods and hardwoods throughout the budworm cycle is required to ensure adequate DNA libraries for the qPCR method and in turn to evaluate the contribution of hardwoods to parasitoid population maintenance and softwood/hardwood coupling.

Evidence for the natural enemies hypothesis comes from the corroboration of similar trends between our different analyses. Parasitoid per capita emergences and abundances were not significantly different along the hardwood gradient (Figure 2 a & b). Nor was parasitoid Chao1 richness different along the hardwood gradient. Nevertheless, there was correspondence of lowered per capita emergence and abundance in the balsam fir dominated plots in 2016 compared to the other forest types (Figure 2 a & b). Furthermore, the balsam fir dominated plots exhibited phylogenetic clustering in 2016 and in the 1980s. Because sister parasitoid species are more likely to share host species or search within the same plant species than distantly related parasitoid species (Ives and Godfray 2006), the observed phylogenetic clustering suggests that environmental filtering dominates over competition (Webb et al. 2002). In this case, the environmental filtering likely occurs due to the differences in caterpillar composition maintained by balsam fir dominated stands compared to stands with greater hardwood content. Consequently, our study provides further hints of the natural enemies hypothesis within the budworm food web.

A full reckoning of the natural enemies hypothesis in the budworm food web requires careful consideration of scale. Our study examined a relatively small scale (plots were 150m by 120m and the Acadia Research Forest is 90km^2) compared to the large distribution of budworm outbreaks. Furthermore, hosts and parasitoids disperse, aggregate, and are influenced by landscape structure at different scales often larger than a few hundred metres (Cronin and Reeve 2005). Therefore, our study may not be able to discern a natural enemies hypothesis signal because the diversity of trees may not influence parasitoid abundance and richness at the scale of less than a kilometre. Indeed, Legault and James (2018) found that the parasitism rate of budworm by *Apanteles fumiferena* was positively correlated with tree diversity at 3km, and the parasitism rate of budworm by *Glypta fumiferana* was negatively correlated with non-host tree density at 15km. Legault and James (2018) suggest that the different dispersal abilities of parasitoids underly how parasitoids respond to landscape structure pattern. *A. fumiferana* is smaller than *G. fumiferana* (~3.5mm compared to ~8.0mm in length) and likely disperse less than *G. fumiferana*. Thus, *A. fumiferana* would be affected by tree composition at smaller scales than G. fumiferana (Legault and James 2018). Similar to our study, Zhang et al. (2020) did not find any difference in parasitism rate of budworm across a hardwood gradient. Again, Zhang et al.'s (2020) plots were 500m^2, much smaller than the determining scale found in Legault and James (2018). Interestingly, although the aggregate measures of parasitism rate, abundance, and richness were not found to be different in our study and Zhang et al.'s (2020) study, phylogenetic clustering in balsam fir dominated plots was consistently found even with small plot scales and two different methods of parasitism sampling (Malaise caught and reared). Therefore, the scale of our plots do seem to be affecting phylogenetic community structure. The aggregate measures may not be affected because either too few replicates were done or compensatory dynamics may be obscuring any effects of local hardwood content on aggregate measures especially parasitism rates as suggested by Royama et al. (2017) and Bouchard et al. (2018). In all likelihood, a mixture of scale dependency, replication, and compensatory dynamics is affecting our ability to find the natural enemies hypothesis, if it exists. Future research will require greater replication over a variety of spatial and temporal scales to understand the parasitoid community's response to hardwood content.

Hardwood trees in forest stands have long been thought to be important to reducing the severity of budworm outbreaks. Rarely, have studies examined the trophic relationships between budworm parasitoids and caterpillars on hardwood trees. In our study, we hope to spark this important research direction by finding that several parasitoids do attack caterpillars on hardwood trees when budworm are rare and budworm on balsam fir when budworm are plentiful. This pattern could be classed as coupling. Because coupling is thought to mute variability, hardwoods trees may be integral to reducing the severity of budworm outbreaks. Further testing of the coupling of softwood and hardwood food webs could use fatty acid analysis because the fatty acid compositions differ between softwoods and hardwoods more than δ13C (Mueller et al. 2012). Furthermore, comprehensive sampling of caterpillars on softwood and hardwood trees combined with qPCR to cheaply quantify the caterpillar's parasitoids could be another effective approach to test the coupling of softwood and hardwood food webs. In theory, hardwood trees maintaining parasitoids populations should translate to finding greater parasitoid abundance and richness in plots with a mixture of softwood and hardwood. We found hints of hardwood content affecting parasitoid communities, but careful testing of the scale of hardwood trees is required. Overall, alongside the knowledge that hardwood dominated stands increases dispersal loss of early instar budworm larvae (Zhang et al. 2020), we have provided further evidence that hardwoods are important for reducing the severity of budworm outbreaks.

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Author contributions

ESE designed the initial studies. ESE, WM, GF, RL, CJGG, and SJD did the field and laboratory work. CJGG did the statistical analyses with assistance from ESE, MAS, SJD, and KSM. CJGG wrote the first draft and all authors contributed to editing the manuscript.

Data accessibility

All sequences and photographs are publically available at http://dx.doi.org/10.5883/DS-ASNBPAR. All data and code (v1.0) to reproduce the reported results are publicly available on GitHub (https://github.com/cgreysongaito/SpruceBudworm_Parasitoids_Hardwood) and have been archived on Zenodo (https://doi.org/10.5281/zenodo.4432484).

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Supporting Information

For “Contribution of hardwood trees to budworm – parasitoid food web dynamics”

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Table 1: List of Malaise caught parastoid species in each group.

<u>Group 1</u>
Apantales fumiferanae
Glypta fumiferanae
Smidtia fumiferanae (Winthemia fumiferanae)
Lypha fumipennis (Lypha setifacies)

<u>Group 2</u>
Actia interrupta
Eumea caesar
Sarcophaga aldrichi
Nilea erecta (Pseudoperichaeta erecta)
Hemisturmia parva (Hemistermia tortricis)
Agria affinis (Psuedosarcophaga affinis)
Compsilura concinnata
Tachinomyia nigricans

Group 3

Meteorus trachynotus

Ceromasia auricaudata (Ceromasia aurifrons)

Nemorilla psyte

Phryxe pecosensis

Madremyia saundersii

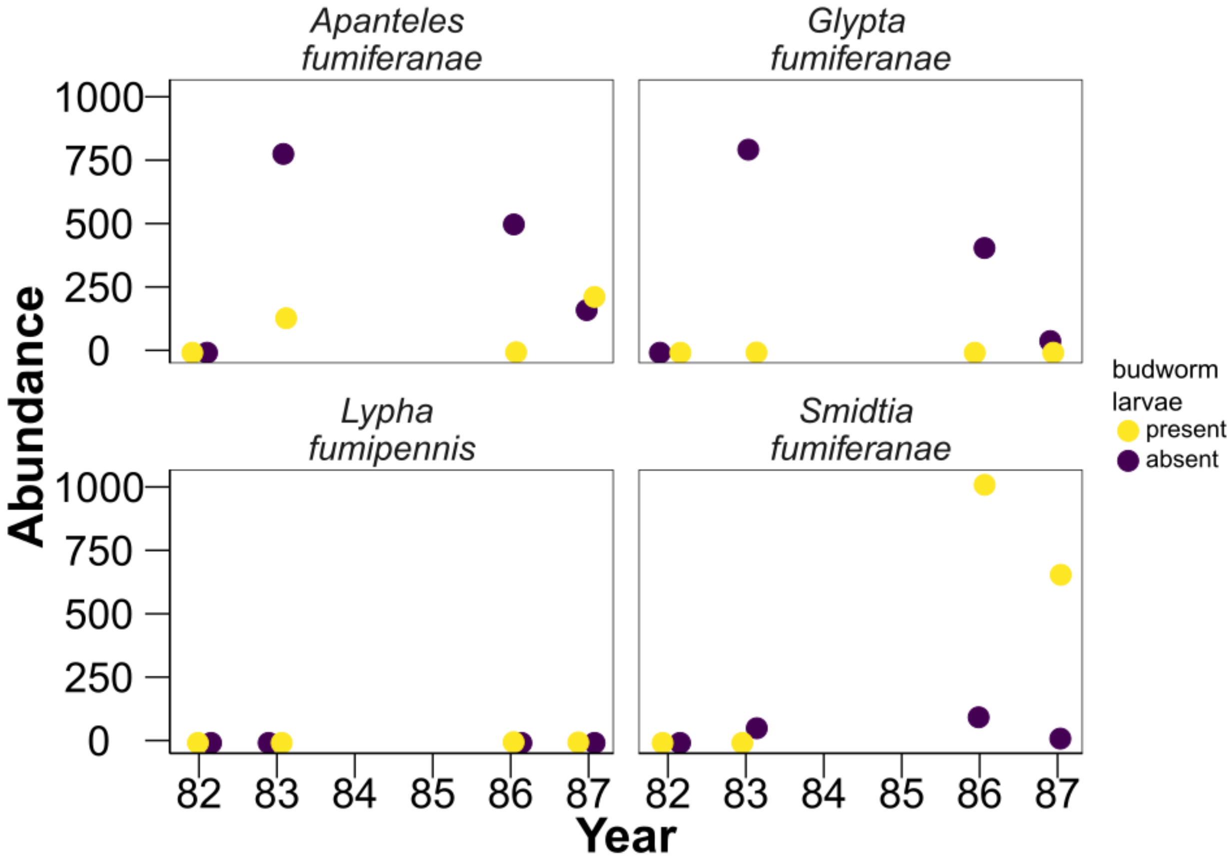

Figure S1 Time series of Malaise caught parasitoid species abundances in group 1.

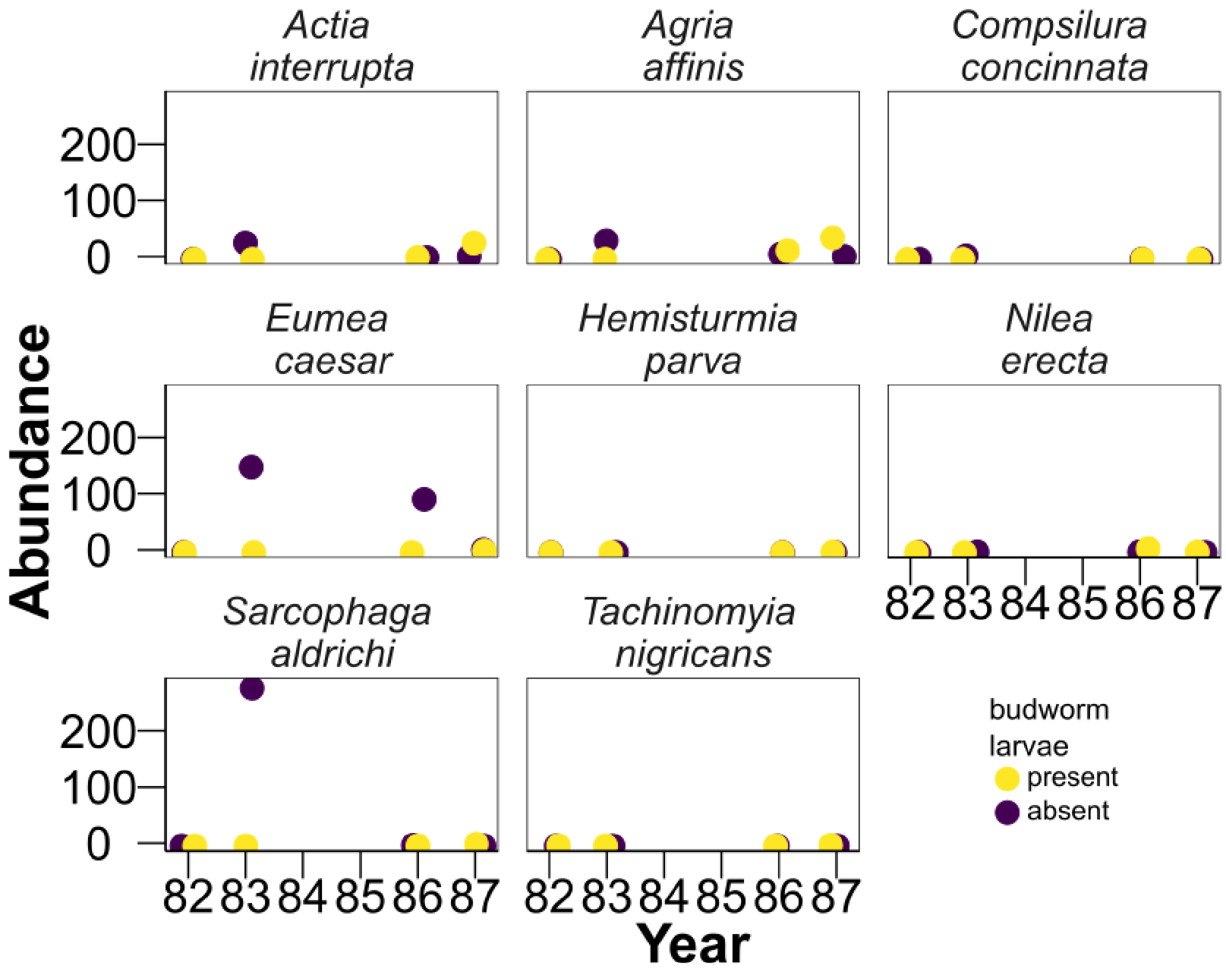

Figure S2 Time series of Malaise caught parasitoid species abundances in group 2.

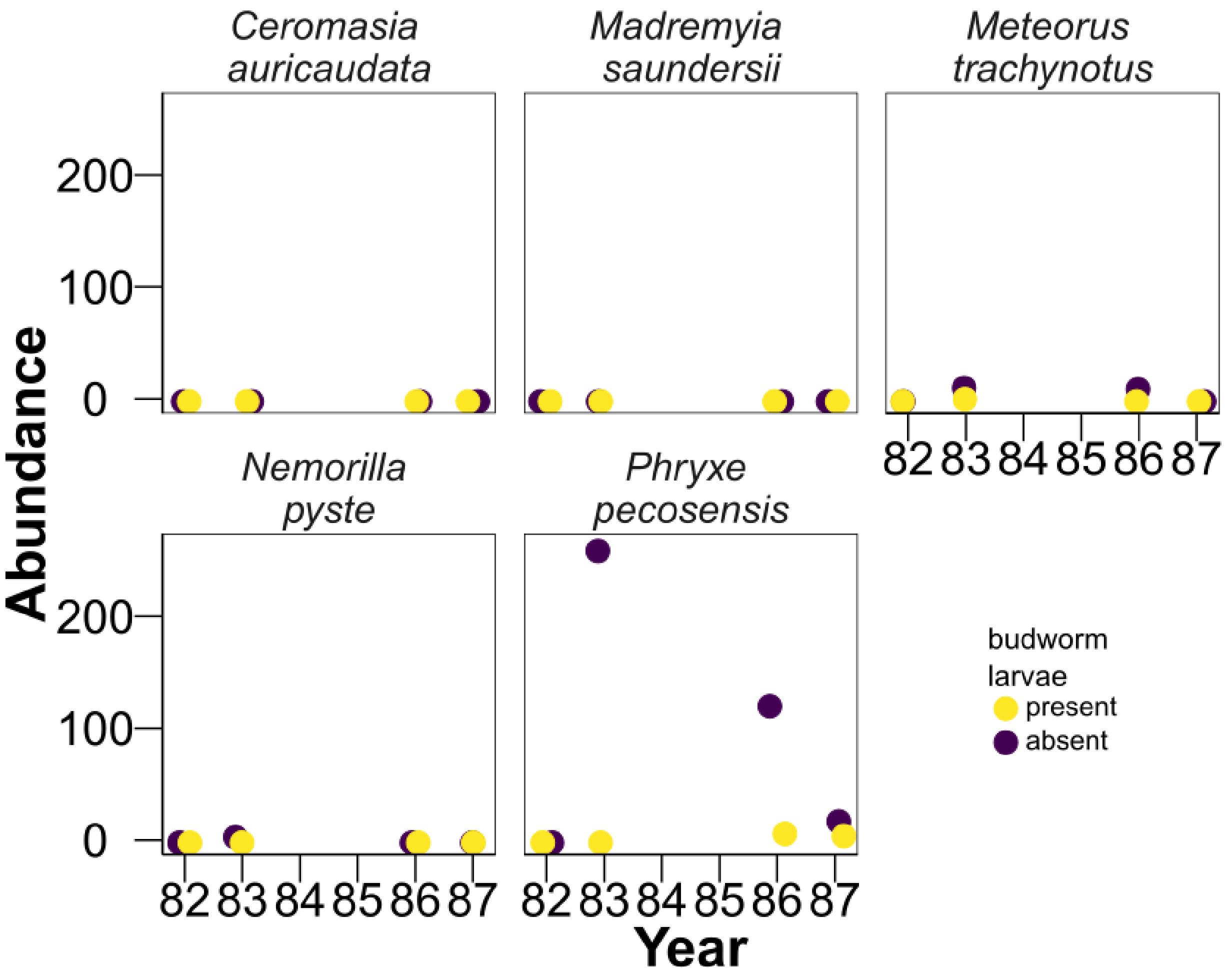

Figure S3 Time series of Malaise caught parasitoid species abundances in group 3.