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Dissemination of Microbial Agents Using an Autoinoculating Device and Several Insect Species as Vectors

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A device for autoinoculating insects with marker dye and active agents, such as biocompetitors of plant pathogens (e.g., *Trichoderma harzianum, Trichoderma polysporum, Bacillus subtilis*) and entomopathogens (e.g., *Bacillus thuringiensis, Beauveria bassiana*) is described. Laboratory tests using a powdered dye indicated that the device worked for sap beetles, house flies, pomace flies, and moths. Quantitative studies with blue dye (as an indicator of dispersal) and some bioactive agents demonstrated that a high percentage of sap beetles became contaminated with the material placed inside the autoinoculator in a short period of time (minutes). In the laboratory, sap beetles carried *B. bassiana* from the autoinoculator to unexposed sap beetles, causing high mortality. In the field, sap beetles entered the baited traps attached to the autoinoculator, became contaminated with the dye, and carried it to damaged corn or apples.

KEY WORDS: ***Carpophilus lugubris; Aspergillus flavus; Trichoderma harzianum; Trichoderma polysporum; Bacillus subtilis; Bacillus thuringiensis; Beauveria bassiana;* sap beetles, biological control, biocompetitors.**

INTRODUCTION

In the biological control of some plant diseases, it is difficult to effectively deliver a competitive biological control agent or "biocompetitor" to the targeted area. A case in point is corn contaminated with the toxigenic fungus, *Aspergillus flavus* Link: Fr., which can be mechanically transmitted by sap beetles. Practical delivery of a biocompetitor to the damaged area of the corn ear remains a challenge because conventional application techniques are typically not cost-effective and damaged kernels are often hidden.

One possible mechanism for targeted delivery is through the use of insects. Contaminating insects and having them spread a material have been reported for insect pathogens (McLaughlin *et al.*, 1969; Shapas *et al.*, 1977; Jackson *et al.*, 1992; Pell *et al.*, 1993; Vail *et al.*, 1993; Lacey *et al.*, 1994) and biocompetitors of plant pathogens [e.g., *Botrytis* (Peng *et al.*, 1992) and *Erwinia* (Thomson *et al.*, 1992; Johnson *et al.*, 1993)].

Sap beetles are best known as mechanical vectors of several different plant pathogens, including fungi that produce mycotoxins (see Dowd, 1991). The dusky sap beetle, *Carpophilus lugubris* Murray (Coleoptera: Nitidulidae), the most common sap beetle in developing corn in the United States, is efficient in locating damaged corn (Connell, 1956; Sanford and Luckmann, 1963). Thus, this insect could serve as a specific vector of biocompetitors to damaged corn, provided there is an effective way to contaminate the beetle. However, no field-based inoculating devices useful for beetles under field conditions have been reported.

To test the feasibility of having sap beetles vector biocompetitive agents to corn as a means of controlling *A. flavus,* it was first necessary to design and field test an "autoinoculative" device. Here, we report on two new devices that permit sap beetles and other insects to become contaminated with a diverse array of materials and then carry them to a desired location. We refer to this process as "autoinoculation" and to the devices as "autoinoculators." Experiments were conducted to test the efficacy of these devices to contaminate insects with an indicator dye and with microbial organisms and subsequently transfer them to the target site.

MATERIALS AND METHODS

The autoinoculating device. Two types of autoinoculator devices were tested (Fig. 1). The first type (Fig. 1a) consisted of a 120-ml (4 oz) polypropylene cup (Baxter Healthcare Corp., McGraw Park, IL) with eight 0.7-cm-diameter holes evenly spaced around the lower circumference and located 0.5 cm from the bottom. Eight 1.5-cm-long plastic tubes with an exit hole diameter of

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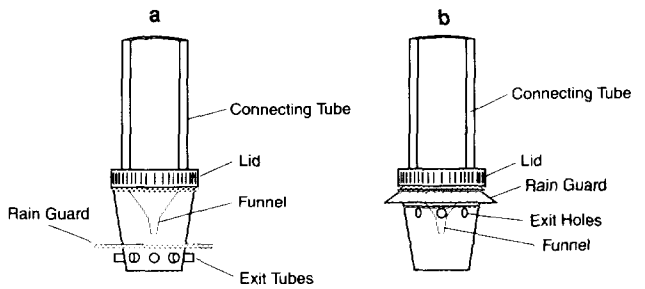

FIG. 1. Devices used to autoinoculate insects. Diameter of connecting tube is 5 cm.

0.4 cm were inserted through the holes flush with the inside wall of the cup. A rain guard (0.3 cm thick, 15.5 × 15 cm polyethylene; McMaster-Carr Co., Chicago, IL) was placed immediately above the eight exit tubes.

The second type of autoinoculator (Fig. 1b) consisted of a polypropylene cup circled near the top with eight evenly spaced 0.6-cm-diameter circular exit holes made with a cork borer. A funnel through which the insects entered the autoinoculator went through the center of a plastic barrier that was flush with the upper margin of the exit holes (the funnel is part of a connecting tube attached to the baited trap; see below). This barrier impeded insects from becoming trapped in the upper portion of the autoinoculator, forcing them to find the exit holes. A circular rain guard 2.5 cm wide, from the edge of the cup to the edge of the guard and made of 1 mm thick plastic, was placed on the exterior surface of the device 5 cm above the exit holes to prevent rain from entering through the exit holes. Hot melted glue was used to cement all parts in place.

The autoinoculator device was screwed onto a lid, which was in turn attached to a connecting tube containing the funnel. The entire unit was then attached to the underside (Fig. 2) of a baited insect trap (Dowd *et al.*, 1992), but other vertical drop traps could be used after suitable modifications. Insects entering the baited trap would drop into the autoinoculator and become contaminated with the agent placed inside. The escape response eventually directed the insect to one of the exit holes.

Laboratory efficacy studies with powdered dye. A fine brilliant blue dye powder (Coomassie brilliant blue R250, Sigma Chemical Co., St. Louis, MO) was used in lieu of an active agent. The dye facilitated observations on potential insect dispersal of powdered materials placed inside the autoinoculators. The dusky sap beetle, *C. lugubris,* was used throughout most of the experiments. Unless otherwise specified, experiments were conducted at room temperature (22 ± 2°C).

The first series of experiments were designed to determine the amount of dye that contaminated the sap beetles and the number of sap beetles that would exit the two types of autoinoculators over a discrete interval of time. Twenty-five sap beetles were introduced through the funnel into an autoinoculator containing 100 mg of dye, using four replicates (i.e., autoinoculators). The eight exit tubes in the first type of autoinoculator had 1-cm caps (with precut holes) glued over them so that 0.5-dr vials could be attached. As a sap beetle entered a vial, the vial was removed, capped, and replaced with a new vial. The experiment was conducted in the laboratory, terminated after 90 min, and repeated three times. For the second type of autoinoculator, beetles were captured with clean forceps after they exited the trap and were placed in vials. The experiment was repeated two times and terminated after 90 min. The dye carried into each vial was dissolved in 1 ml of deionized water and quantified using a standard curve based on spectrophotometer readings at 560 nm (Perkin–Elmer Lambda 4b UV/VIS).

To determine the number of sap beetles exiting an autoinoculator in 1 h, 100 sap beetles starved for 24 h were introduced into the autoinoculator, which was placed in a wind tunnel (2.5 × 1.1 × 1.1 m) with a wind velocity of 0.4 km/h. The autoinoculator was hung 20 cm from the wind source and 60 cm from the bottom of the wind tunnel. At 1 h, the number of insects that had exited was determined by counting those remaining inside the autoinoculator. The experiment was repeated five times for each type of autoinoculator.

Additional experiments were conducted to determine if the autoinoculator would also be useful with other

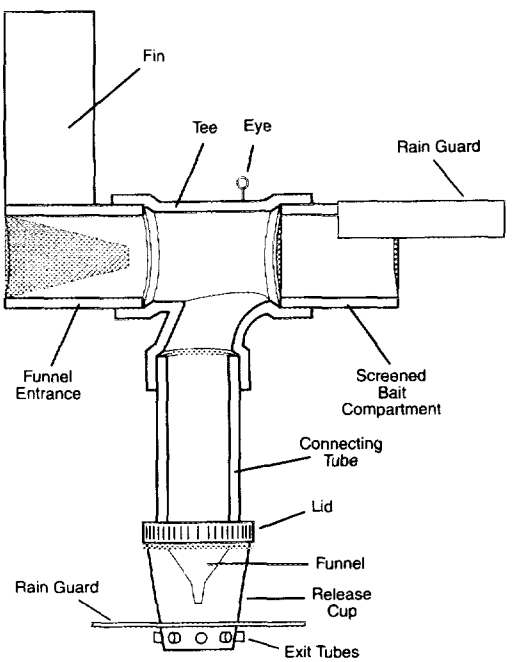

FIG. 2. Autoinoculator device connected to a vertical drop trap (Dowd *et al.*, 1992). Diameter of connecting tube is 5 cm.

types of insects, such as flies and moths. Preliminary tests with these insects indicated that the second type of autoinoculator was more useful, due to flight behavior of these insects (i.e., tendency to immediately move upward once inside the device). To determine the number of adult flies (*Musca domestica* L.; obtained as pupae from Carolina Biological Supply, Burlington, NC) that exited the autoinoculator in 1 h and the percentage carrying dye, three autoinoculators were used, each containing 100 mg of dye. Flies were placed in a freezer for 5 min (to facilitate handling) and 20 were introduced into three separate autoinoculators, each containing 100 mg of dye. Autoinoculators were placed inside plastic bags (25 × 40 cm) to capture exiting flies. The experiment ran for 1 h, after which the bag was placed in the freezer. Flies outside the autoinoculator were counted and placed individually in vials to which 1 ml of water was added to visually determine whether dye had been collected. Similar studies were conducted with pomace flies [*Drosophila melanogaster* (Meigen)] obtained from Carolina Biological Supply. The experimental setup was the same as that just described, except that the number of pomace flies placed in each cup was 30. The experiment was repeated three times, using three replicates (autoinoculators) each time. To determine whether pomace flies had become contaminated those that had exited in one of the trials (with three replicates) were individually placed in a piece of white tissue paper and a drop of water was placed over each insect. A blue color indicated that the insect was carrying the dye. The amount of dye carried by flies was not determined.

For fall armyworm moths [*Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae)], 15 one-week-old moths from laboratory colonies were introduced through a funnel with a 0.9-cm opening into each of three separate autoinoculators containing 250 mg of dye (funnel opening in all previous experiments was 0.6 cm). The exit holes were widened (to allow moths to escape), resulting in rectangular openings ca. 0.8 cm wide and 1 cm high. The autoinoculator and funnel attachment were placed inside individual plastic bags for 1 h, after which the plastic bag was placed in the freezer. The number of moths that had exited the autoinoculator was determined, and the presence of the blue dye was detected as described for pomace flies.

Laboratory efficacy studies with microorganisms (using the first type of autoinoculator). The first series of experiments were designed to determine how many sap beetles exit in 1 h from autoinoculators containing different microorganisms in a powder formulation. Two fungal biocompetitors, *Trichoderma harzianum* Rifai and *Trichoderma polysporum* (Link: Persoon) Rifai (minimum 4.5×10^{10} colony forming units (CFU)/454 g; marketed as Binab T by Binab USA, Inc., Madison, WI), and a concentrated formulation of *Bacillus subtilis* Ehrenberg (Cohn) (2×10^{11} CFU/g; marketed as Kodiak by Gustafson, Inc., Dallas, TX) were used. Powdered formulations of *Bacillus thuringiensis* Berliner subsp. *kurstaki* (32×10^3 international units of potency/mg; marketed as Dipel 2X by Abbott Laboratories, North Chicago, IL) and *Beauveria bassiana* (Balsamo) Vuillemin (3.1×10^{10} CFU/g; Abbott Laboratories) were also used. Twenty-five sap beetles were released in autoinoculators containing 100 mg of each formulation, using three replicates (autoinoculators) per treatment. Experiments were conducted in the laboratory.

A second series of laboratory experiments were designed to determine the amount of powder formulation carried by sap beetles as they exit the autoinoculator. Formulations used were the same as just described. Forty dusky sap beetles were released in each autoinoculator containing 100 mg of the powdered formulation (one autoinoculator per treatment), and the first 15 beetles exiting the autoinoculator were collected in vials. Nineteen beetles were used for *B. subtilis*. The amount of formulation carried into the vial was determined as follows: 1 ml of 0.01% Triton X (wetting agent) was added to the vial containing the insect and vortexed for 5 s to dislodge the powdered formulation from the beetle. The suspension was then transferred to a preweighed vial and placed in an oven for 24 h, and the vial was reweighed. The difference in weight was the amount carried by the insect. Weights were corrected using the mean weight obtained from 10 blank vials containing Triton X and insects that had not been exposed to any powdered formulations.

The final series of experiments were practical demonstrations in the use of the autoinoculator to determine whether the exiting sap beetles could carry the fungal entomopathogen, *B. bassiana,* to entomopathogen-free sap beetles and to assess the mortality on these insects. The experiment was conducted using an 80 × 40 × 50 cm acrylic cage. A 3-cm-wide intake hole in the lower center part of the acrylic wall allowed air to circulate through the cage and exit by suction (5.1 mm Hg) through a tube attached at the opposite end of the cage. In the upwind end of the cage, a funnel was attached to a cup containing the insect's pheromone and host attractant complement (see below). This arrangement resulted in an odor-bearing air current originating in the baited end of the cage and moving to the opposite end of the cage (verified using smoke).

All sap beetles came from colonies maintained in the laboratory. A group of beetles was marked using Testors 1146 silver paint (Testor Corporation, Rockford, IL) on the elytra. A preliminary experiment indicated that painting the elytra did not cause any mortality when compared to unpainted sap beetles. In the first set of experiments, 10 sap beetles were released into a

funnel connected to the autoinoculator. A sporulating culture of *B. bassiana* growing in potato dextrose agar within a 35 × 10 mm petri dish was placed inside the autoinoculator. The *Beauveria* strain, GQ 420503-2 (deposited in the USDA, ARS, NCAUR culture collection as NRRL 22864), originated from *Glishrochilus quadrisignatus* (Say) (Coleoptera: Nitidulidae), collected in field-baited traps. In the upwind end of the cage, 10 marked sap beetles were introduced inside the cup containing the bait. Beetles exiting the autoinoculator followed the odor current to its source where they came in contact with the marked sap beetles in the bait cup. In the second set of experiments, 20 sap beetles were released into the funnel of the autoinoculator. A powder formulation of *B. bassiana* (250 mg of strain AF4; Ciba–Geigy, Vero Beach, FL) was used in the autoinoculator cup. The rest of the experiment was performed as in the first experiment.

Each experiment was repeated three times for each *B. bassiana* strain. For each repetition, the baited cup was replaced with a clean cup and a fresh set of attractants and pheromone-containing septa; this was done to avoid carryover of the entomopathogen from one trial to the next. The controls consisted of 10 sap beetles released into an autoinoculator not containing the entomopathogen, collected, and placed in 100 × 15 mm petri dishes with moistened No. 54 Whatman filter paper (2 ml water/dish). There were three replicates for the control. The moistened chamber (i.e., petri dish and filter paper) provides conditions suitable for *Beauveria* growth, therefore allowing us to assess whether the source of insects used in the experiments might already have been contaminated with an entomopathogen prior to starting the experiments. Once the experiments were terminated (2.5 h after initiation), sap beetles inside the baited cups were transferred to petri dishes with moistened filter paper as described above. All dishes were kept at room temperature. The variables recorded were: (1) the number of unmarked sap beetles exiting the autoinoculator that entered the baited cup in 2.5 h; (2) the percentage of mortality of marked beetles in the baited cup, and (3) *B. bassiana* growth on cadavers for up to 17 days following experiment initiation.

Field tests. For field experiments (University of Illinois, Illinois Valley Sand Field Experiment Station located near Kilbourne, IL), the first type of autoinoculator (Fig. 1a) was attached to a baited trap (Dowd *et al.*, 1992). The bait consisted of a rubber septum impregnated with 500 μg of the *C. lugubris* pheromone (Bartelt *et al.*, 1993) and four individual attractants: apple cider vinegar, 100% ethyl acetate, 100% ethanol, and 97% propanal (Bartelt *et al.*, 1992; Dowd *et al.*, 1992). Twenty milliliters of apple cider vinegar (H. J. Heinz Co., Pittsburgh, PA) was absorbed into 1.5 g of polyacrylamide gel aggregate (Hydrosource, Western Polyacrylamide Inc., Castle Rock, CO) and held in a 20-ml vial covered with a 30 thread/cm cloth. The remaining attractants were individually placed in 5-ml glass vials and released through the appropriate membrane (see below) supported by a 30 × 30 mesh stainless steel screen (McMaster-Carr, Chicago, IL) screwed into a lid with a precut 0.9-cm hole (Pierce, Rockford, IL). For ethyl acetate, the membrane was a 1.5-mm-thick rubber gasket material; for ethanol, 3-mm-thick balsa wood; and for propanal, 1.5-mm-thick high-density silicone rubber (McMaster-Carr). All of these releasers give reliable and consistent rates of release for at least 3 weeks (Dowd and Bartelt, unpublished data). For all field experiments, the attractants in the vials were checked weekly and refilled as necessary. Septa were replaced after 4 weeks.

The first experiment was designed to determine the amount of dye carried by sap beetles into vials attached to the autoinoculator. One hundred milligrams of dye was placed in each of 12 autoinoculators connected to baited traps in the field. The autoinoculators were located at the edge of corn plots, thus allowing sap beetles to enter the trap under natural conditions. The eight exit tubes in each autoinoculator were capped with vials to prevent sap beetles from leaving; therefore, sap beetles could enter the vial, go back into the autoinoculator, and then back into the vial. This allows sap beetles to come in contact with dye several times. The amount of dye in each vial was determined using a spectrophotometer as previously described. The experiment was conducted on two dates, each with 12 replicates.

An additional series of experiments was designed to determine if dye in the autoinoculator was carried to damaged crops by sap beetles. The first experiment, initiated on August 11, 1992, involved corn and was conducted on eight different dates spaced 3–4 days apart with 12 replicates on each date. For each replicate, one milk stage corn ear ca. 6 m away from the autoinoculator (located at the edge of corn plots) was damaged with pruning shears by cutting three times on different locations with the end of the blade. This was done to simulate insect damage which increases the corn ear attractiveness to sap beetles. Due to the low sap beetle mobility levels in the test plot area, 25 beetles were placed in each autoinoculator on each sampling date. The dye was replaced with 100 mg of fresh dye on each sampling date. Sap beetles in the field were also able to enter the autoinoculator and exit on their own accord. On August 25, 1992, a pheromone-containing septum was placed in each damaged ear. This was done to increase the chances for sap beetles that had come in contact with dye to find the damaged ear. Placement of a pheromone septum mimics natural situations in which sap beetles aggregate in damaged

ears, producing pheromones. Ears were harvested 3–4 days after being damaged, taken to the laboratory, and examined under the stereoscope to assess whether the dye was present.

After the initial series of experiments was completed, suitable corn attractive to the sap beetles was no longer naturally available due to the lateness of the season, so corn ears or apple pieces (for a fruit comparison) were placed in the entry portion area of unbaited traps (Dowd *et al.*, 1992), located ca. 2.5–3 m from the autoinoculator. Sweet corn ears were split longitudinally (6 cm long) and the kernels were damaged with pruning shears as previously described. The experiment was conducted on eight different dates, each with 12 replicates. On September 8 and 11, 1992, 75 and 50 sap beetles, respectively, were introduced into each autoinoculator containing 100 mg dye/25 sap beetles introduced. On September 15, no beetles were introduced in the autoinoculator (which contained 500 mg dye) to simulate field conditions where sap beetles will have to enter and exit the trap on their own. During the remainder of the study, flying beetles from the natural population were abundant. This flight activity coincided with the drying of the field corn (rendering it unsuitable for the beetles) and with the emergence of a new generation of adults from the ground. On October 2, 1992, and for 5 consecutive weeks, pieces of apples with three 1-cm circular holes (made to increase attraction to sap beetles) were placed in the unbaited traps adjacent to the autoinoculator.

RESULTS

In the laboratory test with the first type of autoinoculator, 31 ± 1.6% (mean ± SE) of the introduced sap beetles exited within 90 min, carrying 0.26 ± 0.02 mg dye/insect (n = 93). In the corresponding experiment with the second type of autoinoculator, 52 ± 8.9% of the sap beetles exited within 90 min, carrying a mean of 0.16 ± 0.02 mg dye/insect (n = 97). t Tests revealed a significantly higher ($p < 0.05$, t = 2.77) exit rate for the second type of autoinoculator, whereas the amount of dye carried was significantly higher ($p < 0.05$, t = 5.86) when the first type of autoinoculator was used. In the wind tunnel, 78 ± 2.8 and 79 ± 2.5% of the sap beetles exited the autoinoculator within 1 h for the first and second type of autoinoculator, respectively.

In 1 h, 63 ± 8.8% of the house flies emerged from the second type of autoinoculator (Fig. 1b) and 100% of the emerging flies carried dye. Of the total number of pomace flies placed in the autoinoculator, 88 ± 2.6% emerged in 1 h and 99 ± 1.3% carried dye. In 1 h, 51 ± 2.0% of the moths introduced in the autoinoculator had exited, and 100% carried dye.

Experiments with microbial agents indicated that in 1 h, 57 ± 9.3, 75 ± 19.4, 52 ± 0, and 56 ± 8.3% of the sap beetles exited the autoinoculators containing *Trichoderma* spp., *B. subtilis, B. thuringiensis,* and *B. bassiana,* respectively. Sap beetles carried 0.13 ± 0.004 mg (n = 14) of *Trichoderma* spp., 0.47 ± 0.003 mg (n = 19) of *B. subtilis,* 0.07 ± 0.003 mg (n = 15) of *B. thuringiensis,* and 0.07 ± .004 (n = 15) mg of *B. bassiana.* Sap beetles exposed to the microbial agents in the autoinoculator had an obvious powdery coating in all cases. Using the CFUs given by the manufacturer in the stock material (see experiment description), the mean amount of formulation carried per insect was equivalent to 1.3×10^3 CFUs of *Trichoderma* spp. (Binab), 9.4×10^7 CFUs of *B. subtilis,* and 2.2×10^6 CFUs of *B. bassiana.* t Test analyses indicated amounts of all microbial agents picked up by the sap beetles were significantly greater than zero except for *B. bassiana* at $p < 0.05$ (t = 4.24, 14.64, and 2.34 for *Trichoderma* spp., *B. subtilis,* and *B. thuringiensis,* respectively).

For experiments with the *B. bassiana* strain GQ 420503-2, 93 ± 6.7% of the sap beetles released in the autoinoculator had entered the baited cup containing the marked beetles in 2.5 h. Mean mortality of marked sap beetles was 11 ± 0, 37 ± 3.7, and 82 ± 3.7% at days 5, 10, and 15, respectively, after experiment initiation. On Day 17, 78 ± 6.4% of the marked sap beetle cadavers showed *B. bassiana* growth. Although 30 ± 5.8% of the control insects had died in 15 days, none exhibited fungal growth. For strain AF4, 40 ± 5.8% of the sap beetles had entered the cup containing the marked beetles in 2.5 h. On Day 12 after experiment initiation, 81 ± 11.0% of the marked beetles had died with 86 ± 2.9% of the cadavers exhibiting fungal growth. The mortality for the controls was 0%. t Test analysis indicated significantly greater mortality and presence of sporulation at $p < 0.05$ of *B. bassiana* on sap beetles exposed to the GQ 420503-2 strain (t = 22.0 and 19.97, respectively) and the AF4 strain (t = 7.35 and 29.43, respectively) at the final day of examination compared to corresponding unexposed control insects.

In the field, 17 ± 11.25% of the ears (2 ears) contained dye on the first examination date, but no ears were found with dye on the subsequent four dates the ears were examined. After pheromone septa were added to the ears, 17 ± 11.2% (2 ears), 58 ± 14.9% (7 ears), and 17 ± 11.2% (2 ears) of ears damaged contained dye on the subsequent three examination dates. The mean contamination rate for the eight date experiment was 14 ± 2.5%. Sap beetles collected in the damaged ears did not carry dye (as determined by spectrophotometer), except for 1 insect each collected on August 21 and 25 and September 3, 1992, which carried <0.05, 1.44, and <0.05 mg, respectively. On August 18, 1992, 50 ears damaged by deer and raccoons were sampled; 2 were found to contain dye. Fifty-two percent (±12.0) of the corn ears placed inside traps showed traces of dye (with a range of 8 to 100% on individual

TABLE 1

Percentage of Corn Ears and Apples Placed in the Entry Portion Area of Unbaited Traps and Containing Blue Dye Carried by Dusky Sap Beetles Exiting an Autoinoculator (Twelve Replicates per Date in 1992)

Corn ears		Apples	
Date	Percentage of ears with dye[a]	Date	Percentage of apples with dye[a]
9/08	8 ± 8 (1)	10/07	33 ± 14 (4)
9/11	50 ± 15 (6)	10/13	92 ± 8 (11)
9/15[b]	8 ± 8 (1)	10/16	100 ± 0 (12)
9/18	58 ± 15 (7)	10/20	25 ± 13 (3)
9/22	33 ± 14 (4)	10/23	25 ± 13 (3)
9/25	75 ± 13 (9)	10/27	50 ± 15 (6)
9/29	100 ± 0 (12)	10/30	33 ± 14 (4)
10/02	83 ± 11 (10)	11/03	50 ± 15 (6)
		11/06	50 ± 15 (6)
		11/10	25 ± 13 (3)

Note. Values are means ± standard errors.
[a] Number of ears or apples with dye are indicated in parentheses.
[b] Starting on this date, no sap beetles were introduced into the autoinoculators.

collection dates), whereas 48 ± 8.6% of the apples pieces contained dye (with a range of 25 to 100% on individual collection dates) (Table 1).

DISCUSSION

Effectiveness of the Devices in Laboratory and Field

Although a significantly higher percentage of sap beetles exited from the second type of autoinoculator than from the first type, the mean amount of dye carried by sap beetles was significantly higher using the first type of autoinoculator. This difference in dye quantity could be due to dye becoming dislodged from the sap beetle as it crawls upwards toward the exit hole in the second type of autoinoculator, although natural dislodgement during flight may occur in any case. In addition, the amount of dye might have been slightly underestimated for the second type of autoinoculator due to use of forceps (while collecting the insects), which might have retained some dye particles. In the wind tunnel which mimics the continuous movement of traps observed in the field and caused by the wind, the exit rates were almost identical for both types of autoinoculators. In field situations, agitation of the autoinoculator by the wind might increase sap beetle exit rates whereas personal observations indicate that entry rate is not affected by wind (based on high insect captures throughout when both calm and windy weather conditions are prevalent).

Based on dusky sap beetle captures on traps placed in the perimeter of an 18-ha farm and using a 31% exit rate from the autoinoculator, it can be argued that of the total number of sap beetles captured per week, 79, 382, 278, and 199, respectively, would have exited the autoinoculator in 90 min. These insects could be potential carriers of biological control agents. In addition, using these eight dye-free traps, we found that the 12 autoinoculators placed around the farm would contaminate enough insects so that dye could be detected in most of the baited trap cups in the perimeter. In other words, 12 traps could service a 18-ha area. Dye was detected in one trap located ca. 610 m from the closest dye-containing autoinoculator.

The autoinoculator design (Fig. 1) is important when targeting the insect to serve as a disseminator of a biocontrol agent. For example, the first type of autoinoculator (Fig. 1a) was ineffective for house flies, because they would enter the exit tube only for a brief moment and then retreat back into the cup. In addition, they had a tendency to move upward within the cup. Although not tested, the exit rate for pomace flies in the first type of autoinoculator is expected to be very low due to their tendency to immediately fly upward once inside the autoinoculator.

In the late season field experiment, we observed a high variability in the percentage of ears and apples containing dye. This could be due to extensive corn ear damage caused by raccoons and deer at that particular time, making these ears more attractive to sap beetles than those damaged with pruning shears.

Comparisons with Other Autoinoculative Techniques

Different approaches and devices have been used to contaminate insects with entomopathogens or biocompetitors of plant pathogens. Some of these devices have been designed for indoor use. For insects in grain storage facilities, open disks containing sex pheromone and spores of *Mattesia trogoderma* Canning have been used in simulated warehouse conditions for control of the beetle *Trogoderma glabrum* (Herbst) (Shapas *et al.*, 1977). Pyrex cake pans containing a granulosis virus and caged virgin female Indian meal moths [*Plodia interpunctella* (Hübner)], were used to contaminate male moths with the virus (Vail *et al.*, 1993). Other devices are petri dish-like with common exits and entrances designed for contaminating crawling insects such as cockroaches with various pathogens in lab trials (Gunner *et al.*, 1991a,b; Brefka, 1992; Chang and Gehret, 1993; Miller *et al.*, 1993). Cylindrical devices resembling inverted flower pots with large holes cut in the sides have been used to control flies in chicken coops with *Metarhizium anisopliae* (Metschnikoff) Sorokin (Miller *et al.*, 1993).

The number of techniques tested for contaminating insects with entomopathogens and biocompetitors of plant pathogens under field conditions has been more

limited. Early work involved applying viscous baits containing the protozoan pathogens *Mattesia grandis* McLaughlin and *Glugea gasti* McLaughlin to cotton with high clearance spray machinery for control of the boll weevil (McLaughlin *et al.,* 1969). More recently, when plexiglass boxes containing baffles, connected over a large wire net cone trap baited with a sex pheromone were used to contaminate tobacco budworm [*Heliothis virescens* (F.)] male moths with a baculovirus, a range of 0 to 25% mortality of larvae resulted (Jackson *et al.,* 1992). A device composed of a humidification source, multiple baffles, and a pyramidal entry/exit (which also provided some rain protection) that was baited with the sex pheromone of the diamondback moth, *Plutella xylostella* (L.), promoted dispersal of a fluorescent powder to cabbage plants in the field up to 5 m away (Pell *et al.,* 1993). Several hive-based devices have been used to contaminate honey bees (*Apis mellifera* L.) with biocompetitors of fruit pathogens, with varying degrees of effectiveness (Peng *et al.,* 1992; Israel and Boland, 1992; Thomson *et al.,* 1992; Johnson *et al.,* 1993).

In contrast to previously reported devices, our autoinoculator can be used for flying insects both indoors and in the field. It is not as restrictive as having to work with and move entire bee hives; instead, it is compact and portable. Our device appears amenable to a wide range of insects, including flies, moths, and beetles, and can be attached to traps (Dowd *et al.,* 1992; see below) that can capture an equally wide range of insects. Like other moth-based designs, a major advantage of our autoinoculator is that insects fly to the device, enter, and become contaminated with the biocontrol agent, eventually exiting and dispersing it. Field tests have indicated high rates of contamination of sap beetles drawn to the devices and long range dispersal potential. Use of autoinoculative devices with insects with aggregation pheromones, as was done in our study, may provide more effective contamination and dispersal compared to prior tests involving insect sex pheromones because both sexes are involved. Our devices are easy to handle, install, and service. The microbial agent(s) placed inside the autoinoculator is well protected from the elements. Insects entering the devices come in rapid and direct contact with the agent(s) once they enter from an adjoining attractive device.

The baited trap (Dowd *et al.,* 1992) attached to our autoinoculator makes use of specific insect pheromones and/or attractants, therefore restricting the number of insects species that are attracted to the trap. In addition, the baited trap can be modified with different size screen mesh in the entry area to restrict the size of insects entering the trap. As indicated earlier, due to its basic design, our autoinoculator has the potential of being adapted for use with a variety of other commercial traps with vertical funnel drops.

Potential Uses of Autoinoculators

We have shown that the autoinoculator device can contaminate insects such as sap beetles, fruit flies, house flies, and fall armyworm moths with materials placed in the autoinoculator. The dye confirmed that sap beetles can carry it to damaged corn and apples. Depending upon the formulation concentration, individual sap beetles can carry a high number colony forming units of different active agents such as *Trichoderma* spp., *B. bassiana,* and *B. subtilis.* Sap beetles were shown to become contaminated with the fungal entomopathogen *B. bassiana* placed in the autoinoculator and to carry it to uncontaminated sap beetles. This has important practical consequences, especially at the end of the growing season when insects start aggregating and overwintering. By placing *B. bassiana* in the autoinoculator, contaminated sap beetles should carry it to entomopathogen-free aggregating beetles in the field thereby increasing infection rates and mortality.

We suggest that a combination of biological control agents could be used simultaneously in the autoinoculator. For example, a fungal biocompetitor such as *B. subtilis* can be combined with the entomopathogen *B. thuringiensis.* Insect dispersal of *B. thuringiensis* can have dual purposes; it can be useful in pest management in the traditional way, i.e., by killing an insect after ingestion, and also as an antagonist of foliar pathogens (Spurr and Knudsen, 1982). Therefore, sap beetles can carry this organism to corn ears and stalks where it might be consumed by caterpillars and be dislodged on leaves or leaf axils, where it can potentially act as an antagonist of a foliar or stem pathogen. At the same time, insects will be carrying *B. subtilis* to damaged corn ears where it will antagonize colonization by *A. flavus,* a fungus that produces aflatoxins. We have been testing the autoinoculator as a means of preventing aflatoxin contamination in corn. Laboratory and field tests indicate that the autoinoculator can be effectively used to contaminate sap beetles with biocompetitors of *A. flavus,* such as *B. subtilis,* resulting in a reduction in *A. flavus* colonization and aflatoxin concentration (Dowd and Vega, unpublished data).

Combinations of other entomopathogens such as *B. bassiana* and plant disease biocompetitors in the autoinoculator might also be desirable, to increase mortality rates among the dispersing insects. It might also be possible to combine biological control agents with powdered formulations of chitosan (Cuero *et al.,* 1991), which induces the defense response in damaged plants. Dyes can be added to the formulation to monitor dispersal. Current research leading toward the development of granular nematode formulations (Georgis, 1992) could result in their incorporation into pest management programs using the autoinoculator.

The autoinoculator device can be used in biological

control programs aimed at reducing both plant disease via the use of biocompetitors, and insect populations through the use of entomopathogens. It might also be possible to use insects as vectors of weed pathogens. Our autoinoculator device facilitates insect dissemination of biocontrol agents such as entomopathogens, biocompetitors and weed control agents. It can be adapted to a standard funnel-type insect trap and it can be restricted through the use of different size screens and selected pheromones, to specifically attract a particular insect species for transmitting the biocontrol agent.

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