Mortality of Different Life Stages of *Rhagoletis indifferens* (Diptera: Tephritidae) Exposed to the Entomopathogenic Fungus *Metarhizium anisopliae*[1]

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Abstract Effects of the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin on the mortality of different life stages of the western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), were determined in laboratory tests. When teneral adults were exposed to fungal spores mixed in soil (7.63×10^5 and 1.61×10^6 spores/g) or applied to the surface of soil (1.14×10^6 and 2.28×10^6 spores/cm^2) with 13 to 30% moisture, adult emergence was not reduced, but 14.9 to 68.0% of emerging adults were infected at death. When adult flies were exposed to various concentrations of dry spores inside vials, 15 mg (4.59×10^8 spores/10 flies) was the lowest needed for 100% mortality at 7 d post exposure, and resulted in 5.96×10^6 spores adhering to each fly. Females exposed to 1.80 mg (5.51×10^7 spores/10 flies) laid as many eggs as control females between 0 and 3 d post exposure, but due to mortality infected flies laid fewer eggs between 3 and 7 d. Third-instar larvae exposed to treated soil (9.63×10^4 to 4.81×10^6 spores/cm^2) with 20% moisture were not infected.

Key Words Cherry fruit fly, *Rhagoletis indifferens, Metarhizium anisopliae,* infection, mortality

The western cherry fruit fly, Prunus avium (L.) L. *Rhagoletis indifferens* Curran, is the major pest of sweet cherries, in the Pacific Northwest of the United States. Adult flies emerge in May and June from soil beneath host trees and lay eggs in fruit within 2 wks of emergence (Frick et al. 1954). Larvae feed in the fruit for 1 to 2 wks, emerge as third instars that drop onto the ground, and then burrow into the soil to pupate. Pupae remain in the soil until the following May or June, when teneral adults crawl out of the soil. Adults are active for about a month in central Washington, when cherries are on trees and oviposition can occur (Frick et al. 1954).

Because of the zero tolerance for infested fruit, *R. indifferens* has historically been controlled using organophosphate and carbamate insecticides (Zwick et al. 1970, 1975), but the growing concern about their effects on human health and the environment makes the search for biological control agents desirable. Flies in commercial cherry-growing areas are found mostly in abandoned trees or trees in isolated backyards, which are the likely sources of infestations in orchards. Control of flies in these trees using biological agents combined with other methods is a possible alternative to control using insecticides alone. One biological control agent that holds promise in the

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management of subtropical fruit flies in the genera *Ceratitis* and *Anastrepha* is the entomopathogenic fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin (Garcia et al. 1980, 1984, Castillo et al. 2000, Lezama-Gutiérrez et al. 2000, Ekesi et al. 2002, 2003, Dimbi et al. 2003a, 2003b, 2004). However, whether results from studies with subtropical fruit flies can be extrapolated to temperate fruit flies is unknown.

In this series of laboratory studies, the objective was to determine the effects of *M. anisopliae* on mortality of the different life stages of *R. indifferens* under laboratory conditions. Results are discussed with respect to factors affecting the mortalities of each life stage and the possible use of the fungus for fly control. Comparisons between subtropical fruit flies and *R. indifferens* are also made.

Materials and Methods

Fungus source. *Metarhizium anisopliae* was isolated from wireworm larvae, *Limonius canus* LeConte (Coleoptera: Elateridae), that were naturally infected and collected in central Washington in 2001. Cultures were maintained initially on Sabouraud dextrose agar with yeast extract (SDAY) using methods described in Goettel and Inglis (1977). Wax moth larvae, *Galleria mellonella* L., were used to propagate spores used in our tests. Twenty fifth-instar larvae were placed on Whatman filter paper (Maidstone, England) moistened with 2 ml of a *M. anisopliae* spore suspension (10 mg spores/10 ml water + 0.5% Tween® [Fisher Biotech, Fair Lawn, NJ]) in a 9-cm Petri dish and incubated at 25°C until death and maximum sporulation (approximately 10 d). Cadavers were collected, placed on an orbital shaker containing three sieves (in series, 2000, 500, and 125 μm openings [U.S. Standard Sieves, Dual Mfg. Co, Chicago, IL]) and shaken for 10 to 15 min at 300 rpm. Spores exiting the final sieve were collected, weighed, and germination rates determined (>90%, 24 h, SDAY [Goettel and Inglis, 1977]). Spores were stored at 4°C and used within 7 to 10 d of collection.

Fly source and experimental conditions. Flies used in teneral adult experiments were obtained as larvae from infested cherry fruit collected in central Washington in June 2002 and 2003. Cherries were placed on screens in tubs (25 cm wide × 30 cm long × 13.5 cm high) over a 2 to 3 cm layer of moist soil (1:1:1 volumes of sand, peat moss, vermiculite). The peat moss and vermiculite were ground into approximately 0.5 to 3 mm particles, resulting in a soil density of 0.58 g/cm^3. Emerging larvae dropped into the soil and pupated. Pupae were stored in moist soil inside plastic containers at 3°C for 10 and 8 months from 2002 to 2003 and 2003 to 2004, respectively, before experiments. Flies for post-emergent adult and fecundity experiments were collected from infested cherry trees in June and July 2003 or were reared from larvae collected in 2003. Adults were collected in glass vials, then placed inside screen cages containing water and food (dry 20% yeast extract-80% sucrose) on paper strips. All tests were conducted at ambient conditions ranging from 25.5 to 27°C at 30 to 40% RH and under 16 h L: 8 h D photoperiods.

Mortality of adults exposed as tenerals in soil. Five experiments designed to determine the effects of *M. anisopliae* on mortality of teneral adults were conducted, each following the same general methods, but with slight variations. Seventeen days after removing from storage (4 to 7 d prior to first emergence), pupae were spread uniformly on the bottoms of clear round plastic test containers (11.5 cm diam × 7.8 cm high). Soil used in all experiments (same type as for larval collections) was heated at 110°C for 1.5 h, and then cooled. Fungal spores were either mixed in soil and then

placed on top of pupae or applied to soil that was already on top of the pupae. In both cases, there was a 1 to 2.5 cm layer of soil above the pupae. In nature, most pupae are found 2.5 to 10 cm below the soil surface (AliNiazee 1974).

Experiments 1-3 (using emerging adults from larvae collected in June 2002) and experiments 4 and 5 (using emerging adults from larvae collected in June 2003) were conducted using 2 application methods, 4 spore application rates (with 0.25% Tween®), 3 soil moistures, and 4 methods of retaining soil moisture or allowing moisture loss using lids on containers, as shown in Table 1. Application of spores on the top of the soil was achieved by spreading the suspension evenly over the soil surface using a 1-ml pipette (1 mm hole diam). Soil moisture was determined based on weight of water and soil. Depending on the experiment, a lid was used to seal a container throughout an experiment or it was replaced with a perforated lid after first fly emergence. A perforated lid, with a 2.5-cm diam hole covered with organdy cloth, was used to allow gradual loss of soil moisture. In one experiment, Parafilm™ (Pechiney Plastic Packaging, Chicago, IL) was used to seal the lid and containers to reduce moisture loss (Table 1).

In experiment 1, there were 50 pupae per replicate, and in experiments 2 and 3,

Table 1. Methods used in 5 laboratory experiments (Expt) testing effects of *Metarhizium anisopliae* on infection and mortality of adult *Rhagoletis indifferens* exposed as tenerals

Expt	Application method*	Spores/g soil or cm^2 of surface	Amount soil (g)	Soil moisture**	Containers†
1	Mixed	7.63×10^5/g	95	20%	Sealed with lid throughout
2	Mixed	1.61×10^6/g	45	20%	Sealed with lid until first fly emerged, then with perforated lid‡
3	Surface	$1.14 \times 10^6/cm^2$	45	20%	Sealed with lid until first fly emerged, then with perforated lid‡
4	Surface	$2.28 \times 10^6/cm^2$	45	13%	Sealed with lid throughout
5	Surface	$2.28 \times 10^6/cm^2$	45	30%	Sealed with lid and Parafilm throughout

Soil was a 1:1:1 mix (by volume) of sand, peat moss, and vermiculite.

* Mixed-spore suspension added to soil and placed on top of pupae; Surface—spore suspension applied on soil surface using a pipette, pupae 1 cm below surface.

** Immediately after introduction of soil with spores (mixed) or immediately after application of spores (surface).

† Clear round plastic, 11.5 cm diam × 7.8 cm high.

‡ Perforation was 2.5 cm in diam, covered with organdy cloth.

there were 40 pupae per replicate. There were five replicates of the control and treatment for each experiment. In experiments 4 and 5, there were five replicates of 50 pupae for the control and treatment in each of three tests.

In all five teneral adult experiments, adults were collected every 24 h until emergence ended, usually by day 7. Adults were transferred to 0.473-l paper containers with water and food (maximum of 25 flies per container). The numbers of flies that died in the paper containers were recorded every 1 or 2 d for 14 d. Dead flies were dipped in 70% ethanol, and then 5% bleach for about 2 s and rinsed in deionized water to remove or kill spores on their exterior surfaces. Flies were patted dry on paper towels for 5 to 10 s and then placed on water agar inside Parafilm-sealed Petri plates at 25°C. Mycosis (fungal infection) was recorded 7 to 14 d after flies had died.

Mortality of adults exposed after emergence to different fungal concentrations. For concentration experiments, flies were collected using aspirators from field trees and held in the laboratory for at least 5 to 7 d. Flies were exposed to low concentrations of 0, 0.05, 0.10, 0.20, and 0.30 mg of dry spores/vial/10 flies and higher dosages of 0, 5, 10, 15, and 20 mg dry spores/vial/10 flies. Each mg contained a mean of 3.06×10^7 spores (seven samples). Ten flies (8 males:2 females) were placed inside a 2.4 cm wide × 9.3 cm long cylindrical glass vial. (The 8 male:2 female ratio was that normally seen in the field, as females are more difficult to see and catch than males.) Spores were placed on wax paper and weighed using a microbalance (Sartorius, Goettingen, Germany). Flies were chilled at 1.1°C for 3 min to temporarily immobilize them, after which fungus was introduced into the vial. The vial was plugged with a rubber stopper and gently rolled on its side on a flat surface for a few seconds. Flies were left inside vials for 30 min. Control flies were chilled only. Each set of 10 flies was then removed from vials and transferred to a 0.473-l paper container with food and water. Mortality was checked daily for 20 d. Dead flies were handled as in previous experiments. There were five and six replicates of the low and high concentration treatments, respectively.

The numbers of spores adhering to a fly after exposure to the lowest concentration that caused significant mortality (0.30 mg/10 flies) and to the lowest concentration that caused 100% mortality (15 mg/10 flies) after 7 d were determined. Ten flies (8:2 males:females or 2 to 7 males: 3 to 8 females, depending on availability) were treated with spores as before. Flies were transferred to and held in 0.473-l paper containers for 60 min. They were then chilled at 1.1°C for 3 min, removed, and placed in a vial with 5 ml of water with 0.5% Tween. The vial was shaken to remove the spores. Two 20-μl samples were removed from each vial with a pipette, placed on a hemacytometer, and the spores counted. Mean spore counts per fly were determined from five groups of 10 flies for each of the two concentrations.

Effect of fungus on fly fecundity. For the fecundity experiment, 25- to 30-d old laboratory-reared females that had been maintained with males were exposed to spores as previously described. A concentration of 1.80 mg of dry spores/vial/10 females (5.51×10^7 spores) was chosen because the intent was to infect flies, but at the same time keep them alive long enough so they could oviposit. Immediately after treatment, single flies were transferred to 0.473-l paper containers with single males and food and water. One cherry was introduced into a container 2 h later. The cherry was removed after 3 d and replaced with a new cherry. Fly deaths were recorded daily the first 3 d and once at 7 d, when tests ended. Deaths were not checked at 4 to 6 d. Mycosis was confirmed as before. Fruit were examined for eggs. There were 21 control and 20 treated flies.

Effect of fungus on larval and pupal mortality. For the larval experiment, field-infested fruit were collected in June 2003. Fruit were placed on screens above water in tubs. Larvae dropped into the water. They were then removed and allowed to dry. Ten ml of water (control) and spore suspensions were sprayed with a squirt bottle onto the surface of 90 g of soil (1:1:1 volumes of sand: peat moss: vermiculite) in the same type of container used in the teneral adult experiments, to obtain 20% soil moisture. Actively crawling late third instars were dropped onto soil with 9.63×10^4, 4.81×10^5, 9.63×10^5, and 4.81×10^6 spores/cm^2. Containers were sealed with lids for 2 to 4 wks, after which puparia were opened to determine infection. There were five replicates of the control and treatments, each with 20 larvae, in each of two tests.

Statistics. Analysis of variance (ANOVA) was conducted for mortality of teneral adult data. Probit analysis was not performed for concentration-adult mortality data because of insufficient sample sizes (according to Robertson et al. [1984], at least 120 subjects are needed). Instead, linear and quadratic regressions were performed for cumulative percent mortality (square root and arcsine transformed) at 7, 14, and 20 d for both low and high concentration experiments. Fecundity and larval mortality data were analyzed using ANOVA. The Statistical Analysis System (SAS Institute 2001) was used for analyses. Means ± SE are reported.

Results

Mortality of adults exposed as tenerals in soil. There was no effect of *M. anisopliae* on percent adult emergence in experiments 1, 2, and 3 ($P > 0.05$) (Table 2). However, in all experiments, flies were infected at significant levels when exposed to the fungus ($F = 28.3$-88.3; df = 1,8; $P = 0.0004$ to $P < 0.0001$). Most infected flies died 3 to 5 d post emergence. There was greater overall mortality in treated than control flies in experiments 1 and 3 ($F = 130.1$; df = 1,8; $P < 0.0001$ and $F = 5.7$; df = 1,8; $P = 0.0440$), but not in experiment 2 ($F = 1.4$; df = 1,8; $P = 0.2680$) (Table 1).

There was no difference in mortality among tests 1-3 in either experiment 4 or 5 ($P > 0.05$), so data from tests within experiments were combined. There was no effect of *M. anisopliae* on percent adult emergence in experiments 4 and 5 ($P > 0.05$), as emergence from control and treatment groups was equally high (Table 3). However, adults exposed to soil with 13% moisture in experiment 4 showed greater mortality than control flies (infection: $F = 1{,}385.2$; df = 1,4; $P < 0.0001$; mortality: $F = 80.7$; df = 1,4; $P < 0.0001$) (Table 3). This was also the case in flies from soil with 30% moisture in experiment 5 (infection: $F = 583.8$; df = 1,4; $P < 0.0001$; mortality: $F = 109.5$; df = 1,4; $P = 0.0005$) (Table 3). Flies exposed to *M. anisopliae* in soil with 13% moisture showed lower mortality 3 to 5 d post emergence than flies exposed in soil with 30% moisture (data not analyzed because the two experiments were not conducted simultaneously). There were 3.5 and 2% soil moisture losses in experiments 4 and 5, respectively, between 0 and 6 d post treatment, after >90% fly emergence had occurred.

Mortality of adults exposed after emergence to different fungal concentrations. When post-emergent adults were exposed to 0.05 to 0.20 mg concentrations (1.6 to 6.2×10^6 spores/10 flies), mortality was not significant, but when they were exposed to the 0.30 mg concentration (9.3×10^6 spores/10 flies), mortality was greater than in the control at 7 d post exposure (linear regression: $R^2 = 0.128$; $F_{1,23} = 4.5$; $P = 0.0444$), although not at 14 and 20 d post exposure ($P > 0.05$) (Fig. 1A). When flies were exposed to the 5 to 20 mg concentrations (1.53 to 6.12×10^8

Table 2. Effects of *Metarhizium anisopliae* on infection and mortality* of adult *Rhagoletis indifferens* exposed as tenerals in soil with 20% moisture

	Experiment 1**		Experiment 2†		Experiment 3‡	
Parameter	Control	Treatment	Control	Treatment	Control	Treatment
% Fly						
Emergence	30.0 ± 2.1a	24.4 ± 4.9a	21.5 ± 4.7a	24.5 ± 3.5a	21.0 ± 4.4a	26.0 ± 3.4a
% of Flies						
Died Infected	0.0 ± 0.0a	68.0 ± 15.0b	0.0 ± 0.0a	30.1 ± 5.3b	0.0 ± 0.0a	63.2 ± 11.9b
Total % Fly						
Mortality	19.2 ± 6.3a	98.6 ± 1.4b	24.1 ± 3.8a	32.7 ± 5.6a	24.9 ± 8.6a	63.2 ± 11.9b

Means (±SE) followed by same letter within rows and experiments are not significantly different (ANOVA, $P > 0.05$).

* Determined between 1 to 14 d after emergence inside cages.

** 7.63×10^5 spores/g of soil, mixed in soil.

† 1.61×10^6 spores/g of soil, mixed in soil.

‡ 1.14×10^6 spores/cm^2 of soil, applied on surface.

Five replicates, each with 50 (experiment 1) or 40 (experiments 2 and 3) pupae.

Table 3. Effects of *Metarhizium anisopliae* on infection and mortality* of adult *Rhagoletis indifferens* exposed as tenerals in soil with 13 and 30% moisture

	Experiment 4: 13% soil moisture**		Experiment 5: 30% soil moisture**	
	Tests 1-3		Tests 1-3	
Parameter	Control	Treatment	Control	Treatment
% Fly Emergence	77.6 ± 3.5a	73.6 ± 3.4a	80.0 ± 2.6a	80.9 ± 6.2a
% of Flies Died Infected	0.0 ± 0.0a	14.9 ± 0.8b	0.0 ± 0.0a	26.8 ± 2.0b
Total % Fly Mortality	6.0 ± 1.2a	24.5 ± 1.5b	8.7 ± 1.2a	35.8 ± 2.3b

Means (±SE) followed by same letter within rows and experiments are not significantly different (ANOVA, $P > 0.05$).

* Determined between 1 to 14 d after emergence inside cages.

** 2.28×10^6 spores/cm^2 of soil, applied on surface.

Tests 1-3 for each experiment were combined, as no differences existed between them; each test was comprised of five replicates, each with 50 pupae.

spores/10 flies), mortality was significant at 7, 14, and 20 d (quadratic regressions: 7 d: $R^2 = 0.827$; $F_{2,25} = 65.6$; $P < 0.0001$; 14 d: $R^2 = 0.838$; $F_{2,24} = 70.6$; $P < 0.0001$; 20 d: $R^2 = 0.733$; $F_{2,23} = 35.4$; $P < 0.0001$) (Fig. 1B). The 15 mg concentration (4.59×10^8 spores/10 flies) was the lowest needed for 100% mortality at 7, 14, and 20 d post exposure (Fig. 1B). Interestingly, flies covered with spores from this concentration were seen mating in containers. The 0.30 and 15 mg concentrations resulted in $8.53 \times 10^5 \pm 6.44 \times 10^4$ and $5.96 \times 10^6 \pm 1.33 \times 10^6$ spores adhering to each fly, respectively.

Effect of fungus on fly fecundity. Seventeen of the 20 female flies exposed to the fungus were infected. Control and infected flies laid 26.0 ± 4.9 and 34.6 ± 6.5 eggs/fly, respectively, between 0 and 3 d ($F = 1.1$; df = 1,36; $P = 0.3128$). Respective mortalities the first 3 d were 14 and 59%. By 7 d, an additional 18% of control and 100% of infected flies had died. Between 3 and 7 d, control flies (n = 17 after day 3) laid 36.4 ± 8.4 eggs/fly, whereas infected flies (n = 7 after day 3) only laid 0.4 ± 0.4 eggs/fly ($F = 11.6$; df = 1,22; $P = 0.0025$).

Effect of fungus on larval and pupal mortality. No larvae were infected by *M. anisopliae* at any of the four spore concentrations in the two tests of the experiment ($P > 0.05$). Dissections showed that most pupae were alive, bright yellow in color, and had fully formed heads and appendages. Fungus covered the surfaces of the puparia of some pupae. Dead pupae were not infected.

Discussion

The results indicate that application of *M. anisopliae* spores in or on the soil did not reduce adult *R. indifferens* emergence, but that it did cause significant infections and

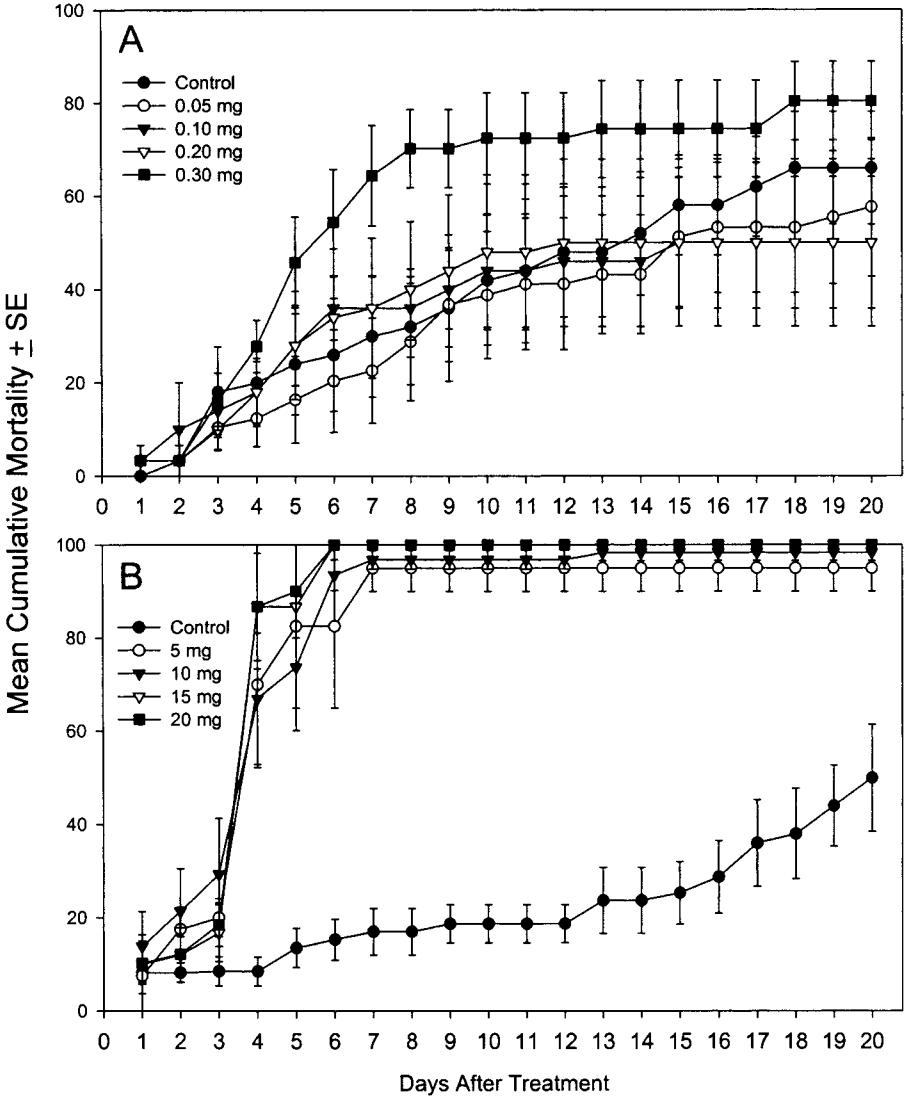

Fig. 1. Cumulative mortality over 20 d of field-collected *Rhagoletis indifferens* adults exposed to (A) low and (B) high concentrations of dry fungal spores of *Metarhizium anisopliae.* Concentrations are mg of spores/10 flies in a 2.4 cm wide × 9.3 cm long cylindrical vial. Each mg contained 3.06×10^7 spores. Experiments at 27°C and 30 to 40% RH.

mortality in flies after emergence. The extracellular enzymes produced by fungi (Charnley 1997) were apparently unable to degrade the chemicals in the puparia of the late-stage pupae, but they must have degraded them in the cuticles of either the teneral or recently-emerged, <3-d old adults. In contrast, emergence of the Mexican

fruit fly, *Anastrepha ludens* (Loew), was reduced by 22% in the field when *M. anisopliae* spores were applied to the soil surface (Lezama-Gutiérrez et al. 2000), and emergence of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), was reduced by up to 60% when spores were mixed in the soil in the laboratory (Ekesi et al. 2003). The mortality of adult *R. indifferens* exposed as tenerals in soil was relatively low compared with *C. capitata,* in which 36 to 100% of emerged adults died when larvae were exposed (Ekesi et al. 2002), although the concentration used (5 ml of 1 $\times 10^8$ spores/ml in 50 g beach sand) was higher than in this study.

Maximum infection rates of *R. indifferens* were 68 and 27% for teneral adult experiments 1, 2, and 3 and experiments 4 and 5, respectively, even though twice the spore concentration (2.90×10^7 spores/ml) was used in experiments 4 and 5. This suggests a possible attenuation of the fungus between experiments conducted one year apart, although germination rates were consistently $\geq$90%. Attenuation of *M. anisopliae* after subculturing on agar has been reported (Latch 1965). Cycling of spores through *R. indifferens* instead of through *Galleria* may maintain virulence, but evidence from an aphid-*Verticillium lecanii* (Zimm.) system (Hall 1980) suggests this would not increase it.

The greater mortality of *R. indifferens* from soil with 30% as compared to soil with 13% moisture indicates adequate moisture is critical for successful infections of teneral adult flies by *M. anisopliae*. The types of soil and moisture content probably interact and influence infection rates by affecting spore germination or percolation through the soil. In *C. capitata,* spore suspensions in sand with only 9% moisture were sufficient to cause high delayed adult mortality (Ekesi et al. 2002).

Metarhizium anisopliae was effective against post-emergent adults of *R. indifferens* only at high concentrations and usually did not cause death until 3 to 5 d after exposure. This suggests that strong defense barriers or mechanisms are present in the cuticle or hemocoel of fully sclerotized *R. indifferens,* that age affects these barriers or mechanisms, or that a fungal isolate with relatively low virulence was used in this study. In 2-d-old *C. capitata,* an LD_{50} value of 5.1×10^3 spores/fly was obtained when spores in aqueous suspensions were topically applied (Castillo et al. 2000), and in 5- to 10-d old *Ceratitis* spp., 90% mortality was obtained when flies were exposed to 4.2×10^5 and 1.0×10^6 dry spores/fly (Dimbi et al. 2003a). These amounts are about 6 to 14 times less than the amounts of dry spores that killed 100% of *R. indifferens* in our studies. However, similar to *R. indifferens, Ceratitis* spp. did not die immediately when infected with lethal concentrations: the time for 90% mortality was 5 to 6 d (Castillo et al. 2000) or 3 to 4 d (Dimbi et al. 2003a). The high spore coverage needed presents a problem in targeting the adult stage of *R. indifferens* in trees for control. The use of baited autoinoculation devices suggested for *Ceratitis* spp. (Dimbi et al. 2003a) is a possibility for bringing spores in contact with the adult flies, but only if more attractive baits and trap designs can be developed.

Infection by *M. anisopliae* did not affect the fecundity of *R. indifferens* up to 3 d post exposure, and seemed to affect it only through increased mortality between 3 and 7 d. This suggests that fungal growth, nutrient exhaustion, and toxicosis (Hajek and St. Leger 1994) had no direct effect on fecundity, but that they indirectly affected it by causing death quickly without making the flies significantly less vigorous 1 to 3 d prior to death. Infected females also mated, supporting this idea. However, as fecundity and mortality were not determined at days 4-6 post-treatment, it is possible fecundity in treated flies was lower than in non-treated flies without regard to mortality during the 2 d. In *C. capitata,* there were 30 to 55% reductions in fecundity 10 d after 1 μl of

M. anisopliae spore suspensions (1×10^2 to 1×10^6 spores/fly) were applied on the ventral abdomen of flies (Castillo et al. 2000), but it was unclear how later mortality accounted for these reductions. Results indicate infecting older, gravid *R. indifferens* does not prevent oviposition, but because females do not lay eggs until they are ≥5 d old (Frick et al. 1954), infecting young females may. *Ceratitis capitata* that were 0 to 7 d old were more susceptible than those 14 d old (Dimbi et al. 2003b).

In contrast to the pre- and post-emergent adults, the larvae and pupae of *R. indifferens* were not susceptible to *M. anisopliae.* The concentrations used may have been too low, the strain used was not virulent to larvae and pupae, or the last larval instar cuticle hardened too quickly for the fungus to penetrate, as *R. indifferens* pupate within 4 to 8 h of exiting fruit (Frick et al. 1954). The fungus covered the puparia of the pupae, but no infections occurred, suggesting it could not penetrate them. In contrast, larvae and pupae of *A. ludens* were highly susceptible to *M. anisopliae* when they were immersed in conidial suspensions for 30 s (Lezama-Gutiérrez et al. 2000), and larvae of three *Ceratitis* spp. were moderately to highly susceptible to *M. anisopliae* mixed in soil, based on pupal mortality (Ekesi et al. 2002) and adult emergence (Ekesi et al. 2003).

The results of this study indicate that *M. anisopliae* can cause significant mortality of two adult stages of *R. indifferens* under laboratory conditions. Of these two stages, the teneral adult probably should be targeted for control because high amounts of fungal spores can be brought into contact with flies in soil more easily than to those in the cherry trees. The potential use of the fungus as a control agent for teneral adult *R. indifferens* will depend on testing and finding more virulent isolates. The isolate of *M. anisopliae* used in our experiments was selected because it is indigenous to the irrigated regions of central Washington, but a more virulent isolate, perhaps obtained from a dipteran, will be required before practical control of *R. indifferens* using *M. anisopliae* can be considered.

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