Entomopathogenic Fungi and Nematodes for Michigan Tree Fruit Management Targeting Plum Curculio (*Conotrachelus nenuphar*)

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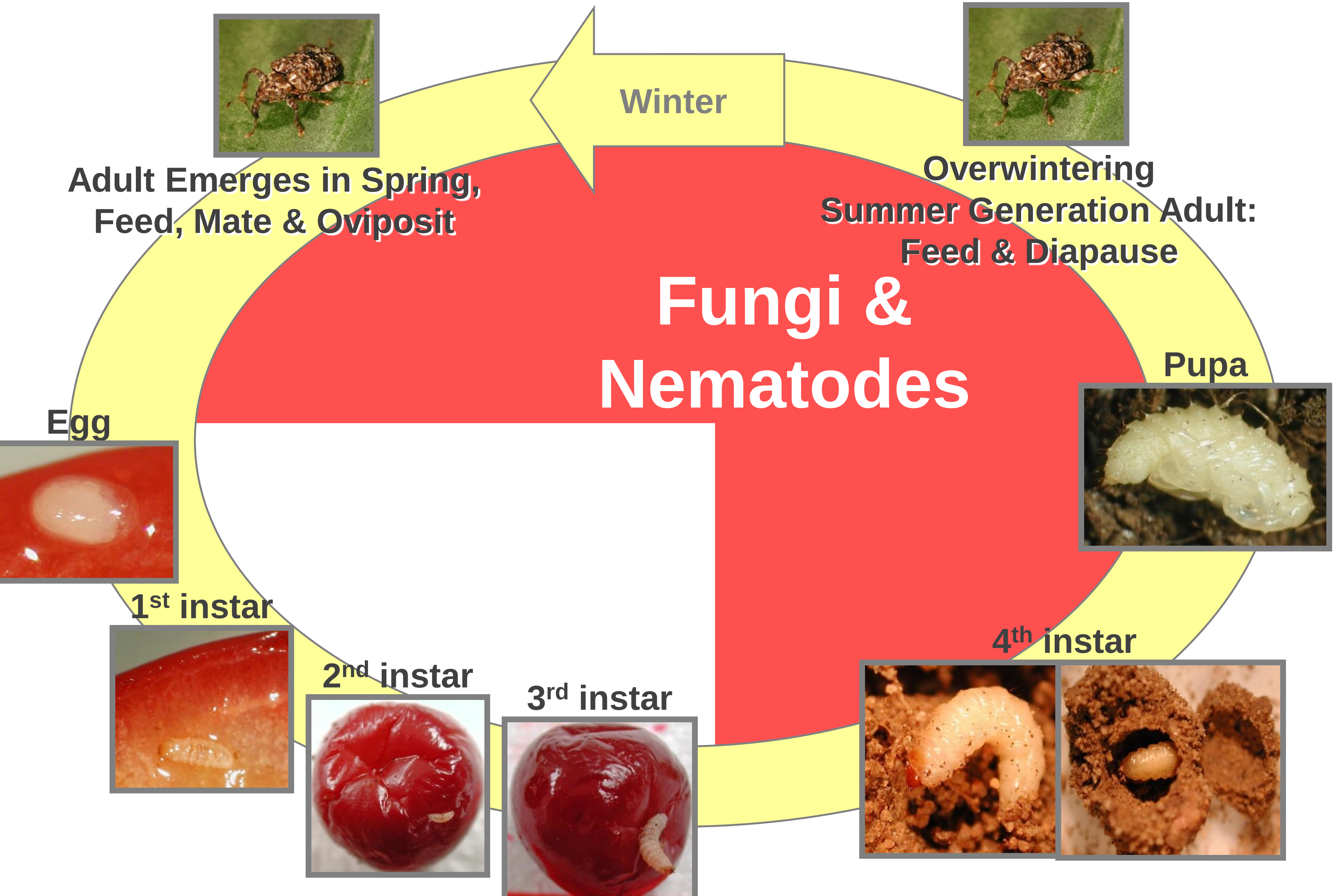
Plum Curculio Background

- **Damage:**
  - Feeding & oviposition scars
  - Zero tolerance for larvae in processed fruit

- **Few Organic Management Tactics:**
  - Repeated kaolin clay coverage
  - Pyganic
  - Livestock

Plum Curculio Life History



Entomopathogens

Nematodes

-Steinernema

-Heterorhabditis

Fungi

-Beauveria bassiana

-Metarhizium anisopliae

Entomopathogens for Plum Curculio

Lab bioassays and field trials with *Steinernema* or *Heterorhabditis* nematodes **(Tedders et al. 1982, Olthof and Hagley 1983, Brossard et al. 1989, Bélair et al. 1998, Shapiro-Ilan et al. 2002, 2004, 2008; Alston et al. 2005, Kim and Alston 2008)**

-Varying LC50s among species & strains

Lab bioassays with *Metarhizium anisopliae* and *Beauveria bassiana* **(Tedders et al. 1982, Alston et al. 2005)**

-Varying LT50s among strains

Field trial with *Beauveria bassiana* **(Jenkins et al. 2006)**

Objectives

1. **Larvae**
   - **Lab (fungi)**
   - **Field (fungi and nematodes)**
2. **Adults**
   - **Lab (fungi)**
   - **Field (fungi)**

Larvae Lab: Experiment Design

Pathogens:	Rates:
-*B. bassiana* GHA	10^5 or 10^6 conidia/ml
-*M. anisopliae* F52	10^5 or 10^6 conidia/ml
-*M. anisopliae* 8270	10^6 conidia/ml
-Control	

-Isolate 8270: locally adapted vs. "new associations"

Exposure:

-Immersed larvae in conidial suspension

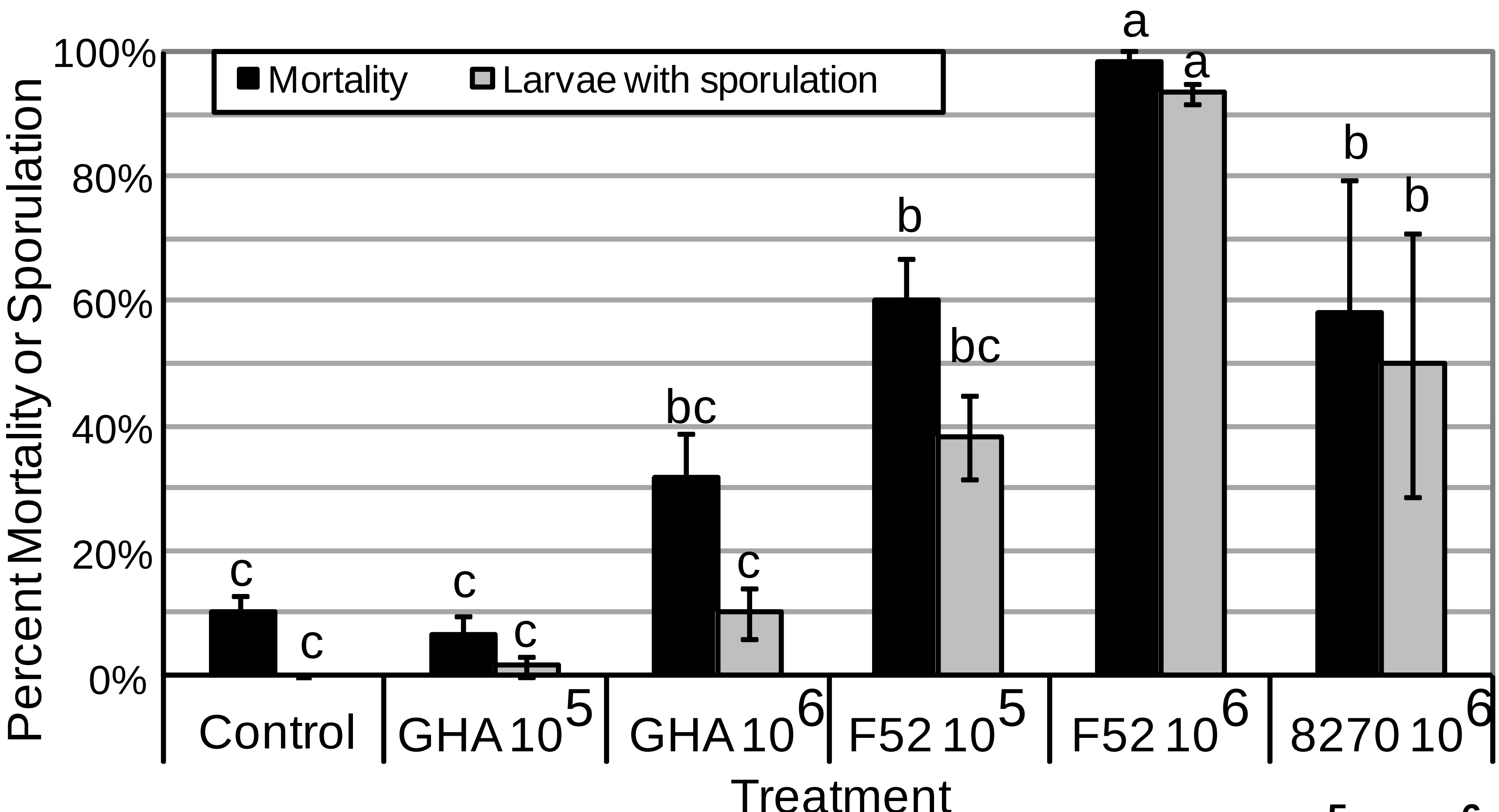
-Larvae held in moist chambers for sporulation

Design:

- 12 larvae per trt; 5 reps

Larvae Lab: Experiment Results

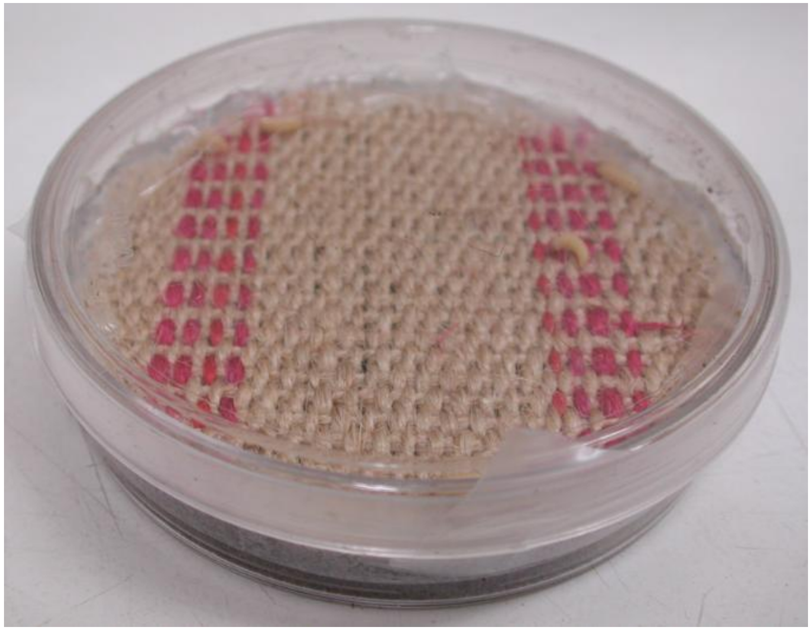
- -Hydrophobicity: conidial adhesion to cuticle: dose levels
- -Consecutive immersion reduced concentration
- -Field: soil texture, temperature, water activity, agrochemical, UV



Mean (SE) percent mortality and percent of larvae with sporulation 14 d after a 10 s immersion in 1x10^5 or 1x10^6 conidia/ml suspensions of *M. anisopliae* F52, *B. bassiana* strain GHA, *M. anisopliae* 8270, or water control. Bars with different letters indicate significant difference among treatments within mortality or within sporulation (Tukey's HSD, α=0.05).

Larvae Lab: Experiment Design

1. Larvae immersed in conidial suspensions
2. **Larvae pass through conidia-treated fabric**



Larvae Lab: Experiment Design

Pathogen:	Rates:
-B. bassiana GHA	Fabric matrix
-Control	

Exposure:

-Larvae placed in arena on fabric for 24 h; allowed to pass through fabric to soil underneath

-Larvae placed in vials with sterile soil for pupation

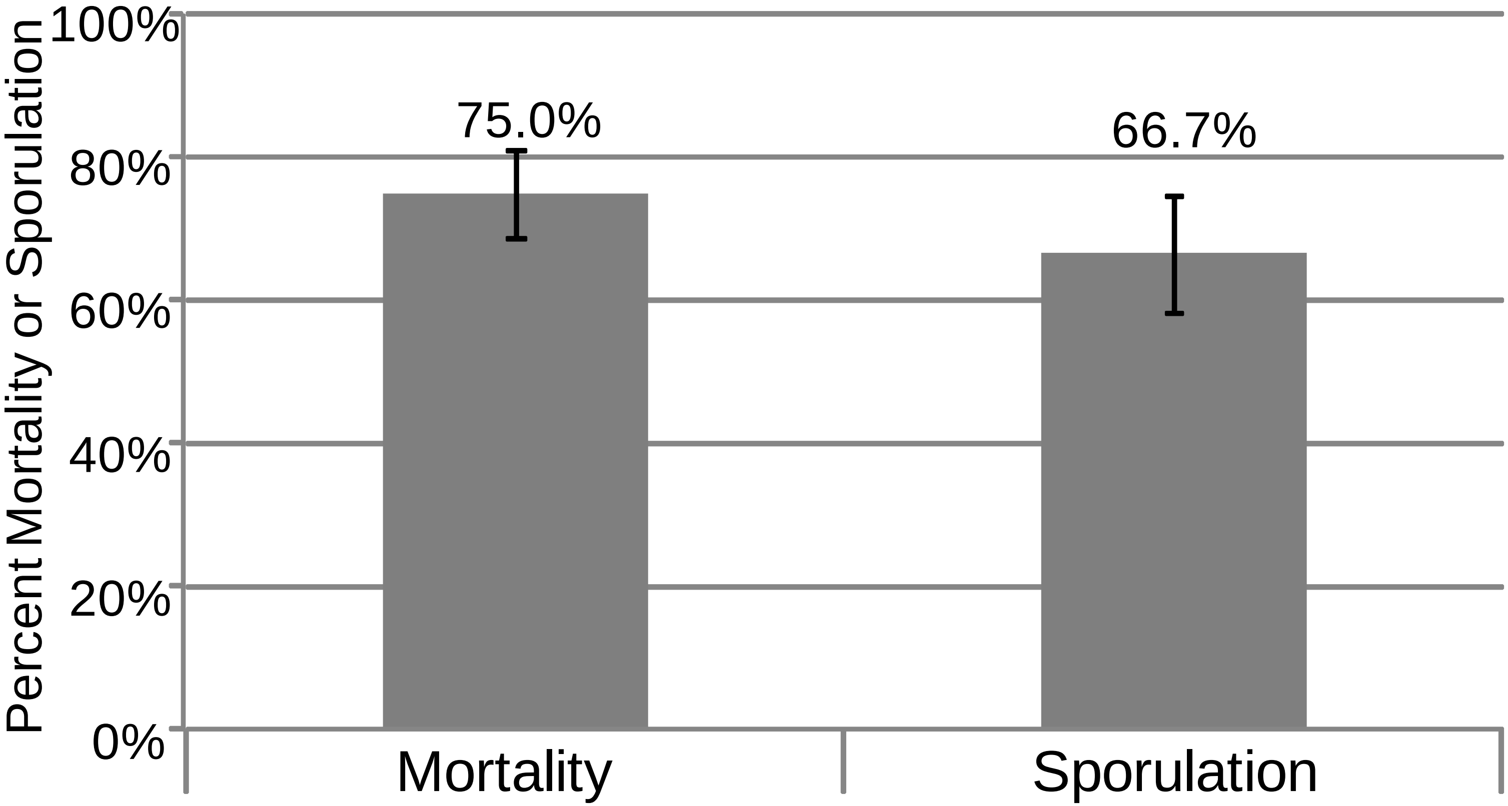
-Evaluated mortality & sporulation after 22 d

Design:

- 5 larvae per trt; 12 reps

Larvae Lab: Experiment Results

- **-8.9% of dead trt individuals pupae; half sporulated**
- **-8.3% of trt larvae failed to burrow; 50.8% of control**
- **-*B. bassiana* establishment under fabric**
- **-Horizontal transmission; alternative hosts**



Percent mortality of and sporulation on last-instar plum curculio after exposure to *B. bassiana*-impregnated fabric as last-instars and subsequent 22 d containment in sterile soil for pupation.

Larvae Field: Experiment Design

-10 larvae placed on surface of enclosed pots installed in orchards

-Soil surface of each pot treated with a pathogen on day 0

-Response variable = Number of adults emerging from pots

Larvae Field: Experiment Design 2006

Pathogen: *B. bassiana* GHA	Rates:
-Mycotrol-O Spray	$2x10^{15}$ conidia/ha
-Conidia on Rice	$2x10^{15}$ conidia/ha
	$2x10^{14}$ conidia/ha
	$2x10^{13}$ conidia/ha
-Control	

Single Timing:

-Introduced larvae to soil <1 h from pathogen application

Design:

- Organic orchards: 2 tart cherry and 2 apple
    - -Spray and only $2x10^{15}$ rate rice in one apple site
- -12 replicate pots per trt, per orchard

Larvae Field: Experiment Results 2006

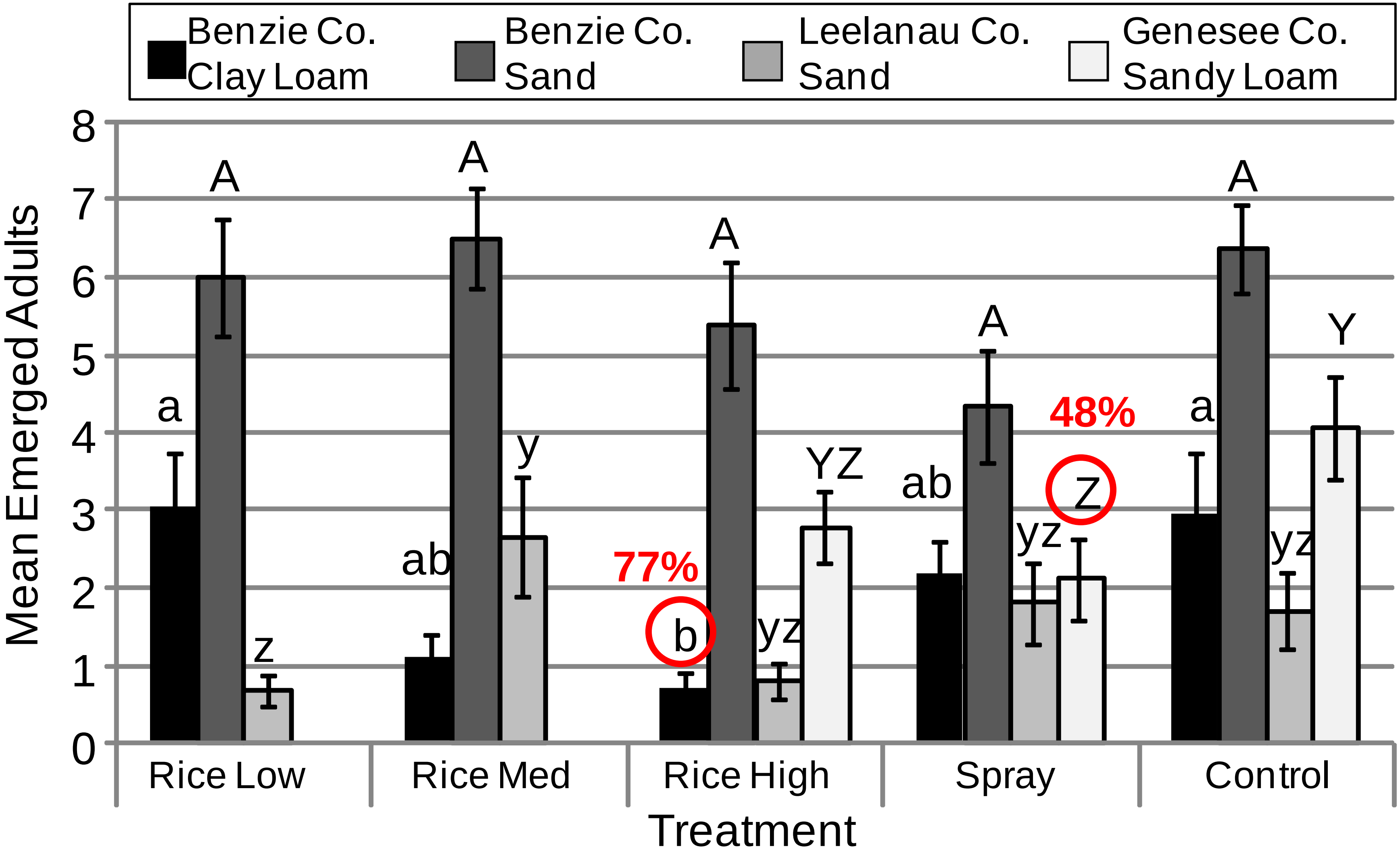


Figure 3.1. Mean (± SE) number of adult plum curculio emerged from *B. bassiana* GHA-treated soil in pots (10 larvae/pot) over a 50-d period in 2006 at four orchards. Rice treatments (*B. bassiana* grown on rice) were not conducted in Genesee Co. Bars with the same letter within an orchard are not significantly different (SNK, alpha=0.05).

Larvae Field: Experiment Design 2007

Pathogens:	Rates:
-*B. bassiana* GHA (unformulated)	$5x10^{13}$ conidia/ha
-*M. anisopliae* F52 (unformulated)	$5x10^{13}$ conidia/ha
-*S. carpocapsae* (All strain, BionemC®)	$4x10^{9}$ IJ/ha
-*S. riobrave* (355 strain, Biovector®)	$4x10^{9}$ IJ/ha
-Control (water)	

Timings:

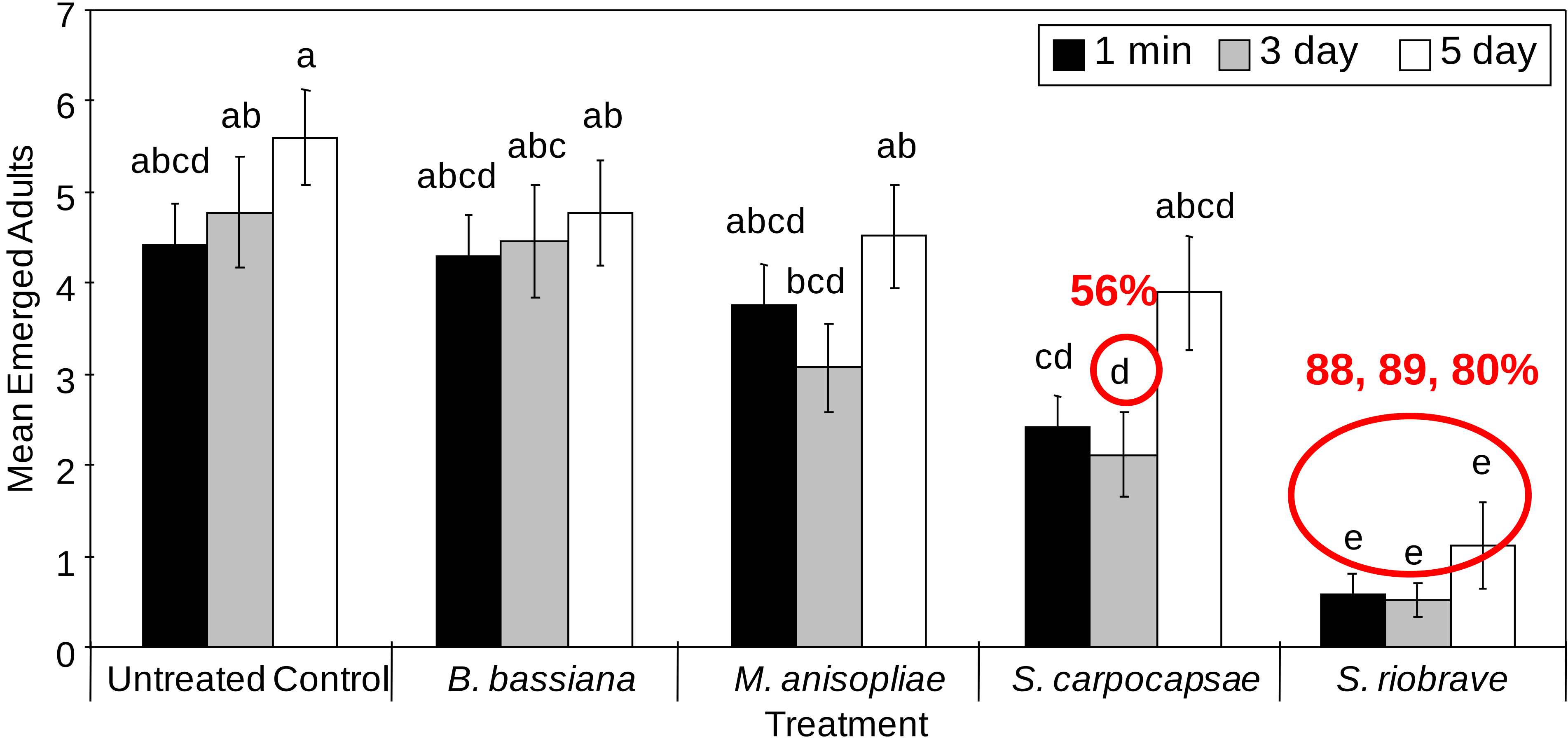
-Introduced larvae to soil 0, 3, or 5 d from pathogen application

Design:

-Two similar transition organic apple orchards

-12-30 replicate pots per trt, per orchard

Larvae Field: Experiment Results 2007



Mean (± SE) number of adult plum curculio emerged from entomopathogen-treated soil in pots over a 50-d period in 2007 at a single orchard. Ten larvae were added to each pot either immediately (1 min), 3 d, or 5 d after entomopathogen application. Bars with the same letter are not significantly different (SNK, alpha=0.05).

Larvae Field: Experiment Design 2008

Pathogens:	Rates:
-*B. bassiana* GHA (Mycotrol-O®)	(1) $5x10^{13}$ conidia/ha
-*H. bacteriophora* (Utah, unformulated)	(2) $1x10^9$ or $4x10^9$ IJ/ha
-*S. riobrave* (355 strain, Biovector®)	(2) $1x10^9$ or $4x10^9$ IJ/ha
-Control (water)	

Timings:

-Introduced larvae to soil -10, -5, 0, 5, 10, 15, or 20 d from pathogen application

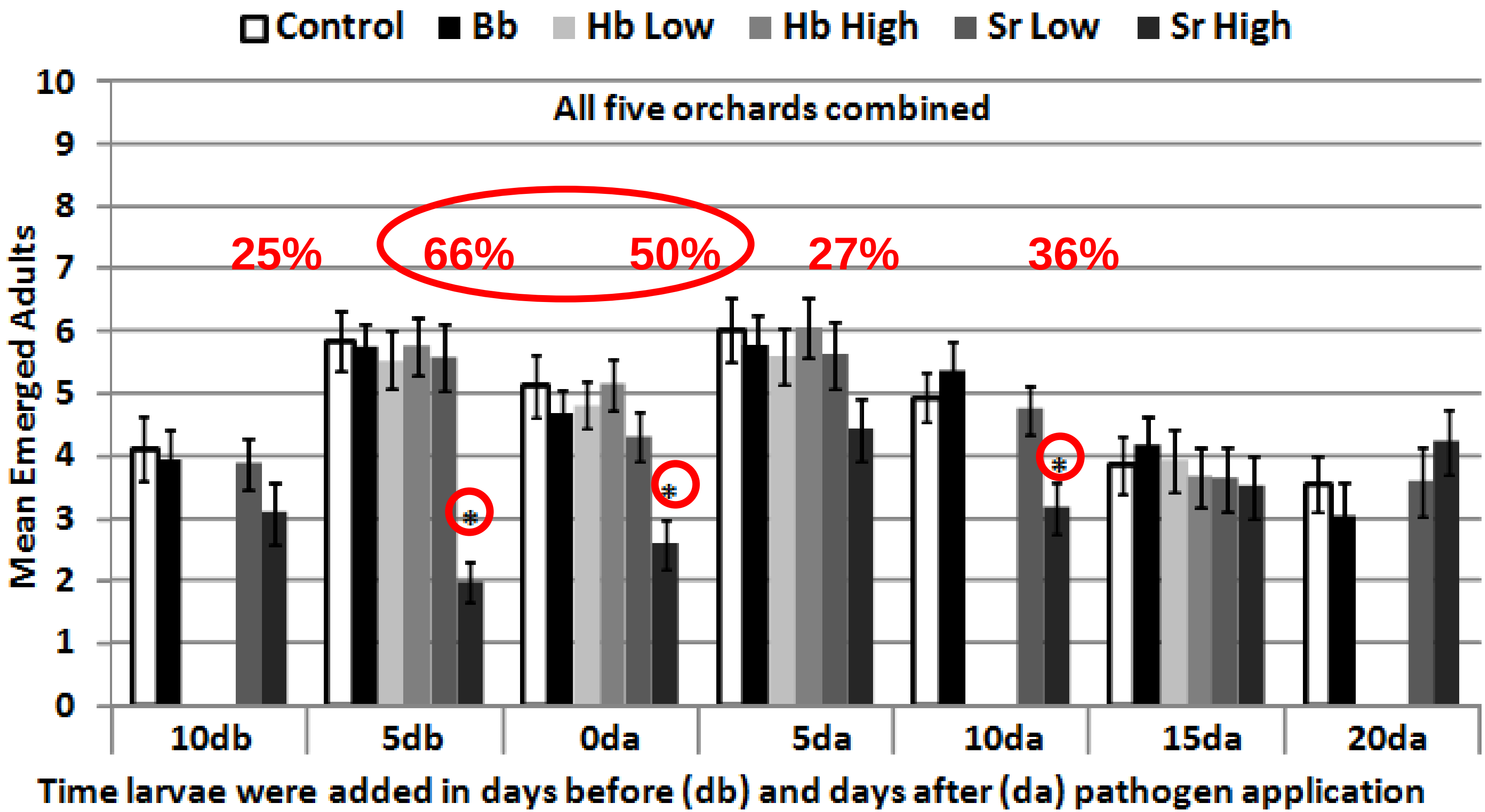
-Hb was not tested for -10, 10, or 20 d

Design:

-3 conventional tart cherry and 2 organic apple

-8 replicate pots per trt, per orchard

Larvae Field: Experiment Results 2008



Average number of adult plum curculios emerging from pathogen-treated soil. Soils were treated with the fungus *Beauveria bassiana* (Bb) or the nematodes *Heterorhabditis bacteriophora* or *Steinernema riobrave* at a low or high rate (Hb Low, Hb High, Sr Low, Sr High). Larvae were placed on soil either -10, -5, 0, 5,10, 15, or 20 days from pathogen application. Bars with an asterisk (*) denote significantly lower means within a day-timing. Note: Hb Low and High were not included for the -10, 10, or 20 day treatments.

Larvae Field: Experiment Results 2008: By Orchard

	Percent Reduction from Control Treatment for *S. riobrave* high rate treatments						
	-10 d	-5 d	0 d	5 d	10 d	15 d	20 d
All Orchards	25	66	50	27	36	8	-18
Loamy Sand	48	89	56	-22	25	-26	12
Sandy Loam	45	69	70	70	41	55	43
Loam	14	65	82	44	36	15	-24
Clay Loam	33	50	4	16	56	4	-79
Loam, High Org.	34	17	21	-10	50	-18	10

Larvae Field: Infested Apple Experiment

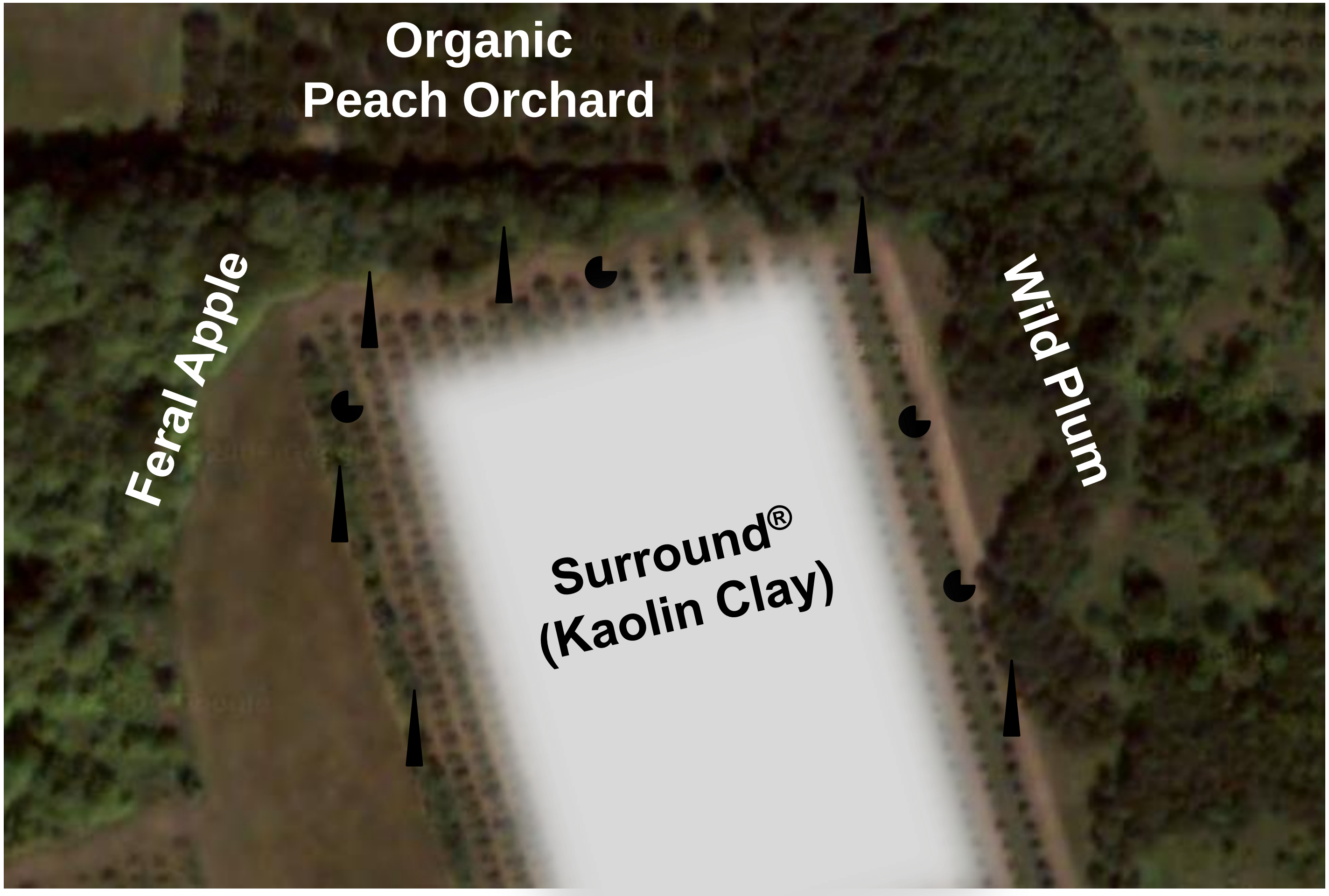
- ***S. riobrave, S. carpocapsae,*** **and *M. anisopliae* failed to reduce plum curculio when applied to soil covered with naturally infested apples**
  - **Single application**
  - **Evaluated by trapping adults:**
    - **Screen Traps**
    - **Within large ground cages**
  - **Design shortcomings: adult dispersal within small plots, late application timing**

Larvae Field: Experiment Discussion

- ***B. bassiana*** **significantly suppressed adult emergence by 48-77% in low sand sites 1/3 yr**
- ***S. riobrave*** **most effective in high sand; larvae introduced -5, 0, or 10 d from pathogen; pupae susceptible**
- **Physical properties of soils**
- **Foraging strategies: "sit-and-wait"carp vs. active bac**
- **Optimal temp ranges:** ***S. riobrave*** **higher**
- **Formulation: gel vs. vermiculite, UV**
- **Fungicides**
- **Water activity: micro-jet sprinkler irrigation in citrus**
- **Experimental Design:**
  - **Inflated efficacy with high larva density (nematode cycling)**
  - **Ant predation**

Larvae Field: Experiment Discussion

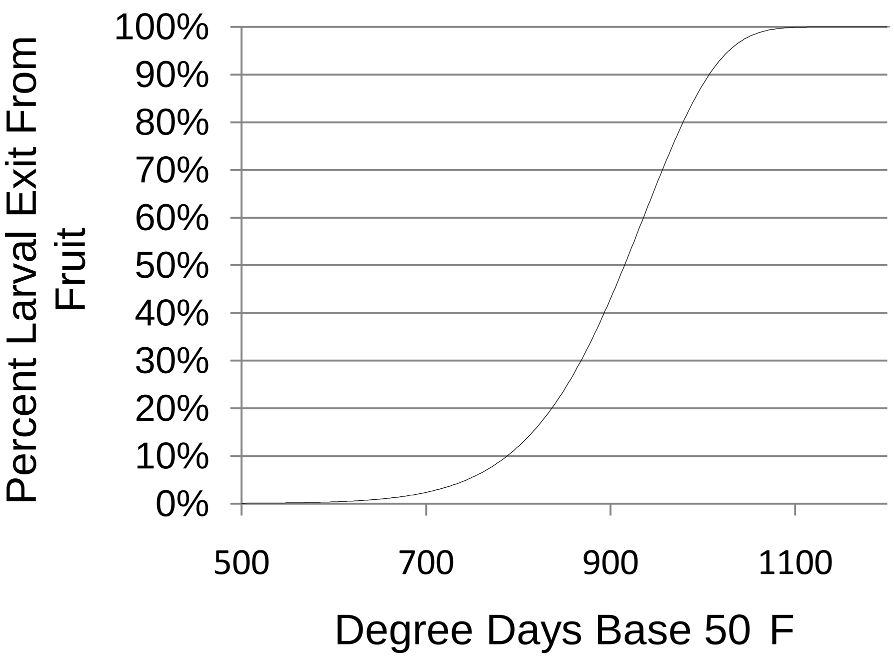
- **Will targeting larvae reduce next summer's damage?**
  - **Spring immigration of adults surviving in refuges: wild hosts, nearby fruit trees, nearby organic orchards**
  - **Reduce cost by concentrating oviposition – combine "push-pull" with oviposition monitoring**



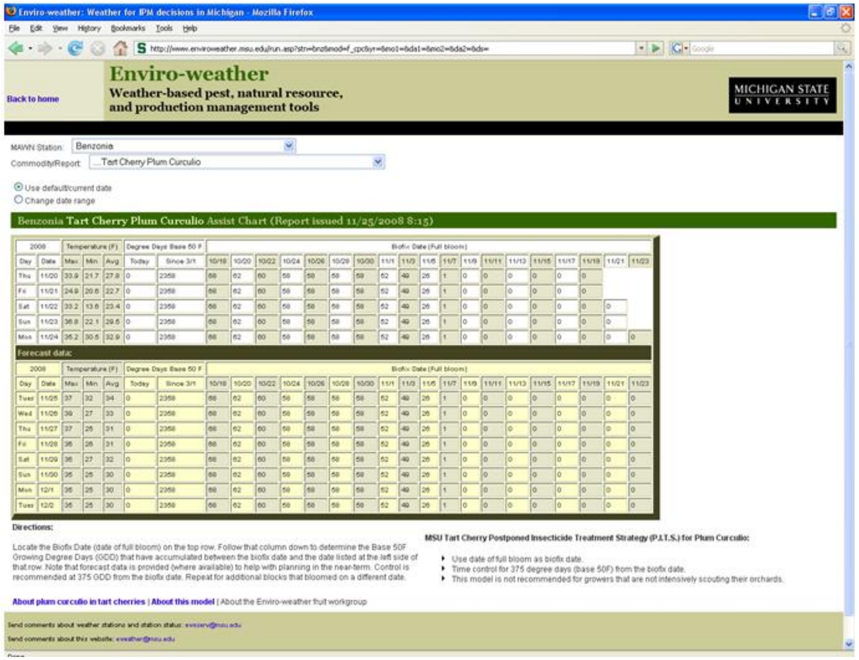
Future Work

- **Timing Recommendations: Larval Phenology**
  - **New York Fruit injury model (Reissig et al. 1998)**
  - **New Jersey Blueberry (Polvarapu et al. 2005)**
  - **West Virginia Tree Fruit (Brown 2005)**
  - **Utah Home Yard Tree Fruit (Kim and Alston submitted)**

MODEL



DELIVERY TO GROWERS



Adults Lab: Experiment Design

1. Aqueous conidial suspensions on soil: field soil or peat
2. Aqueous conidial suspensions on soil: field soil with lower rates
3. Alternative surfaces: Petri dish or wick

Adults Lab: Experiment Design

Pathogens:	Rates:
-*B. bassiana* GHA	$8x10^{14}$ conidia/ha
-*M. anisopliae* F52	$8x10^{14}$ conidia/ha
-Control (water)	

Exposure:

-Introduced adults to medicine cups with loamy sand soil or peat

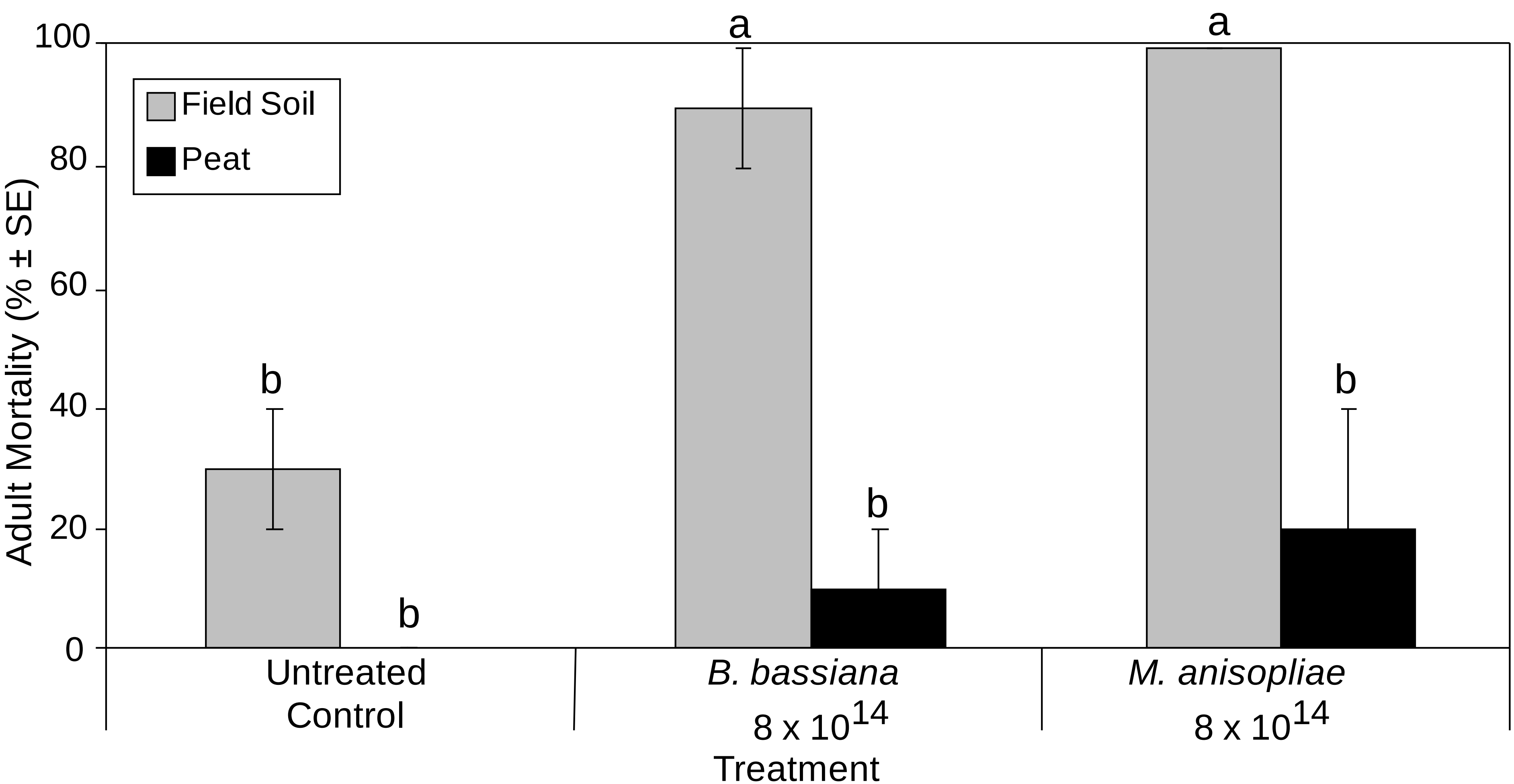
-Observed after 15 d

Design:

-5 cups per trt; 2 reps

Adults Lab: Experiment Results

-Neither fungus effective on peat; both highly effective on loamy sand



Mortality of adult plum curculio exposed to entomopathogen-treated soil or peat at a rate of 8 x 10^{14} conidia/ha after 15 d. Bars with the same letter are not significantly different (SNK, α=0.05).

Adults Lab: Experiment Design

Pathogens: / Rates:

Pathogens	Rates
-B. bassiana GHA	$1x10^{13}$ or $5x10^{13}$ conidia/ha
-M. anisopliae F52	$1x10^{13}$ or $5x10^{13}$ conidia/ha
-Control (water)	

Exposure:

-Introduced adults to medicine cups with loamy sand soil

-Observed after 12 d

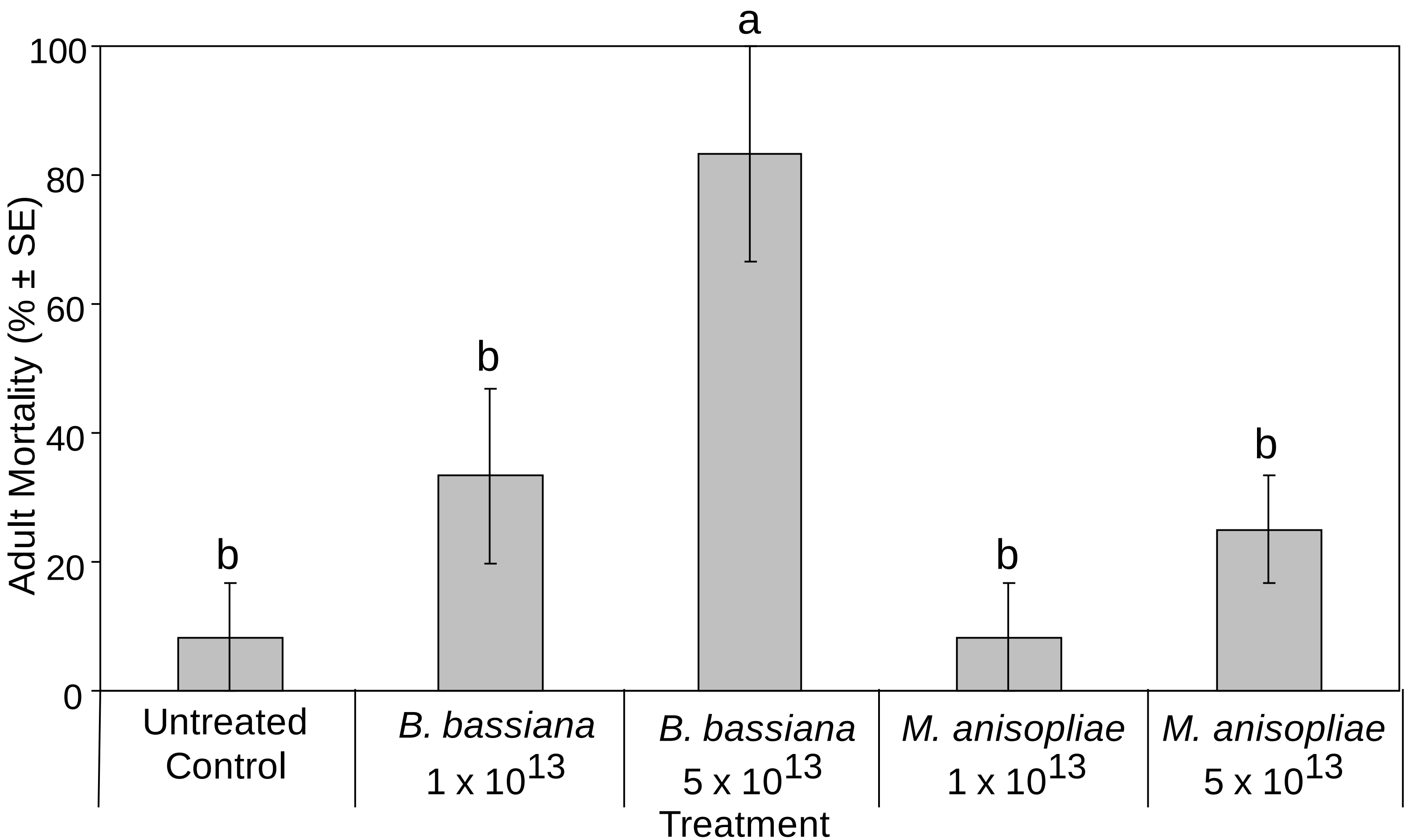
Design:

-3 cups per trt; 4 reps

Adults Lab: Experiment Results

-Fungi are slow-acting; 15, 20, 25, 30 d?

-Constant exposure to fungus-treated soil



Mortality of adult plum curculio exposed to entomopathogen-treated soil (low rate = 1×10^{13} conidia/ ha; high rate = 5×10^{13} conidia/ha) after 12 d. Bars with the same letter are not significantly different (Dunnett's procedure, α=0.05).

Adults Lab: Experiment Design

3. Alternative surfaces: Petri dish or wick

-Autodissemination

- Japanese Beetle, Asian Longhorned Beetle, Sweet Potato Weevil, Diamondback Moth, Brown-winged Bug, Sap Beetle

Adults Lab: Experiment Design

Pathogens:	Surface:
-*B. bassiana* GHA	Dish or wick
-*M. anisopliae* F52	Dish or wick
-Control	Dish or wick

Exposure:

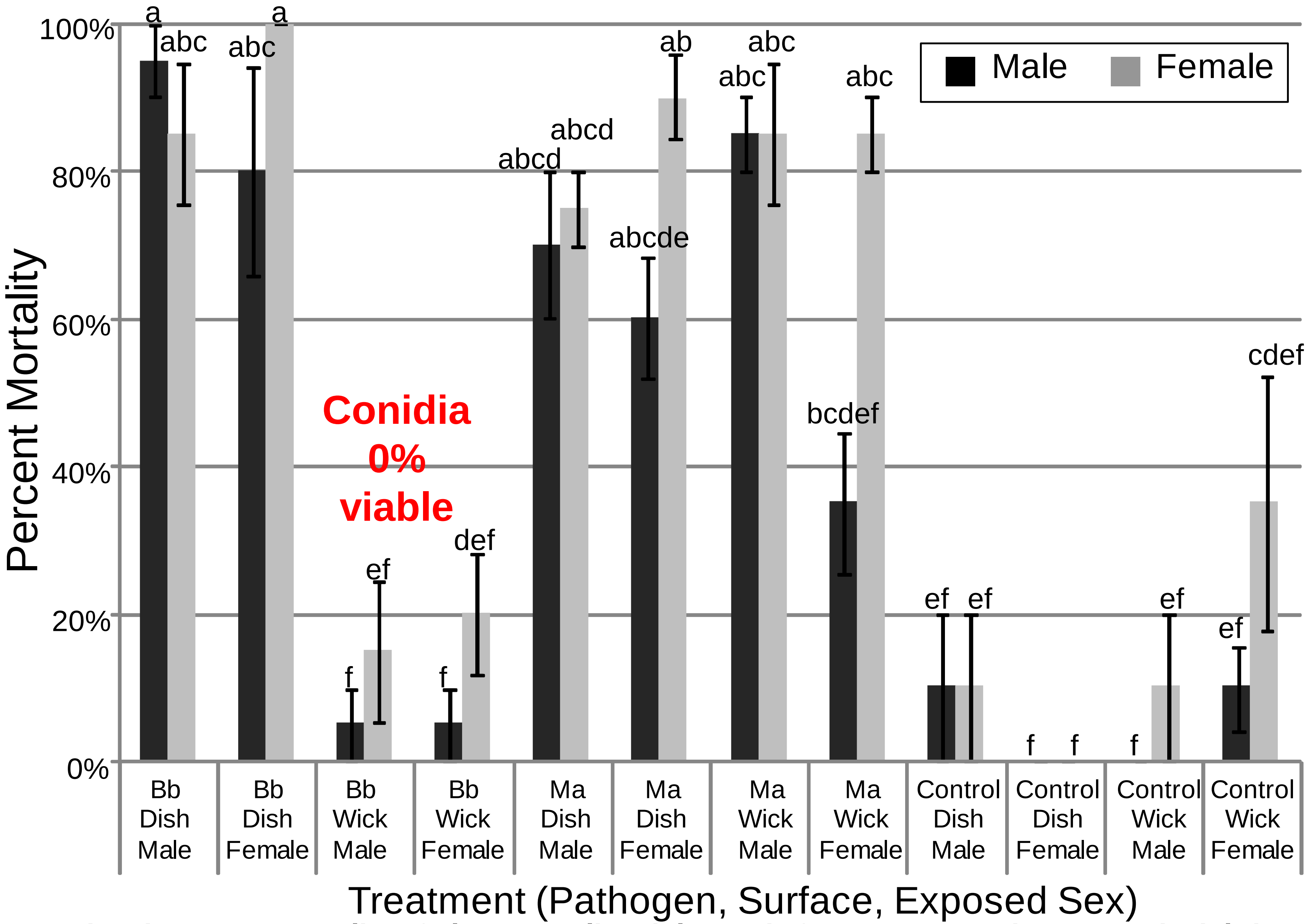
- -Forced adult to walk across surface
- -Introduced adult of opposite sex to soufflé cup
- -Observed once daily for 29 d

Design:

- -5 pairs of adults in each of 4 reps;

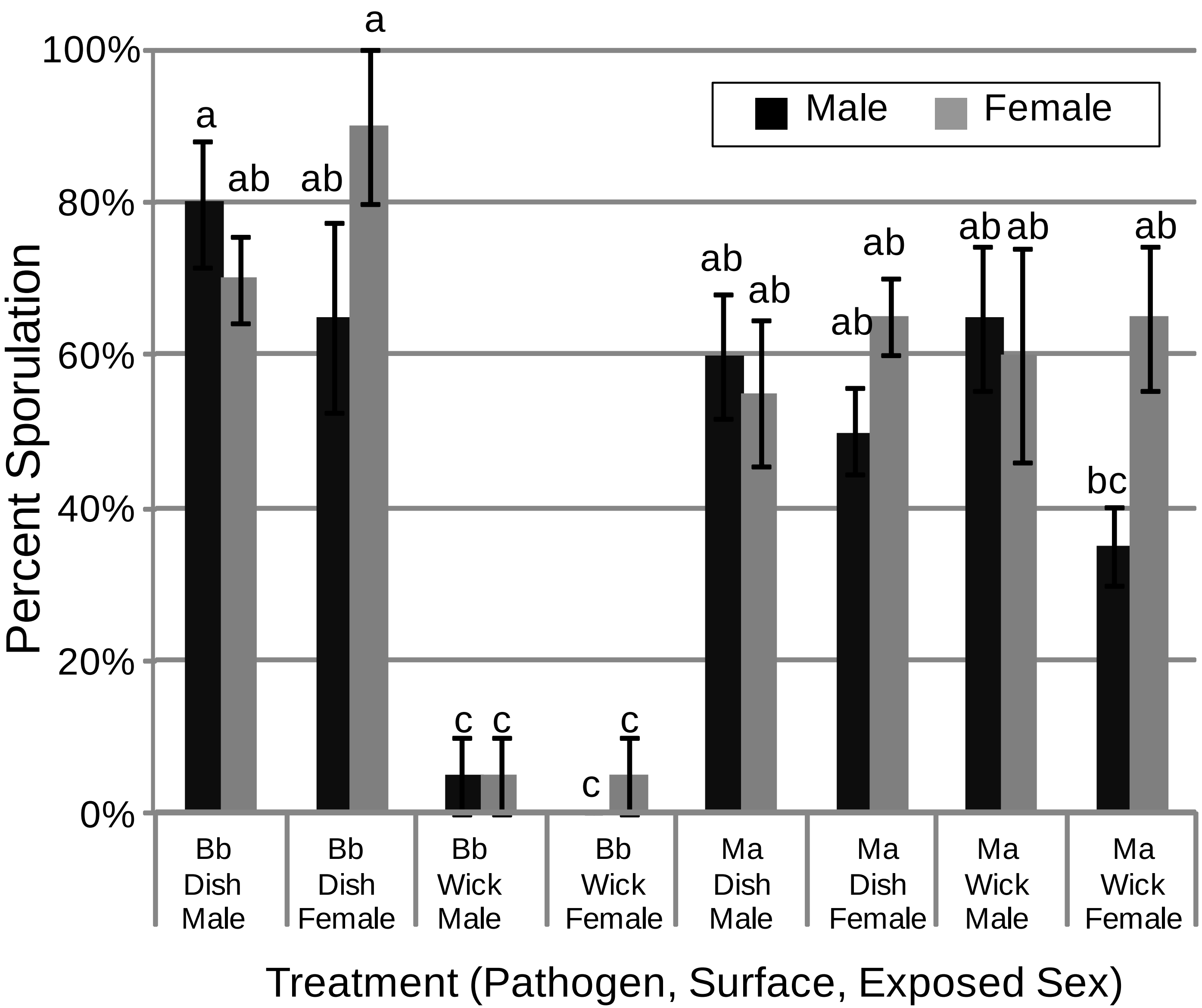
(20 pairs per pathogen x surface x exposed sex)

Adults Lab: Experiment Results



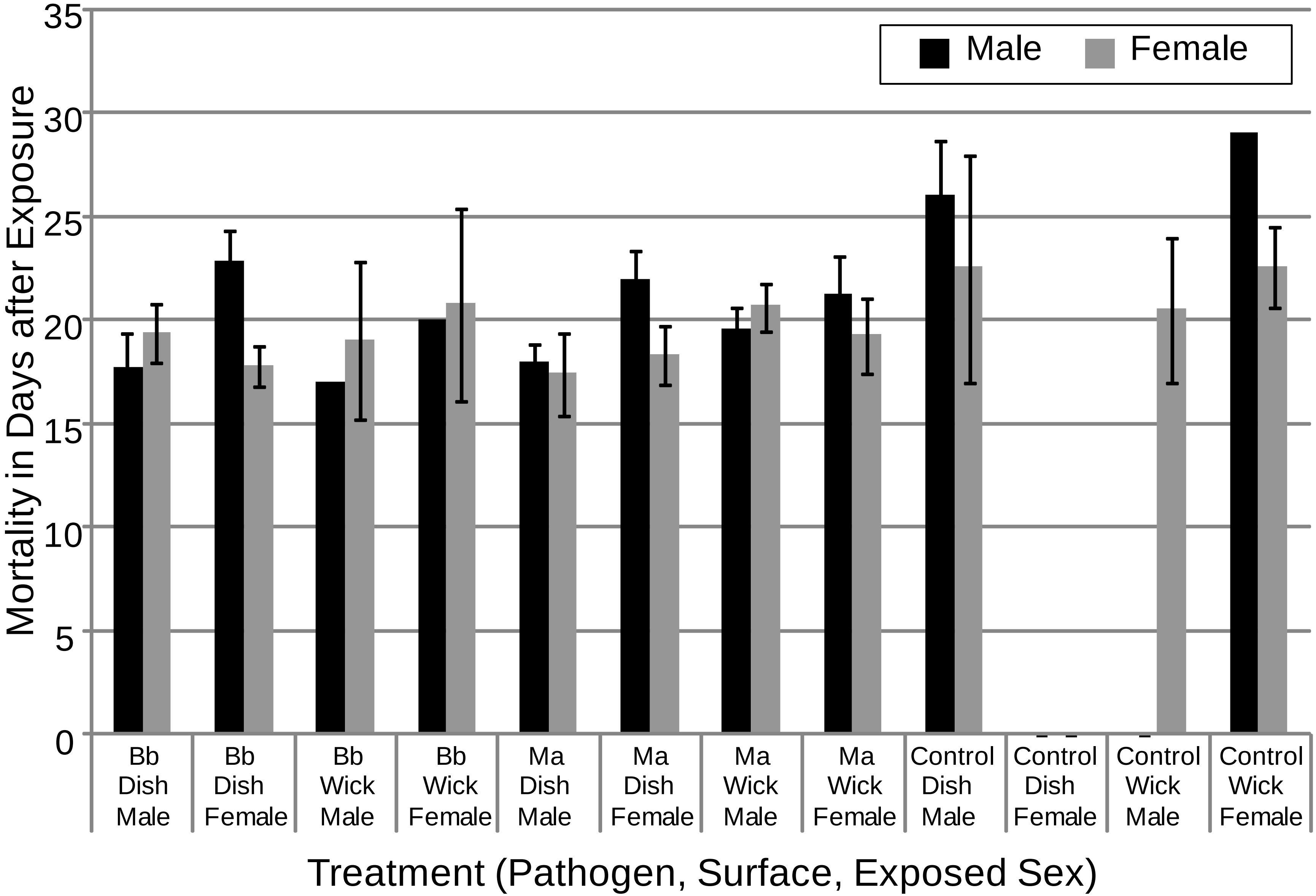
Mean (±SE) percent mortality of plum curculio adults 29 d after treatment. The exposed adult (sex indicated in the x-axis) was held with an unexposed mate of the opposite sex. Bars with same letter indicate that the corresponding means are not significantly different (Tukey, α=0.05).

Adults Lab: Experiment Results



Mean (±SE) percent sporulation of plum curculio adults 37 d after treatment exposure. The exposed adult (sex indicated in the x-axis) was held with an unexposed mate of the opposite sex. Bars with same letter indicate that the corresponding means are not significantly different (Tukey, α=0.05).

Adults Lab: Experiment Results



Mean (±SE) number of days after treatment exposure until mortality of plum curculio adults. The exposed adult (sex indicated in the x-axis) was held with an unexposed mate of the opposite sex.

Adult Lab: Discussion

- **No difference in time until mortality among trts (17-22.7 days)**
  - **Oviposition scarring, larvae reduced?**
- **No difference in mating between treatments (47%)**
- **Conidia dislodged from exposed adult may have contaminated soil**
  - **Hold adult on soil after exposure – dislodgement period**
  - **Time period of physical contact with mate**
- **Duration of contact with soil unknown**
- **Higher baseline female mortality, or do males transmit fungus to females better than females transmit fungus to males?**
- ***B. bassiana* wick conidia viability**

Adults Field: Experiment Design

Pathogen:	Rates:
-*B. bassiana* GHA on barley	$5x10^{13}$ conidia/ha
-*M. anisopliae* F52 on rice	$5x10^{13}$ conidia/ha
-Control	

Single Timing:

-Introduced adults to cages 24 h from pathogen application

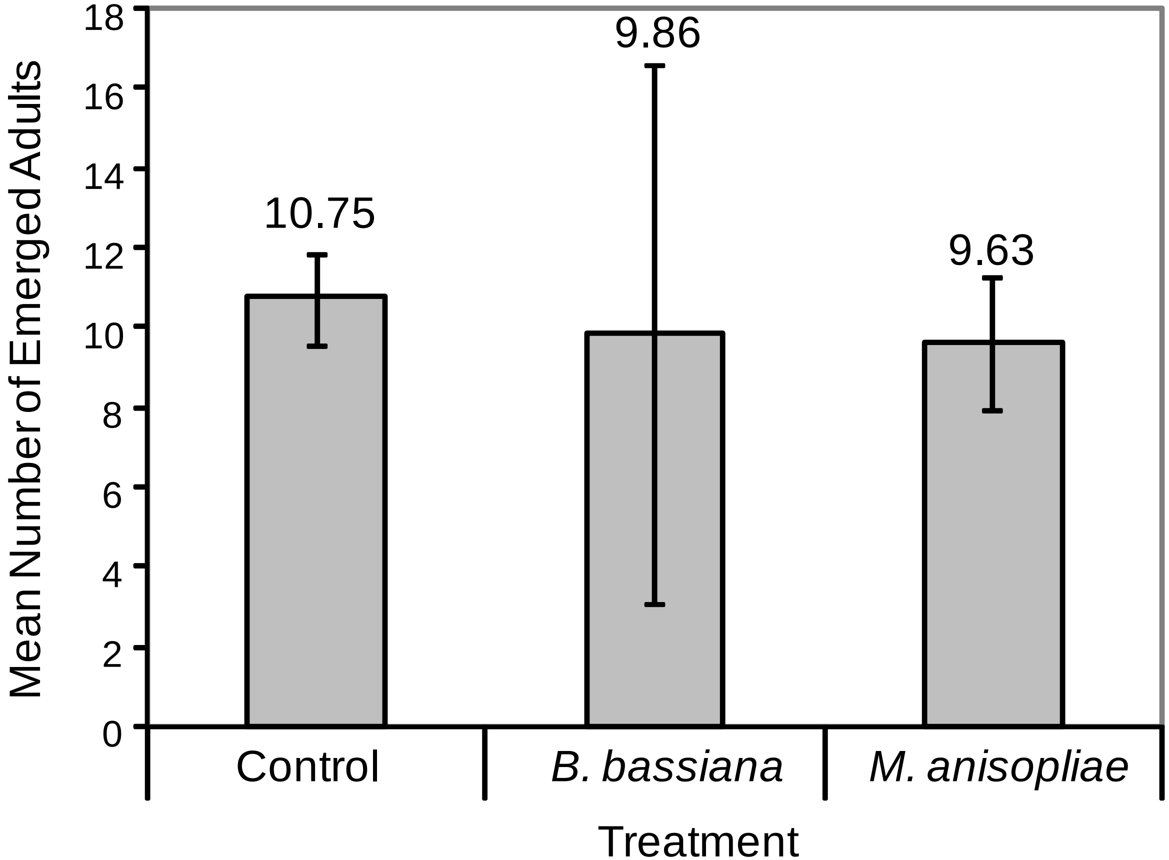
Design:

-One neglected apple orchard

-8 replicate cages per trt

Adults Field: Experiment Results

- **-Optimal conditions for infection: 10-16C, 17-26C; precipitation**
- **-Granular Formulations: European cockchafer, banana root weevil, sugarbeet root maggot, Colorado potato beetle, clover root weevil**
- **-Distribution and host contact problems (2.5 mm granules)**
- **-Spring-tilled granule for Colorado potato beetle**



Mean (±SE) number of emerged adults in the spring following overwintering in cages. Groundcover treatments included a granular formulation of *B. bassiana* or *M. anisopliae*, or an untreated control.

Conclusions

- **Larvae:**
  - **Timing**
  - **Cost & Formulation**
  - **Soil type & water activity**
    - ***S. riobrave* in sands; *B. bassiana* in clays?**

- **Overwintering adults:**
  - **Timing**
    - **Fall or Spring**
    - **Oviposition scarring after exposure?**
  - **Formulation**
    - **Application to soil**
    - **Trap or trunk band**

Acknowledgements

Project GREEEN

Cherries
Not just another berry.

MICHIGAN APPLES

SARE
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The IR-4 Project

Michigan Agricultural Experiment Station
Michigan State University

Enviro-weather
Weather-based pest, natural resource, and production management tools

Mark Whalon, Diane Alston, Dan Nortman, Pete Nelson, Alex Johnson, Willye Bryan, Sam Kim, Karlyn Page, Zach Koan, Michael Hosking, Jeanette Wilson, Chris Arachangeli, Saunte Sutton, Abbra Puvalowski, Jennifer Silveri, Andrew Skwiercz, Rob Brown, Lisa Losievsky, Fred Warner, Richard Humber, Jerry Skeltis, Peach Byler, Denise Ruwersma, and the Farmers

Abstract

Tree fruit growers are seeking effective alternatives to US EPA-mitigated insecticides for managing plum curculio, *Conotrachelus nenuphar* Herbst (Coleoptera: Curculionidae). Formulations and novel delivery mechanisms of entomopathogenic nematodes (*Steinernema riobrave, Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) and fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) were investigated in the laboratory and in Michigan orchards. Mortality of fungus-exposed adults ranged from 38-100% and occurred 17-23 d post treatment in the laboratory. Fall granular fungus formulation applications failed to reduce overwintering survival of adults. Larvae were introduced to orchard soils -10, -5, 0, 5, 10, 15, or 20 d from entomopathogen application. *Steinernema riobrave* consistently reduced adult emergence most effectively in sandy sites. *Beauveria bassiana* exhibited limited effectiveness and sensitivity to environmental and site factors. Both nematodes and fungi were promising tactics against plum curculio, but more investigation is needed to determine if oviposition damage and the second generation can be reduced to economic levels.