Evaluation of Pheromone-Baited Traps for Winter Moth and Bruce Spanworm (Lepidoptera: Geometridae)

Author(s): Joseph S. Elkinton, David Lance, George Boettner, Ashot Khrimian, and Natalie Leva
Published By: Entomological Society of America
DOI: 10.1603/EC09322
URL: http://www.bioone.org/doi/full/10.1603/EC09322
Evaluation of Pheromone-Baited Traps for Winter Moth and Bruce Spanworm (Lepidoptera: Geometridae)

JOSEPH S. ELKINTON, 1,2 DAVID LANCE, 3 GEORGE BOETTNER, 1 ASHOT KHRIMIAN, 4 AND NATALIE LEVA 3

J. Econ. Entomol. 104(2): 494–500 (2011); DOI: 10.1603/EC09322

ABSTRACT We tested different pheromone-baited traps for surveying winter moth, Operophtera brumata (L.) (Lepidoptera: Geometridae), populations in eastern North America. We compared male catch at Pherocon 1C sticky traps with various large capacity traps and showed that Universal Moth traps with white bottoms caught more winter moths than any other trap type. We ran the experiment on Cape Cod, MA, where we caught only winter moth, and in western Massachusetts, where we caught only Bruce spanworm, Operophtera bruceata (Hulst) (Lepidoptera: Geometridae), a congener of winter moth native to North America that uses the same pheromone compound [(Z,Z,Z)-1,3,6,9-nonadecatetraene] and is difficult to distinguish from adult male winter moths. With Bruce spanworm, the Pherocon 1C sticky traps caught by far the most moths. We tested an isomer of the pheromone [(E,Z,Z)-1,3,6,9-nonadecatetraene] that previous work had suggested would inhibit captures of Bruce spanworm but not winter moths. We found that the different doses and placements of the isomer suppressed captures of both species to a similar degree. We are thus doubtful that we can use the isomer to trap winter moths without also catching Bruce spanworm. Pheromone-baited survey traps will catch both species.

KEY WORDS pheromone trap survey, forest defoliator, invasive species

The winter moth, Operophtera brumata (L.) (Lepidoptera: Geometridae), which is native to Europe, has recently invaded the northeastern United States and has caused widespread defoliation particularly in eastern Massachusetts. Beginning in November 2005, we worked with a team of cooperators in seven northeastern states (Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, and Rhode Island) to deploy a grid of Pherocon 1C sticky traps baited with the winter moth pheromone (Z,Z,Z)-1,3,6,9-nonadecatetraene (Roelofs et al. 1982), to delineate the extent of the winter moth infestation across the region (Elkinton et al. 2010). Unfortunately, the winter moth pheromone, as far as it is known, consists of a single compound that is identical to that used by its North American congener, the Bruce spanworm, Operophtera bruceata (Hulst) (Lepidoptera: Geometridae), which is common across the region, although rarely at high density. Thus, traps baited with the pheromone capture both species. In a 2005 winter moth survey across southern New England, we included with the pheromone a 20% blend of (E,Z,Z)-1,3,6,9-nonadecatetraene, an isomer of the pheromone, which research by Underhill et al. (1987) indicated would suppress attraction of Bruce spanworm but not winter moth. Unfortunately, we found that traps baited with 1,000 µg of this blend readily attracted Bruce spanworm, and our traps filled up with both species. Adult males of these two species are difficult to distinguish. We used male genitalia (Eidt et al. 1966) and ultimately DNA analysis to tell them apart (Elkinton et al. 2010). Male moths recovered from sticky traps are messy and difficult to deal with for these analyses; so, when we extended our survey in 2006 and 2007, we used large-capacity Universal Moth traps (“Unitraps”) instead of sticky traps. Males collected in these traps are killed by a fumigant (dimethyl 2,2-dichlorovinylphosphate; DDVP); and, as a result, they are in much better condition for subsequent analysis than those recovered in sticky traps. Furthermore, we found that winter moths captured in sticky traps are often removed by birds and sometimes the birds themselves get stuck in the traps. In 2006 and 2007, we baited these traps with only the pheromone (without inhibitor) because we captured Bruce spanworm in all traps in 2005 despite presence of the inhibitor, except for those traps that saturated quickly with winter moths at sites where it was in outbreak phase.

In this manuscript we report the results of experiments designed to compare the numbers of male winter moths and Bruce spanworm captured in Pherocon
Materials and Methods

Pheromone and Inhibitor Syntheses. The pheromone of the winter moth, (Z,Z,Z)-1,3,6,9-nonadecatetraene, of chemical and isomeric purity was prepared following the approach by Bestmann et al. (1982). Two new syntheses of (E,Z,Z)-1,3,6,9-nonadecatetraene have been developed recently. One synthesis delivers the inhibitor at 96% chemical and isomeric purity (Khrimian et al. 2010). The lures were analyzed by gas chromatography (GC) on a 6890N Network GC system (Agilent Technologies, Santa Clara, CA) equipped with a flame ionization detector and a 6320B capillary column (film thickness, 0.25 μm) that provided a partial separation of geometric isomers (Underhill et al. 1987). The analyses were conducted in a split mode (50:1) using H2 as a carrier gas at 1 ml/min. The oven was programmed from 100°C (5 min) to 240°C at 10°C/min, the injector temperature was 260°C, and the detector temperature was 270°C.

Pheromone Traps and Lures. For our field experiments, we impregnated 19- by 9-mm-diameter gray rubber septa (West Pharmaceutical, Kearney, NE) with 100 μg of (Z,Z,Z)-1,3,6,9-nonadecatetraene (the pheromone) or with different concentrations of (E,Z,Z)-1,3,6,9-nonadecatetraene (the Bruce spanworm inhibitor) as described below. The rubber septa were suspended from plastic strips at the top center of Pherocon 1C sticky traps (Zoecon Corp.) or in the dispenser containers of three large-capacity traps: Universal Moth traps (Great Lakes IPM, Inc., Vestaburg, MI), Multi-pher traps (model MP-3, Bio-Contrôle Services, Sainte-Foy, QC, Canada), and USDA “milk carton” traps (Elkinton 1987). These traps were modified in various ways described below. The large-capacity traps all contained Vaportape II insecticidal strips (Hercon Environmental, Emigsville, PA), which contain DDVP as a killing agent. The traps were deployed in a randomized block design with traps spaced 40 m apart along logging roads or power lines. Each block contained one trap of each treatment. We hung these traps at a height of 1 m from the boles of trees by using metal coat hangers bent so that one end was stapled to the bole and the other end protruded to provide a hook from which we suspended the trap. Males were counted and removed from the traps either daily, or every 2–4 d when the numbers caught were low. In the case of Pherocon 1C sticky traps, we replaced the sticky trap bottoms with fresh ones instead of removing the males. After counting and removing the males, traps within each block were reassigned positions within each block at random with the constraint that no individual trap treatment was assigned to a given position more than once.

Experimental Sites. We ran the experiments along logging roads at Cadwell State Forest in western Massachusetts (in Pelham MA), and along work roads, abandoned roads, and power lines within undeveloped portions of the Massachusetts National Cemetery and adjoining areas of the Massachusetts Military Reservation, Bourne, MA, on Cape Cod. We chose these sites because at Cadwell State Forest we caught exclusively Bruce spanworm and in Bourne, MA, we caught exclusively, or almost exclusively, winter moths. In the survey with pheromone-baited traps at Bourne, Elkinton et al. (2010) caught and identified 22 male winter moths and no Bruce spanworm and 84 winter moths and one Bruce spanworm at various other sites on Cape Cod in 2005 and 2008. In western Massachusetts, Elkinton et al. (2010), caught and identified 221 moths, all of which were Bruce spanworm, including 22 moths from Cadwell State Forest. The nearest capture of winter moths to this site was ~70 km away in eastern Massachusetts.

Statistical Analysis. We used analysis of variance (ANOVA) implemented in general linear model analysis (PROC GLM, SAS 9.3, SAS Institute 1990) to determine whether there were significant fixed factor main effects (date, treatment and block) and interactions among these variables. Nonsignificant interactions were dropped from the final model and Tukey’s honestly significant difference (HSD) test was used to determine the statistical difference in treatment effects. For these analyses the number of males captured per trap was transformed to log10(x + 1) to equalize the variance. In the second and fourth experiment with only two treatments, we used Wilcoxon’s signed rank test for matched pairs to test for treatment effects (PROC UNIVARIATE, SAS 9.3, SAS Institute 1990).

Experiment 1: Trap Design. In the first experiment, we compared male catch at different trap designs as follows: 1) Pherocon 1C sticky trap; 2) milk carton unmodified; 3) milk carton modified; 4) Multi-pher trap; 5) Multi-pher with milk carton body covering the portion below the entry ports; 6) Universal Moth traps with white bottoms; and 7) Universal Moth traps, all green.

Milk carton traps are all green and are used to survey for gypsy moth, Lymantria dispar (L.), across infested portions of the United States (USDA 2008). In the modified milk carton trap, we enlarged the entry ports.
port by cutting out the 25- by 12-mm piece of cardboard that separated the two 25- by 8-mm entry ports on each of the four sides of the trap. In addition, we inserted a square plastic funnel inside the trap such that the top of the funnel was even with or slightly below the bottom of the enlarged entry ports. This modification has been used successfully to trap the moth Dendrolimus superans sibiricus Tschetverikov in Russia (Khrimian et al. 2002). The Multi-pher traps consist of a 20-cm-high by 10-cm- (bottom) to 13-cm (top) -diameter white plastic cup under a 26-cm-diameter green plastic lid. The MP-3 model has five 2.5-cm-diameter round entry ports equally spaced around the cup, just under the lid. By inserting the bottom of the trap (below the entry ports) into a milk carton trap (treatment 5), we converted the trap to a nearly all green trap. The Unitraps come in several colors. We compared catch at all dark-green traps to traps that had the same green on the lids (16 cm in diameter), but the top 7 cm of the trap body was yellow and bottom 11 cm (a removable bucket) was white. With Unitraps, the trap body is suspended on four posts at 25 mm below the lid; moths entering that space can fall through a central funnel into the bucket. In this way, part of our experiment was to evaluate the possible effects of trap color (green versus white or white/yellow) on trap catch in different traps. We ran 10 daily runs of this experiment with winter moth in Massachusetts National Cemetery beginning 28 November 2008 and ending 10 December 2008. Each run consisted of six replicate blocks. For Bruce spanworm at Cadwell State Forest in western Massachusetts, we ran the experiment with six replicates on 7 November 2006 and again on 3 December 2006. On the latter date, we also ran an experiment at Cadwell with six replicate blocks, wherein half the traps of each type were hung as usual from the holes of trees and the other half were hung at the same height from branches at least 2 m from the nearest tree stem. This was done to see whether proximity to the boles had an effect on trap catch.

Experiment 2: DDVP Effect. In the first experiment, all the large capacity traps contained Hercon Vaportape II that releases DDVP as a killing agent, whereas the Pherocon 1C traps did not. To make sure that any difference we detected between catch in the Pherocon 1C traps was not due to the presence or absence of DDVP, as opposed to other aspects of trap design, we ran a test with Pherocon 1C traps with and without a DDVP strip. In the treatment with the DDVP strip, we stapled two strips to the top of the trap, one at each end. Each block contained one trap of each type along the transect.

Experiment 3: Inhibitor Test. In this experiment, we evaluated the effect of adding various concentrations and deployment strategies of the (E,ZZ)-1,3,6,9-nonadecatetraene (the Bruce spanworm inhibitor) to traps baited with the standard lure: 100 µg of (Z,ZZ)-1,3,6,9-nonadecatetraene. For this test we used large capacity Multi-pher traps (white bottoms). In some treatments listed below, the inhibitor was impregnated into a rubber septum and hung alongside of the pheromone at the center of the inside of the trap. In other treatments, the inhibitor was placed at each of the trap entry ports, because experiments by Pivnick et al. (1988), indicated that this approach was especially effective at deterring Bruce spanworm. In these treatments we compared attaching either a standard rubber septum adjacent to, or a 25-mm i.d. rubber ring around, each entry port; the septa and rings either were (treatments 5 and 7, respectively) or were not (treatments 6 and 8) impregnated with inhibitor.

We tested the following treatments: 1) standard: 100 µg on a rubber septum inside the trap; 2) Standard plus 50 µg of EZZ (inside trap); 3) standard plus 500 µg of EZZ inside trap; 4) Standard plus 1000 µg of EZZ inside trap, 5) standard plus inhibitor outside of trap (on five sepa each with 100 µg, one at each hole), 6) standard plus unloaded inhibitor dispensers (five unloaded sepa outside of trap); 7) standard plus inhibitor outside of trap on five rings cut from large sepa, 100 µg each; 8) standard plus unloaded rings cut from large septa, one per hole; 9) inhibitor alone, 500 µg of EZZ inside trap; and 10) a blank trap (no pheromone or EZZ).

We ran the experiment with six replicate blocks daily for winter moth on 28 and 29 November 2008 and on 1, 2, and 3 December 2008 at the Massachusetts National Cemetery; and on 12, 17, and 25 November 2008 at Cadwell State Forest.

Experiment 4: Effect of Inhibitor on Catch in Pherocon 1C Sticky Traps. We tested the effect of the inhibitor on catch in Pherocon 1C sticky traps. All traps were baited with the standard 100-µg lure. The treatment with the inhibitor consisted of four rubber septa each baited with 100 µg of the inhibitor placed

| Table 1. General linear model ANOVA* results for experiment 1 on trap design and for experiment 3 with the inhibitor |
|---------------------------------|------|--------|------|--------|
|                                | F    | df     | P    | F      | df     | P    |
|                                | ratio |        |      | ratio  |        |      |
| **Experiment 1A: trap design** |      |        |      |        |        |      |
| Source                         | Date | 26.46  | 9    | <0.001 | 13.99  | 1    | <0.001 |
|                                | Block | 7.39  | 5    | <0.001 | 7.09   | 5    | 0.009  |
|                                | Trap  | 48.83 | 6    | <0.001 | 33.44  | 6    | <0.001 |
|                                | Date × trap design* | 1.60 | 54 | 0.007  |
| **Experiment 1B: trap design** |      |        |      |        |        |      |
| Source                         | Block | 6     | 0.02 |
|                                | Branch vs. bole | 1 | 0.71 |
|                                | Trap design | 6 | <0.001 |
| **Experiment 3 inhibitor**     |      |        |      |        |        |      |
| Date                           | 10.64 | 4       | <0.001 | 7.64 | 2 | 0.007 |
| Block                          | 3.24 | 5   | 0.005 | 2.01 | 5 | 0.08  |
| Inhibitor treatment            | 10.38 | 9 | <0.001 | 53.02 | 9 | <0.001 |
| Date × treatment*              | 2.00 | 36 | 0.001 |

* SAS Proc GLM: class date, block, treatment; model log 10(Winter moth) or log 10(Bruce spanworm) + 1 = date, block, treatment, date × treatment.

b Winter moth results were obtained at the Massachusetts National Cemetery on Cape Cod, MA, whereas Bruce spanworm results were obtained at Cadwell State Forest in western Massachusetts.

Only interactions significant at P < 0.05 are included.
at the four corners of the top of the trap along with the standard pheromone lure a rubber septum placed at the center of the top of the trap.

Results and Discussion

In the trap design experiment (experiment 1), there were significant date, treatment, and block effects for all experiments (Table 1). For winter moth, the Universal Moth traps with white bottoms caught more moths than any other trap including the Pherocon 1C sticky trap (Fig. 1A) ($P < 0.05$; Tukey’s HSD test). The Pherocon 1C traps captured as many moths as the Universal Moth traps with green bottoms. All the other large-capacity traps caught much fewer moths.

In contrast, with Bruce spanworm (Fig. 1B) the Pherocon 1C sticky traps caught many more moths than any of the large-capacity traps ($P < 0.05$; Tukey’s HSD test). This was also true of the second version of the experiment (Fig. 1C), wherein we compared catch at traps hung as usual from the boles of trees with traps hung at the same height from branches at least 2 m from the nearest tree stem. There was no significant effect of being close or far from the boles of trees (Table 1). Catch was lower in the milk carton traps than in either of the other two large capacity traps.

---

Fig. 1. Number of male winter moths on Cape Cod (A) or Bruce spanworm in western Massachusetts (B and C) captured in pheromone baited traps of different design. All traps were hung next to the boles of oak trees except in C, wherein half the traps were hung from branches at least 2 m from the nearest bole. Bars with the same lower case letters indicate that catch at these treatments were not significantly different from others within the same test at $P > 0.05$ (Tukey’s HSD test).
The significant date × trap interactions in the winter moth test (Table 1) is probably explained by the fact that overall trap catch varied with date due to variation in weather, number of males flying and the efficiency of all these traps, meaning that the proportion of males approaching the traps that get captured, declines gradually as the traps fill up (Elkinton 1987). This is particularly true of the Pherocon 1C sticky traps, which have a much more limited capacity than the other traps.

The evident difference between the two species with regard to the relatively larger catch of winter moths in the Universal Moth traps surprised us. We had expected that the highest catch would be at the Pherocon 1C traps for both species, because previous experience with sticky traps with other species such as gypsy moth indicate that they catch more moths than large capacity traps provided catch is low or that traps are emptied or replaced with sufficient frequency that the sticky surface of the trap does not become covered with moths and lose its stickiness. In contrast to sticky traps with large openings, large capacity traps are harder for males to get into and they therefore catch fewer moths unless the traps are left in place for long enough for sticky traps to become filled. In our experiment, however, the relatively high catch of winter moths in Universal Moth traps versus Pherocon 1C traps (Fig. 1A) compared with Bruce spanworm (Fig. 1B and C) cannot be due to the decline of trap efficiency as the sticky traps became filled, because we caught more Bruce spanworm than winter moths in these experiments.

In the second experiment, the number (mean ± SE) of winter moths captured in the Pherocon 1C sticky traps with the DDVP strip was 28.4 ± 3.0 compared with 25.6 ± 2.6 for Pherocon 1C sticky traps without the DDVP strip, a difference that was not significant (N = 47, S = 114, P = 0.23). For Bruce spanworm, there were 19.4 ± 7.7 males captured in Pherocon 1C sticky traps with the DDVP strip compared with 19.9 ± 4.0 in Pherocon 1C sticky traps without the DDVP strip a difference that was not significant (N = 12, S = 3.5, P = 0.67). The absence of DDVP thus cannot account for the relatively high catch of either species in the Pherocon 1C traps.

![Graph showing number of male winter moths and Bruce spanworm captured in different types of traps](image-url)
The experiments with the inhibitor revealed that higher doses suppressed catch at the traps compared with the three types of control traps baited with the pheromone without inhibitor. (Fig. 2). With both species the lowest catch was at the treatment with the 100 \( \mu g \) of the inhibitor impregnated in a ring at each of the trap entry holes. With winter moth, but not Bruce spanworm catch at this treatment was statistically different \((P < 0.05; \text{Tukey's HSD test})\) from treatments with higher doses of the inhibitor placed at the center of the trap. This treatment had the lowest catch of Bruce spanworm in the results reported by Pivnick et al. (1988). Traps without the pheromone lure with or without the inhibitor caught negligible numbers of moths of either species.

In the fourth experiment, the number (mean \( \pm \text{SE} \)) of winter moths captured in the Pherocon 1C sticky traps with the inhibitor was 25.6 \( \pm \) 2.6 compared with 34.6 \( \pm \) 5.0 for Pherocon 1C sticky traps without the inhibitor, a difference that was significant \((N = 24, S = 84, P = 0.007)\). For Bruce spanworm, there were 2.2 \( \pm \) 0.4 males captured in Pherocon 1C sticky traps with the inhibitor compared with 6.9 \( \pm \) 1.1 in Pherocon 1C sticky traps without the inhibitor, a difference that was significant \((N = 20, S = 93, P < 0.001)\). Although this experiment showed that the inhibitor suppressed catch of both winter moths and Bruce spanworm, these traps still caught substantial numbers of moths of each species. These results explain why Pherocon 1C sticky traps baited with a 1,000-\( \mu g \) solution of 50% pheromone and 20% inhibitor filled with Bruce spanworm at all sites in our 2005 survey of southern New England (Elkinton et al. 2010), except those sites with outbreak densities of winter moth, where that species vastly outnumbered Bruce spanworm.

In conclusion, we recommend that future surveys for winter moths with pheromone traps be done using Universal Moth traps. It seems that traps with light-colored bodies catch more moths than all green traps, although color of trap did not affect catch at the other trap types we evaluated. In addition to catching as many or more winter moths than Pherocon 1C sticky traps, the large-capacity Universal Moth traps yield moths that are in far better condition for subsequent identification of species. Birds will remove moths from the sticky traps and sometimes get captured themselves, but they are unable to access the moths inside the Universal traps. The only problem we have encountered with these traps is that sometimes rainwater accumulates in the bottom and the moths decompose. A different or extended top to the trap might solve this problem. Also, small holes drilled in the bottoms of the trap will let the water escape.

Both species of moths were suppressed to a similar degree by the presence of different doses or positions of the inhibitor \((E,\text{Z},Z)-1,3,6,9\)-nonadecatetraene. Thus, we were unable to repeat the findings of Underhill et al. (1987) and Pivnick et al. (1988) that the presence of the inhibitor would suppress captures of Bruce spanworm without affecting winter moth catches. These previous studies were done in western Canada, where these species may be genetically distinct and respond differently from the populations in Massachusetts. The main difference in our results from these previous studies was with winter moth, however, whose captures were suppressed by the inhibitor in our results, but not in theirs. In recent work, Elkinton et al. (2010) sequenced the barcoding gene \((COI)\) and the nuclear gene \(G6PD\) of winter moths and Bruce spanworm from sites across North America including Massachusetts and western Canada. They found that all winter moths that were examined had identical sequences of these genes, in contrast to substantial variation in Bruce spanworm. These results suggest a common founder effect and genetic similarity among winter moths in North America. Thus, we are unable to explain the difference in our result and those of Underhill et al. (1987) or Pivnick et al. (1988). In some of the trials reported by Pivnick et al. (1988), the inhibitor seemed to suppress catch of winter moth, as well as Bruce spanworm, but not to the same degree. All three studies reported some degree of hybridization between the two species, which implies that genes that governed the response to the inhibitor could be transferred between the species. Whether the inhibitor is a minor component of the pheromone blend of either species is unknown (Underhill et al. 1987). Regardless, our results suggest that the inhibitor is unlikely to play a useful role in an effort to develop a trap that attracts winter moth only, at least in eastern North America.

Acknowledgments

We thank R. Hunkins, S. Lyon, E. Hibbard, J. Cunningham, D. Tessein, and C. Lokerson for help deploying the traps and collecting and counting the moths. We thank S. Lyon for help drafting the figures. We thank the Massachusetts National Cemetery for generously allowing us to use their lands and back roads for the winter moth studies. This research was supported by cooperative agreements with the USDA Forest Service (04-CA-1124225-414), the USDA-APHIS-PPQ (05-8335-0464-CA) and a grant from the Massachusetts State Legislature.

References Cited


Received 26 September 2009; accepted 21 April 2010.