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Autodissemination of *Beauveria bassiana* by Sap Beetles (Coleoptera: Nitidulidae) to Overwintering Sites

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An autoinoculative device was used to test the ability of sap beetles (Coleoptera: Nitidulidae) to carry a specific strain of Beauveria bassiana *(Balsamo) Vuillemin to overwintering sites in a multiyear field study. The device was baited with the pheromone and coattractants for the dusky sap beetle (*Carpophilus lugubris *Murray) and placed in the field in the fall of each year. The introduced strain occurred at high frequency among the* B. bassiana *isolated in the fall of all four years tested (100% of all isolates from 21 of 22 collection dates). The introduced strain of* B. bassiana *was isolated at high frequency from all the* B. bassiana*-contaminated sap beetles recovered from the overwintering traps (100% of all isolates from 13 of 23 trap/beetle species combinations) and was highest after the longest fall exposure. The introduced strain was primarily isolated from* C. lugubris *and* C. antiquus*, but species distribution was also dependent on the overwintering trap design used. Few non-sap beetles species of insects were recovered from the artificial overwintering sites. Although* B. bassiana *was isolated from free flying sap beetles caught in traps in the spring of each year, none were infected with the introduced strain. The autoinoculating device provides selective contamination of sap beetles in overwintering sites when used in the fall. It may be useful in providing some control of sap beetles or other insects where limited numbers of mass overwintering sites (such as tree holes) occur.*

Keywords: Carpophilus, Beauveria, *dispersal*

INTRODUCTION

Sap beetles (Coleoptera: Nitidulidae) are cosmopolitan pests of a variety of fresh fruits and vegetables, as well as some stored products (Hinton, 1945). There is strong evidence that they vector different plant pathogens that can cause disease (Dowd, 1995) and, in some cases,

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produce toxins harmful to humans and animals (Dowd, 1998). The species of economic importance in temperate regions of the US are the dusky sap beetle, *Carpophilus lugubris* Murray; the Freeman sap beetle, *Carpophilus freemani* Dobson; the spotted picnic beetles, *Glischrochilus quadrisignatus* (Say) and *G. fasciatus* (Say); and the strawberry sap beetle, *Stelidota geminata* (Say) (Connell, 1956). Despite recognized involvement in direct damage and pathogen vectoring in maize, vegetables and fruit, sap beetles are generally considered to be of limited economic importance relative to other pests. Pest management strategies for co-occurring pests appear to also control sap beetles in many agricultural crops, such as sweet maize (e.g., Dively *et al.*, 1998; Whalen & Spellman, 1998) or peaches (James *et al.*, 1995a). However, changing agricultural practices, such as the use of pheromone confusion strategies against oriental fruit moths (*Grapholita molesta* (Busck)) (James *et al.*, 1995a) or planting transgenic sweet maize for control of the European corn borer (*Ostrinia nubilalis* (Hübner) (e.g., Dowd, 2000) has reduced insecticide applications, and thereby increased the presence and importance of sap beetles as pests. Development of low input pest management strategies should help sap beetle management in situations where they are of increasing relative importance, as well as in situations where control may be desirable but economically unfeasible by conventional means.

Native biological control agents have been reported for sap beetles, and include parasitoids (Williams *et al.*, 1984) nematodes (Lindegren & Okumura 1973; Vega *et al.* 1994; Dowd *et al.*, 1995), and various pathogens (review, Dowd, 1995). The common insect pathogen *Beauveria bassiana* (Balsamo) Vuillemin has been reported at low levels from *Glischrochilus* spp. sap beetles (e.g., Foott & Timmins, 1979; Peng & Williams 1990). We have isolated *B. bassiana* from various *Carpophilus* species, but the isolates we have tested by allowing the beetles to walk through sporulating cultures have produced only low levels of mortality over a 2-week period (Dowd, unpublished). However, when we tested commercially produced conidia of *B. bassiana* strain AF-4 (originally isolated from citrus root weevil by C. McCoy, University of Florida), 90% of adult *C. lugubris* were killed within 3 days (Vega, unpublished data). Because this strain had been undergoing commercial development at the time of initiation of this study, large quantities of conidia were available. Laboratory assays with autoinoculative release have indicated that autodissemination of *B. bassiana* potentially can be effective for sap beetle control (Vega *et al.*, 1995).

Although the use of insect pathogens has promise, there is some concern about potential nontarget effects (Hajek & Goettel, 2000). For example, there are reports that *B. bassiana* will kill nontarget beneficial insects such as lady beetles (e.g., James & Lighthart 1994). When applied by conventional means (such as sprays), insect pathogens such as *B. bassiana* incur the same costs as chemical insecticides, which may make them uneconomical for use on lower value crops. Evidence that sap beetles overwinter in mass (Dowd, unpublished), like other beetles (e.g., Simpson & Welborn, 1975) suggested that releasing *B. bassiana* after corn harvest in autoinoculators targeting overwintering populations may be a selective and less costly biological control strategy for sap beetles. We report on field studies designed to determine the effectiveness of an autoinoculative device in disseminating a specific *B. bassiana* strain to overwintering sites as a potential low input pest management strategy.

MATERIALS AND METHODS

Entomopathogen

Dry conidia of the AF-4 strain of *B. bassiana* were provided by J. Scott Ferguson (Ciba–Geigy, now Novartis, Vero Beach, FL) from material produced by Mycotech (now Emerald BioAgriculture Corp., Butte, MT). The dry conidia were stored at -20°C until needed.

Because 'wild' isolates of *B. bassiana* occurred at the research site at low frequency (less than 5% of insects surveyed during 1991–1993, Dowd, unpublished data), strain identification was needed to give an indication of whether isolates recovered during the study were AF-4 or other strains. Both RAPD-PCR and isozyme analysis by isoelectric focusing have

been used effectively to detect variation of the same series of different strains of beetle-derived *B. bassiana* (Castrillo & Brooks, 1998). We initially compared RAPD-PCR and 1-naphthyl acetate esterase isoelectric focusing band profiles of the AF-4 strain and wild isolates collected from the proposed research area before the start of the study, and found the isoelectric focusing to be more reliable in distinguishing the AF-4 from 'wild' strains. Although over a dozen bands were often available, major bands at p*I* values of 4.2, 4.5, 4.7, 5.0 and 6.0 were diagnostic, with at most two bands of 'wild' *B. bassiana* strains matching those from AF-4. Thus, isoelectric focusing was used throughout the study to distinguish AF-4 from naturally occurring strains of *B. bassiana* (representative results indicated in Figure 1).

Sap beetles collected during the study were placed individually in 5-ml vials containing 1 mL of 3% water agar. At weekly intervals for up to 2 months after capture, individuals that became infected were examined macroscopically and microscopically for characteristic *B. bassiana* structures. Once *B. bassiana* was detected, the infected insects were refrigerated until the fungus could be isolated. The *B. bassiana* was isolated on semi-selective media (Doberski & Tribe, 1980) supplemented with 0.1% dodine (Chem Service, WestChester, PA). Isolates were then grown from single spores, transferred to slants of potato dextrose agar (PDA) until the colony uniformly covered the surface, and stored at 4°C until use. Material from slants was streaked onto 5-mm diameter Petri dishes containing PDA and incubated at 29°C for 72 h. By that time, the plates were typically well covered with mycelia that had not yet sporulated. It was important to obtain vegetative colonies because preliminary assays indicated isozyme profiles changed once sporulation occurred.

Enzyme Preparation and Isoelectric Focusing

The mycelial mat was scraped off the plate using a sterile spatula and homogenized in 1 mL of pH 7.4, 0.1 M phosphate buffer using a 2-ml ground glass homogenizer (Ace Glass,

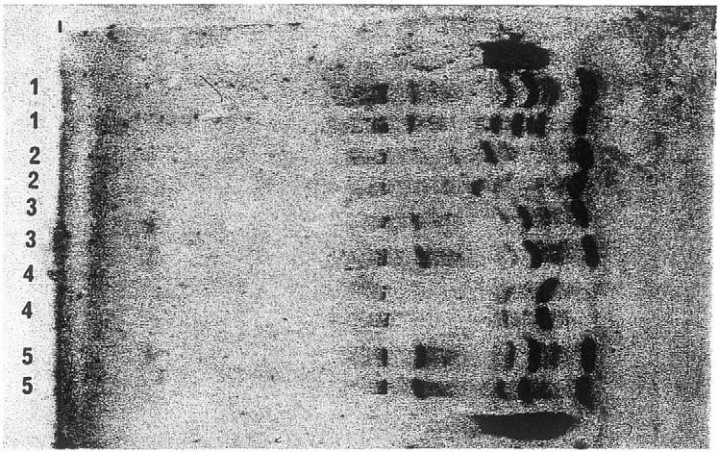

FIGURE 1. Use of 1-naphthyl esterase isozymes separated by isoelectric focusing to match recovered *B. bassiana* strains with the released strain AF-4. (1) Original AF-4 isolate; (2) isolate from sap beetle collected from spring hanging trap in 1997; (3) isolate from sap beetle collected from overwintering trap in 1998; (4) isolate from sap beetle collected from overwintering trap in 1997; (5) isolate from sap beetle collected from overwintering trap in 1998. Isolates (3) and (5) were considered matches with original AF-4. Gel boundaries indicated by upper left bar (pH 9.5) and upper right bar (pH 3.5). Smeared bands in lanes to the upper and lower part of the gel are the marker dye Evan's blue.

Vineland, NJ). When colony growth was less vigorous, the volume of buffer was reduced proportionately. The homogenates were centrifuged at $10\,000 \times g$ for 15 min at 4°C. Protein content of supernatants was determined using the Bio-Rad packaged protein assay according to manufacturers instructions (Bio-Rad, Richmond, CA). Homogenates were diluted as necessary to approximately 1 mg mL^{-1} protein content whenever possible, which covered most cases. A minimum protein content of at least 250 μg mL^{-1} was used. The range of protein contents was reflected by the range of homogenate concentrations of AF-4 standard that were run concurrently and were sufficient to show the diagnostic bands. Isoelectric focusing was performed using wide range precast gels (Ampholine pH 3.5–9.5, Amersham–Pharmacia Biotech, now Amersham Bioscience Corp., Piscataway, NJ) with an LKB Multiphor apparatus according to previously published methods (Dowd, 1994). For routine runs, application strips were split vertically, and 7.5 μL of supernatant were added to each strip. Gels were focused for 45 min at 25 W, the application strips were removed, and focusing continued for another 45 min. Gels were stained for 1-naphthyl acetate esterase activity at room temperature (22°C) using 100 mL of pH 7.4, 0.1 M phosphate buffer, to which 4 mL of a 25 mg mL^{-1} solution of fast blue BB salt (Sigma Chemical Co., St. Louis, MO) in water and 2 mL of a 5 mg mL^{-1} solution of 1-naphthyl acetate (Sigma) in ethanol were added. The staining solution was agitated for 1 h at room temperature, after which banding patterns of unknowns were compared with that of the AF-4. Two separate cultures were tested for each isolate.

Autoinoculation

Autoinoculator design #1 (Vega *et al.*, 1995) was used for all studies, but varied in size from 5 cm (1993) to 10 cm in diameter (1994–1997). The wider diameter design allowed us to use larger bait containers that did not have to be replenished during the release period. Pheromone and coattractant baits were used as described previously (Vega *et al.*, 1995), and included the pheromone and coattractants for *C. lugubris* (Bartelt, 1997). The autoinoculator was inspected weekly so that from 250 to 500 mg of dry conidia could be maintained in the release cup. Dilution plating studies indicated no significant loss in viability during the release period. In 1993, eight autoinoculators were used randomly across the research site and spaced 30–200 m apart but, from 1994 to 1997, a single autoinoculator was used so that distance of dispersal from a single point source could be monitored.

Initially, the autoinoculators were placed in the field immediately after corn harvest, which varied somewhat from year to year but was typically early to mid October. Relatively low recovery rates of *B. bassiana* and temperature limited dispersal of sap beetles (which are typically inactive below 15°C) prompted a change in placement time of the autoinoculator to the first week in October in 1995–1997. Dispersal from the autoinoculator was monitored with two traps from 100 m (1994) to 32 m (1995–1997) north and south of the autoinoculator (the prevailing wind direction) for a weather dependent number of weeks. Poor recovery of *B. bassiana* in the fall of 1994 prompted the relocation of the traps to better determine if beetles were acquiring the fungus. The overwintering traps were put out at the same time as the monitoring traps in 1994 but, due to concerns of interference, they were placed out 2 weeks later. Due to low recovery of *B. bassiana* from overwintering traps in 1996–1997, they were again placed in the field at the same time as the monitoring traps in the fall of 1997. An additional set of traps was set out in 1997 that were 400 m from the autoinoculator to check the potential range of dispersal. Beetles were collected from monitoring traps twice a week when maximum daily temperatures were generally above 15°C. In contrast, when maximum daily temperatures were generally below 15°C, beetles were collected a few days after a daily temperature above 15°C was reached. Traps were removed after a 2-week period with temperatures below 15°C.

Overwintering Traps

From 1994 to 1997, overwintering traps were set up for sap beetles. These were designed to determine whether sap beetles carrying *B. bassiana* would move to an overwintering site and to detect the beetles which may be killed by the pathogen during overwintering in natural sites and thus not be detectable in the spring. In 1994 and 1995 a 'ground' trap was used. The ground trap was made of two pieces of 20-cm long, 10-cm diameter PVC sanitary pipe. Triangular sections of ca 1.5 cm were removed from one end of the base section so that 'teeth' were created that could cut through roots of surface vegetation when the pipe was rotated back and forth while being pushed into the ground. The other end was covered with 80-mesh saran. The center 3-cm of saran was removed and a funnel was cemented to the remaining screen with hot melt glue. The tip of the funnel pointed towards the opposite end (downwards). The same attractant composition used in the fall traps and autoinoculators was placed under the pipe section and the pipe was pushed into the ground until the tip of the funnel was 1 cm above the ground. The second piece of pipe had a disk of coarse aluminum window screen glued over one end, and the other end was taped to the pipe section in the soil using duct tape. The second pipe section was used to help avoid a disruption of the 'trap' by small vertebrates.

Because numbers of the main target, *C. lugubris*, were relatively low compared to other sap beetle species (predominately *Colopterus truncatus* (Randall)) when recovered in the spring in 1995 and 1996, a different trap designed to mimic a stump was used in the fall of 1996–1997. Surveys of *C. lugubris* in early spring of 1995 indicated an association between relatively high numbers of *C. lugubris* and other sap beetle captures when traps were near trees with tree holes, suggesting the *C. lugubris* were using these sites to overwinter. The overwintering 'stumps' were constructed of 14-L dark-colored snap seal plastic garbage cans. A number of 1-cm diameter holes were drilled in the bottom approximately 5 cm apart. A 5-cm diameter hole was cut in the side 10 cm from the top. A modified trap was inserted from the inside so that the trap entrance was flush with the upper hole in the can, and the trap was baited with the standard baits. A 1-m long, 5-cm diameter flat hose was secured to the 'collecting tube' (see Dowd *et al.*, 1992) and clamped shut at the other end using a binder clip. A 12-gauge wire was run through the center of the hose so it would remain partly open. Plastic drain hose was used in 1996, and linen fire hose was used in 1997. The fire hose was used in 1996 because of its ability to 'breathe' compared to the plastic hose. The distal end of the hose was placed at the bottom of the can, and straw was packed around the hose and trap. The bottom of the can was buried 20 cm in the ground.

From 1994 to 1997, both types of overwintering traps were arranged three each along an axis perpendicular to each other with the autoinoculator at the center of the axis. A line of three traps radiated in each direction; at 16, 32 and 64 m from the autoinoculator. The overwintering traps were set up in early to mid October in 1994–1996. Because numbers of *C. lugubris* infected with *B. bassiana* were still relatively low in 1996 compared to fall traps, the overwintering traps were set out at the same time as the monitoring traps in 1997 to extend the autoinoculative exposure period. Overwintering traps were 'harvested' in mid March. The soil enclosed by the ground traps was lifted out, placed in plastic bags, and later sorted for sap beetles. The hose from the stump traps was split and sap beetles were removed. Beetles were held for *B. bassiana* infection assessment as described previously.

Spring monitoring traps were placed around the perimeter of the 16-ha research site as described previously (Dowd *et al.*, 1998). The number of traps used varied during different years, but at least 16 traps were used each year. Approximately the same number of traps were used along the three sides of the research area that bordered woodlands. Traps were generally monitored from mid March through early June. Past surveys had indicated *B. bassiana* was relatively rare in sap beetles after this time. The next sap beetle generation for *C. lugubris* also emerges during late June (Dowd & Nelsen, 1994) so capture after this period would include sap beetles other than those emerging from overwintering. Traps were

harvested within 4 days of the time a daily maximum temperature was 15°C, with a maximum of two collections per week. A maximum of 10 *C. lugubris* per trap were individually caged on 3% water agar. Up to 100 of the remaining live sap beetles from the trap were caged with artificial diet (Dowd, 1987). Beetles were held and examined for colonization by *B. bassiana* as described earlier in the Materials and Methods.

Statistical Analysis

Year to year differences in the percentage infection by the released strain of *B. bassiana* and location effects for recovery of sap beetles from overwintering traps were analyzed for statistical significance using PROC FREQ χ^2 analysis (SAS, 1987). The log likelihood ratio version of the χ^2 statistic was used when values were less than 5.

RESULTS

Fall Releases

The released strain of *B. bassiana*, AF-4, was recovered in fairly high frequency in the fall over multiple years (Table 1), although percentage recovery was dependent on year, species and collection date. Only a few *B. bassiana* contaminated insects were recovered in the fall of 1994, and no strains could be isolated in pure culture for strain determination. In 1995, no *B. bassiana* contaminated insects were collected prior to autoinoculator placement or shortly thereafter. After the autoinoculator was placed in the field, rates of recovery of *B. bassiana* from *C. lugubris* were greater than 20% on three of five sample dates, and all of the *B. bassiana* recovered was AF-4. AF-4 was also recovered frequently from *C. freemani*. In 1996, although fewer sap beetles were collected, a higher percentage of sap beetles were contaminated with *B. bassiana*, and all *B. bassiana* isolates were AF-4. The predominant species trapped were *C. lugubris* and *C. antiquus*. In 1997, the recovery of *B. bassiana* from the traps near the autoinoculator occurred over a combined wider range of sample dates (6) and in higher numbers than other years. Although the number of sap beetles collected was higher in 1997 (167 *C. lugubris*, 156 other sap beetle species) than 1996 (23 *C. lugubris*, 11 other sap beetle species), the percentage of insects contaminated by *B. bassiana* was lower. In 1997, the recovery rates of AF-4 among the isolated strains ranged from 87 to 100%. *C. lugubris* and *C. freemani* were encountered at about a 10 times greater frequency than the other species collected and were the only *B. bassiana*-infected sap beetles. The percentage of sap beetles that were carrying AF-4 in the fall was significantly higher in 1996 compared to

TABLE 1. Total incidence of *B. bassiana* in sap beetles collected during fall release

Number of dates	Traps	Sap beetle species	Beetles trapped	% Beetles with BB	%BB isolated	%BB AF4
1994, 4	2	*C. lugubris*	8	37.5	0.0	–
		Other SB	13	7.7	0.0	–
1995, 6*	2	*C. lugubris*	151	4.0	83.3	100.0
		Other SB	212	2.8	100.0	100.0
1996, 3	2	*C. lugubris*	23	100.0	69.6	100.0
		Other SB	11	91.7	100.0	100.0
1997, 6	2, far	*C. lugubris*	384	2.1	50.0	50.0
		Other SB	6	0.0	–	–
	2, near	*C. lugubris*	169	15.0	55.5	86.7
		Other SB	156	3.8	16.7	100.0

BB, *Beauveria bassiana*; SB, sap beetles. Only traps where sap beetles were captured are reported.
*After the autoinoculator was set up.

1995 and 1997 for both *C. lugubris* alone ($P < 0.001$, $\chi^2 = 82.56$ and $P < 0.001$, $\chi^2 = 44.65$, respectively) as well as for other sap beetles species ($P < 0.001$, $\chi^2 = 53.82$ and $P = 0.013$, $\chi^2 = 6.23$, respectively). A significantly greater number of *C. lugubris* compared to the other sap beetle species carried AF-4 in the fall of 1997.

Overwintering Traps

The percentage of beetles that carried AF-4 was lower in the overwintering traps than for the fall traps. In general, few sap beetles were recovered from traps placed in corn fields compared with traps placed along fence rows or in woods. In the overwintering traps, the most frequently encountered species depended on the year, with *Colopterus truncatus* (Randall) most common in 1995 and 1996, and *C. lugubris* and/or *Carpophilus antiquus* Melsheimer most common in 1997 and 1998. Although the incidence of *B. bassiana* in sap beetles was low in all cases in 1995, AF-4 was present in *C. truncatus* collected from three of 12 traps, up to 32 m from the autoinoculator. Of the *B. bassiana* isolated from infected sap beetles, 42.9% was AF-4 (Table 2). In 1996, the incidence of *B. bassiana* from sap beetles collected from overwintering traps was again low, and 28.6% was AF-4. In 1997, *B. bassiana* was recovered primarily from *C. lugubris* and *C. antiquus*, but also from *G. quadrisignatus* and *C. truncatus*. AF-4 comprised 50.0% of the *B. bassiana* isolated from species other than *C. lugubris*. The trap with the greatest number of contaminated individuals was 64 m from the autoinoculator. Recovery of AF-4 from isolated strains in 1998 ranged from 83.3% (*C. lugubris*) to 100% (other species). Most of the *B. bassiana* was recovered from *C. antiquus*, followed by *C. lugubris* and *C. truncatus*. Most of the infected *C. antiquus* and *C. lugubris* individuals were recovered from a single trap. Infected sap beetles other than *C. lugubris* were recovered from traps up to 32 m away from the autoinoculator. The only cases of a significantly higher percentage of AF-4 than indigenous *B. bassiana* isolates were in 1998 compared to 1996 for *C. lugubris* ($P = 0.004$, $\chi^2 = 8.282$) and 1998 compared to 1995–1997 for the non-*C. lugubris* sap beetles ($P < 0.001$, $\chi^2 = 15.56$; $P < 0.001$, $\chi^2 = 20.64$; $P = 0.002$, $\chi^2 = 9.53$) obtained from overwintering traps. A significantly greater percentage of *B. bassiana* isolates were AF-4 in 1998 for the non-*C. lugubris* compared to *C. lugubris* sap beetles ($P = 0.024$, $\chi^2 = 5.08$). Some significant location effects were noted for beetles collected from overwintering traps in 1998, but this may be due to the relatively high number of infected individuals of all sap beetles collected from a single trap 16 m from the

TABLE 2. Total incidence of *B. bassiana* in sap beetles collected from overwintering traps

Traps with *Beauveria*	Sap beetle species	Beetles trapped	% Beetles with BB	% BB Isolated	% BB Isolated that was AF4
1995					
0	*C. lugubris*	0	–		
5	Other SB	100	9.0	100.0	42.9
1996					
3	*C. lugubris*	46	8.7	0.0	–
5	Other SB	327	2.7	87.5	28.6
1997					
1	*C. lugubris*	5	60.0	33.3	0.0
4	Other SB	37	35.1	21.6	50.0
1998					
6	*C. lugubris*	224	7.1	75.0	83.3
7	Other SB	251	14.3	72.2	100.0

BB, *Beauveria bassiana*; SB, sap beetles. Three of the 1997 traps were destroyed by vertebrates.

autoinoculator. The number of *C. lugubris* ($P = 0.002$, $\chi^2 = 9.76$ and $P = 0.005$, $\chi^2 = 7.79$, respectively) and non-*C. lugubris* ($P = 0.003$, $\chi^2 = 9.04$ and $P < 0.001$, $\chi^2 = 14.91$, respectively) sap beetles contaminated by AF-4 was significantly higher for individuals collected from overwintering traps 16 m from the autoinoculator compared to traps 32 and 64 m from the autoinoculator. Only a few individual insects other that sap beetles (mainly dung flies, Diptera: Anthomyiidae: Scatophaginae) were also found in the overwintering traps over the 4-year period.

Spring Hanging Traps

B. bassiana was recovered from sap beetles collected from hanging traps put up in the spring in several years. Although as many as 50% of the sap beetles captured on specific dates carried *B. bassiana*, none of the isolated strains could be positively confirmed as AF-4. The number of *B. bassiana* isolates from beetles captured in the spring hanging traps in 1994, 1995, 1996, 1997, 1998 and 1999 (no release made in the fall of 1998) examined were 0, 0, 2, 139, 51 and 47, respectively. Per trap captures of sap beetles in spring hanging traps did not decrease during the period of time the autoinoculator was used, although winters were relatively mild for the area.

DISCUSSION

B. bassiana has shown potential as a commercial control agent, and is registered for insects such as Homoptera (Shah & Goettel, 1999). Undesirable nontarget effects of *B. bassiana* have been reported. When an aphid derived strain of *B. bassiana* was applied to alfalfa fields, it caused very high mortality of *Hippodamia convergens* early in the season (James *et al.*, 1995b). However, a strain applied to control *O. nubilalis* in corn fields did not adversely affect *Coleomegilla maculata* DeGeer (Pingel & Lewis, 1996). In our studies, where we were targeting overwintering populations, we found very few nontarget insects associated with the *B. bassiana*-infected sap beetles. Application to overwintering populations through autoinoculative means appears to be a more selective alternative, and may be a preferable application strategy when nontarget beneficial insects are present in crops. The strain we examined (AF-4) was reisolated from sap beetles collected from traps both in the fall during release, and from sap beetles collected from overwintering traps in the spring, but not from sap beetles collected from hanging traps in the spring.

It is not clear why none of the applied strain could be isolated from free flying field populations in the spring. It is possible all *B. bassiana*-infected sap beetles were killed at natural overwintering sites. Although soil, rotten logs, and tree stumps were examined in the area, no naturally overwintering sap beetles were found. Again, this would point to the more selective nature of infection by the autoinoculator released strain when performed in the fall.

Based on mean numbers of beetles collected in hanging traps in the spring, we did not observe obvious population reductions over the period of time *B. bassiana* was used in the autoinoculator. Past studies of sap beetles have indicated high winter mortality of those overwintering in the soil and crop refuse (Connell, 1956). However some adults can successfully pass the winter in soil and in protected places above ground (Connell, 1956), and adults have been found early in the spring in soil at the base of dead elms (Sanford, 1963). However, a relatively large percentage of sap beetles collected from the overwintering stump traps were contaminated with *B. bassiana* compared to our past studies. It is possible a larger proportion of sap beetles overwintered successfully as individuals in the soil during the mild winters that occurred during this study, than might have occurred under normal overwintering conditions.

Dispersal of AF-4 from the autoinoculator in the present study ranged up to 64 m to overwintering traps (greatest distance examined), but in the one year we tested dispersal in the fall, a few AF-4-infected insects were found 300 m away. Greater numbers tended to

occur in overwintering traps placed along or in woodlands. Prior reports of dispersal of *B. subtilis* and/or a blue tracking dye in this area ranged up to several hundred meters (Vega *et al.*, 1995; Dowd *et al.*, 1998).

Overwintering trap design also appeared to influence sap beetle species preponderance. The incidence of AF-4 in different sap beetle species reflected the species composition in the overwintering traps. Ground traps collected primarily *C. truncatus*, which is a potential vector of oak wilt *Ceratocystis fagacearum* (Bretz) Hunt (e.g., Juzwik & French, 1983). The pheromone for this sap beetle species has recently been identified (Cosse' & Bartelt, 2000), and might be used in an autoinoculation strategy. 'Stump'-like overwintering traps collected a higher percentage of *C. lugubris*, especially in 1998. This information suggests that *C. lugubris* may prefer to overwinter above ground when sites are available. This information supports prior observations that numbers of *C. lugubris* collected early in the spring tended to be greater if trees bearing treeholes were near traps (Dowd, unpublished). Certainly, treeholes would be a more stable and reliable overwintering site than ground debris. The early and sustained cold spell in the fall of 1996 appeared to limit sap beetle dispersal into traps compared to that of 1997, as indicated by the numbers of sap beetles obtained in the spring of 1997 versus 1998. In addition, the best recovery of AF-4 from overwintering traps occurred in 1998, which probably reflected the longer period of time the autoinoculator was out in the field when overwintering traps were available compared to the other years, and the larger numbers of sap beetles that were contaminated in the fall.

We have demonstrated that an autoinoculative device can be used to deliver *B. bassiana* to sap beetles and that we could successfully target overwintering populations. Very few other nontarget insects were present, and thus likely to be affected, by the released fungus. Delivery in this manner avoids the cost of conventional application equipment. For sap beetles, this control measure would likely be most effective in severe winters where only populations overwintering in groups in locations such as tree holes would be likely to survive winter kill due to low temperatures. This technique may also be applicable to other insects that overwinter in groups and can be attracted to volatiles.

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