

Exchange of Inoculum of *Beauveria bassiana* (Bals.) Vuill. (Hyphomycetes) Between Adult Flies of the Cabbage Maggot *Delia radicum* L. (Diptera: Anthomyiidae)

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Adult cabbage maggots (*Delia radicum* L.) were exposed to dry conidia of isolates of several hyphomycetous fungi by placing them in a centrifuge tube containing conidia, then releasing them into small screened plastic cages. Mortality was assessed after 48, 120 and 160 h. A *Beauveria bassiana* isolate (P89 from *Musca domestica*) caused the highest mortality after 48 h, resulting in 100% mortality and 100% infection. Isolate L90 (*B. bassiana*) and one *Metarhizium anisopliae* isolate (ARSEF 2521) also caused fatal infection in more than 50% of the flies. To investigate exchange of inoculum, flies were placed in a small container with a dry powder formulation containing *B. bassiana* (Mycotrol®) on the bottom. The flies were removed to small screened cages containing untreated flies. This experiment confirmed the ability of flies to pass inoculum to other flies. In a similar experiment, one treated fly was placed in each cage with one untreated fly. When each fly died, one untreated fly was added to each cage after the dead fly was removed. This study showed that fly to fly transfer of fatal doses of inoculum was possible for a series of at least six flies. When female flies were exposed to the inoculum, then transferred to small cages containing males and an oviposition substrate, no eggs were laid. Further studies are being conducted to develop a system where flies attracted to a trap will be inoculated with the fungus and spread it to a field population.

Keywords: *Delia radicum*, cabbage maggot, root fly, *Beauveria bassiana*, insect-pathogenic fungus, autodissemination

INTRODUCTION

The cabbage maggot (*Delia radicum* L.) is a major pest of crucifers in northern Europe, where it is known as the cabbage root fly. The closely related turnip root fly (*D. floralis*

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Fallen) is of equal or greater importance in northern Scandinavia. The cabbage maggot is also a pest of crucifers in the northeastern USA, but it is not usually among the major pests. When it reaches pest status, it necessitates applications of chemical insecticides, which disrupt the biological control of other pests of crucifers. Therefore, a joint project was initiated between researchers in the USA and Norway to find a biological control agent for these two root fly pests.

Several studies have been conducted to isolate insect pathogens attacking the adult flies (Eilenberg *et al.*, 1994) or the larvae (Klingen *et al.*, 1998) of these species. To our knowledge, no isolate has caused high mortality of juvenile stages of these flies (Meadow, unpublished observations; Vänninen *et al.*, 1999). In one published study, an isolate of *Tolypocladium cylindrosporum* (Gams) applied directly to larvae gave 30% control (Lam *et al.*, 1988).

Adult flies exposed to dry formulations of fungal pathogens, appear to be highly susceptible in some cases (Watson *et al.*, 1995; Meadow *et al.*, 1996). Therefore, tests were conducted to evaluate different species of fungi for control of adults and the ability of the adults to transmit fungi to other adults. Such transmission of pathogens, or autodis-semination, has been suggested for several insect species (Jackson *et al.*, 1992; Lacey *et al.*, 1994, 1995). Successful transmission of the insect-pathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin by honeybees for infection of the pollen beetle *Meligethes aeneus* Fab., although technically not autodis-semination, confirms the ability of insects to transmit fungi for biocontrol (Butt *et al.*, 1998). Since both horizontal and vertical transmission have been reported (Vail *et al.*, 1993; Lacey *et al.*, 1995; Yu & Brown, 1997), experiments were also performed to see if exposure to dry formulations of fungal pathogens affects oviposition and/or progeny.

MATERIALS AND METHODS

In all of the experiments, flies were provided with an excess of dry sucrose and water. The small containers (cages) used in the experiments were translucent rectangular plastic boxes, approximately 12 × 12 × 12 cm. The tops of the boxes were cut away and replaced with a fine meshed muslin to provide ventilation. The flies used in the experiments were taken from a culture held at the New York State Agricultural Experiment Station in Geneva, New York, USA. The flies were reared by presenting mated females with a slice of turnip in a Petri dish bottom containing fine sand. The turnip was to stimulate oviposition and the sand was the oviposition substrate. Eggs were removed weekly and transferred to small food storage cups containing sand and a turnip slice for larval development (25 per cup). Pupation took place in the sand. Pupae were taken from the rearings to provide flies for the experiments. All of the experiments were conducted at the USDA Agricultural Research Service, U.S. Plant, Soil & Nutrition Lab., Tower Road, Ithaca, New York, USA.

Selection of Fungal Isolates

The cultures for this experiment were obtained from the ARS Collection of Entomopathogenic Fungal Cultures (R.A. Humber, Curator, Ithaca, NY, USA) and maintained on Sabouraud dextrose agar plus 2% yeast extract (DIFCO) at 25°C and 15:9 L:D. Spores were harvested by scraping from the surface of 14–21 day-old cultures. Adult cabbage maggots were exposed to dry conidia of isolates of *Beauveria bassiana* (Bals.) Vuill., *M. anisopliae* or *Paecilomyces fumosoroseus* (Wize) Brown & Smith. Two *B. bassiana* isolates were from Cornell University (P89, L90) isolated from *Musca domestica*, one was from Mycotech Corporation (M726), isolated from *Diabrotica undecimpunctata* (ARSEF 201) and passed through a grasshopper, *Melanoplus* sp., and then re-isolated from silverleaf whitefly, *Bemisia argentifolia* (Bradley *et al.*, 1999). The other isolates were from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (Humber, 1992). One *B. bassiana* isolate, 4012, was isolated from *D. radicum*. The *P. fumosoroseus* isolates were from the Diptera *Musca autumnalis* (1626), *M. domestica* (1644, 1645, 1867 and 1868), *Calliphora*

spp. (1646), and an unidentified Dipteran (887). The *Metarhizium anisopliae* isolates were from *Deios* spp. (Homoptera: Cercopidae) (2521), *Galleria mellonella* (Lepidoptera: Pyralidae) (3540) and from soil (4862 and 4865). Flies were exposed to the fungi by placing five flies for 1 min in a 50 ml centrifuge tube containing 0.1 g of conidia scraped directly from culture plates, then released into small screened plastic cages. The cages were placed in an incubator at 20°C with a light regime of L : D 14 : 10 and relative humidity (RH) of 60% ± 20%. There were three replicates in a randomized block design. The three controls were untreated flies. Mortality was assessed after 48, 120 and 160 h. Dead flies were incubated to confirm infection by identifying fungal mycosis on the cadaver. The selection experiment was repeated using only *Beauveria bassiana* isolate P89 and a commercial *B. bassiana* product Mycotrol® (Mycotech Inc.).

Simple Transfer of Inoculum

The commercial *B. bassiana* product Mycotrol® WP (63% *B. bassiana*) was chosen for autodissemination studies based on results of the selection above and because it was readily available. Two newly emerged flies were exposed to the dry WP formulation containing *B. bassiana* by placing the flies in a small container (12 × 12 × 12 cm) with 2 g Mycotrol® WP on the bottom for 2 min. The flies were removed to small screened cages containing three untreated flies. The cages were placed in a glass-fronted incubator at 20°C with ambient daylight (L : D ca. 13 : 11) and RH of 60% ± 20%. Controls were untreated flies. There were four replicates, and the experiment was performed twice. When the flies died, they were incubated for up to 5 days at 20°C to confirm infection by the fungus by placing them individually in a Petri dish with moistened filter paper on the bottom.

Transfer of Inoculum Through a Chain of Individuals

One newly emerged female fly was exposed to a dry powder formulation containing *B. bassiana* by placing it for one min in a small container with 2 g Mycotrol® WP on the bottom. The treated fly was placed in a small cage with one untreated male fly. The cages were placed in a glass-fronted incubator at 20°C with ambient daylight (L : D ca. 13 : 11) and RH of 60% ± 20%. When each fly died, one untreated fly of the same sex as the dead fly was added to each cage after the dead fly was removed. Controls were untreated flies. There were five replicates. Dead flies were incubated to confirm sporulation of the fungus. The experiment was terminated after the third fly in the chain died.

The second time this experiment was performed, it continued until either both flies in a cage were found dead, or when the flies in the corresponding control died.

To investigate the accumulation of the inoculum on the flies after transfer, photographs were taken using Nomarski illumination and fluorescence microscopy, with the body parts of the fly mounted in Calcofluor (Altre *et al.*, 1999). The microscope was an Olympus BH2 with a X100 oil-immersion objective.

Effect on Oviposition

Newly emerged flies were separated by sex on the day of emergence. Within 2 days of emergence, each female fly was exposed to a dry powder formulation containing *B. bassiana* by placing it for 30 seconds in a small container with 2 g Mycotrol® WP on the bottom. The treated female fly was placed with one untreated male fly in a small cage containing an oviposition substrate consisting of a 1 cm thick slice of turnip on a thin layer of slightly moistened fine sand. The oviposition substrate was on a Petri dish cover (10 cm diameter) in the bottom of the cage. Controls were the same, but none of the control flies were exposed to the fungus. There were 10 replicates. The cages were placed in a glass-fronted incubator at 20°C with ambient daylight (L : D ca. 13 : 11) and RH of 60% ± 20%. The experiment was repeated using two female and two male flies per cage, but only five replicates. Oviposition and mortality of the flies were registered after 1 and 2 weeks.

TABLE 1. Mortality of *Delia radicum* after direct contact with fungal conidia

Species	Isolate	Percentage of flies killed by fungus		
		Time post exposure (h)		
		48	120	160
	control	0.0a	0.0a	0.0a
<i>Beauveria bassiana</i>	L90	0.0a	66.6cd	66.6cd
	P89	34.0a	100.0d	100.0d
	M726	0.0a	34.0abc	34.0abc
	4012	6.0a	40.0bc	40.0bc
<i>Paecilomyces fumosoroseus</i>	887	6.0a	6.0ab	6.0ab
	1626	0.0a	0.0a	0.0a
	1644	14.0a	14.0ab	14.0ab
	1645	0.0a	6.0ab	14.0ab
	1646	0.0a	0.0a	0.0a
	1867	0.0a	0.0a	0.0a
	1868	6.0a	6.0ab	6.0ab
<i>Metarhizium anisopliae</i>	2521	6.0a	26.0ab	54.0bc
	3540	14.0a	20.0ab	40.0bc
	4862	0.0a	0.0a	14.0ab
	4865	0.0a	0.0a	0.0a

Means within columns followed by the same letter are not significantly different ($P = 0.05$) by Tukey.

RESULTS AND DISCUSSION

Selection of Fungal Isolates

After 48 h, isolate P89 (*B. bassiana*) caused the most mortality. It was also the only isolate that gave 100% mortality and 100% infection. Isolate L90 (*B. bassiana*) and one *M. anisopliae* isolate (ARS 2521) also caused fatal infection in more than 50% of the flies (see Table 1). The susceptibility of adult cabbage maggot to fungal agents was demonstrated by this experiment. In the selection experiment using only isolate P89 and Mycotrol® all of the treated flies died within 5 days.

Simple Transfer of Inoculum

The flies that were directly exposed to the fungus began to die within 4 days. These flies were removed when dead, thus most of the remaining flies were those that received inoculum indirectly. All of the flies in the Mycotrol® treatment were dead within nine days of the start of the experiment. All of the flies, but one, in the Mycotrol® treatment developed mycoses after incubation. This experiment confirmed the ability of flies to pass fatal doses of inoculum to other flies.

Transfer of Inoculum Through a Chain of Individuals

In the first part of this experiment, all of the inoculated flies died within 5–6 days. The flies that received inoculum from the inoculated flies died within 3–8 days. Flies that were in the third link of the chain were all dead within 6–11 days of their first exposure to the flies of the second link. Figure 1 shows inoculum on different body parts of a female fly that was third in such a chain. The largest amounts of inoculum collected in the leg hairs, possibly due to preening. There were also large amounts of inoculum on the edges of the wings and



FIGURE 1. Photographs showing the inoculum on body parts of a cabbage maggot. The fly pictured was third in a chain of transfer of inoculum. The bright areas show the inoculum on: (A) a leg; (B) the edge of a wing; and (C) the proboscis. (D) Shows germination on the ovipositor.

on the proboscis. This experiment showed that flies can receive a fatal dose of inoculum from flies that in turn received inoculum from other flies.

The inoculated flies in the second part of this study died within 1–3 days of inoculation. Flies received and transferred fatal doses of inoculum to other flies in a chain of, on average, six flies. Because replicates were terminated when both flies in a cage were found dead, or when the flies in the corresponding control died, it is difficult to conclude how many transfers of fatal doses are possible. In one case there were seven transfers of fatal doses, resulting in mortality for eight flies from the initial inoculation of one fly. This study showed that fly to fly transfer of fatal doses of inoculum was possible for a long chain of flies, beginning with one treated fly.

Effect on Oviposition

In the first experiment, with one female and one male fly per cage, no flies died within one week of treatment. During this time, the treated flies laid no eggs, while the untreated flies laid 10.8 eggs per female. At the end of 2 weeks, all of the treated females were dead, as were the males that were caged with them. These flies did not lay any eggs during the experiment. The untreated flies had oviposited 21.9 eggs per female by the end of the experiment.

In the second experiment, with two female and two male flies per cage, no flies died within one week of treatment, as in the first experiment. Again, the treated flies laid no eggs, while the untreated flies laid 4.5 eggs per female. Also in this experiment, all of the treated females were dead at the end of 2 weeks, as were the males that were caged with them, and the treated females did not lay any eggs during the experiment. The untreated flies had oviposited 12.8 eggs per female by the end of the experiment.

That the male flies died when caged with inoculated female flies, confirms the earlier results that flies can transfer fatal doses of inoculum to other flies. Because the inoculated

flies did not oviposit, but were still alive when the untreated flies oviposited, the fungus must have either weakened the females or in some other way inhibited oviposition. More detailed studies should be performed to investigate the effect of the fungus on reproduction.

Developing a Trapping Mechanism for Autodissemination

All of these studies indicate that adult cabbage maggots can transfer fatal doses of inoculum of fungal biocontrol agents within a population. Practical application making use of this transfer will require the flies visiting a source of inoculum and subsequently transporting it to a place where the flies interact or congregate. The cabbage maggot pupates in the soil under the *Brassica* plants on which the larvae develop. The adults spend most of their time in the vegetation around fields of *Brassicaceae* (Hawkes, 1974), feeding on nectar, resting or mating. The females fly into the fields during the day to oviposit, then usually return to the field edges. A trap located in the field margins would be able to function as a source of inoculum for infection of populations of the cabbage maggot.

Traps have been designed for spreading fungal agents in populations of other species, such as the pheromone trap for dispersal of *Zoophthora radicans* Brefeld. (Pell *et al.*, 1993; Furlong *et al.*, 1995) in populations of the diamondback moth, *Plutella xylostella* L. We are currently developing a trap that uses an attractant based on isothiocyanate to lure adult cabbage maggots and turnip root flies, with the aim of inoculating them with fungal agents. A prototype of this trap has been used to catch flies of these species to survey for naturally occurring fungal pathogens (Klingen *et al.*, 2000). The trap proved to be very effective, with large numbers of flies caught, and very selective, with 94–98% of the flies being *D. radicum* or *D. floralis*. The trap has been redesigned for monitoring these two species and it will be further developed for disseminating fungal pathogens on the flies.

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