Research Project Report
California Melon Research Board
For the period of January 1, 2011 to December 31, 2011

TITLE: Mortality of *Blapstinus* Beetles Exposed to Fungal Treated Soil

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OBJECTIVES:

Long-range objectives: Our long range goal is to develop environmentally acceptable control of darkling beetles and other soil dwelling insect pests of melons. 1) We expect to develop formulations of the insect pathogen *Metarhizium brunneum* Petch, [formerly *Metarhizium anisopilae* (Ma)] and possible other insect pathogens into insecticidal formulations. 2) Develop attractants for soil dwelling pests to lure them to the insecticidal formulations 3) Release our findings to industry to develop and market insecticidal formulations for soil dwelling insect control.

Immediate objectives: The objective is to assess the potential for control soil dwelling insect pests of melons by the application of insect pathogenic fungi. The initial target insect will be the darkling beetle (*Blapstinus* spp.), which causes direct damage to melons by feeding at the fruit/soil interface.

SUMMARY OF RESEARCH RESULTS:

There were experiments conducted to evaluate the potential for control soil dwelling insect pests of melons by the application of insect pathogenic fungi in the laboratory and in the field. In the laboratory experiments, three treatments provided significant mortality of beetles after 10 days exposure to treated soil. *Metarhizium anisopliae* Diatomaceous Earth (Ma DE) Granules and Ma Clay granules caused the highest beetle mortality, followed by the Mycotrol O (commercial *Beauveria bassiana*) treatment. Only the *Isaria fumosoroseus* (Ifr) Mat treatment did not cause significant mortality. The field experiments in melon plots at UC Desert Research and Extension Center, had too few *Blapstinus* spp. beetles present in the plots to give very meaningful results.

PROCEDURES AND RESULTS BY EXPERIMENT

Experiment I, Laboratory

Purpose: Evaluate potential soil treatments with entomopathogenic fungi under laboratory conditions for mortality against field collected *Blapstinus* spp. beetles; beetles collected from the UC Desert Research and Extension Center (DREC) near Holtville, CA.

Treatments: Laboratory treatments to match UC DREC field application, Summer 2011.

1. Control
2. *Metarhizium anisopilae* (Ma) Clay granules (CG)
3. *Metarhizium anisopilae* Diatomaceous Earth (DE) Granules
4. *Isaria fumosoroseus* (Ifr) Mats
5. Mycotrol O, commercial *Beauveria bassiana*.

Methods:

1. One ounce shot cups with lids were weighed (90 cups per treatment) (6 July)

2. Greenhouse potting soil (~4 g) was added to each cup and weighted (7 July)

3. Fungal treatments numbers 2, 3, and 4 were added to cups for a 4 day fungal germination period (July 8):
   a. Treatment #2 = 0.0301 MA CG g/cup
   b. Treatment #3 = 0.0302 Ma DE g/cup
   c. Treatment #4 = 3-hole punched disks from filter paper with Ifr, 0.0258 g

4. Mycotrol O was applied to Treatment #5 cups at 1.06×10^8 conidia per cup in 0.25 ml water (July 11).

5. One live beetle was added to each of 90 cups for each treatment (11 July).

6. Beetle mortality was evaluated on 18, 21, and 25 July.

7. The final weight for each cup was record 25 July; then all were refrigerated.

8. Conidia concentrations were evaluated for treatments #2, #3, and #5 (26 July – 2 Aug).
   a. A 10 ml water solution with 0.04% Tween 20 was added to each cup.
   b. Vortex to mix
   c. Spores were counted using a hemacytometer.

9. Randomly selected cups were evaluated from treatments #2, #3, and #5 for colony forming units (CFUs).
   a. 10 cups from each treatment were randomly selected (after step 8 above).
   b. To determine CFUs, samples were serial diluted by 10,000 and plated on selective agar.

Results: Three treatments provided significant mortality of beetles after 10 days exposure to treated soil (Figure 1). Ma DE and Ma Clay granules caused the highest beetle mortality, followed by the commercial Mycotrol treatment. Only the Ifr Mat treatment (#4) did not cause significant mortality. Beetle mortality in the control treatment reached 43% by 14 days after being placed in the cups. Fungal mycosis was obvious for most dead beetles in Ma Clay, Ma DE, and Mycotrol treatments, but not for dead beetles in the Ifr Mat or Control treatments.

Spore counts and CFUs showed that the Ma DE granules produced more conidia than did the Ma Clay granules in this test. We expected the Ma granules to produce about 3.5 x 10^9 conidia per gram of granules and counts indicate that the Ma DE and Ma Clay granules produced 3.91 x 10^9 and 2.30 x 10^9 conidia per gram of granules, respectively. CFU data supported the conidia counts in that Ma DE granules averaged 2.43 x 10^9 CFU per gram of granules and Ma Clay granules were slightly less with 1.65 x 10^9 CFU per gram of granules. As a result, Ma DE and Ma Clay treatments averaged 1.16 x 10^8 conidia per cup and 7.08 x 10^7 conidia per cup, respectively. Mycotrol was added to the soil to provide 1.06 x 10^8 conidia per cup and conidia counts averaged near this expected amount with 1.19 x 10^8 conidia per cup. However, plated sample indicated a much lower CFU count at 1.17 x 10^6 CFU per cup and may explain the lower beetle mortality. Preliminary counts from ten control cups and ten Ifr Mat treatment #4 cups did not provide identifiable conidia and thus were considered to be zero.

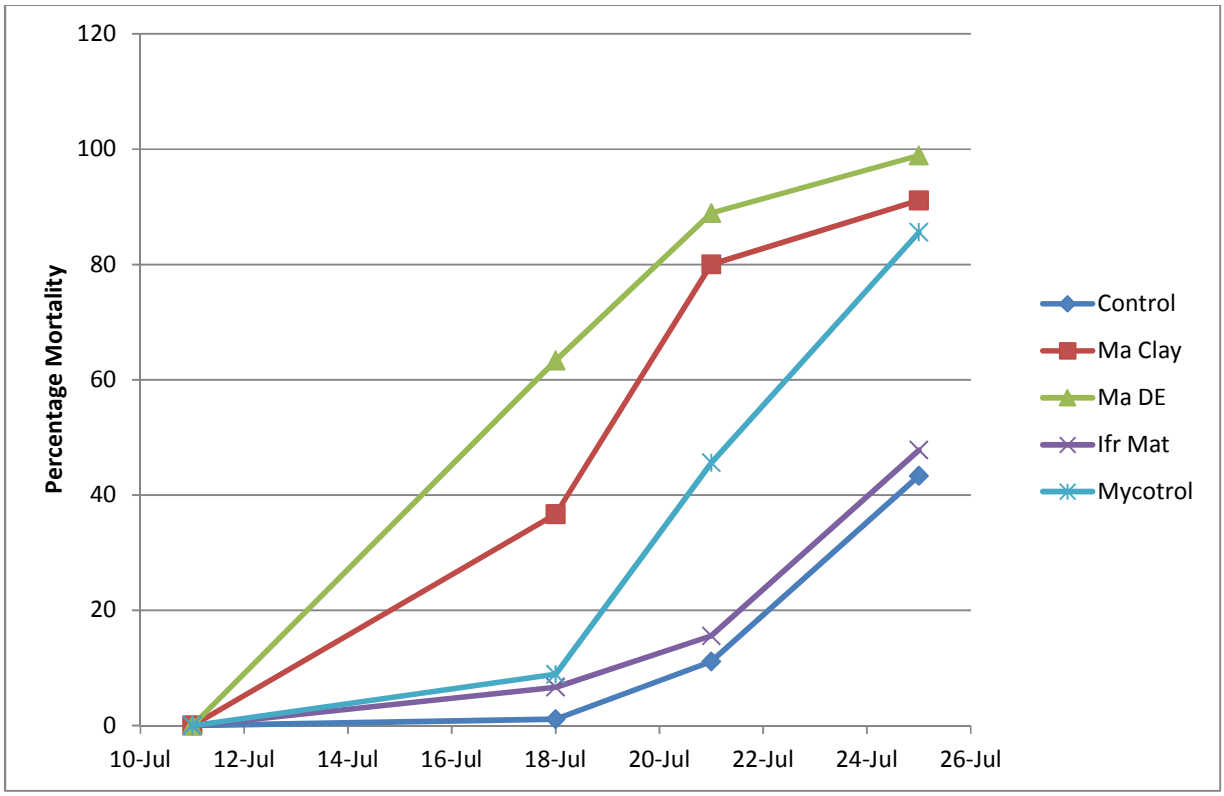

Figure 1. Percentage Mortality of *Blapstinus* spp. beetles exposed to fungal treated potting soil.

Experiment II, Field plots

Purpose: Evaluate potential soil treatments with entomopathogenic fungi for mortality on *Blapstinus* spp. beetles in field planted melons at UC DREC as compared to commercial insecticidal bait formulations used to control darkling ground beetles in commercial melon fields. Treatments are listed in Table 1 below.

Table 1. Darkling Ground Beetle Treatments in Cantaloupe Melons, Holtville, CA, 2011

Treatment	Amount/acre	Amount/plot or amount/3 gal	Application date
1. Sevin Bran bait 5%	20 lb/acre	138.8 gm/plot dry	13 June
2. Ambush Bait 0.05%	20 lb/acre	138.8 gm/plot dry	13 June
3. Botanigard ES	16.0 fl oz	40.6 ml/3 gal	13 June
4. Ma DE Granules	2860 g/acre	46.4 gm/plot dry	13 June
5. Ma Clay Granules	2860 g/acre	46.4 gm/plot dry	13 June
6. Ifr Spray	14.5 L/acre	1244.5 ml/3 gal	13 June
7. Ifr Mat	327mats/acre	20 mats/plot	13 June
8. Check		----------	13 June

Methods:

Plot size: 50' X 13.33' (2 beds/plot on 80" centers); one buffer bed between plots & 10' buffers between blocks. The experimental design was Randomized Complete Block with 4 Replicates.

Granule applications were spread evenly over the plots using a hand-held fertilizer spreader. Foliar sprays were applied using a tractor mounted spray boom with three TJ-60 11003VS nozzle/bed delivering 35 gpa @ 50 psi. Ifr Mat (filter paper) treatments were applied by placing the treated Mats under 20 melons per plot marked with blue flags and 20 Mats with no Ifr were also placed under another 20 melons per plot marked with green flags. All treatments of granules spray and Mats were applied on 13 June, 2011.

In each plot, on 20 and 27 June 2011, 20-melon fruit, still attached to the vine, were examined by looking at the fruit and beneath the fruit on the ground spot and numbers of *Blapstinus spp.* beetles were recorded. On 5 July, 20 melons per plot were examined in each plot for chewing damage from *Blapstinus* spp. beetles. In treatment #7, 20 melons with Ifr Mat treatments and Mat treatments without Ifr were examined for beetle damage.

Results:
There were too few *Blapstinus spp.* beetles present in the field plots to give very meaningful results, but data were collected and are summarized in Tables 2 & 3 below:

Table 2. Number of Darkling Ground Beetle in 20 Melons, Holtville, CA, 20 June 2011

Treatments	Amt/acre	Avg. No. of Beetles/20 melons		% Good Melons
		20 June	27 June	
1. Sevin Bran bait 5%	20 Lb/acre	0.25	0.25	98.75 a
2. Ambush Bait 0.05%	20 Lb/acre	0.00	1.00	100 a
3. Botanigard ES	16.0 fl oz	0.75	0.75	100 a
4. Ma DE Granules	2861 g/acre	0.75	3.00	100 a
5. Ma Clay Granules	2861 g/acre	0.75	0.25	100 a
6. Ifr Spray	14.5 Lb/acre	0.50	1.75	100 a
7. Ifr Mat	327mats/acre	1.00	1.25	88.75 b
8. Check	----------	0.25	1.50	95 a
		P=0.71	**P=0.1854**	**LSD=2.08 P=0.0054**

Mean within columns followed by the same letter are not significantly different by t test; *P*= 0.05

Table 3. *Blapstinus spp.* Beetle per 20 Melons and Percentages of Market Melons, Holtville, CA, 2011

Treatments	Amount/acre	Avg. No. of Beetles/20 melons		% Market Melons (no beetle damage)
		20 June	27 June	
Mat with Ifr	327mats/acre	0.0500	0.0750	88.75
Mat without Ifr	327mats/acre	0.0625	0.0500	82.50
		T=-0.2425 P=0.8164	**T=0.3612 P=0.7304**	**T=0.6702 P=0.5277**