CHEMICAL ECOLOGY

# Trapping of Aethina tumida Murray (Coleoptera: Nitidulidae) from Apis mellifera L. (Hymenoptera: Apidae) Colonies with an In-Hive Baited Trap

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**ABSTRACT** The effectiveness of two lures for trapping the small hive beetle, *Aethina tumida*, by means of in-hive traps was tested by field trials in apiaries located in Florida, Delaware, and Pennsylvania during 2003–2005. Both lures included a mixture (pollen dough) consisting of bee pollen and commercial pollen substitute formulated with or without glycerol and honey. Before it was used in the traps, the dough was conditioned either by the feeding of adult small hive beetles or by inoculation with the yeast *Kodamaea ohmeri* (NRRL Y-30722). Traps baited with conditioned dough captured significantly more beetles than unbaited traps, and traps positioned under the bottom board of a hive captured significantly more beetles than traps located at the top of a hive. In fact, baited in-hive bottom board traps nearly eliminated the beetles from colonies at a pollination site in Florida. However, when these honey bee colonies were moved to an apiary, trap catch increased markedly over time, indicating a resurgence of the beetle population produced by immigration of beetles from nearby hives or emerging from the soil. In tests at three Florida apiaries during 2006, yeast-inoculated dough baited bottom board traps captured significantly more beetles than unbaited traps and traps captured significantly more beetles or emerging from the soil. In tests at three solution produced by immigration of beetles from nearby hives or emerging from the soil. In tests at three solution board traps apiaries during 2006, yeast-inoculated dough baited bottom board traps captured significantly more beetles than unbaited traps, showing the effectiveness of yeast-inoculated dough as a lure and its potential as a tool in managing the small hive beetle.

KEY WORDS Aethina tumida, small hive beetle, honey bee, Apis mellifera, pollen dough

The small hive beetle Aethina tumida Murray (Coleoptera: Nitidulidae) is an important pest of European honey bee colonies in the United States (Elzen et al. 1999, Hood 2004). Adult males and females enter hives where they feed, mate, and lay eggs (Lundie 1940, Ellis and Hepburn 2006, Ellis et al. 2002). The developing larvae are the most destructive stage, feeding on pollen and brood, and contaminating honey with their feces in the process (Lundie 1940, Hood 2004, Neumann and Elzen 2004). When the larvae reach maturity, they enter a wandering phase, leave the hive, and fall to the ground where they burrow into the soil to pupate. Adults emerge from the soil and fly in search of a host, typically a honey bee colony. In the United States, damage to honey bee colonies typically occurs during the summer and is first noticeable when

a frothy liquid leaks from the entrance of heavily infested hives (Lundie 1940, Hood 2004).

Currently, there is no effective tool for monitoring the beetle in managed honey bee colonies. The beetle is attracted to worker honey bee volatiles, composed essentially of alarm pheromones (Suazo et al. 2003, Torto et al. 2005). In laboratory assays, we found that a pollen-honey mixture conditioned by the feeding of either adult male or female beetles releases fermentation products that lure the beetles into traps and that fermentation of the pollen dough is caused by a yeast, NRRL Y-30722, associated with the beetle (Teal et al. 2006, Torto et al. 2007). The objectives of this study were to (1) compare numbers of beetles captured in managed honey bee colonies by bottom board traps baited with conditioned pollen dough to numbers captured by unbaited traps, (2) monitor the temporal pattern of infestation (based on trap capture) in honey bee colonies that were moved from one site to another, (3) compare the effectiveness of baited bottom and top board traps in capturing beetles, and (4) compare the numbers of beetles captured in managed honey bee colonies by modified bottom board traps in a choice type design within the same hive with and without pollen dough inoculated with the yeast isolate NRRL Y-30722 from the beetle.

Environ. Entomol. 36(5): 1018-1024 (2007)

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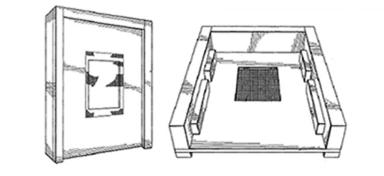
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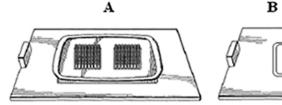
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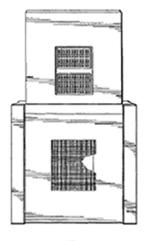
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Fig. 1. Components of the in-hive trap showing (A) a typical Langstroth hive bottom board, with a rectangular opening (18 by 14 cm) in its center and covered with a piece of 4-mesh aluminum screen, (B) underside of bottom board attached to a three-sided frame of two-by-fours with the missing fourth side toward the back of the board and wooden runners attached to the two-by-four frame, (C) underside of plywood panel with the lid of a Rubbermaid egg container and two PCR 96 well plates attached to it, and (D) top face of plywood panel half way under the modified bottom board.

## Materials and Methods

Field Sites. Experiments were carried out in Florida, Delaware, and Pennsylvania, all in apiaries with a previous history of small hive beetle outbreaks. There were four sites in northcentral Florida, one near High Springs in Alachua County and three (sites A–C) located near Lake City in Columbia County. There were three sites in Suffix County, DE (located within 6.4 km of one another) and one in Montgomery County, PA. All of the experiments were conducted between June and October of 2003, 2004, and 2005. In 2006, experiments were conducted at three new sites within 20 km of one another (sites D-F) in Alachua County.

**Traps.** Two types of in-hive traps were used. Both consisted of three parts (Fig. 1). The upper part of type I traps was a typical Langstroth hive bottom board that was modified by cutting a rectangular opening (18 by 14 cm) in its center. This opening was covered with a piece of four-mesh aluminum screen, which allowed beetles to pass through but excluded bees. The modified bottom board was attached to a three-sided frame of two-by-fours, whose missing side was either toward the back or side of the hive. This

opening permitted a plywood panel to slide beneath the bottom board on wooden runners that were attached to the two-by-four frame. In type I, this panel (Fig. 1C), like the bottom board, had an 18 by 14-cm rectangular opening in its center, and when in place, this hole aligned with that in the modified bottom board. The lid of an egg container (Rubbermaid, Huntersville, NC) was attached to the underside of the sliding panel. Two openings cut in the middle of the lid were fitted with polymerase chain reaction (PCR) 96-well plates (USA Scientific, Ocala, FL) that had their conical tips cut off and were positioned with the tips extending downward. The egg trav, which contained the bait and held trapped beetles, snapped into this lid. Because the beetles are attracted to dark shaded areas (Lundie 1940), the egg containers were sprayed with black paint (Rust-Oleum Corp., Vernon Hills, IL) before use to enhance the efficacy of the trap. Three pin holes drilled into each corner cell of the egg container allowed for drainage of water that entered the trap.

Type II traps were a modified version of type I, in which the rectangular opening (with the PCR plates) in the plywood panel (Fig. 1C) was replaced by two holes (each 2 cm in diameter) 12 cm apart (Fig. 1C). These holes led to separate 500-ml plastic containers (Rubbermaid) attached beneath the plywood panel in the same manner as the egg container of the type I trap. Each hole led to a triangular enclosure formed by three strips of wood (0.8 by 0.8 by 6.0 cm) attached to the underside of the plywood panel and covered with seven-mesh screen. Openings (0.5 cm wide) at the apices of each triangle allowed beetles to enter the container below. A petri dish (6 cm in diameter by 2 cm high) for holding bait was glued to the bottom of one container. The petri dish was covered with a tight-fitting lid perforated by  $\approx 60$  pin-holes that allowed the release of odors into the honey bee colony. A soapy solution ( $\approx$ 150 ml) prepared from a household dishwashing detergent (0.25% in water) was placed in each container as a killing agent.

**Bait.** Two types of bait were tested: one consisted of pollen dough fed on (conditioned) by adult beetles and the other consisted of pollen dough inoculated with the yeast (NRRL Y-30722) isolated from the beetle. Experiments 1–3 tested the efficacy of conditioned pollen dough using type I traps, and experiment 4 tested the efficacy of inoculated pollen dough using type II traps.

Conditioned pollen dough was prepared in a room maintained at 26°C by mixing commercially packaged bee pollen (Y.S. Organic Bee Farms, Sheridan, IL), commercial pollen substitute (Bee Pro; Mann Lake, Hackensack, MN), glycerol (Sigma-Aldrich, St. Louis, MO) and warm honey (34°C; 1:12:2: 18) by weight. The dough (8 kg) was conditioned by allowing 800 adult male beetles (4–8 wk old) to feed on it for 1 wk. After conditioning, the dough was thoroughly mixed and ≈100-g portions were scooped with an ice cream scoop into cotton stockinettes (Florida Orthopedics, Miramar, FL). The bagged conditioned pollen dough was transferred to Ziploc bags and stored in a refrigerator at 3°C until used. Traps were baited by placing a bag of conditioned pollen dough and a piece of cotton stockinette (10 by 10 cm) moistened with a 20% glycerol solution ( $\approx$ 20 ml) into the egg container. A piece of moistened stockinette only was placed in unbaited traps.

To prepare inoculated pollen dough, the yeast (NRRL Y-30722) was mass produced under submerged fermentation conditions. Yeast inoculum was propagated initially in 1% peptone and 2% dextrose (PDB) broth at 25°C in a 500-ml shake flask agitated at 150 rpm for 48 h. A batch fermentation vessel (New Brunswick Scientific Microferm) containing 10 liters of PDB was inoculated with seed culture and incubated at a constant temperature of 25°C, aeration of 4 liters/min, and agitation of 600 rpm. After 48 h, the yeast cells were collected by a continuous flow centrifugation (5,000g). Aliquots of the cell pellet were resuspended in a mixture of 5% inositol in autoclaved water, subjected to shelf freezing, and lyophilized. The 10-liter fermentation produced ≈200 g of freezedried yeast. Inoculated pollen dough was prepared by mixing the yeast with Millipore-pure water and pollen patty containing 4% pollen (Global Patties; Airdrie, Alberta, Canada) 1:100:1,000 by wt and allowing the resulting dough to ferment for 3-7 d. Traps were baited by placing inoculated pollen dough ( $\approx 50$  g in a piece of cotton stockinette) in the petri dish on the bottom of the baited container and covering it with the perforated lid.

**Experiment 1: Comparison of Baited and Unbaited** Traps. This experiment tested the effectiveness of conditioned pollen dough in luring beetles into bottom board traps installed under standard Langstroth hives in three Florida apiaries. One test done near High Springs during September and October 2003 involved 10 colonies in single brood chambers, one half with baited and one half with unbaited traps. The traps were checked every other day for 24 d, and numbers of beetles captured in each trap were recorded. The pollen dough lures were replaced every 6 d. Additional tests were done at two sites near Lake City. One test (site A) was run for 12 wk between July and October 2004 and involved 32 colonies in double deep brood chambers supported on pallets, with four hives per pallet. The other (site B) was run between June and October 2005 and involved 12 colonies, 11 in one and a half deep brood chambers and one in a single brood chamber. In both tests, one half the traps at each site were baited and one half were unbaited. The traps were checked, trapped beetles were counted, and lures were replaced weekly. In all three tests, the positions of baited and unbaited traps were reversed halfway through the trapping period.

The totals captured by baited and unbaited traps at each site during each trapping interval were analyzed using SigmaStat 3.1 (Systat Software, Point Richmond, CA). Because the data were not normally distributed (P < 0.05), the Mann-Whitney test (a nonparametric analog of the two-sample *t*-test) (Zar 1999) was used to compare median numbers captured by baited and unbaited traps at each site.

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	Replicates <sup>a</sup>	$\mathrm{Total}^b$	Mean no. captured $\pm$ SE <sup>c</sup>		T / 10	
Apiary site			Bottom board	Top board	F (df)	P
PA	4	50	$9.5 \pm 4.4$	$3.0 \pm 1.0$	1.57 (1,3)	0.30
DE1	7	13	$1.7 \pm 0.5$	$0.1 \pm 0.1$	8.85 (1,6)	0.02
DE2	7	45	$4.0 \pm 1.9$	$2.4 \pm 1.9$	3.7(1.6)	0.10
DE3	7	1,213	$161.3 \pm 83.4$	$12.0 \pm 4.0$	23.4 (1,6)	< 0.01

Table 1. Numbers of *A. tumida* captured by bottom board and top board traps at apiaries in Pennsylvania and Delaware during 4- to 7-wk trapping periods in July and Aug. 2004

One-way repeated-measures ANOVA.

<sup>a</sup> Number of weekly observations (counts).

 $^{b}$  Adult beetles captured by bottom and top board traps combined.

<sup>c</sup> Per week.

Experiment 2: Temporal Pattern of Infestation. The purpose of this experiment was to examine temporal change in infestation of honey bee colonies, including change that may occur when colonies are moved from one site to another. Ten colonies were monitored for 12 wk between November 2003 and February 2004 using baited bottom board traps. All of the colonies were in single brood chambers. During the first 6 wk (27 October to 8 December 2003), the colonies were located at a site south of Lake City (site C) for pollination of cucumbers. On 12 December, the beekeeper moved the colonies to an apiary 40 km north. Trapping continued at the new site for another 6 wk (15 December to 21 January). At both sites, the traps were checked, beetles were counted, and lures were replaced weekly.

Weekly totals were plotted as a histogram and were also fitted to a cubic polynomial to obtain a trend curve using SigmaPlot 9.0 (Systat Software).

**Experiment 3: Comparison of Captures by Baited** Top and Bottom Board Traps. This experiment was conducted in Delaware and Pennsylvania during July and August 2004 to compare the effectiveness of baited in-hive traps used as bottom traps to those used as top traps. Four apiaries with a minimum of 12 hives each were used. Three were located in Delaware and contained overwintered colonies maintained in one and a half brood chambers supported on pallets, with four colonies per pallet. The colonies had been moved from Virginia to Delaware for pollinating water melons. The fourth site was located in Montgomery County, PA, and had colonies in double story deep brood chambers established from bee packages earlier in the year. Treatments were assigned randomly to two groups of four colonies in each apiary. In one group, a baited trap was placed at the bottom of each hive (bottom board traps). In the other, a baited trap was placed at the top of each hive in an empty super (top board trap). Traps were checked weekly for 4 wk at the Pennsylvania apiary and for 7 wk at the three Delaware apiaries. Beetle captures for each treatment group at each apiary were pooled and counted.

Statistical analysis was done with SigmaStat 3.1 (Systat Software). The numbers of beetles captured in top board and bottom board traps were compared within each apiary by one-way repeated-measures analysis of variance (ANOVA), in which the weekly

counts comprised the replicates (Table 1). Apiaries were compared separately, because replication was not equal among sites. The transformation  $\log(x + 1)$  was applied to the beetle counts before analysis as necessary to correct for non-normality and unequal variance.

**Experiment 4: Efficacy of Yeast-Inoculated Pollen** Dough as a Bait in Bottom Board Traps. This experiment was done during the summer of 2006 to test the effectiveness of yeast-inoculated pollen dough in luring small hive beetles into bottom board traps. Type II traps with one baited and one unbaited container were installed under standard Langstroth hives in three Florida apiaries (sites D–F) to provide the beetles in each hive with a two-way choice. The beetles captured in baited and unbaited containers were counted, and the lures were replaced at weekly intervals over a period of 10 wk. Site D had 22 hives, and trapping was done from 16 May to 25 July. Site E had eight hives, and the trapping period extended from 9 June to 18 August. Site F had four colonies, and trapping was done from 5 July to 12 September.

The  $\chi^2$  one-sample test was used to compare the total numbers captured in baited and unbaited containers at each site during each week. Values of  $\chi^2$  were calculated using Microsoft Excel 2002, and  $\chi^2$  probabilities were determined using the probability functions of Statistix 8 (Analytical Software, Tallahassee, FL).

#### **Results and Discussion**

Experiment 1: Comparison of Baited and Unbaited Traps. Baited traps captured significantly more beetles than unbaited traps at all sites (Fig. 2). The total number of beetles captured at a single site ranged from 671 to 1,372. Unbaited traps also captured beetles, but in low numbers, consistent with previous results (Teal et al. 2006).

Experiment 2: Temporal Pattern of Infestation. A total of 456 beetles (156 males and 300 females) were captured during the 12-wk trapping period (Fig. 3). The number of beetles captured declined with time until the hives were moved from the pollination site to the apiary, and the number captured began to rise, reaching a peak in January. This temporal pattern was best described by the cubic polynomial,  $y = 153-8.31x + 0.158x^2 - 0.0089x^3$  ( $R^2 = 0.69$ ). The pattern suggests a

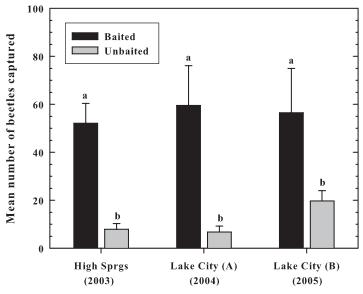


Fig. 2. Captures of adult small hive beetles from honey bee colonies in Florida apiaries with in-hive bottom board traps. Error bars indicate SE, and different letters at each site indicate a significant difference between baited and unbaited traps (Mann-Whitney test, P < 0.05).

declining beetle population at the pollination site, and in fact, the baited in-hive bottom board traps nearly eliminated small hive beetle populations from the colonies. The rebound after the hives were moved suggests renewed infestation of the experimental colonies by beetles from nearby hives or emerging from the soil at the apiary. The eventual leveling off at levels below those observed at the pollination site can probably be attributed to slower development and higher mortality during the winter months. The baited traps captured an average ( $\pm$ SE) of 45.5  $\pm$  15.8 beetles/wk at the pollination site compared with 30.5  $\pm$  6.6 beetles/wk after the colonies were moved to the apiary.

Experiment 3: Comparison of Captures by Baited Top and Bottom Board Traps. The combined total of beetles captured by the top and bottom board traps

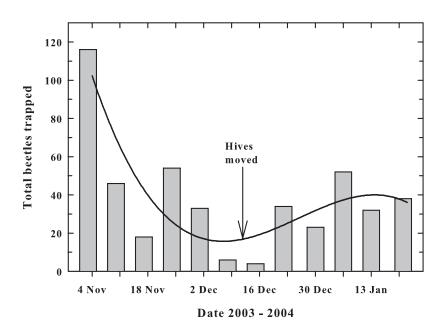


Fig. 3. Captures of adult small hive beetles with baited in-hive bottom board traps before and after the honey bee colonies were moved from a pollination site (site C) near Lake City, FL (3 November to 8 December) to an apiary 40 km north of the pollination site (15 December to 21 January). Arrow indicates date (12 December 2003) when the honey bee colonies were moved to the apiary.

Table 2. Numbers of A. tumida captured by in-hive traps at three beeyards (sites D-F) in Alachua Co., FL, during 10-wk trapping periods in 2006

Week	Baited container		Unbaited container		2	-1-
	Total	$Mean^a \pm SE$	Total	$Mean^a \pm SE$	$\chi^2$	$P^b$
Site D						
1	63	$2.7\pm0.6$	5	$0.2\pm0.1$	49.5	< 0.001
	62	$2.7\pm0.7$	6	$0.3 \pm 0.1$	46.1	< 0.001
2 3	163	$7.1 \pm 1.5$	16	$0.7\pm0.2$	120.7	< 0.001
4	21	$0.9 \pm 0.2$	0	$0.0\pm0.0$	21.0	< 0.001
5	23	$1.0 \pm 0.2$	1	$0.0\pm 0.0$	20.2	< 0.001
6	10	$0.4 \pm 0.2$	1	$0.0 \pm 0.0$	7.4	0.007
7	71	$3.1 \pm 0.6$	15	$0.6 \pm 0.3$	36.5	< 0.001
8	16	$0.7 \pm 0.3$	0	$0.0 \pm 0.0$	16.0	< 0.001
9	10	$0.4 \pm 0.2$	0	$0.0 \pm 0.0$	10.0	0.002
10	10	$0.4 \pm 0.2$	0	$0.0 \pm 0.0$	10.0	0.002
Site E						
1	46	$5.8 \pm 1.0$	7	$0.9\pm0.5$	28.7	< 0.001
2	21	$2.6 \pm 1.0$	2	$0.3 \pm 0.2$	15.7	< 0.001
2 3	11	$1.4 \pm 0.6$	0	$0.0\pm 0.0$	11.0	0.001
4	18	$2.3 \pm 0.5$	0	$0.0 \pm 0.0$	18.0	< 0.001
5	4	$0.5 \pm 0.3$	0	$0.0 \pm 0.0$	4.0	0.046
6	11	$1.4 \pm 0.4$	4	$0.5\pm0.4$	3.3	0.071
7	7	$0.9\pm0.4$	2	$0.3 \pm 0.2$	2.8	0.095
8	16	$2.0\pm0.9$	2 2	$0.3 \pm 0.3$	10.9	0.001
9	15	$1.9 \pm 1.0$	0	$0.0 \pm 0.0$	15.0	< 0.001
10	23	$2.9 \pm 1.5$	0	$0.0 \pm 0.0$	23.0	< 0.001
Site F						
1	42	$10.5 \pm 4.1$	4	$1.0 \pm 0.6$	31.4	< 0.001
2	8	$2.0 \pm 1.2$	0	$0.0 \pm 0.0$	8.0	0.005
2 3	10	$2.5 \pm 1.6$	0	$0.0\pm0.0$	10.0	0.002
4	53	$13.3 \pm 5.4$	1	$0.3 \pm 0.3$	50.1	< 0.001
5	164	$41.0 \pm 33.8$	27	$6.8 \pm 5.8$	98.2	< 0.001
6	52	$13.0 \pm 9.1$	1	$0.3 \pm 0.3$	49.1	< 0.001
7	197	$49.3\pm20.7$	1	$0.3 \pm 0.3$	194.0	< 0.001
8	71	$17.8\pm7.9$	0	$0.0 \pm 0.0$	71.0	< 0.001
9	35	$8.8 \pm 3.8$	0	$0.0\pm 0.0$	35.0	< 0.001
10	13	$3.3 \pm 1.4$	0	$0.0 \pm 0.0$	13.0	< 0.001

<sup>a</sup> Mean no. per hive.

 $b \chi^2$  probability. H<sub>o</sub>, total numbers of beetles captured in the baited and unbaited containers are the same.

varied considerably with location, and more beetles by far were captured at a single site in Delaware than at all the others combined (Table 1). Although the mean number of captures was consistently higher in bottom board traps, the difference was not always statistically significant (Table 1). However, when the total number captured was large, the superiority of the bottom board trap became quite clear. It is not surprising that hive beetles should be lured more readily into bottom board traps, because host-seeking adults enter bee hives mostly through the same entrance at the bottom of the hive used by foraging bees, and they are also known to congregate at the bottom of a hive (Lundie 1940).

Experiment 4: Efficacy of Yeast-Inoculated Pollen Dough as a Bait in Bottom Board Traps. In general, at all the three sites, baited containers captured significantly more beetles than unbaited containers throughout the 10-wk trapping period (Table 2). Total weekly captures by baited and unbaited traps combined ranged from 44 to 449 (site D), 17 to 172 (site E), and 34 to 645 (site F). These results are similar to those of earlier trapping experiments (2003, 2004, and 2005) in which pollen dough conditioned by the feeding of adult beetles was used as bait; that is, yeastinoculated and beetle-conditioned pollen were both effective in luring beetles into the trap. However, there are several advantages to inoculating pollen dough directly by adding yeast over inoculating it indirectly (conditioning) by allowing beetles to feed on it. Direct inoculation with yeast makes it unnecessary to maintain beetle cultures and facilitates a standardized formulation that is simpler to prepare and may be more economical than a synthetic lure.

The attraction of nitidulids to volatiles released from fermenting food is well known (Phelan and Lin 1991, Nout and Bartelt 1998, Bartelt and Wicklow 1999, Mansfield and Hossain 2004, Bartelt and Hossain 2006), and male-produced aggregation pheromones, identified in the presence of these volatiles, have been used to control some of these nitidulids (Bartelt et al. 1993, 1995, Bartelt 1997). Recently, our laboratory studies indicated that the small hive beetle might not require an aggregation pheromone because of its strong association with honey bee colonies and its reliance on the yeast species Kodamea ohmeri (NRRL Y-30722), which, when it grows on bee pollen, mimics the alarm pheromone and related volatiles produced by the bee (Torto et al. 2007), suggesting a different semiochemically mediated interaction for different species within the same family.

In summary, the results of this study show that pollen dough conditioned by the feeding of adult *A. tumida* or inoculated with yeast associated with the beetle is an effective lure for trapping beetles. Furthermore, bottom board traps baited with yeast-inoculated pollen dough and containing a soapy solution showed considerable potential as a monitoring tool in managing small hive beetle infestation in managed honey bee colonies.

### Acknowledgments

We thank A. Platinetty, Jr., C. Skov, and M. Searle at CMAVE, Gainesville, FL, and R. Mitchell at the Delaware Department of Agriculture for providing technical and field assistance that contributed much to the success of the project. We also thank J. Latner, M. Thomas, and J. Bobb for making apiaries available and for cooperation in the study, and the PDA seasonal apiary inspectors for field assistance. This study was supported in part by funds from SARE (OS04-022).

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Received for publication 20 November 2006; accepted 18 June 2007.