Hoplia equina (Coleoptera: Scarabaeidae) and Nontarget Capture Using 2-Tetradecanone–Baited Traps

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ABSTRACT Using bucket traps baited with 2-tetradecanone, the sex pheromone of *Hoplia equina* LeConte, an important pest of cranberries in Massachusetts, we investigated the effect of trap height, color, pheromone load, and lure aging on male capture, as well as captures of nontarget arthropods including pollinators. Male capture was inversely related to height of traps over the four heights tested (0, 20, 60, and 100 cm). Captures increased with increasing pheromone load over the doses of 0, 100, 300, and 600 μg, but captures at the highest load, 1,000 μg, were not significantly different from 300 or 600 μg. *H. equina* captures were strongly diurnal, with a flight period spanning $\approx$6 wk starting in mid-June. Vane color of traps (white, yellow, green, blue, red, black) did not affect *H. equina* capture but significantly influenced capture of nontargets, including pollinators. A bucket trap with the funnel opening at 20 cm, and green (or red) vanes, baited with 600 μg of 2-tetradecanone, was the optimal design for high male capture and low nontarget capture. The low-cost capture of over 50,000 *H. equina* on a 2.4-ha commercial bog in Massachusetts with this lure-trap combination indicates the feasibility of mass trapping for managing established infestations of *H. equina*.

KEY WORDS pheromone, trap color, trap height, cranberry, pollinators

Hoplia equina LeConte is a small (6–8 mm), native melolonthine scarab species whose larvae were first discovered as root feeding pests in cranberries in 1990 but which was correctly identified to species only in 2001 (Zhang et al. 2003). Surveys of Massachusetts cranberry beds in 1994 and 1995 indicated that in those sampled beds where grub species were present, *H. equina* was found in 30% of the beds sampled and at the highest larval densities of all grub species when present (Dunn and Averill 1996). There are no recommended methods of control for the root-feeding larvae of *H. equina.* Because adults emerge and are active during cranberry bloom, pesticide applications pose a risk to pollinators. Numerous trials of pesticides and entomopathogenic nematodes on the larvae have yielded inconsistent and usually disappointing results (D.C.W., unpublished data). Observations by Dunn and Averill (1997) suggested the presence of a sex pheromone in the mating system of *H. equina.* Their work also showed that this species is diurnal and has a 2-yr life cycle.

Using gas chromatography in tandem with electroantennal detection and coupled mass spectroscopy techniques, as well as field trapping in Massachusetts, Zhang et al. (2003) identified 2-tetradecanone as the female-produced sex pheromone of *H. equina.* Unlike most lepidopteran pheromones, which consist of unsaturated 12–18 carbon acetates, alcohols, and aldehydes, this pheromone is a ketone and thus is not subject to regulatory food tolerance exemption (U.S. EPA 1995). However, solid-matrix dispensers (such as retrievable lures) of ketone pheromones and other pheromones that would not be tolerance-exempt in broadcast formulations are exempt from tolerance (U.S. EPA 1994), along with any inerts (U.S. EPA 1993). This consideration, along with the small acreages involved, prompted us to not consider broadcast pheromone formulations for mating disruption, a method that has been successful for two tortricid pests in cranberries (Fitzpatrick et al. 1995, Polavarapu et al. 2001). Recently, another scarab cranberry pest, the Oriental beetle [*Exomala orientalis* (Waterhouse)], has been disrupted experimentally in blueberry and ornamental nurseries with a microencapsulated mixture of enantiomers of (*Z,E*)7-tetradecen-2-one (Polavarapu et al. 2002). Instead, we chose to pursue the prospects for trapping adults using the newly discovered pheromone.

In preliminary field tests, large numbers of bumblebees (*Bombus* sp.) and honey bees (*Apis mellifera* L.) were captured in the white-vaned bucket traps

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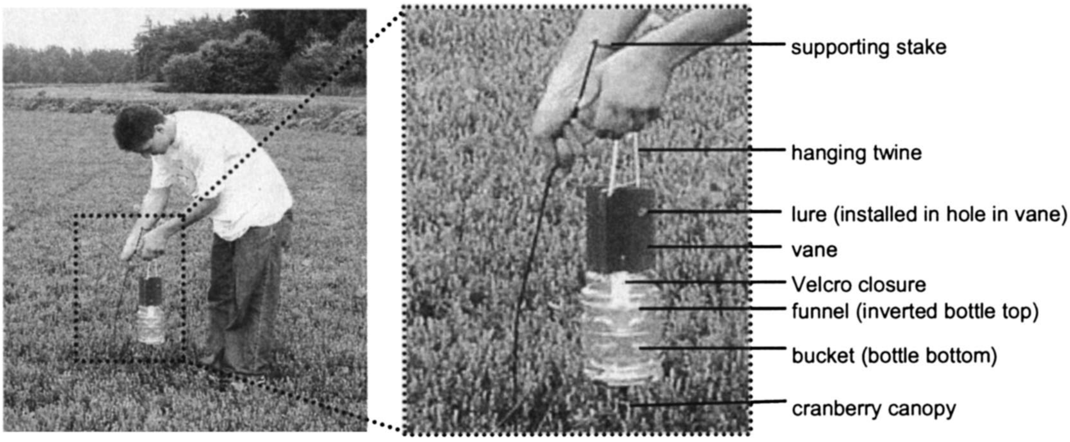

Fig. 1. Bucket trap used in this study, installed at 20 cm height.

used. To optimize trap capture for the target insect while minimizing pollinator and other nontarget captures and to determine the potential for mass-trapping in infested cranberries, we initiated a study to determine the effects of pheromone load, trap height, vane color, lure age, and time of day on captures of male *H. equina* and nontarget species in the field.

Materials and Methods

Trapping experiments were undertaken in 2000 on four commercial cranberry bogs in Plymouth County, MA, and in 2001 and 2002 on a single cranberry bog in the town of Plymouth, consisting of two bog sections totaling 2.4 ha, >80% of which was cultivar 'Howes,' and the remainder was unknown mixed genotypes. The bog was ≈1 km away from other commercial cranberry beds and surrounded by wooded uplands with residential development, except for the south end, which was adjacent to a 5.5-ha pond. Both sections had a history of poor production and in early 2001 were found to be infested with *H. equina* larvae at a number of sample sites. Weather data were recorded at Plymouth Municipal Airport, 1.5 km south of the study site, a National Weather Service permanent location.

Bucket-type traps (Fig. 1) were constructed from 1.89-liter clear polyethylene terephthalate bottles (original "bell bottle" design; Ocean Spray Cranberries, Lakeville, MA) with the top cut off and inverted to form a funnel and reversibly attached to the bottle bottom with strips of Velcro closures stapled to both parts, thus facilitating trap emptying. Drain holes of 2 mm diameter were drilled in the trap bottom to allow drainage of rain. Two corrugated plastic vanes (Kiva Plastics International, Taylors, SC), each 14.6 cm tall by 11.4 cm wide, were connected at right angles to one another and attached to the top of the trap funnel piece. The trap was hung from a green vinyl-coated metal stake by nylon twine attached to the vanes. Rubber stopper septa (5 mm; Thomas Scientific, Swedesboro, NJ) were loaded with various amounts of 2-tetradecanone (Zhang et al. 2003) and were inserted in a hole made in the middle of one vane.

Preliminary Color Test. In 2000, on four sites in Plymouth County, we used six vane colors of corrugated plastic—white, yellow, red, green, blue, and black—with trap opening positioned at 60 cm height and provided with 300 μg 2-tetradecanone on rubber septum lures. The bogs all had known *H. equina* infestations and were located in the towns of Acushnet, Plympton, Plymouth, and Rochester. The traps were set out on 1 or 2 June 2000 and collected twice weekly until termination of the study on 10 July in Acushnet and Plympton and 18 July in Plymouth and Rochester. There was only one trap per color per site; traps were spaced 10 m apart in a single line. Because of differing sample dates and rerandomization, the analysis used trap captures totaled over all dates. All honey bees and bumblebees were enumerated, along with *H. equina.*

Color and Height Test. In 2001, we designed a factorial experiment to test the effects of vane color and height of trap on capture of *H. equina* and other macroarthropods. Six vane colors of corrugated plastic—white, yellow, red, green, blue, and black—were installed at 0, 20, 60, and 100 cm height, as measured from the lip of the funnel entrance. The 0 cm or ground-level trap was installed with the lip <1 cm above the soil level, and the capture chamber was below ground. The 20 cm height corresponded to the bottom of the trap resting on the cranberry canopy. All 24 treatments were provided with 300 μg 2-tetradecanone on rubber septum lures and were completely randomized in four blocks on a 1.54-ha bog section along with 19 additional traps that were green-vaned and installed at 60 cm height. The 115 traps were spaced on a 11.3-m grid covering the entire bog section at a density of 75 traps/ha. The traps were set out on 18 June 2001 and collected but not rerandomized on 19, 20, 21, 23, 24, 25, 26, 28, and 29 June, 3, 5, 9, 12, 20, 23, 26, and 31 July, and 6 August.

Pheromone Loading Test. In 2002, we compared four different pheromone loadings in identical green-vaned 20-cm-high traps baited with 0, 100, 300, 600, or

1,000 μg 2-tetradecanone. Treatments were replicated eight times in a randomized complete block layout, along with 22 additional surrounding traps that were also green-vaned, loaded with 600 μg 2-tetradecanone, and installed at 20 cm height. The 62 traps were spaced on an ≈15.8-m grid covering the entire bog section at a density of 40 traps/ha. They were set out on 4 June and collected and rerandomized on 12 and 20 June, 2, 5, 12, and 19 July, and 2 August. Lures were replaced on 2 July. The grower applied diazinon for lepidopterous pests on 18 July.

Lure Aging Test. In 2001, we tested lures that were field-aged for 20 or 30 d in a randomized complete block experiment including fresh lures and blank lures, replicated seven times, with all loaded lures using 300 μg 2-tetradecanone in traps with green vanes at 60 cm height. Surrounding traps were spaced in a 11.3-m grid for a density of 75 traps/ha. Traps were set out on 18 July and collected on 20, 23, 26, and 31 July.

Diel Capture Pattern. On 24 June 2001, beginning at 1500 hours and continuing for 48 h, we collected each of 115 traps (color/height test and 19 extras) approximately every 3 h to determine the diel periodicity of capture.

Capture data were analyzed using StatView (Windows version 5, SAS Institute 1998) and SAS PC Version 8.2e (for Windows, SAS Institute 2001) by analysis of variance (ANOVA), and for nontarget captures, with $\sqrt{(x + 0.5)}$ transformation to stabilize variance and to adjust for zero counts. Multiple comparisons were made using Tukey's honestly significant difference (HSD) test on transformed values at $\alpha = 0.05$ and nonlinear regression with PROC REG (SAS Institute 2001).

Results

Captures of *H. equina* males were strongly affected by height but not by color. Catch decreased with height, and each of the heights was significantly different in catch from the others. The 60-cm high trap position used in earlier lure studies was clearly not optimal, catching only 20% of numbers caught by 0-cm traps (Fig. 2). Traps failed occasionally because of detachment of the trap bottom or entire trap. Trap positions of 60 and 100 cm were most prone to this failure (8.2 and 6.8% of total trap collections, respectively), significantly more than the 0–1.0% failure rate at the 0- or 20-cm level ($F = 16.08$; df = 3,92; $P < 0.001$). Ground-level (0 cm) traps required digging to install, which damaged the crop, and traps sometimes flooded with groundwater, making collection difficult.

There was a strong dose-response for capture of *H. equina* males to lure loading with the pheromone (Fig. 3). Additional loading of 2-tetradecanone above 300 μg did not increase capture significantly, and 1,000-μg totals did not exceed those for the 600-μg loading. The data suggest a loading near 600 μg 2-tetradecanone to attract maximum numbers of beetles with the current trap design. Nontarget captures did not differ in response to pheromone loading.

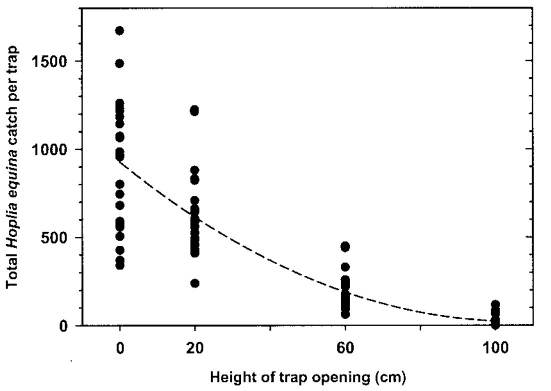

Fig. 2. *Hoplia equina* captures, total per season per trap, $n = 24$ per height, using 300-μg 2-tetradecanone lures, Plymouth, MA, cranberry bog, 2001. Quadratic regression shown with $y = 926 - 17.216x + 0.082x^2$; $R^2 = 0.72$; $F = 119.4$; df = 2,93; $P < 0.0001$.

The 300 μg 2-tetradecanone rubber septum lures were effective at attracting beetles for over 30 d. Aging lures for 20 or 30 d in the field did not significantly reduce trap capture, even though numbers were slightly lower than for new lures (Fig. 4).

Hoplia equina captures began during the second or third week of June, peaking in late June or early July, and decreasing to zero by the end of July. A single diazinon treatment on 18 July 2002 nearly ended captures for the year (only 5 additional captures after >15,000 before spray). As observed in earlier studies (Dunn and Averill 1997), *H. equina* males seek mates (or in this study, trap lures) during the day (Fig. 5). Our 48-h observation period included a photophase that was moderately warm (20.6 increasing to 25°C) and humid (90 decreasing to 62% RH), which corresponded to an extended period of captures into the afternoon after a morning peak. The next photophase

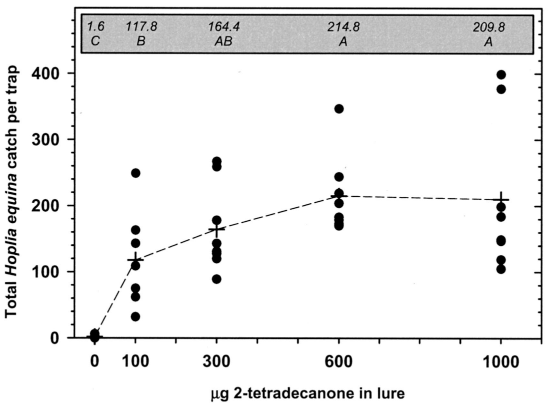

Fig. 3. *Hoplia equina* captures, total per season per trap, $n = 8$ per level of 2-tetradecanone lure loading, Plymouth, MA, cranberry bog, 2001. Levels shown with means and common letters not differing in block-adjusted least-squares means by Tukey's HSD at $\alpha = 0.05$ (Proc GLM, SAS Institute 2001).

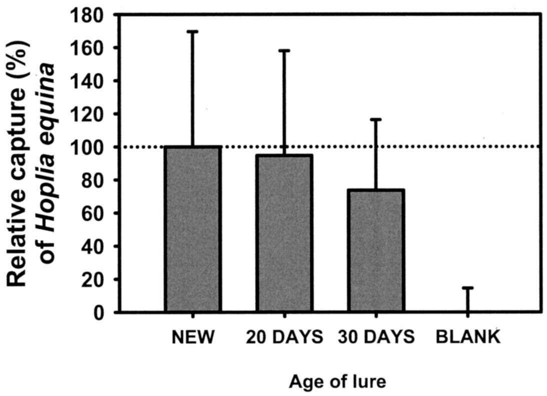

Fig. 4. *Hoplia equina* captures, total per trap relative to new lure, all lures loaded originally with 300 μg 2-tetradecanone except for solvent blank and aged as noted in the field, Plymouth, MA, cranberry bog, 2001. Blank differs from all other treatments, but no loaded lures differ from one another, $F = 4.9$, df = 3,24, $P = 0.0085$; ANOVA with comparison-wise $\alpha = 0.05$.

was warmer and drier (19.4 increasing to 28.3°C, with 93 decreasing to 53% RH), during which beetles were active in mid-morning but then ceased coming to traps. Sunrise was at 0508 hours and sunset was at 20:22 hours, with moon waxing between one-quarter and one-half illumination during the study period.

Nontarget macroarthropods were captured, 605 total in 2001, of which 36.5% were Hymenoptera, almost all Apoidea, and 38.5% were Coleoptera, mostly Scarabaeidae. Considered as a group, nontarget capture showed significant color differences, with white capturing more than all other colors (Table 1; $F = 22.8$, df = 5,72, $P < 0.001$) and also significant but weaker differences with trap height, with 0 cm height capturing significantly >100 cm ($F = 3.9$, df = 3,72, $P = 0.012$) but with only ≈1.6 times as many captures.

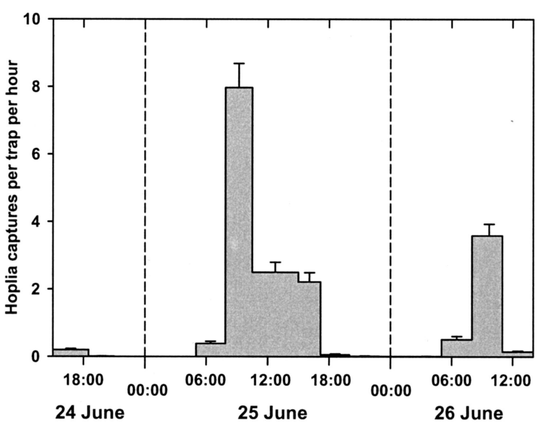

Fig. 5. *Hoplia equina* captures per trap per hour, 24–26 June 2001, $n = 96$, using 300-μg 2-tetradecanone lures and heights and colors of traps as noted in text, Plymouth, MA, cranberry bog, 2001. Each bar spans the starting and collection time along the x-axis, with collection intervals of ≈3 h.

There was no significant color by height interaction for all nontargets.

In both 2000 and in 2001, honey bees were frequently captured (5.0 and 1.1 total/trap, respectively). All four sites in 2000 had commercial hives stationed nearby, but at the site for the 2001 studies, the grower did not station any honey bee colonies. Bumblebees (*Bombus* spp.) were less frequently caught (2.4 and 0.2 total/trap, respectively, in 2000 and 2001). Solitary bees, primarily *Megachile* spp. (Hymenoptera: Megachilidae), were also captured (mean, 0.7/trap) in 2001 and also captured but not enumerated in 2000. Color strongly affected captures of honey bees, bumblebees, and solitary bees (Table 1). White (the color we used in original lure trials, Zhang et al. 2003) was significantly more attractive in 2001 to honey bees and bumblebees (honey bee: $F = 15.8$, df = 5,72, $P < 0.0001$; bumblebee: $F = 3.2$, df = 5,72, $P = 0.011$), and black and blue were also attractive to solitary bees (overall: $F = 6.3$, df = 5,72, $P < 0.0001$). Red, green, and yellow were least attractive. This was consistent with the 2000 data, which showed blue and white to be most attractive, and green and red to be least attractive, for both honey bees and bumblebees (honey bee: $F = 6.4$, df = 5,18, $P = 0.0014$; bumblebee: $F = 4.9$, df = 5,18, $P = 0.0054$). Considering bees as a group in 2001, white caught significantly more than all other colors, black significantly more than green, yellow, or red, and blue significantly more than red ($F = 23.1$, df = 5,72; $P < 0.0001$). Height was also a significant effect ($F = 8.7$; df = 3,72; $P < 0.0001$), with 20 cm height catching an average of twice that of other heights and significantly more total bees than 60 or 100 cm heights. There was no significant interaction between height and color for total bees captured.

Color also influenced the capture of scarabs other than *H. equina.* Most common nontargets were Oriental beetle (mean, 0.4/trap) and Japanese beetle, *Popillia japonica* Newman (mean, 0.7/trap). Elaterids were also commonly captured (mean, 0.5/trap). Nontarget beetles considered as a group showed a significant interaction of color with height effects ($F = 1.9$, df = 15,72, $P = 0.034$). White and yellow traps generally had the most nontarget beetle captures, except that this trend was not evident at the 0 cm height.

Other nontargets captured in >1 per 20 traps included wasps (Hymenoptera: Vespidae) (mean, 0.05/trap; $n = 96$); adult Lepidoptera (mean, 0.2/trap); larval Lepidoptera [mean, 0.1/trap; principally Gypsy moth, *Lymantria dispar* L. (Lymantriidae)]; spiders (Aranae, principally Salticidae; mean, 1.2/trap); and robber flies (Asilidae; mean, 0.1/trap). The latter two groups were observed to prey on *H. equina* aggregating in and near the traps, as were dragonflies (Odonata: Anisoptera) and Eastern Kingbirds (*Tyrannus tyrannus* L.). Caterpillars were only present in the lower two trap heights, 0 and 20 cm, reflecting their ability to crawl into these traps from the cranberry canopy. There was a significant interaction between trap height and color for spider capture ($F = 3.8$; df = 15,72; $P < 0.0001$). Spiders were much more frequently caught in 0 cm high traps (>10-fold other heights),

Table 1. Effect of trap color on mean no. of nontarget captures in bucket traps baited with 2-tetradecanone 300 μg

	2000		2001			
	Apis	*Bombus*	*Apis*	*Bombus*	Solitary bees	All nontargets
White	9.3ab	6.3a	3.8a	0.8a	1.3a	15.1a
Blue	12.0a	6.3a	0.9b	0.1b	1.1a	4.9b
Black	2.8bc	0.0b	1.1b	0.0b	1.4a	5.2b
Yellow	2.5bc	1.3ab	0.4b	0.1b	0.2b	4.5b
Green	2.0c	0.3b	0.4b	0.1b	0.3b	5.3b
Red	1.3c	1.0ab	0.3b	0.0b	0.2b	2.9b

For 2000, $n = 4$ traps per color, and for 2001, $n = 16$ per color, Plymouth County, MA, cranberry bogs. Means followed by different letter in the same column differ at $P < 0.05$ according to Tukey's HSD multiple comparison on $\sqrt{x} + 0.5$ transformed values (means shown are untransformed).

and at this height, they preferred green over all other colors (more than twice the captures of all other colors).

Discussion

Traps proved very selective, capturing >400 *H. equina* males per trap in 2001, with a mean of only 1.4% nontarget macroarthropods. Height influences male *H. equina* capture, whereas color, and to a lesser extent height, influences captures of various nontargets. White-vaned traps and tall traps catch more nontargets relative to the target insect; in the extreme, almost one-half of trap captures were nontargets (46.4%) for the 100-cm-high white-vaned trap. Our original design, a 60-cm-high white-vaned trap, caught 6.6% nontargets and provoked complaints from cranberry growers because of bumblebee and honey bee mortality. We conclude that the optimal design for *H. equina* capture is a green-vaned trap placed at 20 cm height (with trap bottom resting on the cranberry canopy) and baited with 600 μg 2-tetradecanone, placed out at the beginning of June. This design captured the least percentage of nontargets (0.4%) in our 2001 tests. The pheromone lure should be replaced once after 1 mo. Red vanes would also be acceptable, but a red trap might tend to attract the attention of vandals more than a green one.

Honey bees are the principal pollinators of commercial cranberries and many other vital crops. Bumblebees, as well as diverse solitary bees, are increasingly recognized as important alternatives to and/or complementary to honey bee pollination. This is particularly true with cranberries, for which Cane and Schiffhauer (2003) have shown that *Bombus* species are far more effective per individual than *Apis* and that the leafcutter bee *Megachile addenda,* native to New Jersey cranberry bogs, is a faithful and efficient cranberry pollinator. To reduce pollinator mortality, researchers in several crops have endeavored to increase the selectivity of pheromone and other pest-attracting traps. Clare et al. (2000), working in New Zealand apple orchards with sticky traps for tortricid pests, concluded that because of attraction of *Bombus* spp., *Apis mellifera,* and native bees to the standard white as well as blue and yellow traps, green or red traps should be used instead to minimize trapping of pollinators. In sticky traps, the nontarget insects may necessitate more frequent changes of the adhesive trapping surface, and with any imaginable trap design, the capture of nontargets tends to increase the time necessary for, and in some cases the chance for error in, counting or distinguishing target insects.

Many studies have shown that trap design, color, and placement are important in capture of target insects; several comparisons have also shown differential capture of bees and other nontarget species. Bucket traps tend to trap more bumblebees when yellow and/or white colors are used compared with green (Hamilton et al. 1971, Gross and Carpenter 1991, Herman et al. 1994, Meagher 2001). Other beneficial insects such as syrphid flies are more attracted to white than to green or black traps, resulting in the adoption of green-roofed sticky traps for tortricid orchard pests in Germany as early as 15 yr ago (Krause 1990). Using flight barrier traps of different colors for forest scolytids, Dubbel et al. (1985) showed that white traps caught ≈10-fold more Hymenoptera and Diptera than the black, green, gray, or redbrown traps, but captures of nontarget Coleoptera and Heteroptera were not affected. White traps caught far fewer target scolytids in this study, and the authors recommended black traps because "they are less conspicuous in the forest landscape and thus do not disturb the rural esthetics." Color may affect capture of target species whether nocturnal or diurnal (e.g., Mitchell et al. 1989 for noctuids, Hardee et al. 1972 for boll weevil). Conversely, semiochemical lure components may influence nontarget capture. The cause of this attraction is often not clear but may be related to use of the pheromone component(s) by other, often-related species (e.g., Weber and Ferro 1991) to attraction to floral or other plant volatiles (e.g., Meagher and Mitchell 1999) or to use of the target species' pheromone as a kairomone by natural enemies (e.g., Raffa and Dahlsten 1995).

As insect pest monitoring and management using semiochemicals becomes more widespread in agricultural, urban, and forest environments and becomes more integrated into pest management decision-making, researchers should pay early and explicit attention to the nontarget effects to optimize the application of these powerful tactics in managing target pests while minimizing effects on pollinators, natural enemies, and harmless nontarget organisms.

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