Winter Moth Biological Control Report 2018

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March 2018

The winter moth (*Operophtera brumata* L, Geometridae: Lepidoptera), a leaf-feeding inchworm caterpillar native to Europe, invaded eastern Massachusetts a little over a decade ago and is causing widespread defoliation (Elkinton et al. 2010, 2015, Fig. 1). The winter moth has continued to spread west and south across Massachusetts and Rhode Island (Elkinton et al. 2014). Outbreak populations occurred in 2012 for the first time in SE Connecticut and other populations have cropped up in SW Connecticut. Also in 2012, defoliation by winter moth occurred for the first time in coastal Maine (Elkinton et al. 2015).

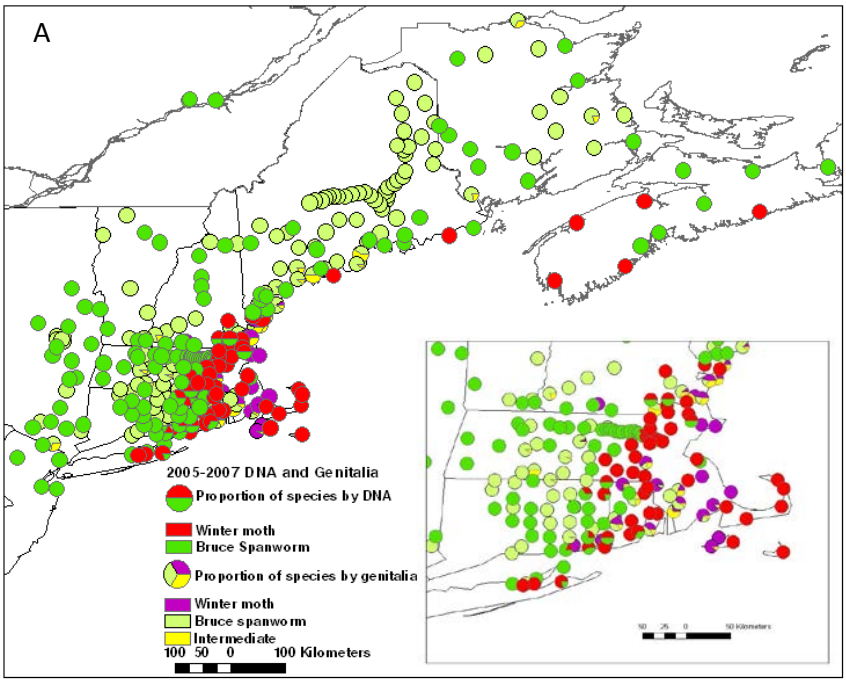

Fig. 1. **Distribution of winter moth and Bruce spanworm in pheromone-baited traps in northeastern North America in 2005-2007**. Unfortunately, winter moths use the same pheromone compound as the native species Bruce spanworm, O*perophtera bruceata.* Adult males of the two species are difficult to distinguish, especially the battered specimens we recover from pheromone traps. Identification of moths is based on male genitalia and the DNA sequence of the COI mitochondrial gene. Winter moth has been in Nova Scotia since the 1930s, but we think it was confined there by the cold winter temperatures in New Brunswick, which prevented spread to the rest of North America until now. (Reprinted from Elkinton et al. 2010, Ann. Ent. Soc. Amer. 108:135-145).

Prior to the current invasion by winter moth to Massachusetts, there had been three previous invasions to North America—to Nova Scotia prior to 1950 and to Oregon and British Columbia in the 1970s. All three prior invasions have been suppressed by the introduction of parasitoids from Europe, in particular the tachinid fly *Cyzenis albicans*, and low-density populations of winter moth now persist indefinitely in these regions, similar to those that exist in most of Europe (Roland and Embree 1995). In Nova Scotia *C. albicans* was introduced in 1954 along with another parasitoid *Agrypon flaveolatum* in

1956. For several years there were no recoveries of these parasitoids, but then in 1959 parasitism by C albicans rose to 10%, then 40% and 60% in 1961 (Fig. 2). *Agrypon flaveolatum* followed along two years later. Winter moth densities collapsed in 1962 (Fig. 2) and have remained in low, non-pest status on forest trees ever since (Embree 1965, 1966, Roland and Embree 1995). This was a famous biological control success, and one of very few applied to pests of forest trees anywhere in the world. It was obvious we should try this in New England.

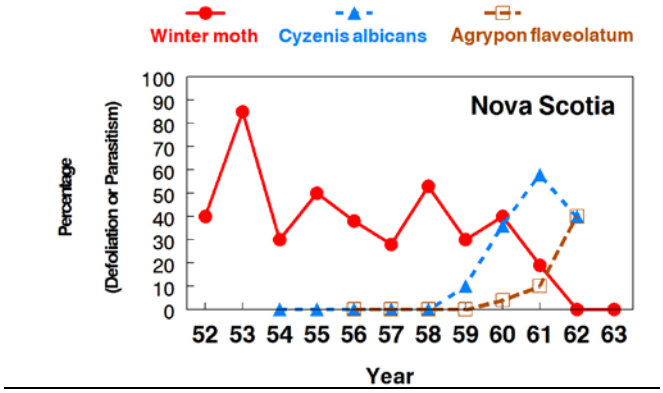

Fig. 2) Defoliation by winter moth and % parasitism by *C. albicans* or *Agrypon flaveolatum* in Nova Scotia in the 1950s following parasitoid release in 1954 (adapted from Embree 1965).

We began our biological control introductions in 2005. . We focused on *C. albicans* and not *A. flaveolatum*, because the latter species is thought to be a generalist attacking the larvae of various moth species. Furthermore, its taxonomic status is uncertain. We have introduced more than 80,000 *C. albicans* distributed across 44 sites in eastern Massachusetts, Rhode Island, Connecticut and Maine (Fig. 3), and so far have established the fly at 32 of those sites. As was seen in Nova Scotia (Fig. 2), it typically takes 3 to 5 years before we recover any *C. albicans* at our release sites. Since there is only one generation per year of both the fly and the winter moth, it takes several years for the 1500-2000 flies we release at a site to catch up with the millions of winter moths that exist at that site. We have now recovered the fly at all 17 of the sites where we released prior to 2012 (Fig. 3) and at 21 of 22 release sites in Massachusetts. At several of those sites, we only documented establishment of *C. albicans* for the first time in 2016. So we expect it will soon be established at all or most of the 44 release sites.

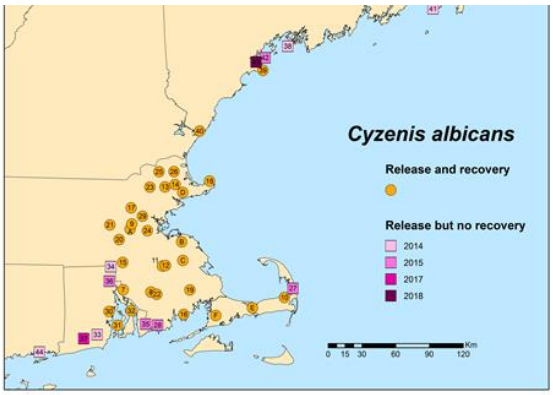

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Fig. 3. *Cyzenis albicans* release and recovery locations in New England 2005-2018.

Prior to 2015, we collected *C. albicans* for release in New England from sites on Vancouver Island in British Columbia, where it had been established in an invasive population of winter moths in the 1970s. Starting in 2015, however, we switched our collection efforts to Wellesley, Massachusetts, given that we have documented high levels of parasitism all across that town and in surrounding towns. Parasitism there is comparable to what we encountered in British Columbia, and it is considerably simpler and cheaper to collect from Massachusetts than from British Columbia. Furthermore, the flies we collect in Wellesley are presumably better adapted to New England climatic conditions, in contrast to those from Vancouver Island, British Columbia, where, for example, it rarely freezes in winter. We collect the fly by collecting late-instar winter moth larvae at sites where parasitism by *C. albicans* is high. We then rear the larvae to the pupal stage. *Cyzenis albicans* pupates inside the winter moth pupae. We rear the fly puparia over the winter and release the adult flies the following spring.

In 2016, we implemented an approach that we had pilot-tested in 2015 successfully, which was to rear the fly puparia in peat moss inside predator-proof boxes that we partially buried in the ground outside. Here the puparia were subject to the same conditions that they would experience in nature. We obtained better fly survival this way than holding them in a laboratory growth chamber, where they tended to dry out even if we attended to them carefully. In addition, the flies can now choose their own appropriate time to emerge from these cages. In previous years, only a fraction of the growth-chamber reared fly puparia successfully emerged as adults in the spring and only some of them lived long enough to lay eggs. In spring 2016, we released 3000 of these over-wintered flies at one site in Arlington, Massachusetts using our new rearing technique (Fig. 2) In 2017 we recovered 3.4% parasitism at this site, the year following release. This result was unprecedented. We have never had such rapid recovery of flies. It strongly suggests that our new technique is a big improvement.

Each year we try to collect 500 late instar winter moth larvae from each of the now 44 previous release sites. These are reared to the pupal stage and dissected in mid-summer in order to document establishment of *C. albicans* and to measure percent parasitism. Collecting samples from each of these sites becomes more and more expensive and challenging with each successive year. Just a few years ago we had six release sites to follow. With 44 sites spread from southeast Connecticut to mid-coastal Maine, sampling requires a much bigger effort and a much bigger crew. To accomplish this task, we have enlisted the help of volunteers to help us collect. In 2016, we had a total of 10 crews and 38 people assembled to collect the parasitized winter moth larvae at 115 sites across four states (60 + sites near Wellesley, MA; see Fig. 5). The collections must be accomplished within a two week period in mid-May; it is a major logistical undertaking. We have perfected the technique of providing each crew with the rearing materials and instructions for how to collect and rear. This is the only way that we can collect the numbers we need in so short a time. In 2016, we collected more than 76,000 winter moth larvae and reared the vast majority of them to the pupal stage. To document percent parasitism, we dissected all pupae to determine what fraction of them had *C. albicans* inside them.

Parasitism at the central release site in Wellesley has fluctuated between 15 and 40% over the past 6 years (Fig. 4). In 2017, both defoliation and the densities of winter moth pupae remained low at that site Fig. 4). This suggests we have now converted winter moth into a non-pest in the areas where *C. albicans* is established. Higher levels of parasitism are seen at all of the other older release sites (Fig. 4). But all this takes time. We suspect that regional densities of winter moth in New England are much higher than any that occurred in Nova Scotia or British Columbia, because the stands of susceptible trees in those regions consisted of much smaller blocks surrounded by non-host conifers.

At each of our earlier *Cyzenis* release sites, and at other permanent non-release sites, we continued to monitor year-to-year changes in winter moth density. We have collected long-term data on density of winter moth life stages (adult females, fecundity, early instar larvae, late instar larvae and pupae) and percent defoliation at selected sample trees (red oaks and red maples). In addition, we have been collecting and rearing many thousands of larvae to look for parasitism and disease. Our intent is to determine the cause of year-to-year changes in population density of winter moths and to document the onset of parasitism by *C. albicans* at the release sites. Levels of parasitism at our older release sites had increased markedly this year compared to last (Fig. 4). Parasitism at Hingham, Wenham,and Hanson MA now exceed 25% (Fig. 4), which is the level at which we started to see effects on winter moth density in Wellesley over the past six years(Fig. 3, 4). At other sites, parasitism is around 10%, but the trajectory of parasitism is upwards at all sites (Fig. 4).

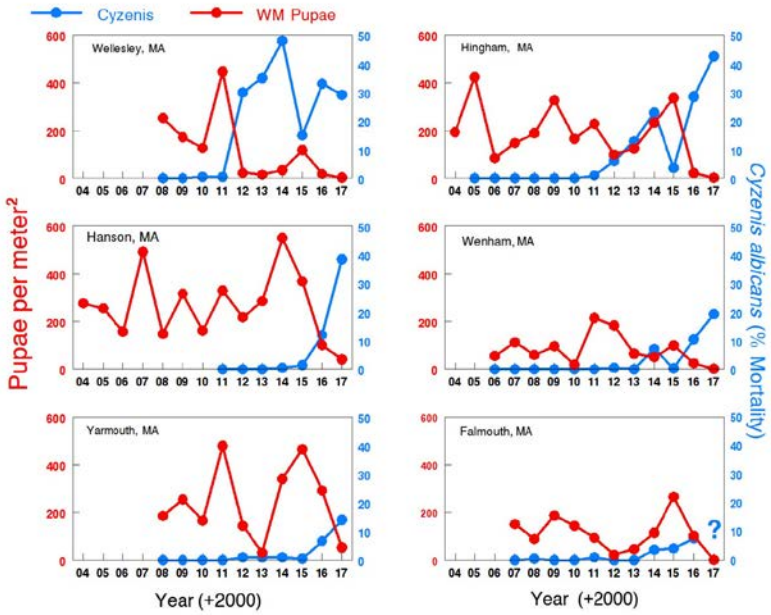

Fig 4. Density of winter moth pupae (red) ,years of release (blue) and percent parasitism by *C. albicans* at six widely spaced release sites in Massachusetts.

In 2014 we showed that high levels of parasitism approaching 40% occurred all across the town of Wellesley, over an area about 2 miles or 3 km in diameter. In 2015 we launched an ambitious effort to document the spread of *C. albicans* along transects extending in six directions over 10 km from Wellesley (Fig. 5). We collected up to 500 larvae at 60 points along these transects (Fig. 5A) totaling more than 25,000 larvae. In 2015 we found larvae infested with *C. albicans* at various locations up to 8 km away along these transects (Fig. 5A). Levels of parasitism fell way off, however, beyond 3 km. In 2016 we sampled these transect points again Fig 5B). In one year *C. albicans* had spread noticeably further than in 2015, extending to the end of five of the six 10 km transects. We estimated that the fly is spreading at about 3 km per year. *Cyzenis albicans* has now spread over most of the western suburbs of Boston, including all sites where winter moth densities are at outbreak levels.

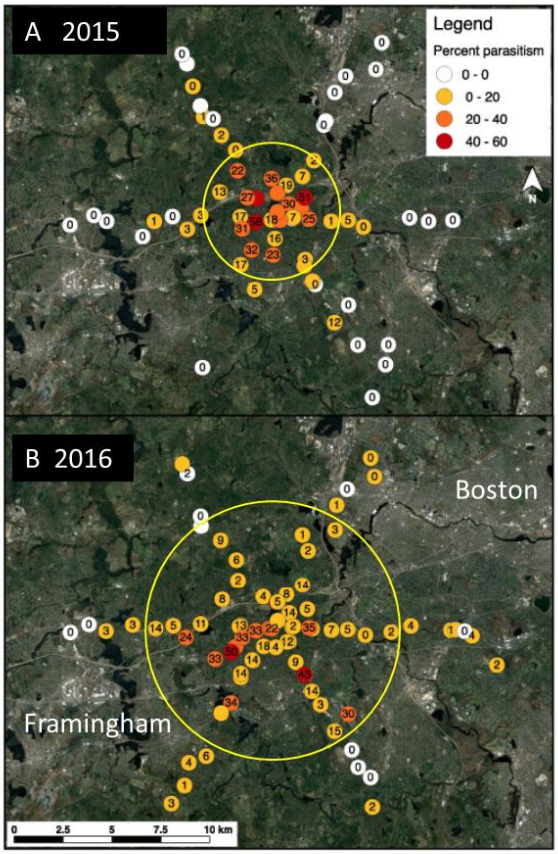

Fig. 5. Percent parasitism (yellow numbers) by *Cyzenis albicans* along transects extending 10 km in six directions from the town of Wellesley in the western suburbs of Boston in A) 2015 and B) 2016. White symbols indicate no parasitism. The yellow circles indicate the mean of the wintermoth distribution indicating a rate of spread of about 3 km a year..

Some years ago Jens Roland (1988, 1990, Roland and Embree 1995) published an intriguing analysis of the successful biological control of winter moth in Canada by *C. albicans*. He claimed that the reduction in winter moth density associated with *C. albicans* parasitism was caused mainly by pupal mortality due to predators and not the direct effect of *C. albicans* parasitism. According to his analysis, this pattern was true both in Nova Scotia and on Vancouver Island. He proposed that the impact of *C. albicans* parasitism was enhanced by the action of soil predators in the form of predatory beetles. He hypothesized that these generalist predators are able to regulate winter moth densities only when *C. albicans* has reduced those densities to a manageable level. Our data from the release site in Wellesley lends support to this idea (Fig. 4). Winter moth densities at this site have declined by 95% over the past six years (Fig. ,4), whereas parasitism by *C. albicans* has varied between 15% and 48 % over the same time interval (Figs. 3,4). That means that parasitism alone cannot account for this large drop in density.

A graduate student in our lab, Hannah Broadley has embarked on predation experiments involving deployment of winter moth pupae at this and other sites to see if Roland's ideas are correct. She has documented high levels of predation by invertebrate and vertebrate predators at these sites. There is a very large community of predatory beetles active at or near the soil surface at these sites. For example, one of our lab technicians identified 29 species of carabid beetles in pitfall traps at these sites.

In addition, Hannah has recovered an ichneumonid pupal parasitoid in the genus *Pimpla* causing high levels of parasitism in winter moth pupae. She has shown that it has multiple generations each year, so the mortality it causes accumulates steadily over the summer and fall. We suspect it may have been the cause of the high pupal mortality of winter moth documented by Jens Roland in Nova Scotia and evidently in our study plots in Wellesley. We have a species ID by one of the world's leading ichneumonid taxonomists, but Hannah's DNA sequences of the CO1 barcoding gene from this species do not match that of the ID, We believe it is an undescribed species. We are pursuing this question with our taxonomic collaborators.

Hannah Broadley's research, and that of Jens Roland, illustrate why it is important to understand the impact of *C. albicans* in the context of all the other causes of mortality occurring in the winter moth system. It is vital that we try to quantify and explain the other factors influencing winter moth densities, in addition to *C. albicans*. This is rarely accomplished in most biological control projects. In the past two years, we and our collaborators published our research on larval mortality of winter moth (Pepi et al. 2016), egg hatch as a function of temperature (Hibbard and Elkinton 2015), hybridization between winter moth and Bruce spanworm (Havill et al. 2016), pathogens of winter moth and Bruce spanworm (Broadley et al. 2016) and the population genetics of winter moth in Europe (Andersen et al 2017). In the coming year, we expect to publish additional papers on the causes of pupal mortality of winter moth and the population dynamics of outbreak winter moth populations. But in the meantime, we believe we have already converted winter moth to non-pest status and hope to continue documenting that fact.

Acknowledgements

We are grateful USDA Forest Service and USDA APHIS for funding and to Richard Reardon, Ron Weeks and Kate Aitkenhead of who helped us obtain funding from these agencies. We are grateful to the many cooperators or student workers who helped us collect and release *C. albicans* and/or samples of winter moths as part of this project.

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