

## Survey for winter moth, *Operophtera brumata* in northeastern United States with pheromone-baited traps, December 2005

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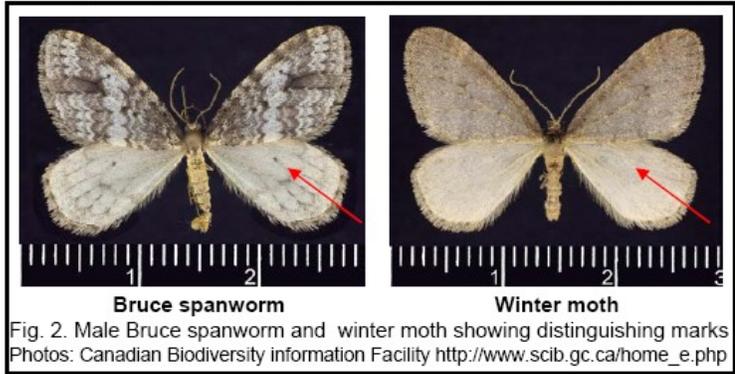
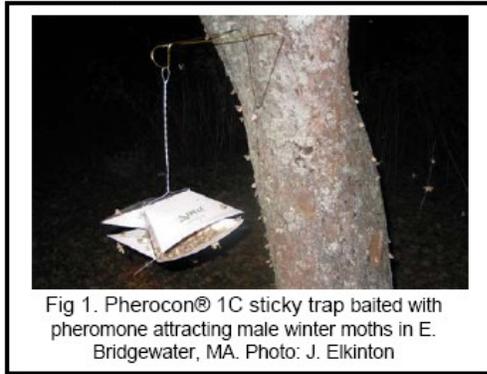
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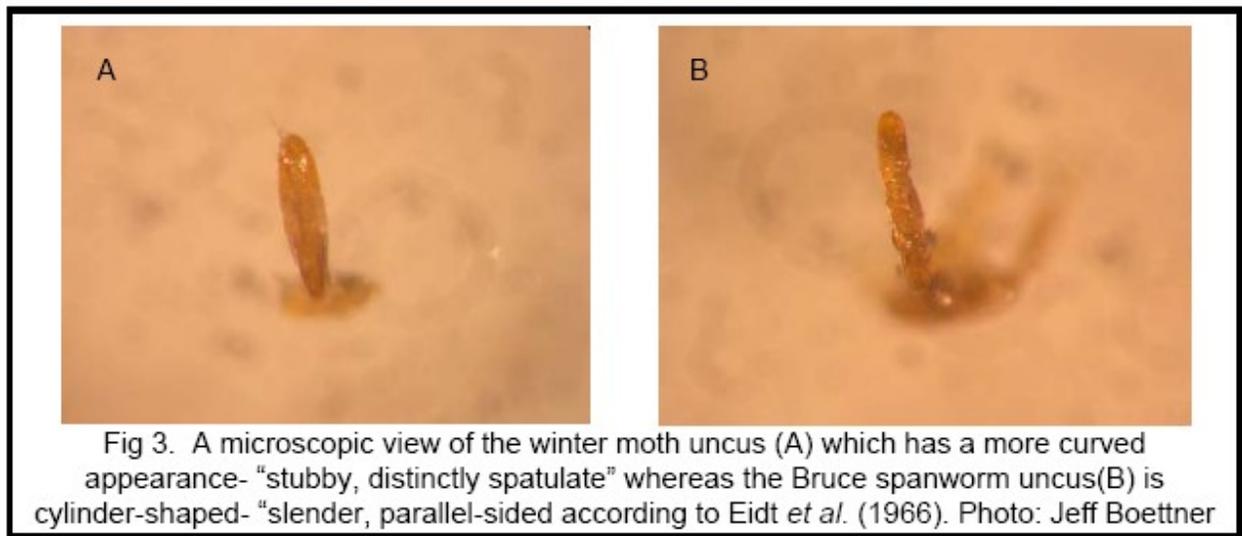
In December 2005, a team of cooperators in seven northeastern states (Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Rhode Island) deployed a grid of pheromone-baited sticky traps (Fig. 1) to survey for winter moth, *Operophtera brumata*, an invasive species from Europe that has been defoliating deciduous trees in eastern Massachusetts for the past several years. Winter moth adult moths emerge (only the males have wings and are capable of flying) in November and December, which is why they are called winter moths. The team used traps baited with sex pheromones to survey for invasive species such as winter moth, because these traps attract male moths even at very low population densities. It is by far the most sensitive available tool to detect incipient populations of invasive species. Unfortunately the winter moth pheromone (Roelofs *et al.* 1982) appears to consist of a single compound that is identical to that used by the Bruce spanworm, *Operophtera bruceata*, a native North American species that is very closely related to winter moth. Thus, traps baited with the pheromone will capture both species. In the 2005 winter moth survey, we included an inhibitory compound with the pheromone, which research by Underhill *et al.* (1978) indicated would suppress attraction of Bruce spanworm, but not winter moth. This did not work however, because the traps filled up with both species.

The problem then became distinguishing the male moths of the two species, which are very similar. Characters described by Troubridge and Fitzpatrick (1993) for distinguishing the two species, such as a black dot on the hindwing of the Bruce spanworm (Fig 2), are not reliable (Dave Wagner, Entomologist, personal communication) and are usually obscured on specimens that have been captured in sticky traps. Dissection of the male genitalia provides a means to differentiate the two species (Eidt *et al.* 1966, Troubridge and Fitzpatrick 1993).



**Distinguishing winter moths from Bruce spanworm based on male genitalia**

Two papers published provide illustrations and descriptions of the genitalia of winter moth and Bruce spanworm (Eidt *et al.* 1966, Troubridge and Fitzpatrick 1993). After dissecting over a thousand potential winter and Bruce spanworm moths from the New England states, Jeff Boettner (Elkinton lab-UMass, Amherst) has found the distinction between the two species to be less clear cut than the published illustrations. He examined the “uncus” for three characters: (1) general shape [see photos below] (2) measurement at the tip of the uncus and [3] measurement at the widest point of the uncus. The photos of the winter moth and Bruce spanworm uncae represent the extreme ends of the spectrum where it is easy to distinguish the two species by shape. Boettner found that many moths had uncae that were intermediate between the two shown below and it was impossible to determine the species



**The observed distribution of winter moths:**

We determined, based on male genitalia, that we trapped winter moths at sites that stretched from the eastern corner of Connecticut, through all of Rhode Island, eastern Massachusetts, coastal New Hampshire, and southern coastal Maine (Fig. 4). We caught winter

moths in areas that were at least 60 mile from any areas known to be defoliated by winter moths. The areas shaded in blue in Fig. 4. indicate towns where we observed defoliation by winter moth in May and June 2005 based on a ground survey conducted by J. Elkinton's crew. Traps further west and north caught exclusively Bruce spanworm. These include other traps not shown on the map placed in New Jersey and then Hudson valley in New York. The traps from Maine also caught mostly Bruce spanworm, which confirms our expectation that the winter moth population in Massachusetts represents a new introduction as opposed to a population that spread from Nova Scotia via Maine. On the other hand, we did catch a quite few 'intermediates' from many sites in Maine and one winter moth at a site near Acadia National Park. Notably one of the main ferry terminals from Nova Scotia is not far from this site. It is quite possible that many of the winter moths we captured originated from sites many miles from where we caught them in traps. In other pheromone trap surveys, such as that done with gypsy moth, males sometimes fly 100 miles or more before capture, particularly if they find themselves over bodies of water. The large number of intermediates in our samples and the overall subjective nature of the identification caused us to seek a more definitive ID based on DNA analysis.

### **Possible hybridization**

The traps with intermediate uncae were particularly common in the regions immediately surrounding the areas of known winter moth infestation (Fig. 4). This pattern suggests that the intermediates might be hybrids between the two species. In examining trap catches from traps baited with the winter moth Bruce spanworm pheromone, Pivnick *et al.* observed intermediate genitalia in 13% (6 out of 46) of the moths at Mill Bay, British Columbia, Canada and 24% (6 out of 25) of the moths at Goldstream, British Columbia, Canada (1988). The published literature does not give a clear description of the intermediate genitalia. Smith and Ring (unpublished) as cited in Underhill *et al.* (1987) have bred winter moth females with Bruce spanworm males in caged experiments and produced viable progeny to the F2 generation. More research is needed to determine if hybridization is occurring in New England, and whether the hybrids will persist or will allow gene flow between the two species.

### **DNA analysis for a definitive identification:**

DNA analysis will provide the best means to differentiate between winter moth and Bruce spanworm and to determine if the two species have hybridized. DNA technology makes it feasible to extract, amplify and sequence DNA for specific genes from a single specimen. Adam Porter at then University of Massachusetts, Amherst, is collaborating with Joe Elkinton on this project and has sequenced a portion of the COI gene from the mitochondrial DNA of six Bruce spanworm and seven winter moth specimens from Massachusetts. Mitochondrial DNA is contributed by the mother; therefore, winter moth COI gene data shows that the specimen is either a winter moth or a hybrid whose maternal line extends back to winter moth. The COI gene sequence demonstrates very clear differences between the winter moth and Bruce spanworm mitochondria. The figure below shows locations in a small portion of the COI gene where the Bruce spanworm sequence (highlighted in pink) is different from the winter moth sequence (highlighted in green). The sequence for the 7 winter moths was identical for all 685 base pairs analyzed and differed by about 7.4% from Bruce spanworm. The entire 685 sequence was identical for all seven winter moths, whereas there was some variation among the Bruce spanworm, as indicated by the blue arrow in upper left corner of the figure. The lack of variation in winter moth (if confirmed by future

analyses) may indicate founder effect or genetic bottleneck: reduced variation in a population that arises from an initial invasion of one or very few individuals.

These DNA sequences show that DNA analysis is without doubt a clear-cut way to distinguish these two moth species. These 13 specimens confirm that our ID based on genitalia is basically reliable at the extremes of the genitalia size distribution. It also shows that we have recovered winter moths or winter moth hybrids from near Worcester, Massachusetts, about 30 miles west of any known winter moth infestation, since three of the winter moths we sequenced came from there. More DNA analysis will be needed to confirm the presence of winter moths from other states and to determine if winter moth is hybridizing with Bruce spanworm. To identify hybrids as such, we will have to sequence genes from the cell nucleus. We are currently attempting to amplify a nuclear gene called G6PD that Adam Porter has used in his studies of hybridization of swallowtail butterflies. The occurrence of hybrids has important implications for the success of the biological control effort versus winter moths.

We have also begun work on an identification system based on restriction enzymes that we hope will provide a reliable and inexpensive way to distinguish between these two species. Restriction enzymes cut strands of DNA at specific base pair sequences. With the CO1 gene sequence in hand, we can identify specific restriction enzymes that will cut the DNA strand of the CO1 gene for winter moth but not Bruce spanworm, or vice versa. The DNA fragments are then run out on an agarose gel yielding different numbers of bands that will allow us to distinguish between the two species without sequencing. In the future if we want to ID a large number of individuals, restriction enzyme analysis will be the way to go.

## References:

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## Cooperating agencies

USDA APHIS PPQ, USDA Forest Service, CT Agricultural Experiment Station  
CT DEP, Division of Forestry, CT State Survey Coordinator - UConn  
RI DEM, Department of Agriculture, RI DEM, Division of Forestry, NJ Dept. of Agriculture  
ME Dept. of Agriculture. MA Dept. of Agricultural Resources, MA Dept. of Conservation and Recreation  
University of Massachusetts, UMass Cooperative Extension

