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ARTICLE

Effect of *Metarhizium anisopliae* (Clavicipitaceae) on *Rhagoletis mendax* (Diptera: Tephritidae) pupae and adults

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Abstract

Blueberry maggot (*Rhagoletis mendax* Curran (Diptera: Tephritidae)) is a pest of blueberries (*Vaccinium* Linnaeus (Ericaceae)). Tephritid flies, including *Rhagoletis* Loew species, are susceptible to entomopathogenic fungi, but mortality levels depend on life stage targeted. We tested *Metarhizium anisopliae* (Metschnikoff) (Clavicipitaceae) strain S54 by application to pupae in the laboratory and using soil drenches in the laboratory and field. We hypothesised that younger (pre-diapause) pupae would be more susceptible to infection than older (post-diapause) pupae. In the laboratory, *R. mendax* emergence was reduced from 80% in the control to 57–60% with *M. anisopliae*. *Rhagoletis mendax* longevity was reduced by two days for both application timings, and mycosed cadavers increased by 9% and 27% with applications to younger and older pupae, respectively, compared to controls. In the field, *R. mendax* emergence was reduced by 50% with application to younger pupae compared to controls and applications to older pupae. The surfactant Silwet L77 caused reduced *R. mendax* emergence when pupae were dipped in suspensions. Even though *M. anisopliae* S54 did not greatly reduce emergence or longevity, infection was successful and younger pupae may be more susceptible than older pupae. Research with other *M. anisopliae* isolates against multiple life stages should be conducted and effects of soil variables on pathogenicity determined.

Introduction

Blueberry maggot (*Rhagoletis mendax* Curran (Diptera: Tephritidae)) is one of the most significant insect pests of lowbush and highbush blueberries (*Vaccinium angustifolium* Aiton and *V. corymbosum* Linnaeus (Ericaceae)) in eastern North America (Rodriguez-Saona *et al.* 2015). Adult *R. mendax* emerge from soil puparia prior to berry ripening, and after a period of feeding and mating, females lay eggs singly in berries (Lathrop and Nickels 1932). Maturing larvae consume fruit tissue, leading to soft and unmarketable blueberries. Prepupal larvae drop from berries and quickly burrow to pupate in the top 1–2 cm of the soil, entering diapause to overwinter (Teixeira and Polavarapu 2005; Renkema *et al.* 2011). Adult *R. mendax*

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are monitored using yellow, baited sticky cards or baited green spheres (Geddes *et al.* 1989; Gaul *et al.* 1995; Teixeira and Polavarapu 2001), with a single capture triggering application of a control, as there is no tolerance for larvae in ripe blueberries.

Blueberry maggot control is achieved with an insecticide application (*e.g.*, phosmet, malathion, acetemiprid, spinosad/GF-120 Fruit Fly Bait) 7–10 days following first fly detection on traps, with reapplication 5–12 days later and throughout the harvest period as long as flies are active (Ontario Ministry of Agriculture, Food, and Rural Affairs 2014). Insecticides are also effective when incorporated into attractive spheres (Liburd *et al.* 1999; Stelinski *et al.* 2001; Barry *et al.* 2004), but sphere field distribution and density need further optimisation (Stelinski and Liburd 2001). Preventative tactics include managing weeds, wild blueberries, and other host plants in and around lowbush blueberry fields (Gaul *et al.* 2002; Collins and Drummond 2004; Renkema *et al.* 2014). In highbush blueberries, organic mulches may impede pupation and emergence success (Pearson and Meyer 1990; Renkema *et al.* 2011, 2012a), and lower infestations have been reported in early season cultivars (Liburd *et al.* 1998). Biological control agents are not commercially available, but the parasitic wasp (*Diachasma alloeum* (Muesebeck) (Hymenoptera: Braconidae)) attacks blueberry maggot larvae (Stelinski *et al.* 2004, 2006), and generalist predatory ground beetles (Coleoptera: Carabidae) can reduce the number of pupating larvae (Renkema *et al.* 2012b, 2013). In order to build an integrated pest management strategy for *R. mendax*, research on new biological control options is needed.

Entomopathogenic fungi such as *Metarhizium anisopliae* (Metschnikoff) (Clavicipitaceae) and *Beauveria bassiana* (Balsam) (Cordycipitaceae) can cause significant mortality to tropical Tephritidae. Lethal and nonlethal effects on all life stages of Mediterranean fruit fly (*Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)) have been particularly well documented (Castillo *et al.* 2000; Lezama-Gutiérrez *et al.* 2000; Ekesi *et al.* 2002, 2005; Quesada-Moraga *et al.* 2006). For Western cherry fruit fly (*Rhagoletis indifferens* Curran (Diptera: Tephritidae)), *B. bassiana* caused mycosis of 20% of buried pupae and 80% of larvae entering sand to pupate (Cossentine *et al.* 2010), and *M. anisopliae* caused high adult mortality but not reduced adult emergence when pupating larvae or teneral adults were exposed to treated soil (Yee and Lacey 2005). Adults of *Rhagoletis cerasi* Loew (Diptera: Tephritidae) were highly susceptible to *B. bassiana* and *M. anisopliae*, but mortality of only about 25% occurred against third instars (Daniel and Wyss 2009). Mortality of apple maggot (*Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae)) larvae due to *B. bassiana* was 35%, and more than 70% of adult flies emerging from exposed pupae died due to infection (Muñiz-Reyes *et al.* 2014). *Beauveria bassiana* was not effective as a soil drench against *R. mendax* when applied shortly before first adult emergence in the early summer (Collins and Drummond 2010), but nothing is known about the use of other fungal pathogens to control *R. mendax*.

Because *R. mendax* are in the soil as pupae for approximately 11 months of the year and entomopathogenic fungi occur in soil, we evaluated the efficacy of *M. anisopliae* against pupae when they were exposed directly to the fungi (pupal dip experiment), through a drench using sterile sand in the laboratory (laboratory drench experiment), and through a soil drench in a lowbush blueberry field (field soil drench experiment). We hypothesised that pupal age influences susceptibility to *M. anisopliae*, testing whether application timing, either to pre-diapause pupae in the fall or to post-diapause pupae in the spring affected adult fly emergence and longevity.

Materials and methods

Laboratory pupal dip

Blueberry maggot pupae were obtained from lowbush blueberries that were harvested on 5 August 2011 from a field near Belmont, Nova Scotia, Canada (45°25′35″N, 63°22′26″W).

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Blueberries were held on screens over large wooden boxes containing sand and gravel into which the larvae that dropped from blueberries pupated. Pupae were floated from the sand and gravel on 23 September 2011 and put in petri dishes with sterilised, moist sand for approximately 100 days at 4 °C to complete diapause.

Metarhizium anisopliae var. *anisopliae* strain S54 cultures were originally obtained from soil collected in southern Alberta, Canada (Entz *et al.* 2005, 2008). Isolates were obtained from soil via washing, dilutions, and plating on dodine-based selective media (Chase *et al.* 1986) during exploration in 2004–2006. Conidia of the S54 strain were produced at the University of Lethbridge (Lethbridge, Alberta, Canada) by growth on potato dextrose agar, followed by inoculation and fermentation of sterile, standard flaked barley (Alberta Barley Commission, Calgary, Alberta, Canada) in 1-kg sterile, aerated mushroom spawn bags (SacO2, Microsac, Nevele, Belgium) from liquid culture or conidia subsampled from colonies grown on potato dextrose agar plates. A total of 800 g of dry conidia was produced for field testing and verification of insect infectivity in experiments in Canada (Entz *et al.* 2008). A portion (approximately 10 g) of this product was provided for the blueberry maggot tests conducted in Nova Scotia. Before shipping, spore viability was checked using selective agar for entomopathogenic fungi. Germination rates over time and among samples were 88–94% in this and all studies with batches of this product. Stock powder was stored at 4 °C before use in the experiment.

Immediately before use, conidia (12 mg) were suspended in distilled water (5 mL) with 0.01% Silwet L-77 (Momentive Performance Materials, Waterford, New York, United States of America) in a 15-mL centrifuge tube that was vortexed and vigorously shaken for five minutes. Conidial concentration was determined with a haemocytometer.

The suspension was poured into a 10-mL sterilised beaker. Twelve pupae were removed from the petri dish and placed on the centre of a square of fine white mesh that was gathered together from the corners and immersed in the suspension for 10 seconds. A few small, sterilised weights were included with the pupae to ensure the mesh sunk in the suspension to fully immerse the pupae. Control groups of pupae were immersed in the same way in distilled water or Silwet only.

Containers (620 mL) (waxed white paper cups; Solo Cup Company, Scarborough, Ontario, Canada) were partially filled to a depth of approximately 5 cm with 170 g of sand (PlaySand, Shaw Resources, Shubenacadie, Nova Scotia, Canada). Sand was dried at 105 °C for 24 hours and then rewetted to 0.125 mL distilled water per gram of sand. A 1 cm deep × 2 cm diameter depression was made in the sand at the centre of each container into which 12 treated pupae were placed in close proximity, but not touching each other, and then covered. There were four replications of each of three treatments: *M. anisopliae* in Silwet, Silwet alone, and water. Containers were placed in a growth chamber at 23 °C, 16:8 light:dark hours, and 60–70% relative humidity and covered with petri dishes (9 cm diameter) containing a small hole for ventilation. After three weeks, the petri dishes were replaced by clear plastic cups (414 mL) (Solo Cup Company, Scarborough Ontario, Canada) with approximately 20 small holes (1 mm diameter) for ventilation that fit tightly into the containers when placed upside down.

The upside-down cups were monitored twice daily for emerged flies, until no flies emerged for three consecutive days. Fly longevity was assessed by removing the inverted cup from the container and transferring each fly to a clear plastic holding cup (120 mL) (ThermoScientific, Waltham, Massachusetts, United States of America). Flies were provided with a moist cotton roll (3.5 cm long; number 2 medium, Mydent International, Hauppauge, New York, United States of America), sugar (1 mg), an amino acid mixture (Nielsen 1965), and vitamins (vitamin diet fortification mixture, Nutritional Biochemical, Cleveland, Ohio, United States of America) placed on lids into which inverted plastic holding cups were screwed. Cups were checked twice daily until fly death. No voucher specimens were retained for the three experiments described in this study.

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Laboratory soil drench

To obtain prepupal larvae, highbush blueberries were picked in late July from a field near Rawdon, Nova Scotia, Canada (45°3′37″N, 63°42′21″W) and held on screens above trays of moist paper. Over a two-week period during mid-August, larvae that fell from the blueberries were collected 3–4 times daily and immediately transferred to the soil surface in containers. Containers used in this experiment were the same as those used in the pupal dip experiment, except that they were partially filled with soil, not sand, from a lowbush blueberry field near Debert, Nova Scotia, Canada (45°26′31″N, 63°27′1″W) that was sieved through a 2-mm screen. Larvae burrowed into the soil where they pupated; 18 larvae were introduced to each container (42 total containers), and larvae that did not immediately burrow were replaced.

Conidial suspensions were prepared as in the pupal dip experiment except they were vortexed at 25 000 rpm for 45 minutes in sterile glass jars (500 mL), followed by shaking vigorously by hand. Because Silwet appeared to cause pupal mortality in the pupal dip experiment, we substituted 0.05% Tween 80 (Sigma-Aldrich, St. Louis, Missouri, United States of America) as the wetting agent. All 42 containers with pupae were treated on 12 September 2012 (pre-diapause), held at 15 °C for two months, and then placed at 4 °C for approximately100 days to complete diapause and treated again on 22 February 2013 (post-diapause). Treatments (10 mL) were applied to containers by dripping water, Tween, or conidial suspensions over the soil surface using a glass pipette. The treatments were (1) water (pre-diapause) + water (post-diapause), (2) Tween (pre-diapause) + water (post-diapause), (3) water + Tween, (4) Tween + Tween, (5) *M. anisopliae* in Tween + water, (6) water + *M. anisopliae* in Tween, (7) *M. anisopliae* in Tween + *M. anisopliae* in Tween. There were six replications of each treatment.

Containers were covered with petri dishes (9 cm diameter) containing a small hole for ventilation and held in a growth chamber, as in the pupal dip experiment. The temperature was reduced by decrements from 23 °C on 13 September to 13 °C on 23 October, after which containers were moved to a cooler at 4 °C to induce diapause in pupae. In order to minimise fungal growth, a solution (3 mL) of methyl paraben (0.6 g) dissolved in 95% ethanol (10 mL) and distilled water (600 mL) was misted over the soil surface in each container on 11 December 2012. On the same day, petri dish lids were replaced with a piece of tulle netting secured over each container with a rubber band. To maintain soil moisture levels, autoclaved water (3 mL) was misted onto soil in each container on 3 January, 30 January, 18 February, 13 March, and 5 April 2013.

On 18 February 2013, containers were moved from the cooler to a growth chamber at 13 °C, 16:8 light:dark hours. On 26 March, cups were fit into containers, as in the pupal dip experiment, except a clear plastic dome lid with a hole (Solo Cup Company, Scarborough, Ontario, Canada) acting as a funnel was placed between the container and the cup so that emerging flies were trapped in the cup. Emerging flies were treated as in the pupal dip experiment, except larger plastic cups (296 mL) with small holes in the top for ventilation were used with half a moist cotton roll and mortality was monitored daily. Fly cadavers were checked for outgrowth and sporulation of *M. anisopliae* within 10 days of death as evidence of mycosis.

Field soil drench

The effect of *M. anisopliae* soil drenches on blueberry maggot emergence was tested in a field experiment in wild blueberry plots at the Wild Blueberry Producers Association of Nova Scotia research farm near Debert, Nova Scotia, Canada (45°26′31″N, 63°27′1″W). An aluminium-framed high tunnel (1.8 m wide × 1.8 m high × 27.4 m long) (MultiShelter Solutions, Palmerston, Ontario, Canada) covered with dark shade cloth was placed over the experimental area. Blueberries were harvested from commercial fields and placed on hardware cloth mesh (1.5 × 1.5 cm openings) nailed to wooden frames that were supported by wooden posts (Fig. 1A). Prepupal larvae exiting blueberries were directed through a funnel attached below the screen to pupate in a 15 × 15 cm area of soil beneath lowbush blueberries. Each area became an experimental plot. On 26 July 2012,

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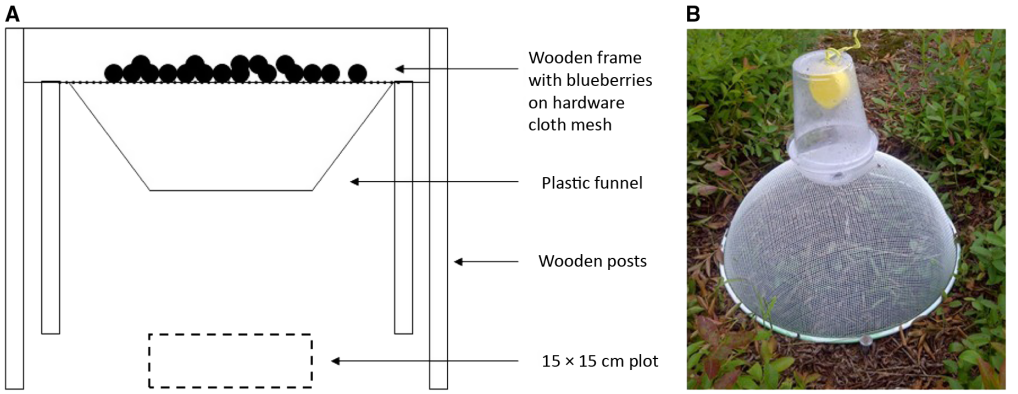

Fig. 1. Structures used in the field soil drench experiment to **A**, infest plots with blueberry maggot pupae and **B**, capture emerging blueberry maggot flies.

179 g of blueberries from a field near Debert, Nova Scotia, Canada (45°26′31″N, 63°27′1″W) were placed on mesh above each plot, and on 1 and 2 August 2012, 1.34 kg and 0.81 kg of blueberries from a field near East Village, Nova Scotia, Canada (45°26′19″N, 63°34′33″W) were placed on mesh above each plot. On 14 September, once blueberries had dried and all blueberry maggot larvae exited blueberries to pupate, the frames and funnels were removed. The blueberry stems in 1 m^2 centred on each plot were pruned to a height of about 5 cm.

The plots ($n = 28$) were created in two rows under the tunnel by splitting each row to create four blocks, each containing seven plots. Treatments were allocated in a randomised complete block design using the same treatments and preparation protocol as described in the laboratory soil drench experiment. On 25 September 2012 and 8 May 2013, treatments (200 mL) were dripped evenly over the 1 m^2 of trimmed blueberry stems using a 1-mL pipette tip secured to the bottom of a glass funnel.

On 28 June 2013, emergence traps were placed over the centre of the 1 m^2, encompassing the 15 × 15 cm area into which blueberry maggot larvae dropped and pupated (Fig. 1B). Traps were a metal mesh dome (bottom diameter = 35 cm) with a hole cut at the apex of the dome to which an overturned clear plastic dome lid with a hole (Solo Cup Company, Scarborough, Ontario, Canada) was glued. A clear plastic cup (120 mL) (ThermoScientific, Waltham, Massachusetts, United States of America) was placed in the dome lid into which a yellow ammonium lure (GL/GL-3000-10, Great Lakes IPM, Vestaburg, Michigan, United States of America) was placed and secured using twist ties through a small hole in the top of the cup. Emergence traps were held down with 10 cm metal spikes, and blueberry foliage was trimmed again so that trap bottoms were flush to the soil surface. Emerged flies were removed daily from traps for one month. Flies were held as described in the laboratory soil drench experiment to assess longevity.

Data analysis

For all experiments, analysis of variance was used to determine treatment effects on the percentage (pupal dip and laboratory drench) or number (field drench) of emerged flies, average longevity of flies, and per cent fly cadavers exhibiting evidence of mycosis. Average longevity was calculated for all flies emerging from each experimental unit in the laboratory and field drench experiments. In the field experiment, no flies emerged from four of seven plots in one block, so this block was excluded from the longevity analysis. Because only two fly cadavers from the field experiment showed evidence of mycosis, per cent mycosis was not analysed. Treatment means were compared using Tukey's honestly significant difference test, $\alpha = 0.05$. Residuals were checked for

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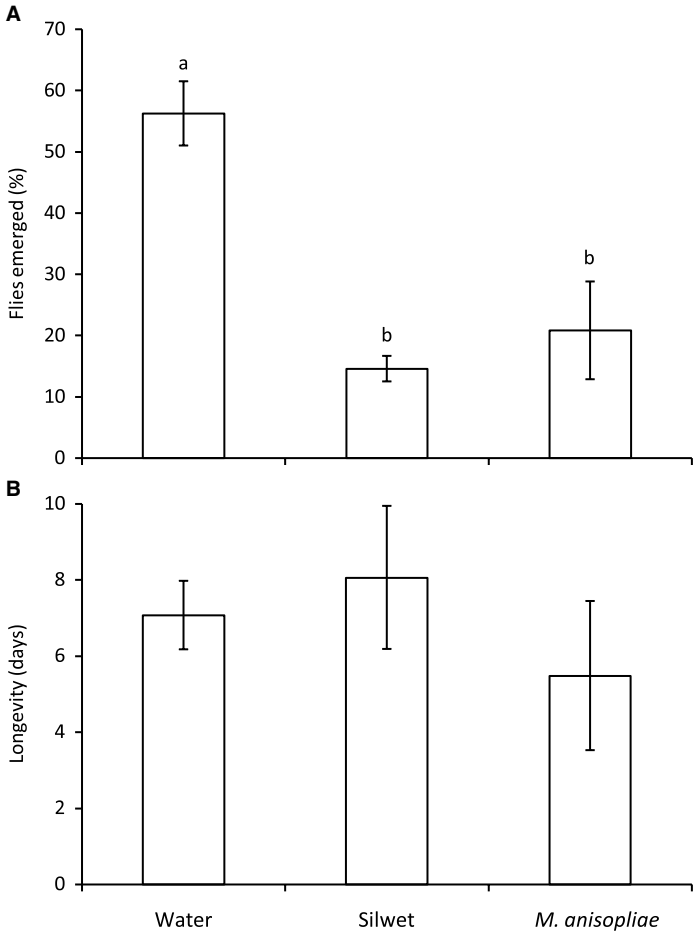

Fig. 2. Mean (standard error of mean) percentage of *Rhagoletis mendax* adult flies emerged **A**, and longevity of emerged flies **B**, when pupae were dipped in water, Silwet (wetting agent, 0.01%), or *Metarhizium anisopliae* + Silwet. Different letters indicate significantly different means using Tukey's honestly significant difference test at $\alpha = 0.05$.

homogeneity of error variance with Shapiro–Wilk test; no data transformation was necessary. JMP statistical software (SAS Institute 2012) was used for all analyses at $\alpha = 0.05$.

Results

Laboratory pupal dip

The treatment used for *R. mendax* pupal dips significantly affected adult fly emergence ($F_{2,9} = 15.86$; $P = 0.001$). Fewer flies emerged from pupae dipped in Silwet and *M. anisopliae* than from those dipped in water (Fig. 2A). Treatment did not significantly affect fly longevity ($F_{2,9} = 0.62$; $P = 0.559$) (Fig. 2B).

Laboratory soil drench

Combinations of pre-diapause and post-diapause treatments significantly affected *R. mendax* fly emergence, longevity, and incidence of mycosis (Table 1). When *M. anisopliae* was applied in Tween 80 to pre-diapause and/or post-diapause pupae, *R. mendax* emergence was significantly

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Table 1. Mean emergence, longevity, and mycosis (standard error of mean) of *Rhagoletis mendax* flies after sequential treatment of 18 pupae buried in soil in containers in a laboratory experiment with drenches of water, Tween 80 (wetting agent), or *Metarhizium anisopliae* in Tween 80 during pre-diapause and post-diapause pupal development ($n = 6$ replicates).

Pre-diapause treatment	Post-diapause treatment	Flies emerged (%)		Longevity (days)		n*	Mycosed flies (%)	
Water	Water	79.6	(9.7)^{a}	12.3	(0.4)^{abc}	75	11.6	(1.5)^{c}
Tween	Water	65.7	(3.3)^{ab}	11.1	(0.5)^{abcd}	66	11.3	(2.6)^{c}
Water	Tween	64.8	(1.9)^{ab}	12.3	(0.5)^{ab}	66	10.4	(2.7)^{c}
Tween	Tween	58.3	(9.1)^{b}	12.5	(0.3)^{a}	62	9.5	(3.9)^{c}
M. anisopliae + Tween	Water	56.5	(4.2)^{b}	10.4	(0.5)^{bcd}	60	20.7	(3.5)^{bc}
Water	*M. anisopliae* + Tween	60.2	(4.8)^{b}	10.0	(0.4)^{d}	64	38.3	(8.5)^{ab}
M. anisopliae + Tween	*M. anisopliae* + Tween	58.3	(4.2)^{b}	10.3	(0.3)^{cd}	63	46.1	(8.0)^{a}
	$F_{6,35}$ P	3.5	0.008	5.8	0.0003		8.8	< 0.0001

Letters indicate significantly different means using Tukey's honestly significant difference test at $\alpha = 0.05$.
*n = Total numbers of *R. mendax* assessed for longevity.

Table 2. Mean number and longevity (standard error of mean) of *Rhagoletis mendax* flies after sequential treatment of pupae buried in soil in a field experiment with drenches of water, Tween 80 (wetting agent), or *Metarhizium anisopliae* in Tween 80 during pre-diapause and post-diapause pupal development ($n = 4$ replicates).

Pre-diapause treatment	Post-diapause treatment	Number of flies emerged		Longevity (days)		n*
Water	Water	2.8	(1.4)	9.4	(1.6)	11
Tween	Water	2.5	(1.2)	10.2	(2.1)	10
Water	Tween	2.0	(1.9)	9.0	(1.1)	8
Tween	Tween	2.0	(1.4)	12.1	(1.8)	8
M. anisopliae + Tween	Water	1.3	(0.4)	9.7	(2.3)	5
Water	*M. anisopliae* + Tween	2.8	(0.9)	13.3	(0.9)	11
M. anisopliae + Tween	*M. anisopliae* + Tween	1.5	(0.7)	13.0	(1.7)	6
	$F_{6,18}$, P	0.7	0.64	1.5†	0.22	

*n = Total numbers of *R. mendax* assessed for longevity.
†Error df = 12 for longevity analysis.

reduced compared to water-only controls (Table 1). The longevity of *R. mendax* flies was significantly reduced by about two days due to soil drenches of *M. anisopliae* to either or both pre-diapause and post-diapause pupae compared to water-only controls (Table 1). Incidence of mycosis was significantly higher on flies from pupae that were exposed to *M. anisopliae* post-diapause compared to water-only controls (Table 1). Applications of Tween 80 had no impact on fly emergence, longevity, and incidence of mycosis compared to water-only controls, except that application to pre-diapause and post-diapause pupae reduced the number of flies that emerged (Table 1).

Field soil drench

Combinations of pre-diapause and post-diapause treatments did not significantly affect *R. mendax* fly emergence or longevity (Table 2). Overall, fly emergence was low, but lowest

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(about 50% less than that in water-only control) when *M. anisopliae* applications were made to pre-diapause pupae (Table 2).

Discussion

Results indicated that *R. mendax* pupae were somewhat susceptible to *M. anisopliae* S54. From the laboratory and field drench experiments results, *M. anisopliae* application to pre-diapause pupae was slightly more effective than to post-diapause pupae and applying at both times did not improve efficacy. Pupae of other *Rhagoletis* Loew species showed little or no susceptibility to entomopathogens that affected adults or larvae (Yee and Lacey 2005; Daniel and Wyss 2009; Muñiz-Reyes *et al.* 2014). Development of pupae within puparium normally occurs in soil where contact with numerous pathogenic organisms has led to structures, mainly the integument, that resist infection (Dubovskiy *et al.* 2013). Examination of *R. pomonella* pupae using scanning electron microscopy has shown conidia attached to pupae, growth of long germination tubes along pupae, but little evidence of cuticular penetration (Muñiz-Reyes *et al.* 2014). However, for *R. indifferens*, a significant proportion of puparium and pupae (pre-overwinter) were mycosed and damaged when exposed to *B. bassiana* (Cossentine *et al.* 2010), and for tropical Tephritidae, with shorter pupation times, high levels of *M. anisopliae* sporulation on puparium have been observed (*e.g.*, Ekesi *et al.* 2002). Therefore, *M. anisopliae* S54, like some other entomopathogens tested against other *Rhagoletis* species pupae, may not be highly virulent, but is able to infect some puparium, depending on cuticle hardness and degree of sclerotisation (St Leger 1993).

Shorter fly longevity and an increased percentage (up to 35% more) of fly cadavers exhibiting evidence of *M. anisopliae* mycosis in the laboratory drench experiment demonstrate that adults are also susceptible to entomopathogens, as with other *Rhagoletis* species (Daniel and Wyss 2009; Muñiz-Reyes *et al.* 2014). Flies may have come into contact with spores as they eclosed from puparia or in soil as they moved to the surface (Ekesi *et al.* 2002). Higher incidence of mycosis in flies emerging from soil when *M. anisopliae* was applied later (to post-overwintering pupae) rather than earlier suggests a reduction in virulence of spores over time. Interestingly, around 10% of fly cadavers in water-only or water and Tween treatments exhibited evidence of *M. anisopliae* mycosis. *Metarhizium anisopliae* caused a two-day reduction in fly longevity, from about 12 to 10 days. From a control perspective, such a short reduction may not be valuable as female flies would still have sufficient time to mate and oviposit in blueberries. However, living, infected flies may transmit pathogens horizontally, thus increasing disease incidence within the population.

In the pupal dip experiment, Silwet alone caused a 40% reduction in fly emergence compared to the control, and addition of *M. anisopliae* did not further reduce fly emergence. Silwet L-77 is an organosilicone surfactant known to be toxic to tephritid pupae, causing reduced emergence of *C. capitata*, *Bactrocera dorsalis* (Hendel) and *B. cucurbitae* (Coquillett) (Diptera: Tephritidae) when used in the range of 0.025–1.0% (Purcell and Schroeder 1996). It has also been toxic to other arthropods in the laboratory, including *Diaphorina citri* Kuwyama (Hemiptera: Liviidae), *Myzus persicae* Sulzer (Hemiptera: Aphididae), and pests of table grape (*Vitis vinifera* Linnaeus; Vitaceae) (Imai *et al.* 1995; Tipping *et al.* 2003; Srinivasan *et al.* 2008). Silwet may be useful as a management tool, but it is difficult to predict toxicity in the field from bioassay results. Results from the laboratory drench experiment showed a small effect of the surfactant Tween 80 on *R. mendax* emergence, particularly when it was applied twice, but no effect on longevity or incidence of mycosis.

The level of pathogenicity caused by an *M. anisopliae* isolate can vary from organism to organism, and different isolates produced variable mortality on the same stage of the same organism (*e.g.*, Ekesi *et al.* 2002). As noted, *M. anisopliae* S54 was isolated from soils in southern

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Alberta, Canada, using a polymerase-chain-reaction-based diagnostic method (Entz *et al.* 2005). Laboratory and field testing indicated that this isolate resulted in high mortality to grasshoppers (Orthoptera: Acrididae), a major pest in that region (Entz *et al.* 2008), and was incorporated into a plan for pest management (Johnson *et al.* 2010). This isolate caused lower and more variable levels of mortality in other insects: 20–60% of sweetclover weevil (*Sitona cylindricollis* Fahraeus (Coleoptera: Curculionidae)), 10–40% of *Lygus* bugs (*Lygus keltoni* Schwartz (Hemiptera: Miridae)), and 0% of Hymenoptera (D.L.J., unpublished data). In the future, testing isolates of *M. anisopliae* from soils in the endemic range (northeastern North America) of *R. mendax* may be fruitful, particularly since in some cases mortality of the soil-dwelling stages has been estimated at up to 60% (Renkema 2011).

Overall, direct application or soil drenching of *M. anisopliae* S54 was not highly effective against *R. mendax* pupae. Results suggest that younger, pre-diapause pupae may be more susceptible than older pupae, and effects on emerging flies should also be investigated. Other factors such as inoculum density, soil texture, temperature, moisture, and microflora can influence pathogenicity (McCoy *et al.* 1992), and effects on other known biological control agents of *R. mendax* (*e.g.*, ground beetle larvae) need to be considered before recommendations for control of *R. mendax* are made.

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