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

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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 measure dispersal, predation, parasitism, and disease. 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 with little mortality from other factors. 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 regulate 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) 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 the enemy release hypothesis 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

67 often oversimplified, or apply to some species and not others.

68 Forest Lepidoptera have been intensively studied with regards to identifying factors
69 that are important drivers of insect population dynamics (Varley and Gradwell 1968; Myers
70 and Cory 2013). Studies on winter moth and autumnal moth (*Epirrita autumnata* Borkh
71 [Geometridae]), in particular, illustrate the complexity of the issue well. For example, ten-
72 year cyclic outbreaks of these geometrids in Fennoscandia have alternatively been proposed
73 to be driven by delayed density-dependent mortality from specialist parasitoids (Tanhuanpää
74 et al. 2002; Klemola et al. 2010), by delayed induced resistance of host plants (Haukioja and
75 Neuvonen 1987), or by generalist pupal predators (Tanhuanpää et al. 1999; Raymond et al.
76 2002). Delayed induced resistance has not been supported as an explanation for geometrid
77 cycles in more recent work (Haukioja 2005; Myers and Cory 2013), while the role of
78 predators and parasitoids has accumulated evidence but remains controversial (Schott et al.
79 2010; Schott et al. 2012; Schott et al. 2013; Myers and Cory 2013). Despite decades of work
80 on these species, a clear explanation of the factors driving winter or autumnal moth
81 population dynamics remains elusive.

82 One aspect of winter moth population dynamics that has rarely been directly
83 investigated, but holds potential significance is larval dispersal. Dispersal has been
84 considered a process of central importance in population dynamics, but as in the case of
85 winter moth, historically has been less studied than other regulatory factors (Cappuccino
86 1995). Density-dependent dispersal occurs in insects (Denno and Peterson 1995), as well as
87 a broad variety of other taxa (Lambin et al. 2001). In insects, density-dependent dispersal
88 has been especially well documented in sap-feeding insects, especially in the orders

Hemiptera and Thysanoptera (Denno and Peterson 1995), but less so in Lepidoptera or other chewing insects (Berger 1992; Herzig 1995; Rhainds et al. 1997; Rhainds et al. 2002).

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).

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 the present study, we investigated the importance of larval dispersal to winter moth population dynamics in New England, 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 2004-2015 (Elkinton et al. 2015) has found larval densities much higher than previous studies (Embree 1965; Varley and Gradwell 1968) and density-dependent mortality during the larval stage. Mortality during the larval stage appears to the main factor affecting variation in population density between years in New England (J. S. Elkinton and G. H. Boettner, unpublished data). 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. Multiple experiments were used to measure different combinations of possible mortality factors during different stages of larval development (see Table 1 for schematic overview).

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 and 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 Massachusetts. During spring 2014 and 2015, eggs were warmed for five 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 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 hrs 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 . Twigs were placed in cups 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 was recorded. This information was recorded for half of the containers after a period of five days following the point at which >80% of larvae had hatched, and for the second half at seven 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 was used to assess the dispersal rates of larvae. 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) spread across four sites in eastern Massachusetts [West Bridgewater (42.021916, -70.982450); Hanson (42.049473, -70.8730180; 42.060583, -70.843865); Freetown (41.794359, -71.053035)] 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, 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 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 decline 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 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. Dispersal 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).

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 right-of-way at Freetown-Fall River State Forest in Freetown, Massachusetts, with natural populations of first and second instar winter moth. Twenty pairs of buds were manipulated with either of two treatments on each of 10 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 30 μm mesh bags designed to allow most first and second instar larvae to disperse but prevented most predation. This size would exclude most predatory insects including ants, wasps and beetles. After six days manipulated buds and 10 pairs of

unmanipulated (control) buds from each sample tree were collected for dissection (total N=300). Differences in final larval densities per single bud 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 a control treatment. The 'no predation or dispersal' treatment consisted of a fine mesh bag (silk screening mesh, 10 µm 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 six to seven 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 defoliated leaves

To assess the effects of previous damage to foliage 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 previously damaged by naturally occurring winter moth herbivory, and was placed in moist floral foam in ventilated 19 liter 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 hours, 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 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 one 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

248 foliar phenolics and the proportion of oxidative phenolics were analyzed using a modified
249 Folin-Ciocealteu assay following the method of Salminen and Karonen (2011) using
250 absorbance measurements from a microplate reader (Spectramax M2, Molecular Devices,
251 California, USA). Total phenolics were calculated using gallic acid standards and species-
252 specific phenolic standards from Sephadex LH-20 gravity column chromatography
253 (Sephadex LH-20, GE Healthcare Bio-Sciences, Pennsylvania, USA), also after Salminen
254 and Karonen (2011). Proportion of oxidative phenolics measurements were read from
255 extracts diluted to 1.0 ± 0.3 mg/ml gallic acid equivalents (due to difficulties with precise
256 dilution). Total nitrogen and carbon analysis of 5 mg of leaf material was conducted with a
257 combustion analyzer (ECS 4010, Costech Analytical Technologies, California, USA) using
258 acetanilide standards. Phenolic, nitrogen, and carbon measures were obtained from a single
259 pooled sample for each sample tree that consisted of two to six leaf clusters per tree in 2014
260 and 20 leaf clusters in 2015. Trichome density was measured using the average number of
261 trichomes intersecting a 1 mm line on 20 leaves from each sample tree. Measures of foliar
262 quality in each year by sample tree and tree species were analyzed for their effect on larval
263 survival in logistic generalized mixed models with site-level and observation-level random
264 effects.

265 ***Statistical analysis***

266 All statistical analysis was conducted in R (R Core Team 2013, version 3.02).
267 Mixed models were run using the lme4 package (Bates *et al.* 2014), and significance tests of
268 mixed models were made using parametric likelihood ratio bootstrap tests with the function
269 PBmodcomp from the package pbkrtest (Halekoh and Højsgaard 2014), except for the early
270 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. Marginal (fixed effects, R^2m) and conditional (fixed and random effect, R^2c) coefficients of determination were calculated for mixed models using the function rsquare.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 log conspecific density (log odds β=-0.022, χ^2 =208.1, P<0.001, Fig. 1), and differed by tree species (χ^2 =36.2, P<0.001). Mortality was almost entirely due to starvation after dispersal: 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 any evidence of cannibalism, and even in those cups mortality due cannibalism was not the main cause of mortality.

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 β=-1.113, χ^2=9.3 P=0.008, R^2m=0.122, R^2c=0.102, 2015: log odds β= -1.461, χ^2=23.2, P=0.001, R^2m=0.123,R^2c=0.123, Fig. 2), and differed significantly between tree species (2014: χ^2=18.4, P=0.003, 2015: χ^2=14.2, P=0.001, Fig. 2). 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 β= -2.239, χ^2=12.4, P=0.0004).

From samples collected in field density monitoring, the percent of dead larvae in leaf samples peaked on 22-Apr (22%) in 2014 and 1-May (3%) in 2015 and decreased as the season progressed (see Fig. S1). Most dead larvae were neonates that failed to establish in buds. No ectoparasitoids or visible endoparasitoids were observed in any larvae, and no adult parasitoids emerged.

Early larval dispersal manipulation and predator exclusion

Larval densities were significantly different between the no dispersal or predation, dispersal only, and control treatments (χ^2=53.7, P<0.001, Fig. 3a). The no predation and no dispersal treatment (cloth bags) had the highest mean densities (8.9±0.45 larvae per two buds), the no predation and limited dispersal treatment (mesh bags) had intermediate densities (5.1±0.79 larvae per two buds), and the full predation and dispersal treatment (unbagged) had the lowest densities (2.5±0.26 larvae per two buds).

Late larval predator exclusion

Over both years of the larval predator exclusion experiment, there was no significant difference in larval survival between the treatments (2013: χ^2=2.762, P=0.243; 2014: χ^2=0.781, P=0.623, Fig. 3b), and overall larval survival was quite high (2013: 80.4 ± 3.9%; 2014: 71.8 ± 3.7%). Number of sample branches where final density was higher than initial density (and for which proportions were adjusted) were not different between treatments (2013 & 2014: χ^2=1.822, P=0.402).

Larval dispersal from defoliated leaves

In the combined analysis of all trials of the larval dispersal from defoliated leaves experiments, the rate of larval dispersal was significantly elevated on defoliated leaves, with

316 35% more larvae dispersing per day from defoliated foliage (χ^2=20.10, P=0.001), with no
317 difference between tree species (χ^2=0.46, P=0.528). All four trials showed the same trend
318 (Fig. 4), though there were differences in significance level between individual trials (Table
319 S4).

320 ***Foliar quality***

321 Proportion of oxidative phenolics were significantly related to proportion of
322 remaining larvae in 2015, but not in 2014, although the fitted effects in both years were
323 negative (2014: log odds β=-1.029, χ^2=25.1, P=0.489, R^2m=0.047, R^2c=0.069, N=22; 2015:
324 log odds β= -1.158, χ^2=66.7, P=0.018, R^2m=0.031,R^2c=0.112, N=26, Fig. 5). None of the
325 other measures of foliar quality were significantly related to larval survival (P>0.05, Table
326 S3).

327 ## Discussion

328 We propose that *ex-situ* mortality after density-dependent dispersal represents the
329 major cause of mortality during the winter moth larval stage, because of the strong density-
330 dependent dispersal of early instar larvae in the laboratory and field, and increased densities
331 when dispersal is prevented in the field. We also propose that larval dispersal is mediated by
332 phenolic oxidative capacity, based on the response of larval dispersal to damaged foliage,
333 and the relationship between the phenolic oxidative capacity of host tree foliage and larval
334 dispersal rates in the field. Furthermore our results are the first to suggest a negative
335 relationship between larval performance and pH 10 phenolic oxidative capacity as measured
336 using methods from Salminen and Karonen (2010).

337 The significant declines in larval density in outbreaking 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.

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 (Fig. 2; 3-11 May 2014 & 1-8 May 2015), 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 show that predators have little impact in high-density winter moth populations, and that top-down regulation by predation is probably not an important cause of larval mortality in North America. 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) . As such densities, we suspect avian predators are saturated. This is probably what caused low rates of predation in our studies.

The significantly greater densities of the dispersal and no predation treatment (mesh bags excluding predation but allowing most dispersal) in the early larval dispersal manipulation and predation exclusion experiment could indicate that predation occurs on early larval instars alongside dispersal. However, the increased density in this treatment may have been because larvae could not exit bags once they reached third instar or were deterred by the bag as earlier instars. Because of this the mesh bags likely reduced the amount of dispersal, though not as much as cloth bags. 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.

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 die of any cause during mass rearing for biological control release monitoring (2013: 1.1%, 2014: 3.2%, from numbers of cadavers found in rearing, H. J. Broadley, J. S. Elkinton and J. P. Burand, unpublished data). Of those, only some are infected with disease. The 28% infection rate of larvae by *O. brumata* nucleopolyhedrovirus reported by Burand et al. (2011) represents the proportion of larvae infected out of the number of larvae that had died in rearing; although percent larval mortality in rearing was not recorded for the years of that study, but was likely also small.

Even in the absence of any apparent regulation from disease, parasitism, or predation, 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). This pattern holds 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). 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 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 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 herbivores (Ayres *et al.* 1996). 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 present study, to the authors' knowledge, is the first to show evidence suggestive of anti-herbivore effects from the oxidative capacity of phenolics in foliage.

Conclusion

Density dependent mortality during the larval stage appears to be the main factor driving variation in winter moth population densities (J. S. Elkinton and G. H. Boettner, unpublished data). If the major cause of winter moth larval mortality is *ex-situ* mortality after the dispersal of early instars in response to conspecific density and oxidative phenolics as our results show, then this represents the main factor regulating winter moth population

densities in the absence of its co-evolved natural enemies. Dispersal has not previously been considered a major regulating factor of winter moth population densities, and our present study adds to the growing body of evidence showing that density-dependent dispersal is an important density regulating factor in insect populations (Denno and Peterson 1995).

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. In any case, our results provide preliminary evidence confirming the suggestion by Salminen and Karonen (2011) that the oxidative activity of phenolics in a pH 10 environment is likely to have biologically significant effects on herbivores.

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Tables

Table 1. Schematic table overviewing experiments in this study. Factors and stages of development examined in each experiments are represented, to illustrate how experiments collectively cover different mortality factors and combinations of factors over the entire larval stage.

615 Table 1

Experiment	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 defoliated leaves			✕ ■	✕ ■	✕ ■

616 Factors examined:

617 Dispersal ✕ Cannibalism ● Predation ◆ Host plants■

Figures

Figure 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 days, 7 days (red oak and red maple), and 10 days (apple).

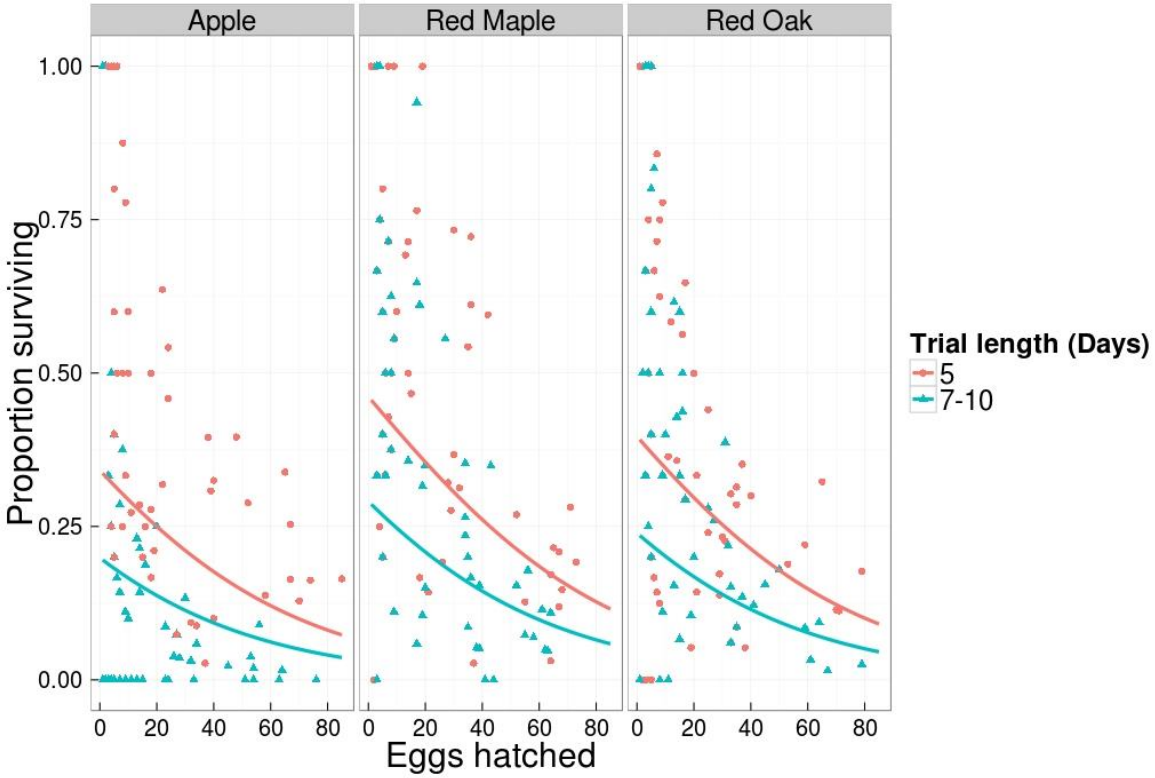
Figure 2. Time series of log winter moth larval densities (left) by sample tree (narrow lines) and overall mean (broad line), and predicted proportion of larvae remaining (survival) by density from proportional logistic mixed models of density dependent winter moth larval dispersal (right), in 2014 (top) and 2015 (bottom). Host species are shown by color and line type. In 2015, the apple and red oak regression lines are nearly identical and are overlapping. Log densities for time series were generated from log of number of larvae per bud plus one.

Figure 3. Results from predator exclusion experiments. Mean and ±1 standard error of (a) larvae per 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. 'No predation or dispersal' treatments prevented all dispersal and predation (cloth bag [a], fine mesh bag[b]), 'no predation and limited dispersal' allowed most dispersal but no predation (fine mesh bags [a]), 'no avian predation, full dispersal' excluded bird predation (bird netting [b]), and 'full predation and dispersal' allowed all predation and dispersal (unbagged).

Figure 4. Mean and ±1 standard error of daily proportion of larvae dispersed from defoliated and undefoliated leaves in four trials (see Table S4).

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

641 Figure 1



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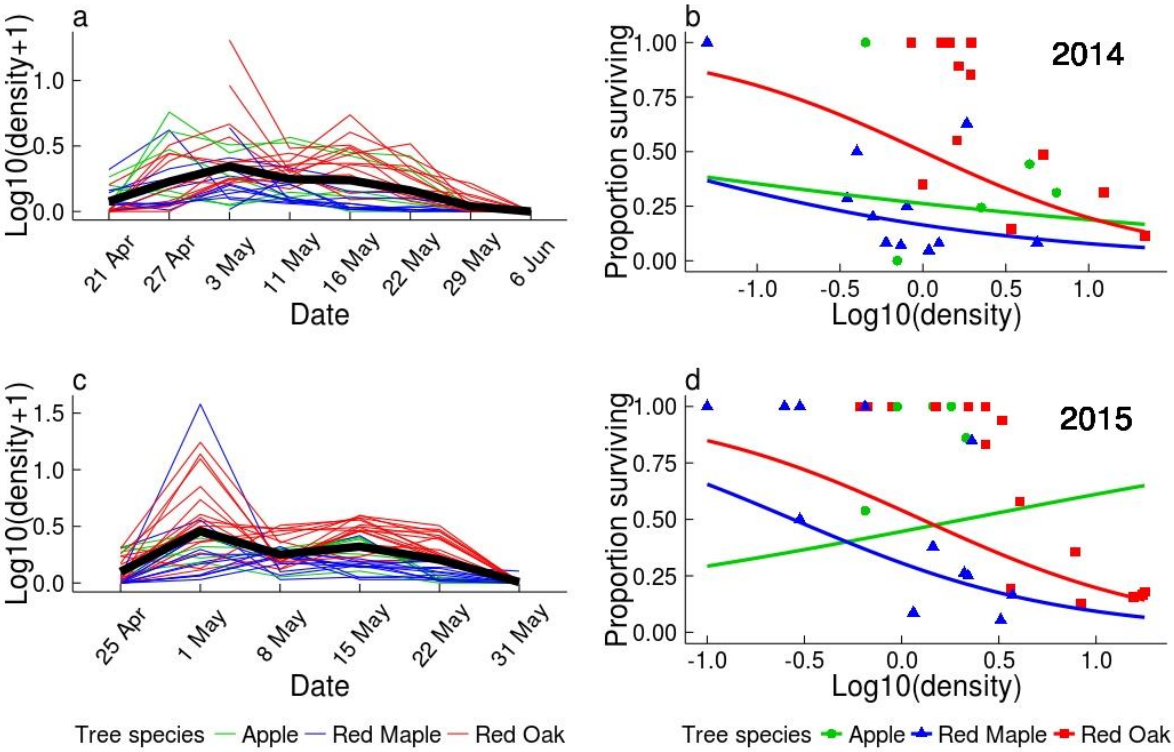

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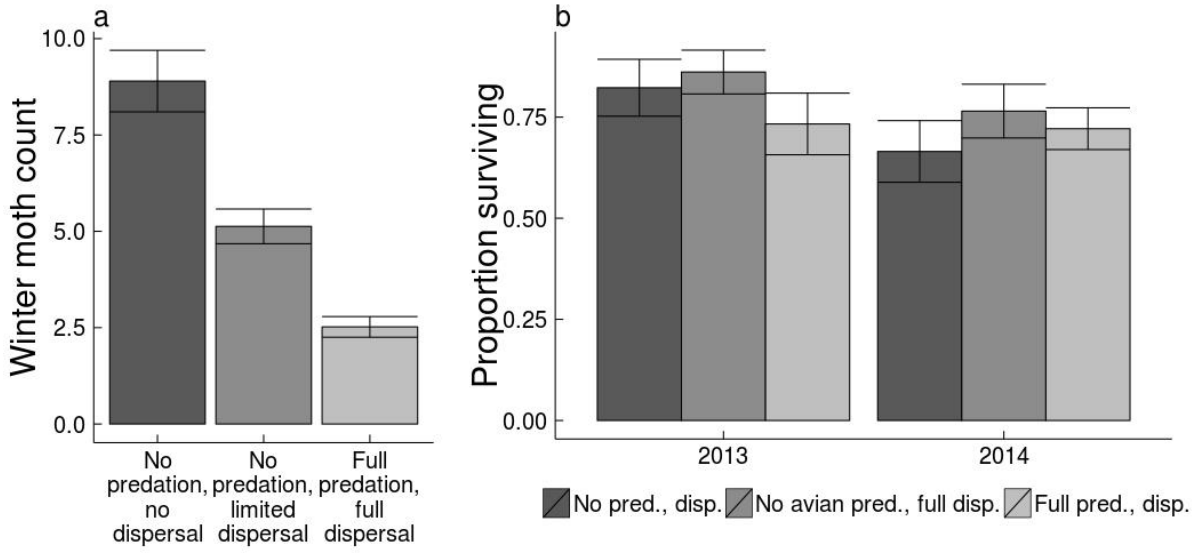

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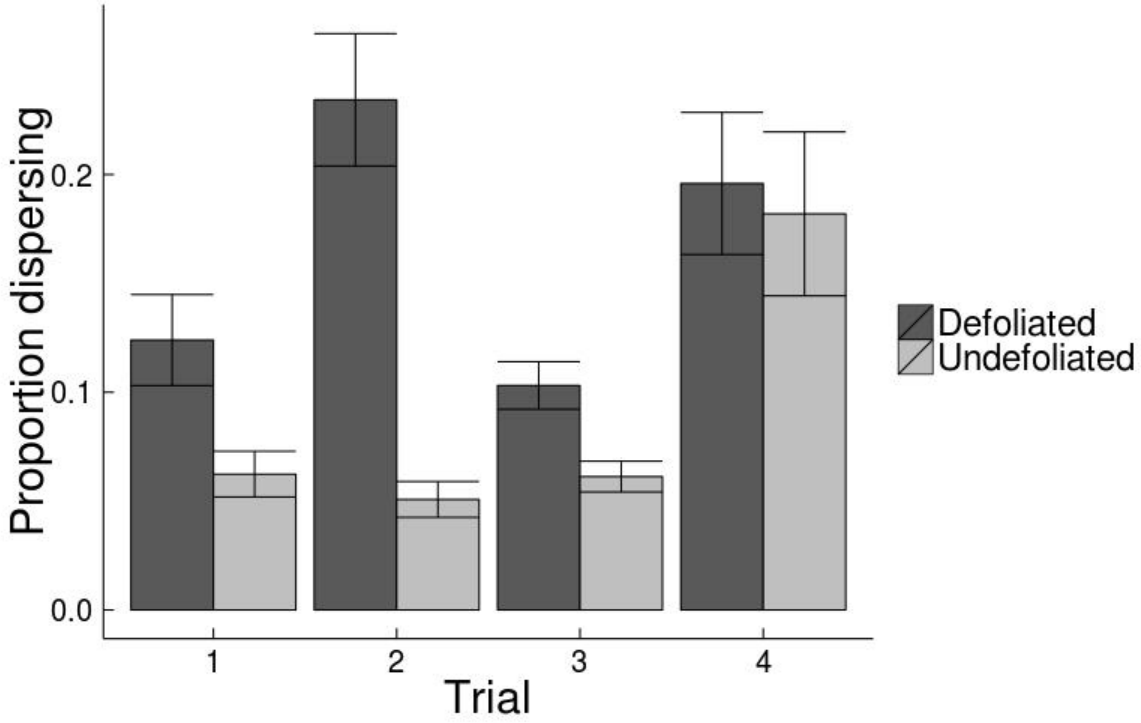

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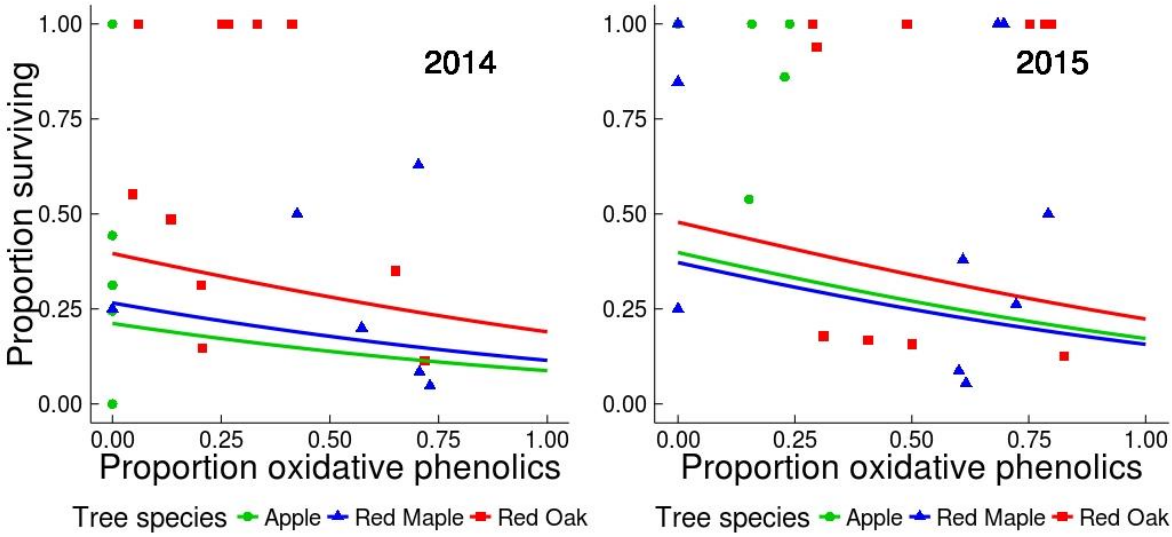
697 Figure 5



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Oecologia (2017) 185:533–535
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ERRATUM

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

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Erratum to: Oecologia (2016) 182:499–509 DOI 10.1007/s00442-016-3689-z

There was a coding error in the original paper resulting in incorrect model parameter estimates and in some cases incorrect model conclusions. The error was the specification of logistic models (using the glm and glmer functions) in *R* as cbind (survived, total) instead of cbind (survived, dead). The differences between the originally published and correct models of those affected are detailed.

Results

Laboratory density manipulation

The laboratory density manipulation model has different parameters, but the direction and significance of effects is unchanged. The following are the correct numbers: In the laboratory experiments, larval survival in cup trials significantly decreased with increasing conspecific density (log odds $\beta = -0.031$, $\chi^2 = 191.2$, $P < 0.001$, Fig. 1), and differed by tree species ($\chi^2 = 97.6$, $P < 0.001$) and measurement date ($\chi^2 = 34.0$, $P < 0.001$). Fitted survival probabilities (least-square means, from lsmeans package, Lenth 2016) were 31% for red oak, 40% for red maple, and 26% for apple, and 46% lower at 7–10 days than at 5 days.

Field density monitoring

The main model of the paper, from field density monitoring, of density-dependent larval dispersal, also has different parameters, but the direction and significance of effects is unchanged. The following are the correct numbers: In the field, proportion of larvae remaining on sample trees decreased significantly with increasing initial density in both years (2014: log odds β: red oak = −2.187, red maple = −4.553, apple = −2.114, $\chi^2 = 11.1$ $P = 0.032$, R^2 m = 0.190, R^2c = 0.238, 2015: log odds β: red oak = −6.792, red maple = −4.873 apple = 4.708, $\chi^2 = 22.5$, $P < 0.001$, R^2m = 0.126, R^2c = 0.126, Fig. 2), and differed significantly between tree species (2014: $\chi^2 = 20.9$, $P = 0.005$, 2015: $\chi^2 = 15.3$, $P < 0.001$, Fig. 2). Fitted survival probabilities (least-square means) were 94% for red oak, 14% for red maple, and 43% for apple in 2014, and 98% for red oak, 42% for red maple, and 98% for apple in 2015. Parametric bootstraps were used only for the tests of the effects of log density and tree species in 2014, and all others were conducted using Wald Chi square tests because of the failure of parametric bootstraps and profile likelihood confidence intervals.

Late larval predator exclusion

For the 2013 results, the treatments in the late larval predator exclusion experiment model now become significant according to parametric bootstrap tests (i.e., models with treatment included as a predictor are significantly better than those without) in 2013; however, effects of individual treatment

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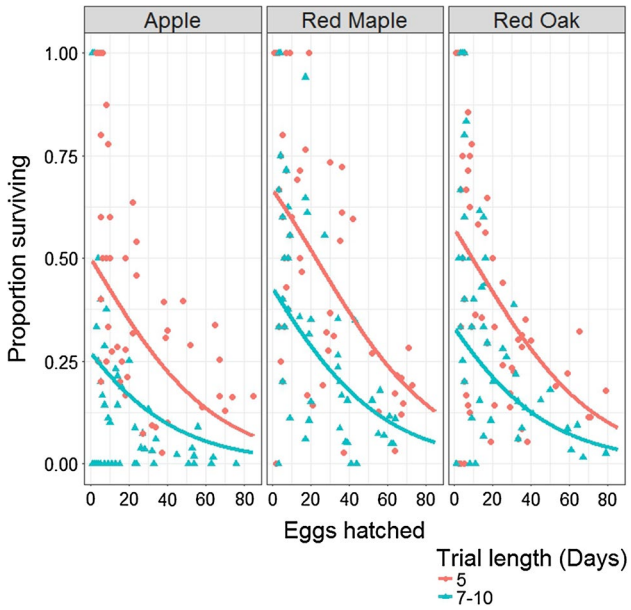

Fig. 1 Updated figure of laboratory density manipulation experiment, showing corrected regression lines

levels are not significant according to 95% profile likelihood confidence intervals. All effects remain non-significant in 2014. The corrected results, by years: in the larval predator exclusion experiment, models with treatment included were significantly better in 2013 but not 2014 (2013: $\chi^2 = 62.5$, $P < 0.001$, 2014: effect: $\chi^2 = 3.9$, $P = 0.152$), though treatment levels were not different in 95% profile likelihood confidence intervals in either 2013 or 2014 (2013: log odds β: no predation or dispersal = 2.95 [CI 0.84–1.48], no avian predation and full dispersal = 2.78 [CI 0.84–1.48] full predation and full dispersal = 1.79 [CI −0.81 to 3.07], $P < 0.001$, 2014: log odds β: no predation or dispersal = 2.95 [CI 0.34–2.07], no avian predation and full dispersal = 2.78 [CI −0.04 to 0.42], full predation and full dispersal = 1.36 [CI −0.03 to 0.47]).

Foliar quality

The foliar quality models are changed. The main changes are that the effects of pH 10 oxidative phenolics on dispersal are no longer significant in either year, carbon–nitrogen ration is now significantly related to dispersal in 2014, and the 2015 models of percent nitrogen and percent carbon fail to converge. The corrected results are summarized in the corrected Table S3, and a plot of the relationship of carbon–nitrogen ratio to larval survival rates in 2014 in Fig. 3.

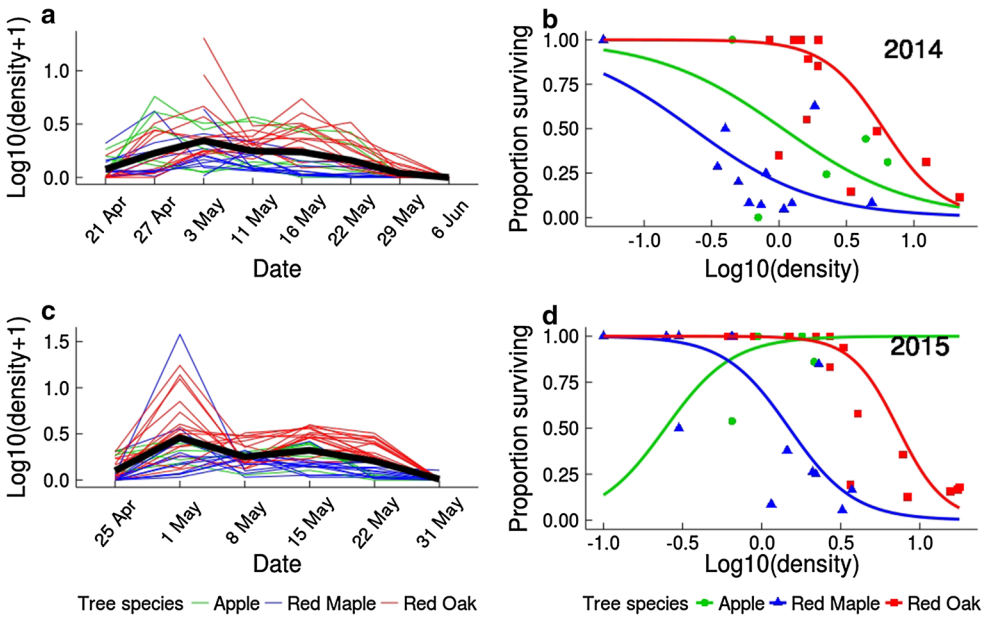

Fig. 2 Updated figure of field monitoring experiment, showing corrected regression lines

Table S3 Year, sample size, slopes, marginal r-squared, conditional r-squared, likelihood ratio test chi squared values, parametric bootstrap significance tests, and Wald χ^2 tests from models of effects of leaf quality on declines in larval density. P-values in bold are significant to the 0.05 level

Variable	Year	N	β (log odds)	R^2m	R^2c	χ^2	P	P_{wald}
Percent phenolics, gallic acid equivalents	2014	26	−0.040	0.1800	0.1800	9.349	0.824	
Percent phenolics, self-standard equivalents	2014	26	−0.018	0.1798	0.1798	9.335	0.820	
Proportion oxidative phenolics	2014	22	-2.679	0.1958	0.1958	26.797	0.582	
Percent phenolics, gallic acid equivalents	2015	33	0.111	0.0554	0.1738	0.431	N/A	0.512
Percent phenolics, self-standard equivalents	2015	33	0.050	0.0559	0.1696	0.410	N/A	0.522
Proportion oxidative phenolics	2015	26	−2.799	0.0964	0.3182	1.329	N/A	0.249
Percent nitrogen	2014	27	−2.250	0.2965	0.2965	14.512	0.052	
Percent carbon	2014	27	−0.013	0.2220	0.2220	10.627	0.364	
Ratio percent carbon: percent nitrogen	2014	27	0.855	0.3380	0.4426	16.189	**0.024**	
Ratio percent carbon: percent nitrogen	2015	24	−0.333	0.1177	0.1177	0.328	N/A	0.567
Trichomes per linear mm	2015	33	0.120	0.1213	0.2203	2.840	N/A	0.092

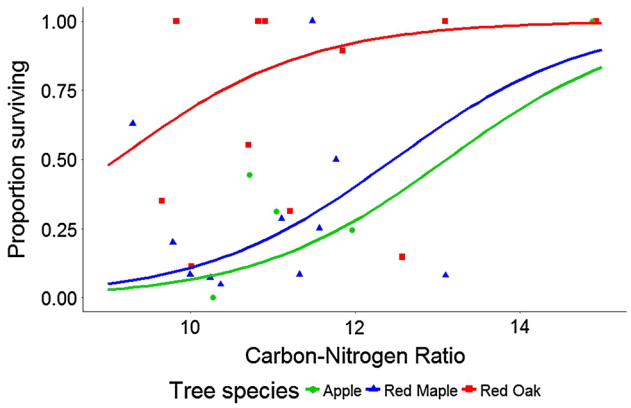

Fig. 3 Graph illustrating the relationship between carbon–nitrogen ratio and predicted larval survival in 2014

Discussion

The model results for the laboratory density manipulation and field monitoring model results have not changed significantly. The effect of treatment in models of late larval predation have become significant in 1 year, though the estimates of individual treatment level effects remain non-significant.

The pH 10 oxidative phenolics are no longer significantly related to larval dispersal rates in 2015 as they were in the original model. This new result, however, does not change the main conclusions of the paper. We did not emphasize the importance pH 10 oxidative phenolics because the evidence for its effect was quite weak, as is the new evidence for the effect of carbon–nitrogen ratio. The claim of the paper that larvae are responding to foliar quality rests on the larval dispersal from defoliated leaves experiments, the model results of which remain unchanged because it was specified correctly unlike the other logistic models.

In summary, the major conclusions of the paper, 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' (from the abstract) remain unchanged, though we now no longer have evidence to suggest that pH 10 oxidative phenolics could be the active component of foliar quality driving larval dispersal.

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