Native generalist natural enemies and an introduced specialist parasitoid together control an invasive forest insect

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Abstract
Specialized natural enemies have long been used to implement the biological control of invasive insects. Although research tracking populations following biological control introductions has traditionally focused on the impact of the introduced agent, recent studies and reviews have reflected an appreciation of the complex interactions of the introduced specialist agents with native generalist natural enemies. These interactions can be neutral, antagonistic, or complementary. Here we studied the invasive defoliator winter moth (Operophtera brumata) in the Northeast USA to investigate the role of native, generalist pupal predators along with the introduced, host-specific parasitoid Cyzenis albicans. Prior research in Canada has shown that predation of winter moth pupae from native generalists increased after C. albicans was established as a biological control agent. To explain this phenomenon, the following hypotheses were suggested: (H₁) parasitoids suppress the winter moth population to a density that can be maintained by generalist predators, (H₂) unparasitized pupae are preferred by predators and therefore experience higher mortality rates, or (H₃) C. albicans sustains higher predator populations throughout the year more effectively than winter moth alone. We tested these hypotheses by deploying winter moth pupae over 6 years spanning 2005 to 2017 and by modeling pupal predation rates as a function of winter moth density and C. albicans establishment. We also compared predation rates of unparasitized and parasitized pupae and considered additional mortality by a native pupal parasitoid. We found support for the first hypothesis; we detected both temporal and spatial density dependence, but only in the latter years of the study when winter moth densities were low. We found no evidence for the latter two hypotheses. Our findings suggest that pupal predators have a regulatory effect on winter moth populations only after populations have been reduced, presumably by the introduction of the host-specific parasitoid C. albicans.

KEYWORDS
biological control, biotic resistance, Cyzenis albicans, Operophtera brumata, parasitoid, population dynamics, predation, winter moth
INTRODUCTION

Invasive species increasingly threaten national and global agriculture, human health, and biodiversity and stability within ecological systems (Hooper et al., 2005; Marsh et al., 2021; Paini et al., 2016). Of invasive species, insects are particularly devastating, costing a minimum of US$70 billion per year globally (Bradshaw et al., 2016) and countless ecological effects (Kenis et al., 2009). Mortality from imported biological control agents and natural enemies already present in the invaded range can each play a vital role in diminishing the destructive effect of invasive insects. However, complicated and novel dynamics arise in an ecological system when new species are introduced and intraguild and population dynamics shift (Kenis et al., 2009). Invasive herbivore insects, such as winter moth, are affected by and influence populations of predators already in the system (Bompard et al., 2013; Pintor & Byers, 2015; Rosenheim, 1998).

The importance of specialist parasitoids (insect parasites of another insect) as drivers of population dynamics, density dependence, and top-down forces of outbreaking invasive species is supported by applied biological control (Kenis et al., 2017; Murdoch et al., 1985; Snyder & Ives, 2001), long-term population studies (Berryman, 1996; Myers & Cory, 2013; Tanhuuanpaa et al., 1999), and theoretical models (Hassell, 2000; Ruohomaki et al., 2000; Tanhuuanpaa et al., 1999). However, more often than not, control of an invasive or outbreaking insect will involve complex interactions among many regulatory factors (Heimpel & Mills, 2017; Myers, 2018). The field of classical biological control (or importation biological control) traditionally has focused on the role of the introduced, specialist natural enemy. However, several recent studies and reviews have suggested that both specialist parasitoids and generalist predators can each inflict strong top-down effects on insect herbivores (e.g., Klemola et al., 2014; Symondson et al., 2002; Vidal & Murphy, 2018) and that specialists and generalists can interact to maintain populations at low densities (e.g., Hajek & Eilenberg, 2018; Hochberg et al., 1990; Murphy et al., 2018, Snyder & Ives, 2003). This focus highlights the importance of specialist parasitoids in controlling invasive species but at the expense of understanding the role of these species along with native natural enemies already present in the system.

Winter moth, Operophthera brumata L. (Lepidoptera: Geometridae) was an important invasive pest of hardwood forests in Canada and in the USA (Elkinton et al., 2015; Roland & Embree, 1995) and is an example of successful classical biological control (Elkinton et al., 2021; Hajek & Eilenberg, 2018; Kenis et al., 2017; Van Driesche et al., 2008). It also a well known model species for understanding population dynamics (Grubb & Whittaker, 2013; Varley et al., 1973). Winter moth is native to Europe and can be a major defoliator, particularly in its invasive ranges in North America and in sub-Arctic birch forest in northern Fennoscandia. Detailed studies of the population dynamics have been made in the pest’s native range in Europe (Tenow et al., 2013; Varley et al., 1973) and in its introduced ranges (Elkinton et al., 2015; Roland & Embree, 1995).

In the native range, pupal mortality was found to be the most important regulatory factor (Varley et al., 1973). However, direct density-dependent predation on pupae has only been detected when population densities of winter moth are low to medium (Hassel & May, 1986; Roland, 1994; Varley & Gradwell, 1968). It appears that when populations of winter moth are low, generalist predators (e.g., shrews, other small mammals, or carnivorous beetles such as carabid or staphylinid beetles) can respond to increasing densities with increasing predation rates (Roland, 1994), but only up to a population density threshold. Above this threshold, in high-density, outbreak populations, the opposite trend (inverse density-dependent predation) has been found (Raymond et al., 2002); there is more food available than the predators can consume and their effect saturates (Appendix S1: Figure S1).

Cyzenis albicans Fallén (Diptera: Tachinidae) is a specialist parasitoid of winter moth also native to Europe. Cyzenis albicans deposits its eggs on leaves and winter moth caterpillars that feed on those leaves ingest the eggs and are parasitized. Cyzenis albicans is attracted to leaves defoliated by winter moth and each individual egg that is deposited has a better chance of being consumed by a potential host if the host population density is high (Hassell, 1980). Notably, C. albicans is not considered an important mortality factor in the winter moth’s native range, but it was one of a few parasitoids released in Nova Scotia to control the invasive populations there (Embree, 1965). Subsequently, because it is a specialist parasitoid and could establish and reproduce in its introduced range, it was selected as a classical biocontrol agent for release in British Columbia (Roland & Embree, 1995) and most recently in Northeast USA (Elkinton et al., 2021).

Between the 1930s and 1970s, winter moth was accidentally introduced from Europe into Nova Scotia, British Columbia, and Oregon. A more recent introduction of winter moth to North America has taken place in the Northeast USA (Elkinton et al., 2014, 2015). The suppression of winter moth in Canada is attributed to the introduction of C. albicans and another parasitoid, Agrypon flaveolatum Gravenhorst (Hymenoptera: Ichneumonidae) (Hassell, 1980). In 2005, a biological control effort began in the Northeast USA using releases of C. albicans that were collected from British
Columbia (Elkinton et al., 2015). Only *C. albicans* was released because it is a specialist on winter moth, whereas the host range and taxonomy of *A. flaveolatum* is uncertain. *Cyzenis albicans* has now successfully established across much of this new winter moth range and a corresponding decline of winter moth population densities and defoliation has been noted (Elkinton et al., 2021).

Following the introduction of parasitoids and ensuing winter moth population decline in Nova Scotia, the introduced parasitoids *C. albicans* and *A. flaveolatum* were assumed to be responsible (Hassell, 1980). However, subsequent studies, in both Nova Scotia and British Columbia, suggested that mortality from native natural enemies along with mortality from introduced parasitoids together were necessary to regulate winter moth densities (Roland & Embree, 1995). Here we apply the definition of a regulated population presented by Varley et al. (1973); a regulated population is one that tends to return to an equilibrium density following any departure from this level. Predators on pupae were found to be particularly important (Horgan et al., 1999; Pearsall & Walde, 1994; Roland & Embree, 1995), but native or resident parasitoids also have been noted (Broadley, 2018; Humble, 1985). Roland (1990) showed that pupal mortality in both Nova Scotia and British Columbia increased after parasitoid establishment, suggesting that predation on pupae was somehow synergized by the presence of *C. albicans*. It was not known why predation on the pupae increased with parasitism rates, but Roland (1990) proposed several hypotheses regarding how *C. albicans* parasitism and predation on the winter moth pupae might interact (Figure 1): (H1) parasitoids suppress the winter moth population to a density that can be maintained via pupal predation by native generalist predators, (H2) unparasitized pupae are preferred by predators and therefore experience higher mortality rates, or (H3) having *C. albicans* in the system sustains generalist predators that attack winter moth pupae by being present in the soil over the winter and spring, when winter moth pupae are not present.

We tested the hypotheses outlined by Roland (1990) by quantifying the interactions of *C. albicans* and generalist predators on winter moth population dynamics in Massachusetts. In this system, large changes in winter moth densities (>90% reduction) have been documented with a relatively modest percentage parasitism (10%–45%) (Elkinton et al., 2021), suggesting that parasitism alone is

![Figure 1](https://example.com/figure1.png)

**Figure 1** Schematic showing our hypotheses for how the introduced parasitoid *C. albicans* may affect predation rates on winter moth pupae by generalist predators in winter moth’s introduced range (as described in Roland, 1990). Synergy between the two factors may arise from one or more of three mechanisms: (H1) introduced parasitoids suppress the winter moth population to a density that can be maintained by generalist pupal predators; (H2) unparasitized winter moth pupae are preferred by predators over parasitized pupae and therefore experience higher mortality rates, or (H3) *C. albicans*, which is present in the soil over winter and sustains predator populations throughout the year more effectively than winter moth alone, which, in turn, results in high mortality on the pupae in sites where *C. albicans* is present. The two lines shown in H1 show two possible senarios when pupal densities are low.
not responsible for declines in winter moth densities and instead acts with predation to regulate the population. To empirically test the three hypotheses, we (1) analyzed pupal mortality before and after establishment of *C. albicans* and as a function of winter moth pupae density, (2) compared mortality rates between parasitized and unparasitized pupae, and (3) analyzed mortality on pupae and tested for a difference in the predator community with or without *C. albicans*.

**METHODS**

**Study organisms**

Winter moth, *Operophtera brumata* L., is a geometrid moth native to Europe and northern Asia and can be an important defoliator, particularly in its invasive ranges in coastal North America (Nova Scotia, British Columbia, and New England) (Elkinton et al., 2015; Roland & Embree, 1995) and in sub-Arctic birch forest in northern Fennoscandia (Jepsen et al., 2008). Winter moth caterpillars hatch in synchrony with bud-break of their host plants, a broad range of deciduous trees, particularly oak and maple. The caterpillars feed on the foliage in early spring before dropping to the soil in mid to late May to pupate. Winter moth has a long pupal period (6–7 months during the summer, representing most of its life) and it pupates in the top layer of soil or leaf litter. They emerge as adults in early winter, from early November through early January in western Europe and North America, at which point they mate and lay their eggs in bark cervices. These eggs overwinter and the cycle repeats with one generation per year.

Winter moth is associated with several parasitoids. *Cyzenis albicans* is a parasitoid that co-evolved with winter moth. It deposits its eggs on leaves, and any subsequent caterpillars that feed on those leaves ingest the eggs. The fly maggot develops inside the caterpillar, eventually killing it, and pupates inside its host’s pupa. Like winter moth, *C. albicans* has one generation per year, but the adult flies emerge the following spring rather than in the early winter when winter moth adults emerge. *Agrypon flaveolatum* was introduced as a classical biological control agent to populations of winter moth in Canada (Hassell, 1980), but its role in population control and its host specificity are uncertain. *Pimpla aequalis* Provancher (Hymenoptera: Ichneumonidae) and a related *Pimpla* sp. are native or resident parasitoids that has been found associated with winter moth pupae in the Northeast USA (Broadley et al., 2019). They are generalists that spilled over from other hosts to attack winter moth and *C. albicans* pupae. Unlike *C. albicans*, which has only one generation a year, both *Pimpla* species are likely to have multiple generations on pupae over the summer and can therefore respond quickly to high densities.

**Deployment of pupae**

Pupae were deployed in 2005 and yearly from 2013 through 2017 to estimate mortality from predation over the winter moth pupal period. The first deployment in 2005 was conducted prior to the establishment of *C. albicans*; the first recovery of *C. albicans* in Massachusetts was not until 2010 (Elkinton et al., 2021). This first pupal deployment used the same deployment methods as the later deployments except as specified below. We deployed unparasitized winter moth pupae and *C. albicans* puparia (winter moth pupae parasitized by *C. albicans*), both reared from spring larval collections. Methods for pupal collection, rearing, and storage are described in Appendix S1: Section S1. Pupae were deployed in eastern Massachusetts at eight study sites (outlined in Table 1), which were also used for long-term assessment of winter moth population dynamics (Elkinton et al., 2015, 2021). Sites are dominated by northern red oak (*Quercus rubra*), which is winter moth’s favored host tree. Five deployments (one every 3 weeks) were completed in 2005, 2013, and 2014; three deployments (one every 6 weeks) were completed in 2015–2017. In 2005, 40 winter moth pupae were deployed in each consecutive deployment, whereas in all the subsequent years (2013–2017) 100 winter moth pupae and 50 *C. albicans* puparia were both deployed by burying them intermingled at each site in each consecutive deployment. To evaluate season-long mortality compared with the cumulative mortality calculated from the consecutive deployments, we deployed an additional 200 winter moth pupae at two sites (Table 1; sites B and D) from 12 June to 20 October 2014. To evaluate mortality of *C. albicans* puparia over the winter, we deployed 100 puparia at sites B and D from 26 October 2013 to 3 April 2014.

Deployed pupae were attached to small burlap squares (approximately 4 cm², one pupa per square) using beeswax, as described in Broadley et al. (2018). Pupae on burlap were placed haphazardly 2.5 cm below the soil surface under the drip line of a red oak at each study site (Table 1; Appendix S1: Figure S2). The deployment depth was chosen to mimic natural pupa placement (Hollday, 1977). Study sites coincided with winter moth long-term study sites and reflected a range of winter moth and *C. albicans* establishment histories (Elkinton et al., 2014, 2021). Due to the limited supply, only select sites received deployments of *C. albicans* puparia (Table 1).
To test for an effect of our deployment method compared with previously published mortality estimates using a wire tag method (Buckner, 1969; East, 1974; Horgan & Myers, 2004; Raymond et al., 2002), in 2005 we compared the mortality of pupae attached to wire tags compared with pupae attached to burlap squares. We buried 30 wired pupae and 30 burlap-attached pupae at two sites for 2 weeks. We analyzed these data using a generalized mixed model (GLM) with a logit link and with treatment and site as fixed factors.

Estimating site pupal densities and parasitism by *C. albicans*

To estimate winter moth pupal density and percentage *C. albicans* parasitism at each site, 16 plastic buckets (16 cm wide × 28 cm long × 10 cm high) were filled 3 cm deep with sifted peat moss and placed under each study tree in late May, before prepupal winter moth caterpillars began to spin down from the tree canopies. Each bucket was placed at a randomly selected distance between the tree stem and the edge of the tree canopy, along one of eight evenly spaced directions radiating from the tree stem. This is the same red oak that was used for the pupal deployment. Parasitism rates on winter moth by *C. albicans* were estimated both from the proportion of parasitized pupae from these pupal bucket collections and from collections of 100–500 late instars collected at each site. The collections were made by knocking caterpillars off adjacent branches 1 week before the onset of pupation. The larvae were reared in groups of up to 500 in 20-L buckets with screen tops. In July, after *C. albicans* had completed development, we partially opened the earthen cocoons to determine whether they had been parasitized. After scoring them, the cocoons were closed again. The parasitized pupae were easily distinguished from the unparasitized by their small size, darker color, and smoother texture of the cuticle.

Pupal mortality estimates

After each deployment, pupae were retrieved and stored in a growth chamber (Percival Scientific, Inc.) at 12°C in the dark until analysis. We characterized the fate of the

<table>
<thead>
<tr>
<th>Study site</th>
<th>Latitude, longitude</th>
<th>Winter moth pupa deployments</th>
<th>C. albicans puparium deployments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Co-op Extension, Hanson, MA</td>
<td>42.048889, -70.873806</td>
<td>1.00 (200) 0.88 (500) 0.96 (500) 0.99 (300) 1.00 (300) 0.72 (300)</td>
<td>0.88 (200) 0.98 (300)</td>
</tr>
<tr>
<td>B. Maquan St., Hanson, MA</td>
<td>42.060694, -70.844167</td>
<td>... (750) 0.80 (700) 0.99 (300) 0.98 (300) 0.81 (300) 0.77 (300) 0.83 (350) 1.00 (200)</td>
<td>0.98 (200) 0.85 (300)</td>
</tr>
<tr>
<td>C. Pondview Dr., Falmouth, MA</td>
<td>41.626417, -70.580417</td>
<td>... (500) 0.81 (500) 0.77 ... (300) 0.97 (300) 0.79 ...</td>
<td>... ... 0.92 (300)</td>
</tr>
<tr>
<td>D. Centennial Park, Wellesley, MA</td>
<td>42.308444, -71.266778</td>
<td>... (750) 0.78 (700) 0.94 (300) 0.84 (300) 0.93 (300) 0.87 (300)</td>
<td>0.87 (350) 0.98 (200) 0.80 (300)</td>
</tr>
<tr>
<td>E. Wompatuck SP, Hingham, MA</td>
<td>42.208333, -70.853056</td>
<td>1.00 (200) 0.89 (500) 0.92 (500) 0.97 (300) 0.78 (300) 0.79 (300)</td>
<td>... ... 0.59 (200) 0.59 (300)</td>
</tr>
<tr>
<td>F. Route 6, Yarmouth, MA</td>
<td>41.686167, -70.287722</td>
<td>... (500) 0.91 (500) 0.81 ... (300) 1.00 (300) 1.00 (300)</td>
<td>... ... 1.00 (300)</td>
</tr>
<tr>
<td>G. Center St., W. Bridgewater, MA</td>
<td>42.020806, -70.982306</td>
<td>0.93 (200) 0.86 (500) 0.88 (500) ... ... ... ...</td>
<td>... ... ... ...</td>
</tr>
<tr>
<td>H. Bare Cove Park, Hingham, MA</td>
<td>42.238222, -70.913389</td>
<td>1.00 (200) 0.83 (500) 0.99 (500) ... ... ... ...</td>
<td>... ... ... ...</td>
</tr>
</tbody>
</table>

Notes: Number of pupae deployed (samples size) is indicated in parentheses. Predation rates are presented as a proportion ranging from 0 to 1.
pupae as intact, preyed upon, parasitized, or diseased. Predation was assumed for pupae that had been removed from the burlap square and for pupae with only the crushed cuticle remaining, holes chewed in them, and evidence of teeth or claw marks in the wax, as shown in Broadley et al. (2018). Parasitism by *Pimpla* wasps was assumed for pupae with characteristic wasp emergence holes and pupae that yielded wasps as shown in Broadley et al. (2019). No data on *Pimpla* wasps were recorded in 2005 and 2013, because we had not yet identified the characteristic emergence holes that these wasps had left in the pupal cuticle, and we did not hold the pupae to allow for wasp development. To allow for the development of pupal parasitoids, seemingly intact pupae were stored in a growth chamber over the winter and were re-examined in the spring. They were stored at 12°C until the beginning of December, at 9.5°C until the end of December, and at 4°C until late March when the temperatures were increased 4°C every 3 days to 22°C at which point the pupae were kept at room temperature. The pupae were kept in the dark with no day/night cycle and sprayed with a sodium propionate solution (5 g sodium propionate/l of water) once a month to prevent mold. We excluded diseased pupae in our mortality estimates because the desiccation or mold probably occurred because of the rearing conditions; these pupae accounted for a small proportion of mortality (<6%).

Predation rates and parasitism by *Pimpla* wasps were calculated for each pupal deployment. Predation rate was calculated as the total number that were removed divided by the total number of pupae deployed. The proportion parasitized by *Pimpla* was calculated as the number of pupae parasitized by the wasps divided by the number of pupae that remained after predation. This method of calculating percentage parasitism estimates the underlying mortality rate of contemporaneous mortality factors and incorporates the fact that parasitism rates can be obscured by predation rates because predation typically occurs on the pupae whether or not they were parasitized (Elkinton et al., 1992). Because previous research had found no relationship between the proportion of pupae parasitized by *Pimpla* wasps and the proportion lost to predation (Broadley et al., 2019), we assumed the estimate of *Pimpla* parasitism determined after predation acted on the deployment would be the same as that prior to predation. We calculated predation and parasitism rates separately for the winter moth pupae we had deployed compared with the deployed *C. albicans* puparia.

Annual cumulative (life-stage long) survivorship (\(S_i\)) values were calculated as the product of successive survivorships of each pupal deployment (\(S_c = S_1 \times S_2 \times S_3\) multiplied as such for the number of deployments involved, where \(S_1\), \(S_2\), and \(S_3\) are the survivorships of each successive deployment) and the cumulative mortality values (\(P_c\)) were calculated as (\(P_c = 1 - S_i\)). To analyze mortality rates of deployments across years and months, even when the number of days deployed varied (from 19 to 45), we standardized mortality proportions to the mean deployment duration of 31 days. We estimated the daily survival rate (\(S = S_1^{1/n}\) where \(n\) is the variable number of days the pupae were deployed (ranged from 19 to 45 days, mean of 31 days). The daily survivorship (\(S\)) was raised to an exponent of 31 for the expected survivorship over a standardized 31 days (\(S_{31} = (S)^{1/n} \times 31\)). To estimate pre-experimental mortality of the pupae (\(S_{1a}\)), we used the estimated daily survival of the first deployment (\(S_{1a} = S_{1b}^{1/n}\) where \(n\) is the number of days the first deployment was out and raised this to the estimated number of days of the winter moth pupal period that elapsed before the onset of the study. Based on prior research (Elkinton et al., 2015), we estimated the start of the pupal period to be 1 June.

**Predator exclusion and community experiments**

In 2013 and 2014, we conducted a predator exclusion study to evaluate the relative contribution of generalist small mammal and beetle predators to pupal mortality. Using duplicate sites (Table 1; sites A and D), we ran one round in 2013 (12 August to 20 October) and two in 2014 (26 June to 11 August and 18 August to 30 October) for a total of six deployments. For each deployment, 100 pupae were buried in a 100 m by 100 m grid with one pupa placed every 1 m² in the array. The pupae were attached to burlap squares (4 cm²) and these were secured to the bottom of one of three cage treatments: cages with 3.2 mm, 6.4 mm, or 12.7 mm square openings or a control (just the wire mesh bottom of one of the other three cages). The cages were placed 2–3 cm into the ground and covered with soil and leaf litter. At the end of each deployment, the pupae were characterized as intact, preyed upon, parasitized, or diseased.

In 2013, to characterize the invertebrate predator community, two pitfall traps were placed at all study sites used that year and checked weekly. The pitfall traps were each made from a 0.91 L plastic cup, buried flush to the surface, covered with a lid elevated 5 cm above with a wire stand, and partially filled with 70% ethyl alcohol solution. All beetle larvae, adult staphylinid and carabid beetles, and any small mammal by-catch were counted. Carabid beetles were identified to species when possible. Tabulated results are available in the corresponding Dryad Digital Repository (Broadley et al., 2022a).
**Statistical analyses**

All analyses were run in R 3.4.4 (RCoreTeam, 2013) using RStudio, version 1.1.442 (RstudioTeam, 2015). The data files are available from Dryad Digital Repository (Broadley et al., 2022a) and the R code are available from Zenodo (Broadley et al., 2022b). For each analysis, the full model was run initially (including site, year, and deployment effects). The model was evaluated for evidence of skew in the residuals or outliers, and any insignificant predictors ($p > 0.05$) were dropped sequentially starting with the largest $p$-value until the best fit model was selected using AIC comparisons. We checked for overdispersion and, when noted, we applied a quasibinomial or quasipoisson distribution. For the logistic regression, proportions were weighted by the total number of pupae evaluated and a pseudo-$R^2$ was calculated (deviance null model – deviance fit model/deviance null model). All graphical data were displayed using `ggplot2` (Wickham, 2009).

To analyze total pupal mortality, predation on pupae, and pupal parasitism across the years and study sites, we used a logistic GLM with the cumulative mortality by each mortality factor as the response with year and site as fixed effects. To test for a significant difference among sets of the mortality factors (predation and parasitism), we ran multiple comparisons tests of the means (Tukey contrasts) using the package `multcomp` (Hothorn et al., 2008) with year and site as factors. We considered seasonality of predation by analyzing monthly mortality rates over the pupal period with year and site effects.

To test for direct density-dependent effects, we analyzed the cumulative mortality by predation on the winter moth pupae against the site’s background density of pupae. We included year and site effects (temporal and spatial density dependence, respectively). To compare the strength of density dependence of predation in the current study to that in the United Kingdom and British Columbia, we converted the pupal mortality from predation to a $k$-value (where $k = -\log_{10}$ proportion surviving), regressed this against the corresponding winter moth density ($\log_{10}$ pupae/m²), and evaluated the slope of this relationship against results previously reported from England (Varley et al., 1973) and British Columbia (Roland, 1994). To test for delayed density dependence, we analyzed the mortality from predation against the prior year’s densities. As Roland and Myers (1987) described in their evaluation of a delayed density effect, we plotted the residuals of the fit model of the mortality from predation against the same year’s density estimates plotted against the prior year’s density.

We used a logistic GLM to compare the mortality from predation of winter moth pupae parasitized by *C. albicans* compared with unparasitized winter moth pupae. We ran the analysis first using the annual cumulative mortality experienced by both pupae type just over the winter moth pupal season (June to October) and then including mortality of the *C. albicans* puparia deployed over the winter. We also compared predation rates of winter moth pupae in sites with or without *C. albicans* establishment and across a *C. albicans* parasitism gradient. To compare the relative mortality of the predator exclusion treatments, we used a logistic regression with deployment and treatment as fixed effects. Last, we tested for a correlation between predation rates and the abundance of carabid beetles, staphylinid beetles, beetle larvae, and small mammals at each site.

**RESULTS**

**Winter moth pupal mortality**

In total, 14,500 winter moth pupae were deployed, and 13,525 burlap squares were successfully retrieved after their deployment period and evaluated (Table 1). For *C. albicans* puparia, 3700 were deployed and 3507 retrieved. Predation accounted for most of the pupal mortality (Figure 2). There was no consistent increase or decrease in pupal predation from 2005 to the end of the study in 2017; however, the rate of pupal predation varied across years (pseudo$R^2 = 0.54$, $df = 38$, $p = 0.011$), with rates in 2013 and 2017 significantly lower than 2005 (Appendix S1: Figure S3 A; $p = 0.011$ and $p = 0.031$, respectively). There were no significant effects by site (Appendix S1: Figure S3B; $p > 0.29$). The background densities of winter moth varied significantly ($p = 0.0066$; $F_{3,26} = 4.1564$) over the study period (Figure 2) with significantly low winter moth densities in the last year of study when *C. albicans* had established across all sites. Parasitism by pupal parasitoids was significantly lower than predation ($p = 0.0001$) but ranged from ~20% to 40% of mortality on pupae left behind after predation; parasitism by *Pimpla* wasps was most notable. Predation rates showed some seasonality across the winter moth pupal period (June to October); there was a significant effect of Julian day (January 1 as day 1, Appendix S1: Figure S4; pseudo$R^2 = 0.37$, $df = 128$, $p < 0.0001$), with year and site effects ($p < 0.0001$ and $p = 0.001$, respectively). Peak mortality from predation occurred in the third week of August with an average of 50% of pupae lost to predation every 31 days.

There was no effect of deployment method (deploying the pupae on burlap squares versus tethering the cocoons using a wire tag) on pupal mortality; an equal number of pupae were depredated (58% of 60 pupae) from each deployment method. Therefore, we can compare across
studies that have deployed pupae using these different methods. However, we do not claim that our experimental predation rates are equal to the rates on naturally occurring pupae. Instead, these rates are an experimental test of predation rate that are comparable across treatments, years, sites, and studies.

Density dependence of winter moth pupal mortality

Winter moth pupa density had a significant effect on mortality from predation ($\text{pseudo} R^2 = 0.50$, $df = 24$, $p < 0.018$) with a significant effect of year ($p = 0.004$) and site ($p = 0.026$; Figure 3). There was no evidence of delayed density dependence when regressing the current year’s rate of predation to the prior year’s winter moth density (Appendix S1: Figure S5A; $p = 0.63$) nor by regressing the residuals of the model of the current year’s predation correlated with the current year’s density of pupae against the prior year’s winter moth density (Appendix S1: Figure S5B; $p = 0.81$ and $p = 0.10$ for the model with year and site effects, respectively).

Comparative mortality on winter moth pupae versus \textit{C. albicans} puparia

Predation rates on \textit{C. albicans} puparia were similar to those of winter moth pupae when compared over the
same interval (winter moth’s pupal stage, June to October) or when including the additional mortality *Cyzenis albicans* puparia experienced overwintering (June to April; Appendix S1: Figure S6; pseudo-$R^2 = 0.67$, $df = 19$, $p = 0.32$). Although not significantly different, the mean mortality of *C. albicans* puparia was slightly lower than that of winter moth pupae. However, restocking puparia for each new deployment interval may have increased the estimate of predation.

**Effect of *C. albicans* presence on winter moth pupal mortality**

*Cyzenis albicans* established in some sites earlier than others (e.g., as early as 2010 for Wellesley, MA [site D] or as late as 2014 for Hanson, MA [sites A and B]). We compared predation rates on winter moth pupae as a function of winter moth density and *C. albicans* establishment status (sites with or without *C. albicans*). The effect of *C. albicans* establishment was significant, with lower rates of predation after *C. albicans* establishment (Figure 4; $p = 0.012$). Simultaneously, pupal predation was also only significantly related to density after *C. albicans* establishment (Figure 4; pseudo-$R^2 = 0.38$, $df = 33$, $p = 0.0027$). No correlation was detected between predation rates and percentage *C. albicans* parasitism (Appendix S1: Figure S7; pseudo-$R^2 = 0.29$; $df = 36$; $p = 0.90$ and pseudo-$R^2 = 0.32$; $df = 31$; $p = 0.25$ for estimates using pupae and larval collections, respectively). We converted the cumulative mortality to a $k$-value ($k_{pred}$) and plotted that against winter moth density (log pupae/m²) as has been done by others (East, 1974; Roland, 1994; Varley et al., 1973); the slope of that relationship was 0.27 (Appendix S1: Figure S8, $R^2 = 0.28$).

**Predator exclusion and community experiments**

There was a significant effect of predator exclusion cage mesh size treatment (Figure 5; pseudo-$R^2 = 0.48$; $df = 41$; $p < 0.0001$) with decline in predation rates on the pupae with decreased mesh size. To a lesser extent, deployment date ($p = 0.0017$) was also significant but it may be that the act of replacing the pupae midway through the study period may have affected the results. No significant effect ($p > 0.41$) was found when regressing predation rates against the abundance of each taxonomic group of predators (Appendix S1: Figure S9).

**DISCUSSION**

In this study, we evaluated the role and interaction of contemporaneous mortality factors acting on the pupal stage of winter moth that together maintain the population densities of invasive winter moth in Massachusetts at a

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**FIGURE 4**  Total season-long mortality from predation (as a proportion ranging from 0 to 1) of winter moth pupae prior to *C. albicans* establishment compared with *C. albicans* establishment regressed against the corresponding pupal density. Pupal density had a significant effect on the resulting predation with *C. albicans* establishment (black circles, black trendline), but was not significant prior to *C. albicans* establishment (open circles). Cumulative mortality of all sites and years with data (please refer to Table 1) is shown.

**FIGURE 5**  Proportion of pupae that were preyed upon across predator exclusion cage treatments with different mesh sizes. The images show the likely predators (staphylinid, beetle larvae, carabid, and small mammals) for each mesh size. Proportion data (y-axis) had a maximum range of 0 to 1. Error bars show standard errors and letters indicate post hoc multiple comparisons.
stable and reduced level. The two focal mortality factors are generalist pupal predators and the introduced, specialist biological control agent, *C. albicans*. The results from this study suggest that in the Northeast USA, the introduction of *C. albicans* suppresses populations of the invasive winter moth to densities that can then be maintained by generalist predators and parasitoids. Only after *C. albicans* was established did we detect a positive (or direct) density-dependent relationship between winter moth density and mortality from predation (Figure 4). Temporal and spatial density dependence in pupal predation rates were detected and effects were strongest in the most recent year of study (Figure 3), when the population of winter moth was at its lowest for the study years since long-term life-table studies in Massachusetts commenced in 2004 (Elkinton et al., 2021). These findings lend support to the earlier hypothesis (Roland, 1990; Roland & Embree, 1995) that the introduction of *C. albicans* suppresses the winter moth population to a density that can be maintained by native, generalist natural enemies.

The underlying biology of specialist and generalist natural enemies can render each capable of contributing strong, but different, top-down forces on their prey (Hassel & May, 1973; Murphy et al. 2018, Varley et al., 1994, Vidal & Murphy, 2018). Generalist predators can inflict the strongest top-down control on a prey species when the population is at a low to medium abundance (Campbell & Sloan, 1978). At low population levels, this background mortality from predation may be enough to maintain the population below an outbreak threshold (Elkinton et al., 1996). When the prey population is at high densities, the generalist predator numerical or functional response may be insufficient to maintain density-dependent mortality. In this case, the generalist predators are considered saturated by the food abundance (prey density) and further increases in food availability may cause predation rates to decline (Figure 1). In contrast, specialists generally thrive when prey populations are at high densities; the tight linkage of specialist natural enemies to their prey allows the specialists to mount a strong numerical response to the prey population density (Murdoch, 1994; Snyder & Ives, 2003).

This trend was detected in the long-term population studies conducted on winter moth in Nova Scotia (Embree, 1965) and in British Columbia (Roland, 1994); mortality in the pupal stage did not govern the survivorship between generations until *C. albicans* was established. We note, however, a very important difference between our predation rates, which declined and became density dependent following the establishment of *C. albicans*, in contrast with the predation rates reported by Roland (1994), which increased in Nova Scotia (Oak Hill) and in British Columbia (Mt. Tolmie) and became density dependent after the population decline of winter moth associated with the introduction of *C. albicans*. A likely explanation for this difference is that the predator community at the sites in Nova Scotia and British Columbia may be less robust than at our sites in Massachusetts such that the predators were well above the saturation threshold when winter moth was at outbreak densities. In contrast, at our sites in Massachusetts, predation rates were likely to be at peak values close to, but not above, the saturation threshold. Indeed, when compared with other invasive or outbreaking populations of winter moth at or above a predator saturation threshold (please refer to data presented in Appendix S1: Figure S1), the winter moth population densities recorded in Massachusetts before 2015 were similar. However, after *C. albicans* was established, they dropped to densities comparable with the densities in England, where winter moth is native and not a serious pest. The overall comparatively high rates of predation we reported, even before *C. albicans* was introduced, may also explain why winter moth populations had declined even with modest levels of parasitism (10%–30%) reported in Elkinton et al. (2021) compared with the higher levels of parasitism reported in Nova Scotia and British Columbia. Notably, the rates of *C. albicans* parasitism now in Massachusetts are similar to those in the endemic, low-density winter moth populations in Europe (Varley et al., 1973). Winter moth densities were lowest in the final year of the study (Figure 2) when density dependence was the strongest (Figure 3).

When regressing *k*<sub>pred</sub> against log pupae/m<sup>2</sup> to compare the strength of the density-dependent relationship to that found in early studies in England and in British Columbia, we found that the strength of this relationship (0.27 from our study) was similar or slightly stronger in England (0.27; East, 1974 and 0.35; Varley et al., 1973) where winter moth and *C. albicans* are native. This finding again suggests that now that *C. albicans* has established in Massachusetts, the population dynamics are now similar to those of winter moth in its native range. In contrast, the slope of this relationship was much stronger, close to compensatory, in British Columbia after the establishment of *C. albicans* there (0.87; Roland, 1994). This suggests that the dynamics experienced by the population of winter moth in Massachusetts, with a lesser strength density dependence, is predicted to take longer (more generations) to stabilize at equilibrium (Varley et al., 1973). In fact, it has taken over a decade for winter moth populations to decline and stabilize in Massachusetts (Figure 2; Elkinton et al. 2021). However, it should be noted that to determine definitely whether pupal predation on winter moth causes a low-density equilibrium, one would need to analyze both the fecundity and mortality of other life stages.
(e.g., overwintering mortality and larval mortality) to complete a full life-table analysis. We have collected these data at each of these study sites and will explore this question in a follow-up manuscript.

Predation was by far the main source of winter moth pupal mortality, resulting in a loss of almost 90% of deployed pupae (Figure 2). The rates of pupal mortality from predators were overall similar to those found in prior studies in British Columbia (Horgan et al., 1999; Horgan & Myers, 2004; Roland, 1998) and Nova Scotia (Macphee et al., 1988; Pearsall & Walde, 1994), but only for the lower density years following C. albicans establishment at these sites (Roland, 1994). The predation rates we recorded were similar to those reported from western Europe (Hansen et al., 2009; Heisswolf et al., 2010; Varley et al., 1973), but were higher than some other estimates of pupal mortality in northern Europe from outbreak winter moth populations or high elevation sites (Klemola et al., 2009, 2014; Pepi et al., 2017). This further supports that idea depicted in Figure 1 that, at high densities, the predator saturation point can be surpassed resulting in relatively low predation rates. Although we tested for a numerical response by predators by looking for evidence of delayed density dependence, no lagged effect was detected.

Similar to Roland (1990), we found mortality by native pupal parasitoids to be the next highest mortality factor for winter moth pupae after predation (Figure 2). Although Roland (1990) did not identify the native pupal parasitoid wasp or wasps, we identified the wasps in this study as Pimpla spp., including P. aequalis and another closely related Pimpla sp., as was reported earlier in Broadley et al. (2019). We do not have data on parasitism by Pimpla wasps in 2013 because we only learned to recognize them as a component of pupal mortality in 2014. From this study, we do not have enough data to determine whether these wasps are increasing in density in response to the presence of winter moth. From a prior study (Broadley et al., 2019), it was found that, in areas where winter moth is present, the wasps showed positive density dependence but simultaneously they also showed very high densities in sites far outside the current range of the winter moth. Nonetheless, we can consider these wasps another generalist mortality source along with the pupal predators. Similarly, recruitment of native parasitoids was noted by Vindstad et al. (2013) on geometrid populations in northern Norway as their range expanded northward. Also, similar to previous findings with winter moth (Cunningham et al., 1981; Donahue et al., 2018; Pearsall & Walde, 1994; Roland & Embree, 1995), fungal or viral mortality accounted for a very small portion of mortality in our study.

Considering Roland’s second hypothesis, we did not find significant differences in the predation rate between unparasitized versus parasitized pupae (Appendix S1: Figure S6). We found no significant difference when comparing the two pupal types for the duration of the winter moth pupal period (June–October), nor when we included the additional mortality C. albicans experiences over the winter as a pupa. We did not restock puparia deployed over the winter as we had done with our other estimates and acknowledged that this may have shifted resulting estimates. Other studies have found that C. albicans puparia suffered lower mortality than winter moth pupae (Horgan & Myers, 2004; Roland, 1990), others found no difference (Hassell, 1969), and still others reported that C. albicans puparia experienced higher mortality rates (Horgan & Myers, 2004). A choice experiment in a laboratory setting in which predators are given parasitized and unparasitized pupae would help to provide an answer. Even if a difference in predation rates on these two types of pupae existed, it is not at all clear what impact, if any, there would be on the predation rates of unparasitized pupae. We found little additional mortality to C. albicans puparia during the late fall, winter, and early spring when cold temperatures probably limit predation by invertebrates (hypothesis 3). These low rates of mortality contrast with the predictions made by Hassell (1969) but align with predictions made by Horgan and Myers (2004).

There has been much debate in the literature about which predators are most important in applying top-down mortality on winter moth populations (e.g., East, 1974; Frank, 1967; Horgan & Myers, 2004; Roland, 1990), but likely predator communities vary greatly across the range of winter moth and play different roles depending on the host density, environment, and season. The relative role of each predator must be assessed for each region. The results of our predator exclusion study and pitfall traps show that both the mammalian and invertebrate component of the predator community each inflict substantial predation on the pupae, and they do so in an almost even proportion (Figure 5). This contrasts with what was found in British Columbia (Roland, 1990), where the smallest group of predators (predators between 0.5 and 1.5 mm) appeared to be the dominator predator guild. Here in the Northeast USA, it appears that both predator guilds contribute to the top-down regulation of winter moth densities; having both size factions of predators working together probably make this community of predators more influential than that acting on the British Columbia population of winter moth, as was noted above. Furthermore, the fluctuation in density of individual predator species, but consistent mortality caused by all components of the predatory community, obscures any obvious association between densities of particular taxa and predation rates.
Overall, the findings from this study lend support to the hypothesis that pupal predators have a regulatory effect on winter moth populations, but only now that the population has been reduced, presumably by the introduction of the host-specific parasitoid *C. albicans*. This research provides evidence for classical (or importation) biological control of specialized natural enemies working in tandem with native natural enemies to stabilize an outbreaking invasive population. These findings are important for understanding population dynamics, particularly what regulates insect outbreaks, and highlight the combined importance of specialist parasitoids and generalist predators as additive drivers of insect population dynamics that together regulate population outbreaks.

**AUTHOR CONTRIBUTIONS**

HJB, JSE, GHB, and BS planned and carried out the experiments. HJB and BS processed the samples. HJB performed the analysis, made the figures, and wrote the manuscript with input from all authors.

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**CONFLICT OF INTEREST**

The authors certify that they have no conflict of interest in the subject matter or materials discussed in this manuscript.

**DATA AVAILABILITY STATEMENT**

Data (Broadley et al., 2022a) are available in Dryad at https://doi.org/10.5061/dryad.1g1jwstrt. Analysis code (Broadley et al., 2022b) and metadata (Broadley et al., 2022c) are provided in Zenodo at https://doi.org/10.5281/zenodo.5219083 and https://doi.org/10.5281/zenodo.5219085, respectively. Code used for this manuscript is not novel and is all drawn from existing functions and packages.

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**REFERENCES**


SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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