

# Density-dependent effects of larval dispersal mediated by host plant quality on populations of an invasive insect

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Received: 1 February 2016 / Accepted: 22 June 2016  
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**Abstract** The success of invasive species is often thought to be due to release from natural enemies. This hypothesis assumes that species are regulated by top-down forces in their native range and are likely to be regulated by bottom-up forces in the invasive range. Neither of these assumptions has been consistently supported with insects, a group which includes many destructive invasive species. Winter moth (*Operophtera brumata*) is an invasive defoliator in North America that appears to be regulated by larval mortality. To assess whether regulation was caused by top-down or bottom-up forces, we sought to identify the main causes of larval mortality. We used observational and manipulative field and laboratory studies to demonstrate that larval mortality due to predation, parasitism, and disease were minimal. We measured the response of larval dispersal in the field to multiple aspects of foliar quality, including total phenolics, pH 10 oxidized phenolics, trichome density, total nitrogen, total carbon, and carbon–nitrogen ratio. Tree-level declines in density were driven by density-dependent dispersal of early instars. Late instar larvae dispersed at increased rates from previously damaged as compared to undamaged foliage, and in 2015 field

larval dispersal rates were related to proportion of oxidative phenolics. We conclude that larval dispersal is the dominant source of density-dependent larval mortality, may be mediated by induced changes in foliar quality, and likely regulates population densities in New England. These findings suggest that winter moth population densities in New England are regulated by bottom-up forces, aligning with the natural enemy release hypothesis.

**Keywords** Population dynamics · Density-dependence · Trophic interactions · Tannins · Ballooning

## Introduction

Human activity has resulted in the purposeful or accidental introduction of non-native species worldwide, some of which reach far higher densities in their introduced range than in their native range (Mack et al. 2000). This phenomenon is commonly considered to be due to the absence of natural enemies that regulate densities in the native range, as proposed by the enemy release hypothesis (Keane and Crawley 2002). This hypothesis is based on the assumption that most species are regulated by top-down factors such as predators, disease, or parasitoids in their native range, and implies that such species are more likely to be regulated by bottom-up factors in their introduced range. The lack of top-down control for invasive species has been a central justification for the introduction of non-native natural enemies for biological control, and an abundance of clear cases of thorough control of invasive pest species after the introduction of natural enemies from their region of origin—particularly by specialist parasitoids—exist [e.g., the control of red scale (*Aonidiella aurantii* Maskell [Diaspididae]) on citrus by *Aphytis* spp. (Murdoch 1994)

Communicated by Klaus Fischer.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00442-016-3689-z) contains supplementary material, which is available to authorized users.

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and winter moth (*Operophtera brumata* L. [Geometridae]) by *Cyzenis albicans* Fall. (Tachinidae) and *Agrypon flaveolatum* Gravenhorst (Ichneumonidae) (Roland and Embree 1995)]. These successes have helped lead to a general hypothesis that populations of insect herbivores are regulated by specialist parasitoids (e.g., Berryman 1996). However, the evidence that parasitoids drive population dynamics of native insect species and that natural enemy release is a primary driver of invasiveness is inconsistent (e.g., Rosenheim 1998; Myers and Cory 2013 for the role of specialist parasitoids, and Colautti et al. 2004 for the importance of the enemy release hypothesis). This suggests that such assumptions about how populations are regulated are often oversimplified, or apply to some species and not others.

Forest Lepidoptera have been intensively studied with regards to identifying factors that are important drivers of insect population dynamics (Varley and Gradwell 1968; Myers and Cory 2013). Studies on winter moth and autumnal moth (*Epirrita autumnata* Borkh [Geometridae]), in particular, illustrate the complexity of the issue well. For example, 10-year cyclic outbreaks of these geometrids in Fennoscandia have alternatively been proposed to be driven by delayed density-dependent mortality from specialist parasitoids (Tanhuanpää et al. 2002; Klemola et al. 2010), by delayed induced resistance of host plants (Haukioja and Neuvonen 1987), or for local outbreak propensity to be controlled by generalist pupal predators (Tanhuanpää et al. 1999; Raymond et al. 2002). Delayed induced resistance has not been supported as an explanation for geometrid cycles in more recent work (Haukioja 2005; Myers and Cory 2013), while the role of predators and parasitoids has accumulated evidence but remains controversial (Hansen et al. 2009; Schott et al. 2010, 2012, 2013; Myers and Cory 2013). Despite decades of work on these species, a clear explanation of the factors driving winter or autumnal moth population dynamics remains elusive.

One aspect of winter moth population dynamics that has rarely been directly investigated, but holds potential significance is larval dispersal. Dispersal has long been considered a process of central importance in population dynamics, but as in the case of winter moth, historically has been less studied than other regulatory factors (Cappuccino 1995). Density-dependent dispersal occurs in insects (Denno and Peterson 1995), as well as a broad variety of other taxa (Lambin et al. 2001).

Lepidopteran species commonly disperse as early instar larvae by ballooning on silken threads with wind currents that transport them to new host plants (Bell et al. 2005). Passive dispersal strategies like ballooning can lead to heavy mortality, since the ability of larvae to land on a suitable host is largely due to chance (Cox and Potter 1986;

Terry et al. 1989). For such behavior to occur, it is expected that the possible benefits of dispersal must outweigh the costs. Evolutionary models predict that dispersal can increase individual fitness when competition for resources is sufficiently high in the potential disperser's high-density local population, even if dispersal carries a high risk of mortality (Travis et al. 1999). In the case of winter moth, which is a generalist in deciduous forests, the chances of finding a suitable host plant are likely to be favorable.

Mortality of larvae if hatch is not closely synchronized with budburst, has widely been considered to be an important factor that affects population fluctuations in winter moth (Embree 1965; Varley and Gradwell 1968; Holliday 1977; Visser and Holleman 2001) and other spring-feeding lepidopteran populations (Feeny 1970; Hunter and Elkinton 2000; Jepsen et al. 2009), although there is some evidence to the contrary (Hunter et al. 1991; Dewar and Watt 1992; Kerslake and Hartley 1997). Winter moth larvae hatch in early spring and disperse by ballooning onto opening buds of deciduous trees and feed on young leaf tissue. Some authors (Embree 1965; Varley and Gradwell 1968) have suggested that dispersal of winter moth larvae occurs only immediately after hatch, but Edland (1971) showed that winter moth larvae can continue to balloon through the second instar. The possibility of population-level effects from larval dispersal after the beginning of feeding has so far remained unexplored.

In this study, we investigated the importance of larval dispersal to winter moth population dynamics in New England (northeastern United States), where it has been present as an outbreaking invasive species since at least the early 1990s, at times causing severe defoliation (Elkinton et al. 2010). Long term monitoring in New England from 2004 to 2015 (Elkinton et al. 2015) found larval densities much higher than previous studies (Embree 1965; Varley and Gradwell 1968) with strongly and consistently density-dependent mortality during the larval stage (J. S. Elkinton and G. H. Boettner, unpublished data). At the highest densities there were often as many as 20 larvae per bud, but these numbers declined to one or two larvae per leaf cluster by the end of the larval stage (J. S. Elkinton and G. H. Boettner, unpublished data). At lower densities, there were one or two larvae per bud and these numbers remained unchanged over the larval stage. These latter results were similar to those described by Holliday (1977) on apple trees (*Malus domestica* L.) in England. To investigate the causes of density-dependent declines in population density during the larval stage, we measured density declines due to dispersal, predation, parasitism, and disease in the field. We also examined the response of larval dispersal to density of conspecifics and to host plant foliar quality.

## Materials and methods

### Laboratory larval density manipulation

To measure causes of early instar winter moth larval mortality across a range of densities, we conducted laboratory rearing experiments. Winter moth adults reared from June 2013 to 2014 collections of larvae on Vancouver Island in British Columbia, Canada, were bred in the laboratory, and resulting eggs were used for rearing experiments (all other experiments were conducted with larvae from Massachusetts, USA). To create a range of densities, eggs were counted into groups of 5, 10, 20, 40, and 80 and stored at 1 °C. These reflect the natural range of densities found in individual buds in long term monitoring in Massachusetts. During spring 2014 and 2015, eggs were warmed for 5 days at 20 °C until they turned blue, signifying imminent hatch. Counted groups of eggs were then attached to twigs with a single developing bud using a small piece of marking tape and placed in plastic containers (drink cups, 8 cm diameter top × 15 cm depth, Fabri-Kal, Kalamazoo, MI, USA) and ventilated with fine mesh. Twenty replicate containers of each density treatment were set up, except for the 5 egg treatment which had 40 replicates. Containers were kept at 20 °C under 14 h per day of artificial light. Twigs of red oak (*Quercus rubra* L.), red maple (*Acer rubrum* L.), and apple (*Malus domestica* L.) were collected from Amherst, Massachusetts, when buds had expanded sufficiently to expose green tissue and were available for winter moth larvae to enter and feed. A single twig with a single bud was placed in each cup and embedded in moist plaster of Paris (for the apple trials) or set in water with a layer of paraffin wax solidified on the surface, to hydrate twigs and prevent death of larvae by drowning.

For each container, number of eggs hatched, live and dead larvae, head capsules, and location of larvae in buds or on container sides were recorded from observation under a dissecting microscope. This information was recorded for half of the containers after a period of 5 days following the day on which >80 % of larvae had hatched, and for the second half at 7 days (red oak and red maple trials) or 10 days (apple trial) after >80 % hatch. Number of head capsules was used to assess cannibalism; presence of detached head capsules above the number of second instar larvae (each of which would leave a head capsule from molting) was considered to be evidence of cannibalism. The location of dead larvae in the bud or on the inside of the cup was noted and used to assess whether larvae died while feeding or after leaving the bud. Proportion of surviving larvae relative to initial density and host species was analyzed with logistic regression using a quasibinomial distribution to correct for overdispersion.

### Field density monitoring

To assess dispersal rates in the field, 20 buds or developing leaf clusters (originating from a single bud) were collected weekly from each of 5 apple, 11 red maple, and 13 red oak trees (total  $N = 29$ , number of each species based on availability at field sites) spread across four sites in eastern Massachusetts [West Bridgewater (42.021, −70.982); Hanson (42.049, −70.8730; 42.060, −70.843); Freetown (41.794, −71.053)] from 12-Apr to 6-Jun-2014. The same sample trees at the same sites along with two additional red oak and red maple sample trees at Freetown (total  $N = 33$ ) were sampled from 25-Apr to 31-May-2015. Each leaf cluster was dissected under a microscope (for samples containing instars 1–3, afterwards without), and the number of live or dead winter moth larvae and the instar of each larva was recorded. An additional two to four bud or leaf clusters in 2014 were collected in a pooled sample at every sample tree and date, and brought back to the laboratory and frozen at −20 °C for subsequent chemical analysis. In 2015, pooled leaf material from 20 buds or leaf clusters from each sample tree that had been collected for density counts was frozen at −80 °C for chemical analysis.

To assess the relationship between density and dispersal, a period of the larval stage within which to measure declines in density was identified. The density of larvae in buds climbs at the beginning of the season as larvae hatch, and as buds develop sufficiently for larvae to enter. Towards the end of the larval stage the number of larvae per leaf cluster declines, as larvae drop off foliage to pupate in the soil beneath the host tree. Therefore, data from the beginning and end of the larval stage was not considered in our analyses. To determine the beginning of the period within which to measure dispersal, first, average larval densities per bud cluster for each week were calculated. Second, the date of peak average larval density for the majority of sample trees of each host species was determined (In 2014, this was 3-May for red maple and red oak and was 27-Apr for apple. In 2015 this was 1-May for all tree species). Third, the proportion of larvae remaining was measured as a proportion of total larval count from 20 leaf clusters from each tree on a date before the beginning of pupation (16-May-2014 and 15-May-2015), out of the total initial (peak) larval count from that tree. Some sample trees had more larvae in samples before pupation than at the initial larval count. These results were likely due to sample error, or from later immigration of larvae onto sample trees. These counts ( $N = 6$  in 2014,  $N = 10$  in 2015) were changed to the same value as the initial counts for those sample trees. Disappearance rates of winter moth larvae in response to initial density and tree species of each sample tree was analyzed using a logistic generalized mixed model. Site was included as a random effect, and an observation-level

random effect (sample tree) was also included in the model to account for overdispersion (Elston et al. 2001). An interaction between larval density and tree species was included in the model. The overall decline in density of all trees from peak density to before pupation was also assessed using a Poisson generalized mixed model, with the same random effects.

### Early larval dispersal manipulation and predator exclusion

To experimentally assess the relative importance of predation and dispersal in observed declines of early instar winter moth larvae in the field, we set up a predator exclusion and dispersal manipulation experiment in May 2015. This was conducted on trees along a pipeline right-of-way at Freetown-Fall River State Forest in Freetown, Massachusetts, with natural populations of first and second instar winter moth. Twenty groups of two buds were manipulated with either of two treatments on each of ten red oak trees on 2-May, approximately at peak larval densities. The ‘no dispersal or predation’ treatment ( $N = 100$ ) consisted of cloth bags designed to prevent larval dispersal and predation. The ‘dispersal only’ treatment consisted of 300  $\mu\text{m}$  mesh bags designed to allow most first and second instar larvae to pass through (as demonstrated in preliminary laboratory tests), but prevented most, if not all predation. This mesh size would exclude most, if not all, spiders and predatory insects including ants, wasps and beetles. In contrast, standard window screen has a mesh size of  $\sim 600\text{--}700\ \mu\text{m}$ , or twice as coarse. After 6 days manipulated buds and ten pairs of unmanipulated (control) buds from each sample tree were collected for dissection under a microscope (total  $N = 300$ ). Differences in final larval densities per two buds by treatment were analyzed using a Poisson generalized mixed model, with treatment by sample tree as a random effect, and an observation-level random effect (group of two buds) to account for overdispersion.

### Late larval predator exclusion

To assess the predation rates on late instar winter moth larvae in the field, predator exclusion manipulations were conducted at Freetown-Fall River State Forest May 2013 and 2014, with natural populations of fourth and fifth instar winter moth larvae. Red oak trees were selected, and the number of larvae and leaf clusters on a single section of branch were counted in situ, and one of three treatments were applied by block: no predation, no avian predation and full dispersal, and full predation (control). The ‘no predation or dispersal’ treatment consisted of a fine mesh bag (silk screening mesh, 10  $\mu\text{m}$  mesh) which was intended to exclude all predation and prevent larval dispersal. The

‘no avian predation and full dispersal’ treatment consisted of a wire tomato hoop encased in coarse mesh (bird netting, 1.5 cm mesh) intended to allow larval dispersal and invertebrate predation but to prevent avian predation. The ‘full predation and full dispersal’ treatment consisted only of a wire tomato hoop, which allowed larval dispersal and all predation. Replicates (2013,  $N = 59$ ; 2014,  $N = 45$ ) were grouped into blocks of three with one tree randomly assigned to each treatment. After 6–7 days, leaf clusters from treated branches were removed, taken to the laboratory and frozen, and the number of larvae per branch counted. The proportion surviving was compared across treatments in a logistic generalized mixed model with block as a random effect to account for spatial non-independence. Similar to the field monitoring of larval density, some sample branches had more larvae per branch at final count than at the initial count; such differences were assumed to be due to sample error or undercounting of initial densities, and these counts (2013,  $N = 33$ ; 2014,  $N = 11$ ) were adjusted to the same value as the initial counts for that sample branch.

### Larval dispersal from damaged leaves

To assess the effects of damage to foliage earlier in the same season on larval dispersal rates, during May 2013–2015 foliage was collected haphazardly from red maple and red oak trees with undamaged leaves, and with foliage damaged the same year by naturally occurring winter moth herbivory such that roughly one-third of foliage was missing, and was placed in moist floral foam in ventilated 19 L buckets, separated by tree species (see Table S4 for details of experimental design for individual trials: total replicates inclusive of all trials was 354). Late instar larvae were collected from the field and placed on foliage in each bucket. Every 24 h, the numbers of larvae on the side, bottom, or lid of the bucket were counted, and the larvae returned to the foliage. The proportion of dispersing larvae (larvae that moved off leaves) was then compared across treatments using logistic generalized mixed models, with bucket as a random effect to account for non-independence due to repeated measurements of individual buckets in 2013 and 2014, and year as a random effect in the overall model of all years.

### Foliar quality

To determine the relationship between foliar quality and larval dispersal rates in the field density monitoring experiment, samples collected from sample trees 1 week after peak larval density were analyzed for multiple aspects of foliar quality. Phenolic content, oxidative phenolics, nitrogen content, and carbon content from

11-May-2014 samples and the same data plus trichome density from 8-May-2015 samples were measured, as follows: leaves for chemical analysis were freeze-dried and ground with a mortar and pestle. Total foliar phenolics and the proportion of pH 10 oxidative phenolics (the proportion of phenolics that oxidize when held at a pH of 10 for 90 min) were analyzed using a modified Folin–Ciocalteu assay following the method of Salminen and Karonen (2011) using absorbance measurements from a microplate reader (Spectramax M2, Molecular Devices, California, USA). Total phenolics were calculated using gallic acid standards and species-specific phenolic standards from Sephadex LH-20 gravity column chromatography (Sephadex LH-20, GE Healthcare Bio-Sciences, Pennsylvania, USA), also after Salminen and Karonen (2011). Proportion of pH 10 oxidative phenolics measurements were read from extracts diluted to  $1.0 \pm 0.3$  mg/ml gallic acid equivalents (due to difficulties with precise dilution). Total nitrogen and carbon analysis of 5 mg of leaf material was conducted with a combustion analyzer (ECS 4010, Costech Analytical Technologies, California, USA) using acetanilide standards. Phenolic, nitrogen, and carbon measures were obtained from a single pooled sample for each sample tree that consisted of two to six leaf clusters per tree in 2014 and 20 leaf clusters in 2015. Trichome density was measured using the average number of trichomes intersecting a 1 mm line on 20 leaves from each sample tree. Measures of foliar quality in each year by sample tree and tree species were analyzed for their effect on larval survival in logistic generalized mixed models with site-level and observation-level random effects.

### Statistical analysis

All statistical analysis was conducted in R (R Core Team 2013, version 3.02). Mixed models were run using the lme4 package (Bates et al. 2015), and significance tests of mixed models were made using parametric likelihood ratio bootstrap tests with the function PBmodcomp from the package pbrtest (Halekoh and Højsgaard 2014), except for the models of population declines from field monitoring, and the early larval dispersal manipulation and predation exclusion experiment, for which Wald Chi-square tests were used to calculate  $P$  values because of model convergence failure with PBmodcomp. Interactions were not included except in analyses of larval dispersal in the field monitoring. Marginal (fixed effects,  $R^2_m$ ) and conditional (fixed and random effect,  $R^2_c$ ) coefficients of determination were calculated for mixed models using the function r-squared. GLMM from the package MuMIn (Nakagawa and Schielzeth 2013). Plotting was implemented in R using the ggplot2 package (Wickham 2009).

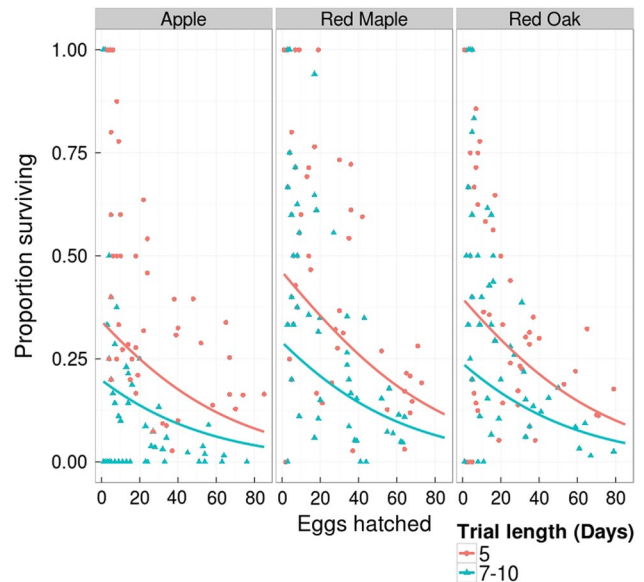
## Results

### Laboratory larval density manipulation

In the laboratory experiments, larval survival in cup trials significantly decreased with increasing conspecific density (log odds  $\beta = -0.022$ ,  $\chi^2 = 208.1$ ,  $P < 0.001$ , Fig. 1), and differed by tree species ( $\chi^2 = 36.2$ ,  $P < 0.001$ ) and measurement date ( $\chi^2 = 110.1$ ,  $P < 0.001$ ). Fitted survival probabilities were 39 % for red oak, 46 % for red maple, and 34 % for apple, and 67 % lower at 7–10 days than at 5 days. Mortality appeared to be almost entirely due to starvation after dispersal, because 97.2 % of recovered dead larvae had crawled out of buds and died on the inside of the cup. In all laboratory trials, there was negligible evidence of cannibalism. Less than 10 % of the cups had evidence of cannibalism.

### Field density monitoring

In the field, proportion of larvae remaining on sample trees decreased significantly with increasing initial density in both years (2014: log odds  $\beta$ : red oak =  $-1.403$ , red maple =  $-0.832$ , apple =  $-0.428$ ,  $\chi^2 = 9.3$ ,  $P = 0.008$ ,  $R^2_m = 0.122$ ,  $R^2_c = 0.102$ , 2015: log odds  $\beta$ : red oak =  $-1.564$ , red maple =  $-1.460$ , apple =  $0.667$ ,  $\chi^2 = 23.2$ ,  $P = 0.001$ ,  $R^2_m = 0.123$ ,  $R^2_c = 0.123$ , Fig. 2), and differed significantly between tree species (2014:



**Fig. 1** Logistic regressions of larval survival in each cup by initial number of hatched larvae per cup from laboratory larval density manipulation experiments, on three host species, after trial lengths of 5, 7 days (red oak and red maple), and 10 days (apple) (color figure online)



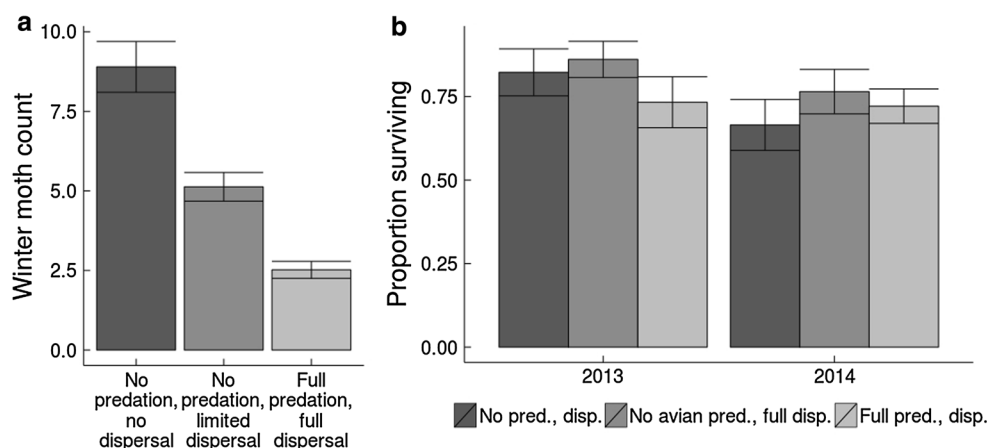
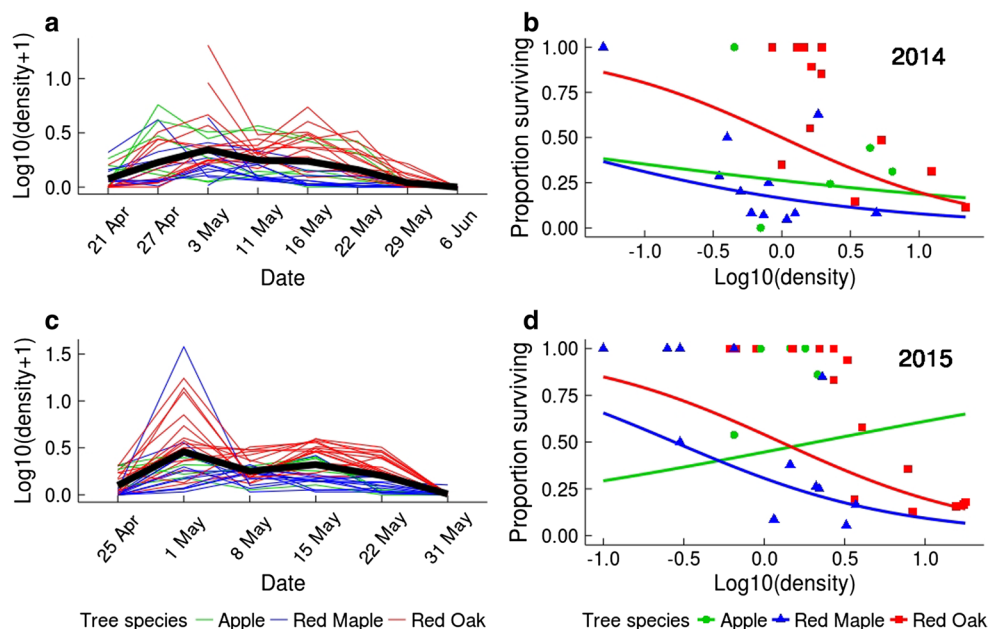
$\chi^2 = 18.4$ ,  $P = 0.003$ , 2015:  $\chi^2 = 14.2$ ,  $P = 0.001$ , Fig. 2). Fitted survival probabilities were 50 % for red oak, 16 % for red maple, and 26 % for apple in 2014, and 54 % for red oak, 31 % for red maple, and 45 % for apple in 2015. Interactions between density and tree species were not significant in either year ( $P > 0.05$ ). Sample trees which had higher final than initial densities and which were adjusted for this analysis were clustered among trees with low initial density; the likelihood of a sample tree to have higher final than initial density declined with increasing initial density (binomial GLM; log odds  $\beta = -2.239$ ,  $\chi^2 = 12.4$ ,  $P = 0.0004$ ). There were significant overall declines in density in both years (2014:  $\chi^2 = 59.8$ ,  $P < 0.001$ , 2015:  $\chi^2 = 59.8$ ,  $P < 0.001$ ), differing significantly by species (2014:  $\chi^2 = 33.7$ ,

$P < 0.001$ , 2015:  $\chi^2 = 33.7$ ,  $P < 0.001$ ). Densities declined on average by 57 % in 2014 and 54 % in 2015.

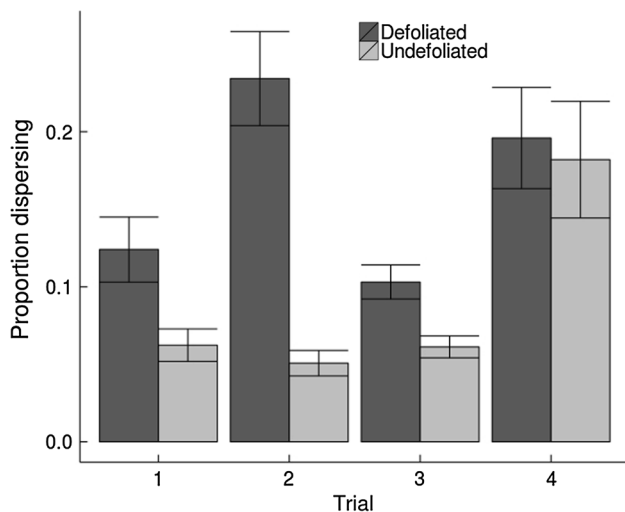
### Early larval dispersal manipulation and predator exclusion

Larval densities per two buds were significantly different between treatments ( $\chi^2 = 53.7$ ,  $P < 0.001$ , Fig. 3a). The full dispersal and full predation (unbagged) had densities 72 % lower than the no predation and no dispersal treatment (cloth bags) and 49 % lower than the no predation and limited dispersal treatment (fine mesh bags). The limited dispersal treatment had densities 57 % lower than the no dispersal treatment.

**Fig. 2** Time series of log winter moth larval densities (left) by sample tree (narrow lines) and overall mean (broad line), and predicted proportion of larvae remaining (survival) by density from proportional logistic mixed (right), in 2014 (top) and 2015 (bottom). Host species are shown by color and symbol type. Log densities for time series were generated from log of number of larvae per bud plus one (color figure online)



**Fig. 3** Results from predator exclusion experiments. Mean and  $\pm 1$  standard error of **a** larvae per single bud by treatment of early larval dispersal manipulation and predator exclusion experiment, and **b** larval survival by year and treatment of late larval predator exclusion experiment



**Fig. 4** Mean and  $\pm 1$  standard error of daily proportion of larvae dispersed from damaged and undamaged leaves in four trials (see Table S4)

### Late larval predator exclusion

Over both years of the larval predator exclusion experiment, there was no significant difference in larval survival between the no predation or dispersal, no avian predation and full dispersal, and full predation and full dispersal treatments (2013:  $\chi^2 = 2.762$ ,  $P = 0.243$ , 2014:  $\chi^2 = 0.781$ ,  $P = 0.623$ , Fig. 3b). Number of sample branches where final density was higher than initial density (and for which proportions were adjusted) were not different between treatments (2013 and 2014:  $\chi^2 = 1.822$ ,  $P = 0.402$ ).

### Larval dispersal from damaged leaves

In the combined analysis of all trials of the larval dispersal from damaged leaves experiments, the rate of larval

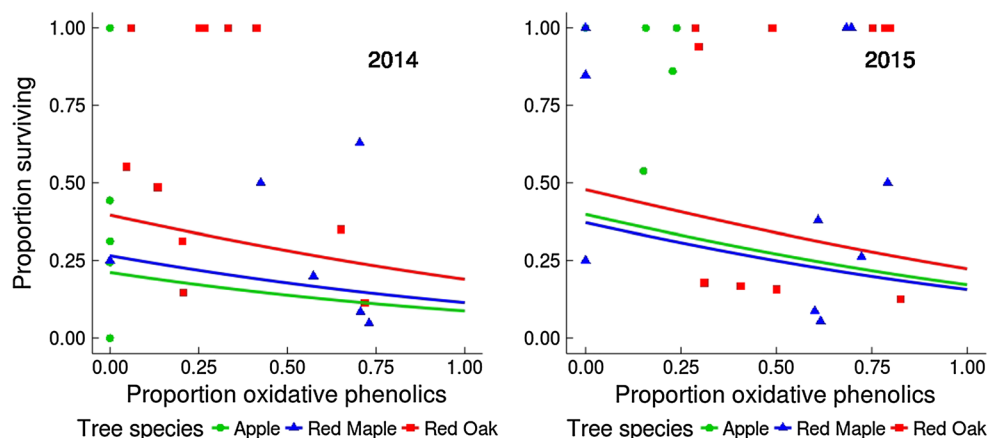
dispersal was significantly elevated on defoliated leaves, with 35 % more larvae dispersing per day from damaged foliage (Fig. 4,  $\chi^2 = 20.10$ ,  $P = 0.001$ ), with no difference between tree species ( $\chi^2 = 0.46$ ,  $P = 0.528$ ).

### Foliar quality

Proportion of pH 10 oxidative phenolics were significantly related to proportion of remaining larvae in 2015, but not in 2014, although the fitted effects in both years were negative (2014: log odds  $\beta = -1.029$ ,  $\chi^2 = 25.1$ ,  $P = 0.489$ ,  $R^2m = 0.047$ ,  $R^2c = 0.069$ ,  $N = 22$ , 2015: log odds  $\beta = -1.158$ ,  $\chi^2 = 66.7$ ,  $P = 0.018$ ,  $R^2m = 0.031$ ,  $R^2c = 0.112$ ,  $N = 26$ , Fig. 5). None of the other measures of foliar quality were significantly related to larval survival ( $P > 0.05$ , Table S3). In the pH 10 oxidative phenolics models, tree species was not significantly related to larval survival in either year ( $P > 0.05$ ).

### Discussion

We propose that ex situ mortality after density-dependent dispersal represents the major cause of mortality during the winter moth larval stage in these outbreak populations, because of the strong density-dependent dispersal of early instar larvae in the laboratory and field, and increased densities when dispersal is prevented in the field. We also propose that larval dispersal may be mediated by phenolic oxidative capacity, based on the response of larval dispersal to damaged foliage with constant larval density, and the relationship between the phenolic oxidative capacity of host tree foliage and larval dispersal rates in the field (see Table 1 for schematic diagram of examination of different factors over the larval stage). Furthermore, as far as we know, our results are the first to suggest a negative



**Fig. 5** Proportion of larvae remaining on sample trees after dispersal by proportion of pH 10 oxidative phenolics of analyzed leaf material from sample trees, in 2014 and 2015

**Table 1** Schematic table over-viewing experiments in this study

<i>Experiment</i>	Instar				
	1st	2nd	3rd	4th	5th
Laboratory density-dependence	×	×	×		
	●	●	●		
Field density monitoring and foliar quality	×	×	×	×	×
	■	■	■		
Early larval dispersal manipulation and predator exclusion	×	×	×		
	◆	◆	◆		
Late larval predator exclusion			◆	◆	◆
Larval dispersal from damaged leaves			×	×	×
			■	■	■

Factors examined:  
Dispersal × Cannibalism ● Predation ◆ Host plant quality ■

Factors and stages of development examined in each experiment are represented, to illustrate how experiments collectively cover different mortality factors and combinations of factors over the entire larval stage

relationship between larval survival and pH 10 phenolic oxidative capacity.

The significant declines in larval density in outbreking populations of winter moth presented here contrasts with the behavior of low-density populations. Multiple studies including classic work by Varley and Gradwell (1968) in England and by Roland (1994) in British Columbia, Canada, provide evidence that low-density populations of winter moth are regulated by density-dependent predation during the pupal stage. Similar to Holliday (1977), who showed that there was negligible mortality of winter moth during the larval stage in a low-density population in England, we generally observed no significant changes in density over the larval stage on sample trees with low densities of larvae (Fig. 2). In contrast, when densities were high, we observed a large drop in larval density during early instars. In the present study we suggest that dispersal mortality is an important regulatory factor in winter moth population dynamics in New England, where winter moth is in near continuous outbreak status, and it is likely that the same process could result in mortality during outbreaks when they occur in the native range.

As with most other work on the population-level effects of insect dispersal (e.g., Rhainds et al. 2002), we were unable to account for the fate of larvae after they disperse. Dispersal may not necessarily constitute mortality, as we have suggested. However, since the average population density of all sample trees declined during the weeks in which most dispersal occurred, it does not appear that dispersal simply represents the redistribution of spatially heterogeneous population densities to a more even distribution. Instead, dispersal appears to cause localized

regulation of larval densities on high-density trees through larval mortality caused most likely by starvation or predation occurring after dispersal. The occurrence of some higher final than initial densities in the field density monitoring and late larval predation experiments suggests that some immigration of larvae may occur. However, in the former experiment increases in density were concentrated to sample trees with low initial densities, which both suggests that such results are more likely to have been generated by sample error, and that if larval immigration occurs it is limited enough in scale that initial larval densities must be low for it to have an observable effect. For the latter experiment, the lack of a difference in the occurrence of density increases between closed and open treatments suggests that increases were an artifact of measurement error.

The lack of differences in survival between late instar larvae predator exclusion treatments and controls suggest that predators have little impact on late instars in high-density winter moth populations. Roland et al. (1986) observed density-dependent predation by a flock of pine siskins (*Spinus pinus* Wilson) on a population of winter moth in British Columbia. However, they argued that bird predation was unlikely to be an important regulator of larval density, due to the inconsistent presence of bird predators, and the lack of any numerical response of birds to winter moth food resources due to territorialism. Winter moth larval densities at these high-density sites often exceed 100,000 larvae per tree (J. S. Elkinton and G. H. Boettner, unpublished). At such densities, we suspect avian predators are saturated. This is probably what caused low rates of predation in our studies. Embree (1965) studied outbreak populations of



winter moth in Nova Scotia and also concluded that avian predators were saturated at higher densities.

In the early larval dispersal manipulation and predation exclusion experiment, the cloth bag which prevented all dispersal and predation resulted in three and a half fold higher densities than the control treatment which allowed all predation and dispersal (Fig. 3b). The intermediate treatment with a 300 µm mesh bag resulted in densities that were about twice as high as controls. We believe this result was mostly due to reduced dispersal, because larvae could not exit mesh bags once they reached third instar and could only do so with some difficulty as earlier instars. Furthermore, these bags sheltered larvae from wind currents necessary for ballooning. Some of this treatments effect may have been due to reduced predation, but the 300 µm mesh (twice as fine as standard window screen) would exclude most, if not all predatory wasps, flies, spiders, ants or beetles, i.e., most if not all conceivable insect or arachnid predators. In addition, since we have shown that there is negligible predation on late instar larvae, which are probably most desirable to predators, it seems unlikely that there would be significant predation on early instar larvae which are 1–2 mm in length and feed sheltered inside of buds. However, it remains a possibility that there may be some very small predators involved in the early larval disappearance that we have documented.

The absence of parasitism or other mortality in later instars in the field monitoring samples reflects the fact that in New England winter moth lacks parasitoids and pathogens with significant population-level impacts, as shown in biological control release monitoring collections taken from 2004 to present (J. S. Elkinton and G. H. Boettner, unpublished data). Similarly, mortality from disease is negligible. Few larvae collected in Massachusetts die during mass rearing (2013: 1.1 %, 2014: 3.2 %) and of those, only some are infected with disease (H. J. Broadley, J. S. Elkinton and J. P. Burand, unpublished data). Burand et al. (2011) reported a 28 % infection rate of larvae by *O. brumata* nucleopolyhedrovirus; however, this value represents the proportion of larvae infected out of the number of larvae that had died in rearing. The percent larval mortality in rearing was not recorded for the years of that study, but was very small.

Complete defoliation by winter moths of red oak and red maple trees is rare in New England (J. S. Elkinton and G. H. Boettner, unpublished data), and was also rare for Garry oaks (*Quercus garryana* Douglas ex Hook) in British Columbia before the release of biological control agents there (Roland and Myers 1987). Limited defoliation occurs even if the larvae establish at high densities at the beginning of the feeding season, and may be a result of early instar larval dispersal in response to high

densities of conspecifics even when there is still abundant foliage available. The choice by larvae to disperse can confer a fitness advantage if the risk of mortality from remaining is sufficiently high relative to the likelihood of finding a suitable host (Travis et al. 1999). We suggest that if there are high densities at the beginning of the season, caterpillars have a better chance of survival by dispersing and potentially finding a suitable host rather than remaining and starving. Dispersal behavior would seem likely to result in larval densities tracking host plant carrying capacity, as has been observed in some other herbivores (Cappuccino 1995; Solbreck 1995). In this case such tracking is clearly imperfect, because often there is only moderate defoliation on trees that experience high dispersal rates. This suggests that dispersal may in part be caused by reduced host plant quality induced by damage from high herbivore densities, a process that has precedence with other cases of density-dependent dispersal in insects (Denno and Peterson 1995). The larval dispersal from defoliated leaves experiment, together with oxidative phenolics data, provide evidence that winter moth larval dispersal may be mediated by induced host plant defense, although proportion of oxidative phenolics was only related to larval survival rates in 2015 and not 2014.

Phenolics have long been considered to have a defensive role for plants against insect herbivores (e.g., Feeny 1970; Schultz and Baldwin 1982). Early work assumed that the primary function of tannins was herbivore resistance through protein precipitation, a mechanism which has not been consistently found to effect insect herbivores (Ayres et al. 1997). More recent work by Appel (1993) and Salminen and Karonen (2011) has suggested that tannins may have anti-herbivore effects through oxidative activity in high pH guts (i.e., most insect herbivores) and protein precipitation in low pH guts (i.e., mammalian herbivores). The different oxidative activity by different tannins shown by Salminen and Karonen (2011) mean that measures of total phenolics are unlikely to have ecological significance for insect herbivores. In this study, we used pH 10 oxidative phenolics as a measure of tannins groups that we would expect to more likely effect insect herbivores.

If winter moth larval dispersal is triggered by pH 10 oxidative phenolics, as our results suggest, then we can conclude that winter moth populations are regulated by a bottom-up process, confirming the assumptions of the natural enemy release hypothesis. However, further work, such as laboratory leaf-painting dispersal studies with phenolic extracts of greater or lesser oxidative capacity, would be necessary to conclusively demonstrate that the pH 10 oxidative capacity of foliage is the mechanism that causes winter moth larvae to disperse.

**Acknowledgments** The authors thank David Prodanas, Grace Pold, and Kristen DeAngelis for assistance and use of laboratory space and equipment, Donald Adams, Deborah Swanson, and the Massachusetts DCR for use of field sites, Juha-Pekka Salminen and Scott McArt for advice on phenolic analyses, and Jeremy Andersen, Jeffrey Lombardo, Lynn Adler, Roy Van Driesche, Jens Roland, Jane Jepsen, Ole Petter Vindstad, George Boettner, Toomas Tammara and Tero Klemola for comments on the manuscript. Funding for this work was provided by Cooperative Agreements 14-8225-0464 from the USDA APHIS and 13-CA-1140004-236 from the USDA Forest Service.

**Author contribution statement** AAP, JSE and HJB conceived and designed the experiments. AAP and HJB performed the experiments. AAP conducted the analyses and wrote the manuscript. HJB and JSE provided editorial advice.

## References

- Appel HM (1993) Phenolics in ecological interactions: the importance of oxidation. *J Chem Ecol* 19:1521–1552
- Ayres MP, Clausen TP, MacLean SF et al (1997) Diversity structure and antiherbivore activity in condensed tannins. *Ecology* 78:1696–1712
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Soft* 67:1–48. doi:[10.18637/jss.v067.i01](https://doi.org/10.18637/jss.v067.i01)
- Bell JR, Bohan DA, Shaw EM, Weyman GS (2005) Ballooning dispersal using silk: world fauna, phylogenies, genetics and models. *Bull Entomol Res* 95:69–114. doi:[10.1079/BER2004350](https://doi.org/10.1079/BER2004350)
- Berryman AA (1996) What causes population cycles of forest Lepidoptera? *Trends Ecol Evol* 11:28–32. doi:[10.1016/0169-5347\(96\)81066-4](https://doi.org/10.1016/0169-5347(96)81066-4)
- Burand JP, Kim W, Welch A, Elkinton JS (2011) Identification of nucleopolyhedrovirus in winter moth populations from Massachusetts. *J Invertebr Pathol* 108:217–219
- Cappuccino N (1995) Chapter 1—novel approaches to the study of population dynamics. In: Price PW, Cappuccino N (eds) *Population dynamics*. Academic Press, San Diego, pp 3–16
- Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? *Ecol Lett* 7:721–733. doi:[10.1111/j.1461-0248.2004.00616.x](https://doi.org/10.1111/j.1461-0248.2004.00616.x)
- Cox DL, Potter DA (1986) Aerial dispersal behavior of larval bagworms, *Thyridopteryx ephemeraeformis* (Lepidoptera: Psychidae). *Can Entomol* 118:525–536
- Denno RF, Peterson MA (1995) Chapter 6—density-dependent dispersal and its consequences for population dynamics. In: Price PW, Cappuccino N (eds) *Population dynamics: new approaches and synthesis*. Academic Press, San Diego, pp 113–130
- Dewar RC, Watt AD (1992) Predicted changes in the synchrony of larval emergence and budburst under climatic warming. *Oecologia* 89:557–559
- Edland T (1971) Wind dispersal of winter moth larvae *Operophtera brumata* L. (Lep., Geometridae) and its relevance to control measures. *Nor Entomol Tidsskr* 18:103–105
- Elkinton JS, Boettner GH, Sremac M et al (2010) Survey for winter moth (Lepidoptera: Geometridae) in northeastern North America with pheromone-baited traps and hybridization with the native Bruce spanworm (Lepidoptera: Geometridae). *Ann Entomol Soc Am* 103:135–145. doi:[10.1603/AN09118](https://doi.org/10.1603/AN09118)
- Elkinton JS, Boettner GH, Liebhold AM et al (2015) Biology, spread and biological control of winter moth in the eastern United States. USFS FHTET 2014-07
- Elston DA, Moss R, Boulinier T et al (2001) Analysis of aggregation, a worked example: numbers of ticks on red grouse chicks. *Parasitology* 122:563–569
- Embree DG (1965) The population dynamics of the winter moth in Nova Scotia, 1954–1962. *Mem Entomol Soc Can* 46:1–57
- Feeny P (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* 51:565–581
- Halekoh U, Højsgaard S (2014) A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models—the R package pbkrtest. *J Stat Softw* 59:1–30
- Hansen NM, Ims RA, Hagen SB (2009) No impact of pupal predation on the altitudinal distribution of autumnal moth and winter moth (Lepidoptera: Geometridae). *Environ Entomol* 38:627–632
- Haukioja E (2005) Plant defenses and population fluctuations of forest defoliators: mechanism-based scenarios. *Ann Zool Fenn* 42:313–325
- Haukioja E, Neuvonen S (1987) Insect population dynamics and induction of plant resistance: the testing of hypotheses. In: Barbosa P, Schultz JC (eds) *Insect outbreaks*. Academic Press, San Diego, pp 411–423
- Holliday NJ (1977) Population ecology of winter moth (*Operophtera brumata*) on apple in relation to larval dispersal and time of bud burst. *J Appl Ecol* 14:803–813
- Hunter AF, Elkinton JS (2000) Effects of synchrony with host plant on populations of a spring-feeding lepidopteran. *Ecology* 81:1248–1261
- Hunter MD, Watt AD, Docherty M (1991) Outbreaks of the winter moth on Sitka Spruce in Scotland are not influenced by nutrient deficiencies of trees, tree budburst, or pupal predation. *Oecologia* 86:62–69
- Jepsen JU, Hagen SB, Karlsen S, Ims RA (2009) Phase-dependent outbreak dynamics of geometrid moth linked to host plant phenology. *Proc R Soc B Biol Sci* 276:4119–4128. doi:[10.1098/rspb.2009.1148](https://doi.org/10.1098/rspb.2009.1148)
- Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy hypothesis. *Trends Ecol Evol* 17:164–170
- Kerslake JE, Hartley SE (1997) Phenology of winter moth feeding on common heather: effects of source population and experimental manipulation of hatch dates. *J Anim Ecol* 66:375–385
- Klemola N, Andersson T, Ruohomäki K, Klemola T (2010) Experimental test of parasitism hypothesis for population cycles of a forest lepidopteran. *Ecology* 91:2506–2513
- Lambin X, Aars J, Piernney SB (2001) Dispersal, intraspecific competition, kin competition, and kin facilitation: a review of the empirical evidence. In: Clobert J, Wolff JO, Nichols JD et al (eds) *Dispersal*. Oxford University Press, New York, pp 110–122
- Mack RN, Simberloff D, Lonsdale WM et al (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecol Appl* 10:689–710
- Murdoch WW (1994) Population regulation in theory and practice. *Ecology* 75:271–287
- Myers JH, Cory JS (2013) Population cycles in forest lepidoptera revisited. *Annu Rev Ecol Evol Syst* 44:565–592. doi:[10.1146/annurev-ecolsys-110512-135858](https://doi.org/10.1146/annurev-ecolsys-110512-135858)
- Nakagawa S, Schielzeth H (2013) A general and simple method for obtaining  $R^2$  from generalized linear mixed-effects models. *Method Ecol Evol* 4:133–142. doi:[10.1111/j.2041-210x.2012.00261.x](https://doi.org/10.1111/j.2041-210x.2012.00261.x)
- R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Raymond B, Vanbergen A, Watt A et al (2002) Escape from pupal predation as a potential cause of outbreaks of the winter moth, *Operophtera brumata*. *Oikos* 2:219–228

- Rhainds M, Gries G, Ho CT, Chew PS (2002) Dispersal by bagworm larvae, *Metisa plana*: effects of population density, larval sex, and host plant attributes. *Ecol Entomol* 27:204–212
- Roland J (1994) After the decline: what maintains low winter moth density after successful biological control? *J Anim Ecol* 63:392–398
- Roland J, Embree DG (1995) Biological control of the winter moth. *Annu Rev Entomol* 40:475–492
- Roland J, Myers JH (1987) Improved insect performance from host-plant defoliation: winter moth on oak and apple. *Ecol Entomol* 12:409–414. doi:[10.1111/j.1365-2311.1987.tb01022.x](https://doi.org/10.1111/j.1365-2311.1987.tb01022.x)
- Roland J, Hannon SJ, Smith MA (1986) Foraging pattern of pine siskins and its influence on winter moth survival in an apple orchard. *Oecologia* 69:47–52
- Rosenheim JA (1998) Higher-order predators and the regulation of insect herbivore populations. *Annu Rev Entomol* 43:421–447. doi:[10.1146/annurev.ento.43.1.421](https://doi.org/10.1146/annurev.ento.43.1.421)
- Salminen JP, Karonen M (2011) Chemical ecology of tannins and other phenolics: we need a change in approach. *Funct Ecol* 25:325–338. doi:[10.1111/j.1365-2435.2010.01826.x](https://doi.org/10.1111/j.1365-2435.2010.01826.x)
- Schott T, Hagen SB, Ims RA, Yoccoz NG (2010) Are population outbreaks in sub-arctic geometrids terminated by larval parasitoids? *J Anim Ecol* 79:701–708. doi:[10.1111/j.1365-2656.2010.01673.x](https://doi.org/10.1111/j.1365-2656.2010.01673.x)
- Schott T, Ims RA, Hagen SB, Yoccoz NG (2012) Sources of variation in larval parasitism of two sympatrically outbreaking birch forest defoliators. *Ecol Entomol* 37:471–479. doi:[10.1111/j.1365-2311.2012.01386.x](https://doi.org/10.1111/j.1365-2311.2012.01386.x)
- Schott T, Kapari L, Hagen SB et al (2013) Predator release from invertebrate generalists does not explain geometrid moth (Lepidoptera: Geometridae) outbreaks at high altitudes. *Can Entomol* 145:184–192. doi:[10.4039/tce.2012.109](https://doi.org/10.4039/tce.2012.109)
- Schultz JC, Baldwin IT (1982) Oak leaf quality declines in response to defoliation by gypsy moth larvae. *Science* 217:149–151
- Solbreck C (1995) Chapter 14—long-term population dynamics of a seed-feeding insect in a landscape perspective. In: Price PW, Cappuccino N (eds) *Population dynamics*. Academic Press, San Diego, pp 279–301
- Tanhuanpää M, Ruohomäki K, Kaitaniemi P, Klemola T (1999) Different impact of pupal predation on populations of *Epirrita autumnata* (Lepidoptera; Geometridae) within and outside the outbreak range. *J Anim Ecol* 68:562–570. doi:[10.1046/j.1365-2656.1999.00305.x](https://doi.org/10.1046/j.1365-2656.1999.00305.x)
- Tanhuanpää M, Ruohomäki K, Turchin P et al (2002) Population cycles of the autumnal moth in Fennoscandia. In: Berryman AA (ed) *Population cycles: the case for trophic interactions*. Oxford University Press, New York, pp 142–155
- Terry I, Bradley JR, Duyn JW (1989) Establishment of early instar *Heliothis zea* on soybeans. *Entomol Exp Appl* 51:233–240. doi:[10.1111/j.1570-7458.1989.tb01234.x](https://doi.org/10.1111/j.1570-7458.1989.tb01234.x)
- Travis JMJ, Murrell DJ, Dytham C (1999) The evolution of density-dependent dispersal. *Proc R Soc B Biol Sci* 266:1837
- Varley G, Gradwell G (1968) *Population models for the winter moth*. Blackwell Scientific Publications, Oxford
- Visser ME, Holleman LJM (2001) Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proc R Soc B Biol Sci* 268:289–294. doi:[10.1098/rspb.2000.1363](https://doi.org/10.1098/rspb.2000.1363)
- Wickham H (2009) *ggplot2: elegant graphics for data analysis*. Springer, New York