Efficacy of Entomopathogenic Fungi for Control of the Cabbage Maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae)

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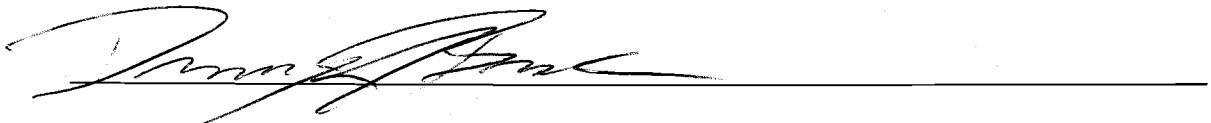

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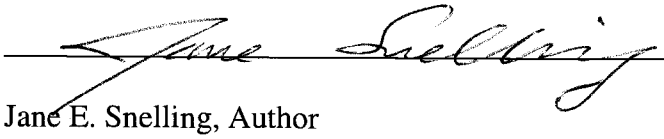

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Literature Review

Delia radicum life biology and identification

Cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae), is a major pest of cruciferous crops in temperate regions of North America, Europe, and Asia (Whistlecraft et al., 1985). Cruciferous crops include important vegetables such as broccoli, cabbage, turnip, rutabaga, daikon, and canola. *Delia radicum* larvae also infest a wide range of cruciferous weeds such as wild radish and mustard (Finch et al., 1977). Damage is caused by larval root feeding. If not controlled, larval feeding can weaken or stunt developing plants, reduce yields and kill plants outright. In crops of rutabaga and turnips, larval feeding can easily render crops unmarketable (Mackenzie et al., 1987).

The *D. radicum* life cycle takes approximately 5-7 weeks. Climatic conditions may allow as many as three generations of *D. radicum* each year in the Pacific Northwest (Dreves, personal communication 2004). Flies begin to emerge in early April and a final flight can take place as late as October. Some climatic regions, such as coastal northern California, allow *D. radicum* to persist all year as long as host plants are available (Johnsen and Gutierrez, 1997).

Identifying *D. radicum* adults, larvae, and pupae can be difficult for the untrained eye. The flies are small (5 mm), gray and look much like a common house fly (Berry, 1998). Features distinguishing the fly include wing venation, long prealar on the scutellum and specific arrangement of hairs on the hind tibia, and dark grey stripes along the thorax (Brooks, 1951). Male flies emerge 4-8 days before the females. When females emerge, they feed on pollen and nectar from flowering plants for carbohydrates and protein before their mating period (2-5 weeks). Females begin laying eggs within 7 days of mating. Eggs are laid around the base of wild and domesticated cruciferous crops

and in soil cracks (2-5 cm). Eggs are approximately 1 mm in length, white and elongated. Depending on soil temperature, eggs hatch within 3-10 days (Berry, 1998).

There are three instars in which the larvae range from 1-9 mm in length. First instars burrow into the soil and feed along the outside of the roots and root hairs of crucifers. As the larvae mature, they burrow into the root to feed with hook-like mouthparts until pupation. *Delia radicum* larvae can be distinguished from other Anthomyiids by posterior turbercles, which consist of 14-16 lobes of median turbercles with a forked apex (Capinera, 2001; Ritchot et al., 1994). Larvae feed inside the root for 18-22 days (Harris and Svec, 1966) and crawl out into the soil to pupate. Most pupate in the top 5 – 7.5 cm of soil (Finch and Skinner, 1980). Some larvae may pupate in the root if soil conditions are dry. Pupae are brown, elongate, and about 6-8 mm in length. Pupae remain in the soil for 12-25 days before they eclose (Harris and Svec, 1966).

Control Measures

Control of *D. radicum* in crucifer crops currently depends on the use of chemical insecticides such as pyrethroids, botanicals, and organophosphates. The most commonly used insecticide is the organophosphorous insecticide, chlorpyrifos. Chlorpyrifos has been used to control Coleoptera, Diptera, Homoptera and Lepidoptera pests in soil and on foliage of a wide range of crops (Tomlin, 1994). Chlorpyrifos is non-phytotoxic to most plant species, non-systemic, and cannot be absorbed from the soil into the roots. The stability of chlorpyrifos is compromised by high pH, presence of copper, and alkaline materials. Chlorpyrifos displays contact, stomach, and vapor mode of action (Tomlin, 1994). Like other organophosphates, the insecticidal action of chlorpyrifos is due to the inhibition of the enzyme acetylcholinesterase. This results in the accumulation of the

neurotransmitter, acetylcholine, at nerve endings of its target host. Excessive transmission of nerve impulses causes mortality in the target pest.

Chlorpyrifos is registered under several trade names for use on food crops as a soil treatment for several soil insect pests. Lorsban (4E and 15G formulations) are the most common products used in the Pacific Northwest. Lorsban can be applied as an over-the-row band, broadcast, or infurrow treatment to the soil surface for the control of larvae. It is recommended to incorporate the chemical into the soil to achieve a longer lasting effect. Only one application of Lorsban per season at planting is allowed as specified by the label. Lorsban 4E is a water based spray and Lorsban 15G is a granular formulation. Both forms of Lorsban can be applied infurrow with seeds at time of planting. If granular or liquid formulations are applied too close to the seed during hot dry weather, seedling damage may occur. Lorsban use does not ensure a damage free crop or a larvae free field, the treatment only reduces *D. radicum* numbers. The residual activity of Lorsban is not effective in controlling adults. Diazinon is also suggested as a pre-plant insecticide but has not shown efficacy in recent years. One application of Lorsban per season for control of cabbage maggots fails to provide adequate residual activity for larval control for long season crops (Getzin, 1985).

Preventative and cultural controls of *D. radicum* include row covers, tillage, and crop rotation. Polyethylene plastic or polypropylene fabric row covers, such as Reemay, can provide a barrier to discourage egg laying. Row covers are not effective in fields that are already infested with *D. radicum*, as overwintering pupae eclose and the adults mate and lay eggs under the covers (Fisher, 2004). Tillage after a crop is harvested can turn overwintering pupae deeper into soil so adults can not reach the surface after eclosing and/or pupae can be destroyed mechanically by rototilling (Finch and Skinner, 1980).

Crop rotation is a practice used to avoid planting in close proximity to other cruciferous crops. This allows levels of *D. radicum* to decrease before crucifer crops are planted in the same area.

Entomopathogenic Fungi

Entomopathogenic fungi have long been recognized for their ability to infect insects. The infection of silkworms and cicadas by entomopathogenic fungi, *Cordyceps* and *Isaria*, were noted by the Chinese a thousand years ago (Roberts and Humber, 1981). Fungal pathogens infect an insect host by penetrating and proliferating inside the hemocel. The insect is killed by being deprived of soluble nutrients in its hemolymph, the invasion and digestion of tissues and/or by the release of toxins from the fungus. Two species of entomopathogenic fungi that have been tested extensively for use against foliar and soil insects are *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) (Roberts and Humber, 1981; Butt et al., 1992; Majchrowicz et al., 1990; McDowell et al., 1990; Ramoska et al., 1985). *Metarhizium anisopliae* and *B. bassiana* belong to the phylum Deuteromycotina, and the class Hyphomycete. Hyphomycetes are asexual and characterized by production of naked conidia free on the mycelia.

Metarhizium anisopliae and *B. bassiana* infect hosts in similar ways. Infection begins with the attachment of conidia to the insect cuticle. Ingestion of conidia may also be a route for infection (Bruck and Lewis, 2002a). The conidia germinate on the cuticle at high humidity (>90%) (Roberts, 1977). Although environmental conditions may be dry, the microclimate on the insect cuticle often provides enough humidity for germination. Germination inhibitors and stimulators on the cuticle can effect conidial

germination. For instance, some bacteria produce compounds which inhibit conidial germination on the insect cuticle (Schabel, 1976). Once a conidium has germinated, the germ tube can penetrate the cuticle directly or an appressorium may be formed. An appresorium is a structure that attaches to the insect cuticle and utilizes an infection peg to force its way through the cuticle. Penetration of the cuticle requires enzymatic and physical activity. Entomopathogenic fungi have been shown to produce proteases, chitinases, and lipases to break down the insect cuticle (Roberts, 1980). If the insect molts before the germ tube penetrates the cuticle, the insect can rid itself of the fungus and be spared from infection (Fargues and Vey, 1974). Once inside the insect, the fungi invade the hemocel, producing hyphae and yeast-like hyphal bodies. Entomopathogenic fungi often produce toxins which aid in overcoming the insect's body before infection of organs begins. Many of these toxins are depsipeptides which contain proteases. Toxins are thought to be responsible for host mortality. Death of the host is preceded by behavioral changes such as tremors, loss of coordination, or climbing to an elevated position which can occur as soon as 48 hours after infection with high rates of inoculum (Baird, 1954). Sporulating insect cadavers can serve as an inocculum reservoir in periods of adverse conditions. At low levels of humidity, infected cadavers will not sporulate, but as humidity levels increase, the hyphae will emerge from the cadaver and sporulate. Dispersal of conidia takes places by wind (Roberts and Humber, 1981), water (Bruck and Lewis, 2002b), or by insect movement (Bruck and Lewis, 2002a).

Metarhizium anisopliae occurs in two different forms based on conidial size: *Metarhizium anisopliae* var. *anisopliae* (3.5-9.0 μm) and *M. anisopliae* var. *major* (9.0-18.0 μm). The conidia are green, slightly oblong and bean shaped. *Metarhizium anisopliae* conidia form in large aggregates which stay on or in the soil rather than

becoming airborne (Roberts and Humber, 1981). *Metarhizium anisopliae* is a frequent pathogen of soil insects. The smaller form of *M. anisopliae* has a wide range of hosts including termites and mosquito larvae. The first attempts to control insects with *M. anisopliae* were carried out by Metchnikoff (1879) and Krassilstchik (1888) on wheat cockchafer, *Anisoplia austriaca* (Coleoptera: Scarabaeidae), and sugar beet curculio, *Cleonus punctiventris* (Coleoptera: Curculionidae), respectively. At least partial control was achieved. *Metarhizium anisopliae* var. *major* is host specific and primarily infects scarab beetles. This form of *M. anisopliae* has been used to control rhinoceros beetle larvae, *Oryctes nasicornis* L. (Scarabaeidae: Dynastinae), a pest of coconut trees in the South Pacific (Roberts and Humber, 1981).

Metarhizium anisopliae strain F52 (Earth BioSciences, New Haven, CT) is currently EPA registered as a biopesticide. Current regulations designate use sites as terrestrial non-food sites such as greenhouses, nurseries, and residential and institutional lawns. The isolate F52 is not labeled for use in areas where it could contaminate water. Current target pests include ticks, beetles, root weevils, flies, gnats, thrips, and some termites. There are currently three F52 products registered: Taenure Granular Bioinsecticide, Tick-EX G, and Tick-EX EC. Tick-EX G and Tick-EX EC are labeled for the control of ticks. These products have been registered for one year and will be granted full registration if proven effective.

Beauveria bassiana infects a wide range of hosts and survives in the soil as a saprophyte. Conidia of *B. bassiana* are small ($\leq 3.5\mu m$ diameter), globose, white, and form singly on denticles (Humber, 1997). They are dispersed in a dry and single form, and easily carried by wind (Roberts and Humber, 1981). Insects on foliage or tree trunks are often infected by *B. bassiana* conidia as it has an extensive host range (Roberts, 1980).

Beauveria bassiana is the most frequently isolated entomopathogenic fungus of infected insects collected in the field (Roberts and Humber, 1981).

The infection methods of *B. bassiana* are not unlike that of *M. anisopiae*. *Beauveria bassiana* utilizes physical and chemical means to penetrate the cuticle. In the infection process chitinases, lipases, and proteases break down chitin, lipids, and proteins of the insect cuticle. The enzyme chitinase is more than likely employed first (Roberts and Humber, 1981). Studies have shown that individual enzymes produced by *B. bassiana* cannot dissolve the cuticle alone. An appressorium may be needed to aid in cuticle penetration.

Agostino Bassi established the germ theory of disease in animals based on his studies of *B. bassiana* (Steinhaus, 1956). Since, *B. bassiana* has been evaluated for control of a variety of insect pests. In the U.S., the cereal pest *Blissus leucopterus* (Hemiptera: Lygaeidae) was treated with *B. bassiana* in early 1890's. Although promising, this project was not successful (Roberts, 1980). *Beauveria bassiana* has been used successfully in Russia since 1956 for the control of Colorado potato beetle (Coleoptera: Chrysomelidae) (Kendrick, 1992). The *B. bassiana* strain GHA (Emerald BioAgricultural Corporation, East Lansing, MI) has been registered by the EPA for insect control since 1995. Uses include all food and feed uses, rangeland, forests, greenhouses, and homes. Target pests include whiteflies, aphids, weevils, borers, grasshoppers, and diamondback moths. *Beauveria bassiana* may be harmful to bees so caution must be taken to keep away from bee hives or areas where bees actively feed (http://www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet_128924.htm, 2004).

As of 1999, there were seven approved mycoinsecticide products containing *B. bassiana*. The common mycoinsecticides, produced by Emerald BioAgricultural

Corporation, include Mycotrol O, BotaniGard, and BotaniGard ES are used to control whiteflies, aphids, and thrips. Mycotrol O is used on crops such as vegetables, melons, tree fruits and tree nuts, and organic crops. BotaniGard is used in horticultural, nursery, and vegetable crops. The company Troy Biosciences, Phoenix, AZ also produces *B. bassiana* mycoinsecticides.

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Efficacy of Entomopathogenic Fungi for Control of the Cabbage Maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae)

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Introduction

The cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae) is a serious pest of cruciferous crops (i.e. broccoli, cabbage, turnip, rutabaga) in North America and Europe (Coaker and Finch, 1971; Whistlecraft et al., 1985). The adult flies lay eggs around the base and in cracks at the soil surface around wild and domesticated cruciferous plants and in cracks at the soil surface. Larvae can seriously reduce crop quality and yield by feeding directly on the roots (Jensen et al., 2002). In root crops such as rutabaga and turnip, maggots can render the crop unmarketable if more than slight feeding damage is evident at harvest.

Although some cultural controls such as crop rotation, row covers, and cultivation are used, current cabbage maggot control measures depend almost solely on the use of chemical insecticides (Jyoti et al., 2001). The most commonly used pesticide in the Pacific Northwest is chlorpyrifos (i.e. Lorsban), an organophosphate. Environmental scrutiny, strict regulation, and the potential for chemical resistance have raised an interest in pursuing alternative control methods.

An overlooked method for controlling the cabbage maggot may be the use of the entomopathogenic fungi, *Metarhizium anisopliae* (Metchnikoff) Sorokin (Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae). *Metarhizium anisopliae* and *B. bassiana* have been evaluated for control against other pests such as corn rootworm (Krueger et al., 1997), flea beetle (Butt et al., 1997), onion maggot (Majchrowicz et al., 1990), and chinch bug (Ramoska and Todd, 1985).

The objectives of these experiments were to determine the efficacy of *M. anisopliae* and *B. bassiana* in controlling *D. radicum* in laboratory soil bioassays at economic field rates, identify the most virulent isolate and determine the LD_{50} and LD_{95} of the most virulent isolate.

Materials and Methods

Spore stock

Three isolates of *M. anisopliae* (TM109, MA1200, and F52) and one isolate of *B. bassiana* (GHA) were used in laboratory soil bioassays to determine their virulence against *D. radicum*. The isolates TM109 and MA1200 were obtained from Dr. Stephan Jaronski at USDA-ARS, Sidney, MT; Earth BioSciences, New Haven, CT (F52), and Emerald BioAgricultural Co., Lansing, MI (GHA). The isolates MA1200 and GHA were used as dry spore powder (prepared by Dr. Stephan Jaronski). Dry spores were stored in the freezer at – 20°C in capped vials. The isolates TM109 and F52 were cultured weekly on potato dextrose agar (PDA).

Spore viability

Spore viability was evaluated the day before spore suspensions were prepared for bioassays. Five ml of spore suspension (~ 1×10^6 spores/ml) were prepared and spread plated onto PDA and incubated overnight in complete darkness at 28°C. After 16-24 hrs, plates were evaluated for percentage of spore germination by indiscriminately observing 100 spores with a compound microscope (400X magnification). A spore was considered to be viable if the germ tube was twice the length of the spore. Assessing spore viability prior to performing the bioassays allowed for adjustment of the total spore concentration to obtain the desired concentration of viable spores/ml for use in the soil bioassays.

Spore suspensions

Spore suspensions were prepared by aseptically adding dry spores to 10 ml sterile 0.1% Tween 80 and aseptically scraping spores from PDA plates. Spores were agitated by drawing the solution up in a 10 ml pipette and expelling the solution back into the beaker repeatedly. The spore solutions were then mixed with a vortex for 2-3 minutes and sonicated if spore aggregates remained. Spores were removed from PDA plates by adding 5 ml of sterile 0.1% Tween 80 solution to the plate and gently scraping with a flamed loop. The solution was then poured off into a test tube, mixed with a vortex and sonicated to release spores from aggregates.

Soil Bioassay

Assays were performed using field soil (loamy sand) collected from turnip fields in the Northern Willamette Valley of Oregon. Before use, the soil was autoclaved (1.1 K/cm^2, 121 °C) for 2 hrs, left overnight and then autoclaved for an additional hr. After autoclaving, the soil was placed in a drying oven at 70°C for 24 hrs and stored in zip lock bags until use. *Delia radicum* larvae were collected from turnip and rutabaga fields in the Canby, Oregon area. Larvae were carefully removed from infested turnips and rutabagas in the laboratory and placed in petri dishes lined with moistened filter paper containing slices of turnip. Larvae were held at 4°C for up to two days before use. Spore suspensions were prepared and the concentration adjusted with the use of a hemacytometer. The viability of each isolate (determined the previous day) was adjusted when preparing suspensions.

The inoculation rates used in the soil bioassays were based on economical field application rates of infurrow and broadcast application of entomopathogenic fungi

(Jaronski, personal communication 2003). An infurrow application can be economically applied to achieve 5×10^{14} spores/hectare providing an effective rate of 3.85×10^{6} spores/g soil. A broadcast application can be economically applied at 5×10^{13} spores/hectare providing an effective rate of 3.85×10^{5} spores/g soil. Twenty grams of autoclaved and oven dried soil were inoculated with each fungal isolate to achieve 3.85×10^{6} and 3.85×10^{5}spores/g soil at 15% moisture. To allow for uniform incorporation, spore suspensions were added to the soil in a 237 ml plastic cup and mixed until homogenous with a sterile spatula. The soil was then poured into a 30 ml cup. Five second instar *D. radicum* were added to each cup and covered with a lid. The cups were placed in plastic baskets containing damp paper towels and placed inside sealed gallon zip lock bags to maintain moisture. The assays were incubated in complete darkness at 21°C. After 24 hrs, turnip slices were added to the cups to serve as a food source for the larvae. This was done to ensure larval movement throughout the soil. Assays were incubated for 14 d. After 14 d, assays were evaluated for the number of dead, infected and live larvae. Mortality was adjusted based on the amount of larvae that died in the control group. The experiment was arranged in a randomized complete block design, replicated four times and contained an untreated control of 0.1% Tween 80 solution. The assay was repeated on October 4, 7, and 14 and on November 4 and 11, 2003. Two assays were performed on November 4, 2003.

An additional assay (November 19) took place at the USDA-ARS, Sidney, MT laboratory. *Delia radicum* larvae and bioassay soils were sent to Dr. Stephan Jaronski and a single assay was performed. The spore suspensions and soil bioassay were performed as described previously with the exception that GHA was not evaluated.

LD_{50} and LD_{95}

Because F52 was the superior isolate tested, we established the LD_{50} and LD_{95} concentration of F52 against *D. radicum* in soil. Spore suspensions were prepared as described previously to achieve 1×10^7, 1×10^6, 1×10^5, and 1×10^4 spores/g soil at 15% moisture in 70 g of autoclaved and oven-dried soil. Spore suspensions were added in a 237 ml plastic cup and incorporated with a sterile spatula. Ten second instar *D. radicum* were added to each cup and covered with a lid. Larvae were maintained in an incubator in complete darkness at 21°C. After 24 hrs, slices of turnip were introduced to the cups to serve as a food source. The cups were incubated for a total of 14 d. At the end of the experiment, the numbers of dead, infected, and live larvae were recorded. This assay was performed twice.

Data Analysis

Data from the bioassays were non-parametric and were analyzed using a Chi-Square analysis (SAS Institute 1999). The Chi-Square analysis was performed separately for the high and low dose of each bioassay to determine if there were any significant differences in larval mortality between isolates. A 2×2 Fisher's Exact Test was used to determine if mortality differences between isolates at the same rate were significantly different from each other or the control (SAS Institute 1999). Data from the lethal dose assays were analyzed with the Probit Analysis (SAS Institute 1999). The reference probability used throughout the data analysis was $P \leq 0.05$.

Results and Discussion

Laboratory Soil Bioassays

Of the six bioassays performed and evaluated at USDA-ARS, HCRL, data from four assays were used for statistical analysis. Due to high larval mortality in the control treatment, the other two assays were not included in the analysis. The mean number dead larvae were calculated for each treatment. A Chi-Square analysis was used to determine if there were any significant differences between the mean number of dead larvae within the high and low dosages and control (SAS Institute, 1999). In assays which there were significant differences between treatments, a 2 × 2 Fisher's Exact test was used to determine any significant differences between individual pairs of isolates (SAS Institute, 1999).

Fungal concentrations at the high dose (3.85×10^6 cfc/g soil) consistently killed more larvae than at the low dose (3.85×10^5 cfc/g soil). Isolates F52 and MA1200 were the most consistent isolates at the high dose with F52 being the more virulent isolate (Table 1). In the four assays, F52 and MA1200 at the high dose showed consistently higher levels of larval mortality, 61-89% and 35-75%, respectively, than the control. At the low dose, F52 consistently killed significantly more larvae than the other isolates. The isolate MA1200 at the low dose was significantly different than the control group in one assay, while TM109 and GHA were not significantly different than the control in any assay (Fig 1, 2, 3, 4).

Delia radicum larvae and bioassay soils were sent to USDA-ARS, Sidney, MT and a single assay was performed by research entomologist, Stephan Jaronski. The assay was performed as described previously with the exception that GHA was not evaluated.

Results from the USDA-ARS Sidney, MT assay were different from the assays run at the USDA-ARS, HCRL, Corvallis, OR. Significant differences in mortality from the control were shown for all isolates at the high and low doses (Fig 5). At the high dose, TM109 was the most virulent isolate compared to F52 being the most virulent in the four Corvallis assays. The isolate TM109 was followed by F52 and then MA1200 in percentage of larval mortality at the high dose. Not only did TM109 kill a mean of 94% of *D. radicum* larvae at the high dose. In the four assays performed in Oregon, TM109 killed in a range of 0-50% larvae. F52 killed 89% of *D. radicum* larvae at the high dose (10% higher than the highest mean from the previous assays.) Possible reasons for the differences in data could be explained by the fact that *D. radicum* larvae were sent from Oregon to Montana and that the spores used in the assays were grown in the Sidney laboratory. It is possible that the larvae were stressed from shipment and therefore more susceptible to being infected. It may also be possible that the spores being used in the Sidney lab were fresher and more infective than spores used in Corvallis.

LD_{50} and LD_{95} Assays

Two replications of LD_{50} and LD_{95} assays using the isolate F52 were evaluated for mortality and analyzed using Probit Analysis (SAS Institute 1999). The LD_{50}, based on $\log_{10}$ (dose), was 3.13×10^6 spores/g soil. This dose falls within the range of economic fungal field application rates (infurrow application). The LD_{95} of the F52 was 1.81×10^8, well above the economic range for in-furrow or broadcast applications.

Conclusion

The *M. anisopliae* and *B. bassiana* isolates tested were pathogenic to second instar *D. radicum* larvae. Vanninen (1999) also found *M. anisopliae* to be more effective in killing *D. radicum* larvae than *B. bassiana*. Isolates F52, MA1200, and TM109 caused higher rates of mortality than the commercially used *B. bassiana* isolate, GHA. All three isolates of *M. anisopliae* have potential to be use in cabbage maggot control programs. Although mostly ineffective in the Corvallis bioassays, TM109 also proved to have significantly more larval mortality than the control, according to Sidney, MT assays. Currently registered on a two year trial basis, isolate F52, with a LD_{50} within economic application rates, may prove to be an effective tool in integrated management plan for *D. radicum*. Alternative controls for *D. radicum* are necessary but more research with F52 is needed before it can be registered for commercial use in the field.

Summary and Implications

It is recognized that there is an increasing need for alternative control methods for the control of *D. radicum* in longer growth period cruciferous crops. Although chlorpyrifos is used successfully on short season crops (e.g. radish), it does not persist in the soil long enough to provide sufficient control for crops that are in the ground 90-120 days. With chlorpyrifos being the predominant control used, insect resistance is likely to be a problem in the near future. In 2003, *Delia radicum* larvae in Canada were laboratory tested and found to be chlorpyrifos resistant. A multi-tool approach to control *D. radicum* could include the use of entomopathogenic fungi. In laboratory soil bioassays, *M. anispoliae* isolate F52 has shown potential as a *D. radicum* control option, killing an average of 74% at a rate economical for infurrow application. Although soil amendments of *M. anisopliae*, like any other control option, will never give 100% control of larvae, it could offer one more tool to curb insect resistance to cholorpyrifos.

Further research on the efficacy of *M. anisopliae* as an insect pest control is needed. Field trials using *M. anisopliae* would need to be performed to see how it would react in a non-laboratory environment. Other factors that need to be considered are fungal application timing and application methods, interactions with chlorpyrifos, interactions with fungicides, and *M. anisopliae* persistence and viability in the soil.

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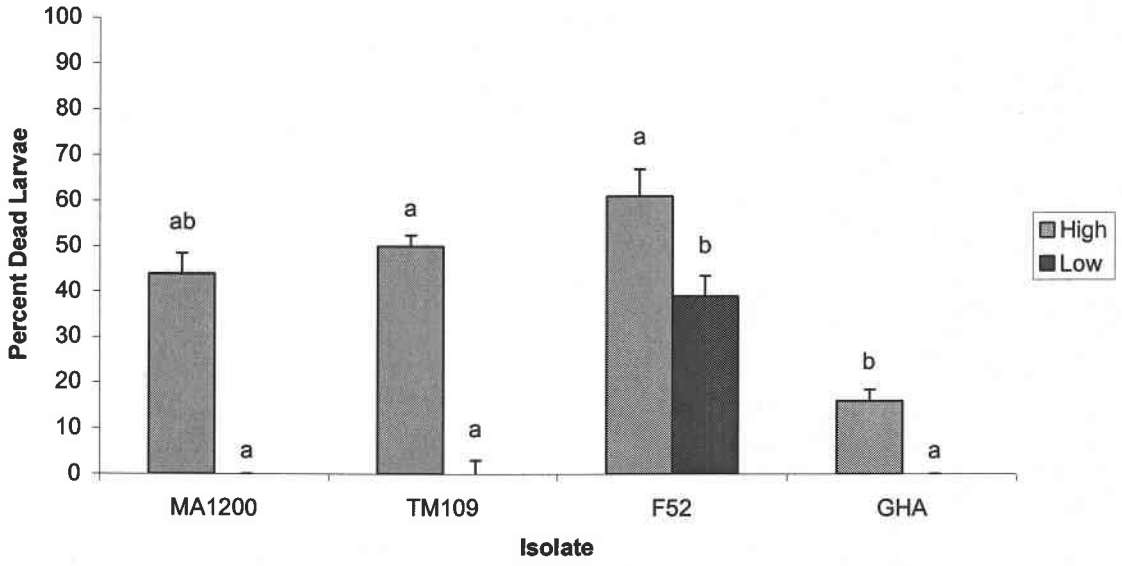

Fig.1. Mean mortality from the bioassay performed 10/7/03 of second instar *Delia radicum* after 14 d exposure to *Metarhizium anisopliae* and *Beauveria bassiana* isolates (3.85×10^6 and 3.85×10^5 spores/g soil). Isolate means at the same concentration that share the same letter are not significantly different ($P \leq 0.05$) as determined by Fisher's Exact Test (SAS Institute, 1999).

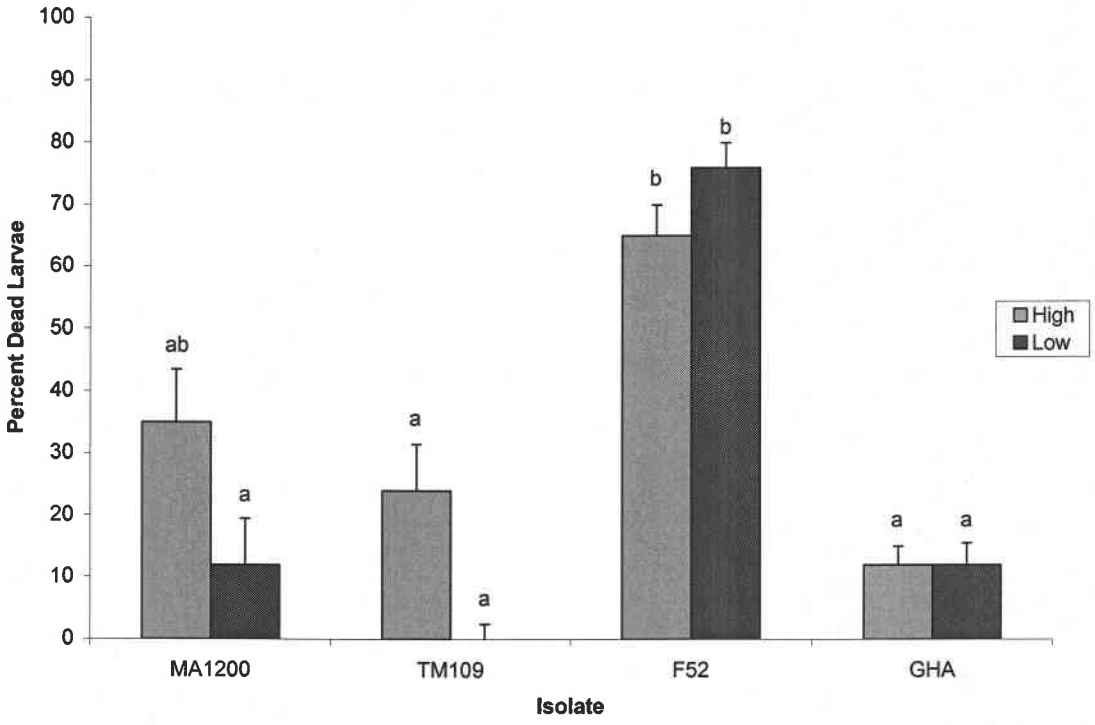

Fig. 2. Mean mortality from the bioassay performed 10/14/03 of second instar *Delia radicum* after 14 d exposure to *Metarhizium anisopliae* and *Beauveria bassiana* isolates (3.85×10^6 and 3.85×10^5 spores/g soil). Isolate means at the same concentration that share the same letter are not significantly different ($P \leq 0.05$) as determined by Fisher's Exact Test (SAS Institute, 1999).

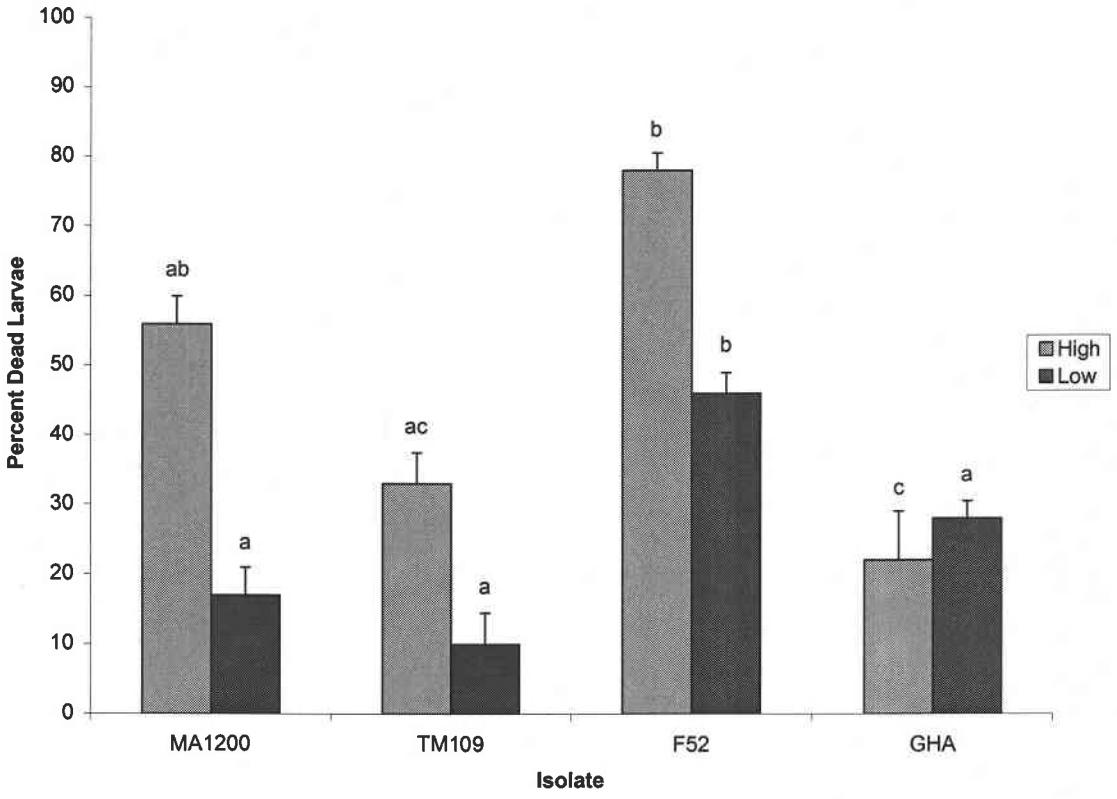

Fig. 3. Mean mortality from the first bioassay performed 11/4/03 of second instar *Delia radicum* after 14 d exposure to *Metarhizium anisopliae* and *Beauveria bassiana* isolates (3.85×10^6 and 3.85×10^5 spores/g soil). Isolate means at the same concentration that share the same letter are not significantly different ($P \leq 0.05$) as determined by Fisher's Exact Test (SAS Institute, 1999).

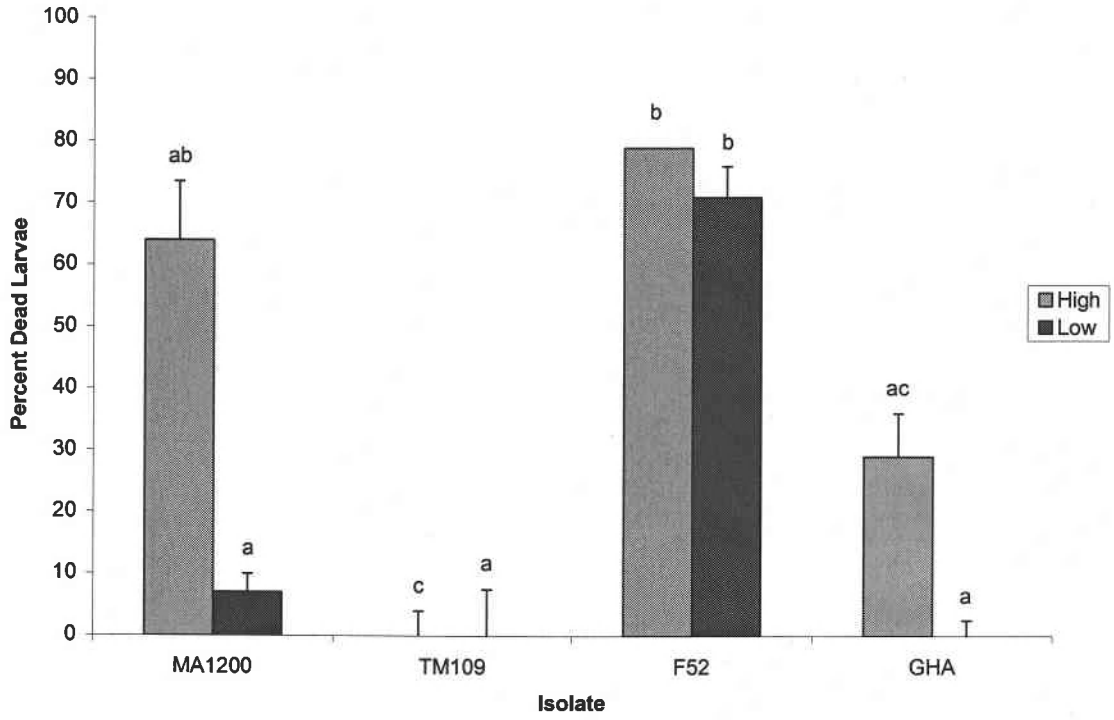

Fig. 4. Mean mortality from the second bioassay performed 11/4/03 of second instar *Delia radicum* after 14 d exposure to *Metarhizium anisopliae* and *Beauveria bassiana* isolates (3.85×10^6 and 3.85×10^5 spores/g soil). Isolate means at the same concentration that share the same letter are not significantly different ($P \leq 0.05$) as determined by Fisher's Exact Test (SAS Institute, 1999).

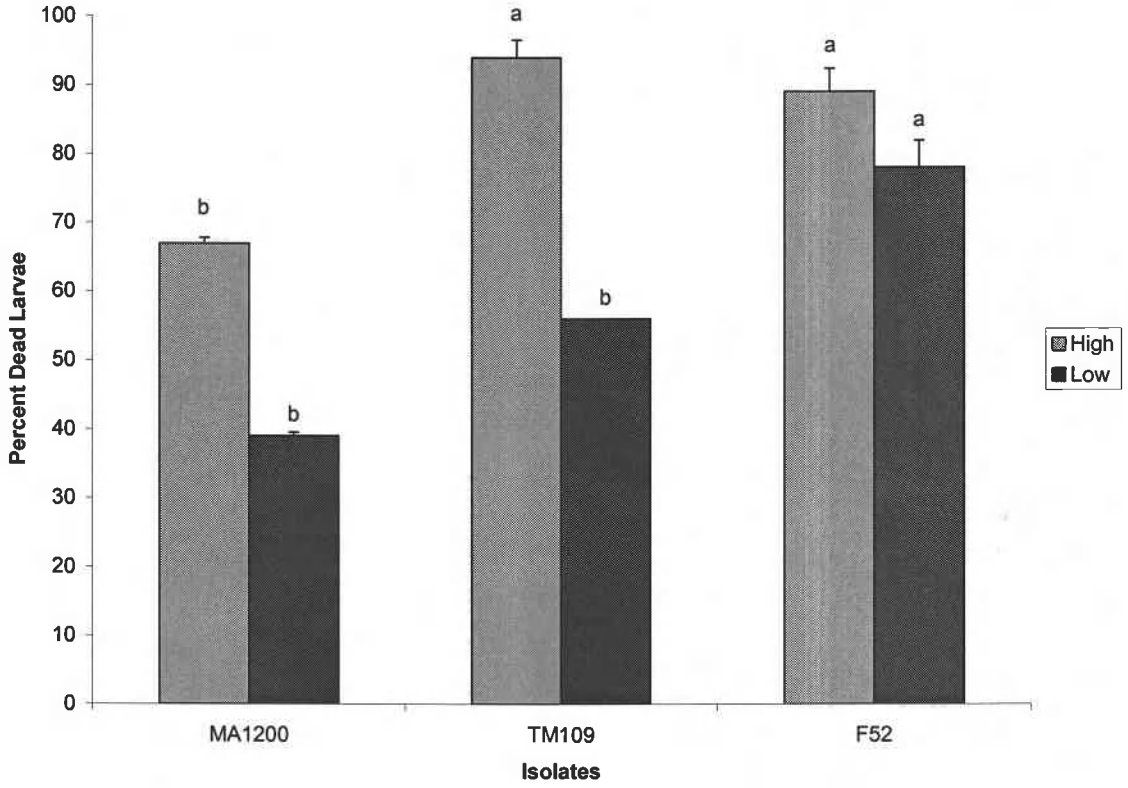

Fig. 5. Mean mortality from the bioassay performed 11/19/03 of second instar *Delia radicum* after 14 d exposure to *Metarhizium anisopliae* and *Beauveria bassiana* isolates (3.85×10^6 and 3.85×10^5 spores/g soil). Isolate means at the same concentration that share the same letter are not significantly different ($P \leq 0.05$) as determined by Fisher's Exact Test (SAS Institute, 1999). This assay was performed at the USDA-ARS Sidney, MT laboratory by Dr. Stephan Jaronski.

Table 1. Range and mean mortality of *D. radicum* larvae at the high (3.85×10^{6}) and low (3.85×10^{5}) doses shown for each fungal isolate of *Metarhizium anispoliae* and *Beauveria bassiana*. USDA-ARS, HCRL, Corvallis, OR.

Isolate	**High** Dose % Mortality Range	**High** Dose % Mortality Mean	**Low** Dose % Mortality Range	**Low** Dose % Mortality Mean
MA1200	35-64	50	0-17	10
TM109	0-50	27	0-13	3
F52	61-79	71	38-71	58
GHA	12-29	20	0-28	10

Data was corrected for mortality in the control groups. Mean mortality from all four bioassays performed is shown for overall death in high and low doses.

Table 2. Mean larval mortality at high (3.85×10^{6}) and low (3.85×10^{5}) doses shown for each fungal isolate of *Metarhizium anispoliae*. Assay performed by Stephan Jaronksi, USDA-ARS, Sidney, MT.

Isolate	**High** Dose % Mean Mortality	**Low** Dose % Mean Mortality
MA1200	67	39
TM109	94	67
F52	89	78

Data was corrected for mortality in the control groups. Mean mortality from the bioassay performed is shown for overall death in high and low doses.