California Melon Research Board
2012 FINAL Report

TITLE: Biological control for soil dwelling insect pests of melon crops

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OBJECTIVES:

Immediate objective: The objective is to assess the potential for control soil dwelling insect pests of melons by the application of insect pathogenic fungi, *Metarhizium anisopliae* (*M.a.*). 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:
Experiments conducted to evaluate the potential for control of a soil dwelling insect pests of melons (darkling beetles *Blapstinus* spp.) by the application of an insect pathogenic fungi (Ma) in granular and liquid formulations both in the laboratory and in the field. In the laboratory experiment, *M.a.* treatments provided varying levels of beetle mortality, but higher *M.a.* concentrations in the soil provided greater beetle mortality than the untreated control mortality. However, the granular formulations provided greater beetle mortality than the *M.a.* broth. The field experiments in melon plots at UC Desert Research and Extension Center had too few *Blapstinus* spp. beetles in the plots to give any meaningful results.

Experiment I, Laboratory

Purpose:
Evaluate *Metarhizium anisopliae* (*M.a.*) soil treatments for mortality of field collected *Blapstinus* beetles; the beetles were collected from the UC Desert Research and Extension Center (DREC) near Holtville, CA.

M.a. treatments were chosen to match 4 of the treatments applied by Eric Natwick to field plots, Summer 2012. Six application rates per treatment (including a no treatment control) were

applied to potting soil, but only the three highest rates and control were evaluated for fungal concentrations and insect mortality.

1. *M.a*. microclerotia clay granules, rates: a) 0.0625, b) 0.0125, c) 0.0025, d) 0.0005, e) 0.0001 and f) 0 g/cup
2. *M.a*. microclerotia clay granules with antibiotics, rates: a) 0.0625, b) 0.0125, c) 0.0025, d) 0.0005, e) 0.0001 and f) 0 g/cup
3. *M.a*. microclerotia fermentation broth , rates: a) 0.1968, b) 0.0394, c) 0.0079, d) 0.0016, e) 0.0003 and f) 0 ml/cup
4. Met52 EC, Novozymes commercial product, rates: a) 0.0398, b) 0.0080, c) 0.0016, d) 0.0003, e) 0.0001 and f) 0 g/cup)

Methods:

1. Greenhouse potting soil (2 g/cup) was added to one ounce shot cups with lids (5 cups per treatment/rate). (July 10)
2. Water (2 ml) was added to cups receiving granule treatments 1 and 2 to match the liquid added to treatments 3 and 4 (below). (July 11)
3. Treatments 1, 2, and 3 were added to the cups at the rates noted above. (5 cups per treatment/rate) These trays were allowed to incubate for 7 days to allow conidial production from the microsclerotia. The application rates of broth were diluted and applied in 2 ml water per cup. (July 11)
4. Met 52 EC (Treatment 4) was added to the cups at the rates noted above. For each of the rates, the Met52 EC was diluted and applied in 2 ml water per cup. (5 cups per treatment/rate) (July 18)
5. Added 6 live beetles to each cup. (July 18)
6. Evaluated beetles for mortality on July 30. (Mortality data for the "d" and "e" rates were not different than the control ("f") rate and are not reported.)
7. Evaluated cups for conidia concentrations. (August 6 and 7)
    a. Added 20 ml water with 0.04% Tween 20 to each cup.
    b. Vortexed to mix
    c. Counted spores using a hemacytometer.
    d. The conidia counts for the "c" rate were not significantly different than the control ("f"). The "d" and "e" rates were even lower, and are not reported.
8. Evaluated cups for colony forming units (CFUs)
    a. Serial diluted liquid used for condia counts 10x, 100x, 1000x and 10,000x.
    b. Pipetted serial dilutions onto selective agar, incubated 72h and counted fungal colonies.
    c. The CFUs for the "c" rate were not significantly different than the control ("f"). The "d" and "e" rates were even lower, and are not reported.

Results:

Beetle mortality in the control treatments was high, so the mortalities for the *M.a.* treatments were adjusted using Abbott's formula. The highest application rate (0.0625g/cup) of the two granule treatments provided the highest beetle mortality after 12 days of exposure to treated soil. Beetle mortality (Abbott's corrected) was 72.4% for the granule treatment 1, and 65.5% for the granule treatment 2 that contained antibiotics. Beetles exposed to the broth treatment 3 and the Met52 EC treatment 4 had significantly lower mortalities at all application rates when compared with those exposed to granule treatments. The highest beetle mortality achieved for the broth treatment was 17.2% and the highest mortality for the Met52 EC was 28.6% (Figure 1). Fungal mycosis was observed in dead beetles found in all of the *M.a.* treatments, although fewer dead beetles exposed to broth treatments exhibited mycosis. No fungal mycosis was observed in the dead beetles found in the control cups.

Conidia counts and CFUs, for the highest application rate, showed that the granules produced significantly more conidia in the soil than did the Broth and Met52 EC treatments, however, treatments produced fewer conidia than expected (Figure 2). Conidia counts and CFUs matched well among all treatments. Conidia counts for the granule treatment 1 were 32.3% of the expected number and CFU counts were 30.5% of the expected value. For the granules with antibiotics, treatment 2, conidia counts were 38.4% of expected and CFU's were 27.8% of expected. Counts for broth treatment 3 were lower with conidia counts at 18.9% of expected and CFU's at 18.9% of expected. The Met52 EC counts were the lowest at 1.6% of expected conidia number and 3.5% of expected CFU number. The lower conidia counts and CFUs for the broth and Met52 EC treatments could explain the lower mortality for these treatments. The lower conidia counts and CFUs for the Met52 EC treatment could partly be explained by application techniques as spores were observed sticking to the sides of the containers used to dilute the product before application.

Conclusions:

Blapstinus beetles are susceptible to infection by *M.a.* applied to soil environments as expressed by higher mortalities with higher application rates, correlation between beetle mortalities and fungal counts (conidia and CFU), and by observed fungal growth from dead beetles exposed to *M.a.* treatments, but not seen for dead beetles in the controls.

Beetle mortalities suggest higher application rates of granule treatments are required to provide sufficient conidia necessary for control of beetles in field environments and that granule formulations have greater potential for providing beetle control compared with the two liquid applications.

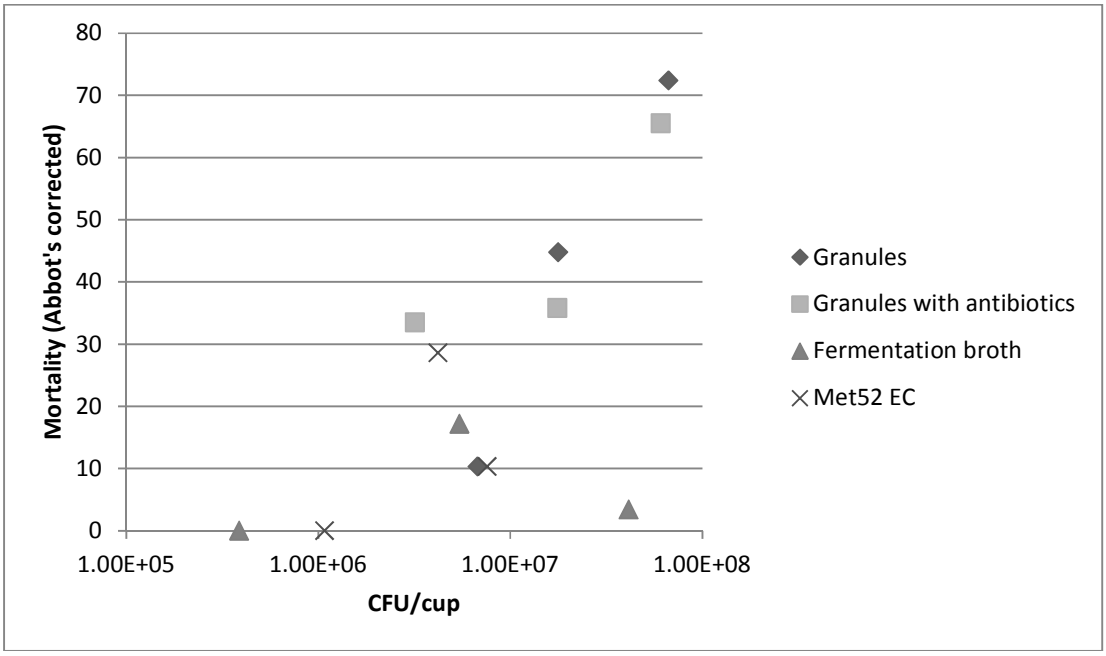

Figure1: Percentage mortality of *Blapstinus* beetles exposed to treated soil versus CFU per cup.

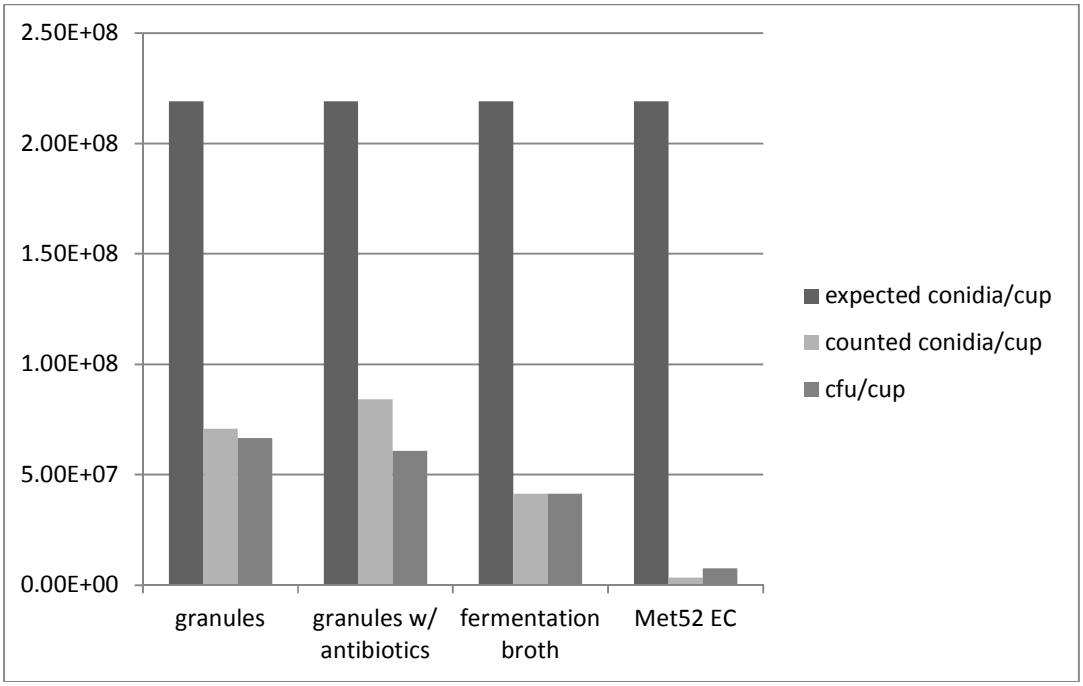

Figure2: Expected number of conidia, counted conidia and CFUs per cup for highest rate of *Metarhizium anisopliae* applied to potting soil.

Experiment II, Field plots

Purpose:
Evaluate in-the-field mortality from granular and liquid treatments with Ma formulations 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. Treatment List for Darkling Ground Beetle in Cantaloupe Melons, Holtville, CA, 2012.

Treatment	Amount/acre	Application date
1. Sevin Bran bait 5%	20 lb	31 May
2. Ambush Bait 0.05%	20 lb	31 May
3. Met_{52} EC	130.68 fl oz	31 May
4.MaGranules w/antimicrobial	2860 g	31 May
5. Ma Granules w/o antimicrobial	2860 g	31 May
6. Ma Broth Spray	10 L	31 May
7. Met_{52} granules	36 lb	31 May
8. Check	----------	31 May

Methods:

Plot size: 50 ft X 13.33 ft (2 beds/plot on 80 inch centers); one buffer bed between plots and 10 ft 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 five TJ-60 11003VS nozzle/bed delivering 43 gpa @ 30 psi. All treatments were applied on 31 May, 2012.

On 25 May and 4 June 2012, the *Blapstinus spp.* beetles beneath 20 melon fruit in each plot were counted and numbers were recorded. On 11 June, the numbers of *Blapstinus spp.* beetles beneath 25 melon fruit per plot were recorded. The percentages of *Blapstinus spp.* beetle damaged fruit per 20 fruit were recorded on 4 June and per 25 fruit on 11 June 2012.

Results:
There were too few *Blapstinus spp.* beetles present in the field plots to give any meaningful results, but data were collected and are summarized in Tables 2 and 3 below. There were no differences among the treatments for the numbers of *Blapstinus spp.* beetles per fruit, nor for percentages of *Blapstinus spp.* beetle damaged fruit.

Table 2. Number of Darkling Ground Beetle per Melon Fruit, Holtville, CA, 2012.

Treatments	Amount/acre	25 May	4 Jun	11 Jun
Sevin Bran bait 5%	20 lb	0.050	0.013	0.050
Ambush Bait 0.05%	20 lb	0.013	0.000	0.050
Met_{52} EC	130.68 fl oz	0.013	0.050	0.050
Ma Granules w/antimicrobial	2860 g	0.013	0.063	0.050
Ma Granules w/o antimicrobial	2860 g	0.038	0.013	0.050
Ma Broth Spray	10 L	0.025	0.038	0.010
Met_{52} granules	36 lb	0.025	0.000	0.040
Check	----------	0.025	0.063	0.000

There were no differences among the means within columns via ANOVA, *P*=0.05.

Table 3. Percentages of Darkling Ground Beetle Damage Melon Fruit, Holtville, CA, 2012

Treatments	Amount/acre	4 Jun	11 Jun
Sevin Bran bait 5%	20 lb	0.00	0.0
Ambush Bait 0.05%	20 lb	0.00	4.0
Met_{52} EC	130.68 fl oz	0.00	2.0
Ma Granules w/antimicrobial	2860 g	0.00	0.0
Ma Granules w/o antimicrobial	2860 g	0.00	3.0
Ma Broth Spray	10 L	0.00	0.0
Met_{52} granules	36 lb	0.00	0.0
Check	----------	0.00	0.0

There were no differences among the means within columns via ANOVA, *P*=0.05.