Biological Control of White Grubs in Turf with Microsclerotial Granules of *Metarhizium brunneum* (Mb) (formerly *Metarhizium anisopliae*).

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Objectives:

1. Determine relative susceptibility of three common grub species to the fungus
2. Compare several biopesticide formulations for control efficacy when applied under field conditions
3. Determine rates and timing of applications for optimal grub control under field conditions

White grubs cause damage to turf grass by feeding on plant roots and may result in plant death. Control often relies on the application of chemical insecticides at relatively high rates. As governments adopt new legislation restricting chemical insecticide applications, turf grass managers face increasing constraints for pest control and have only a few non-chemical options available to control insect pests. Biological insecticides of *Metarhizium* (Mb) fungus are commercially available for control of white grubs. Current commercial products commonly contain the infective spore (conidia) as the active agent, but are costly and may be ineffective when applied to control soil pests. As an alternative, liquid and granular formulations of Mb made with microsclerotia (capable of producing infective conidia after application to the field) may be better suited to target soil-dwelling insects. This research evaluates the efficacy of these prototype formulations applied under field conditions for control of white grubs.

Figure 1. Masked chafer adults killed by *Metarhizium* fungus and showing mycosis in the form of hyphal growth (white mycelia) and formation of conidia

Objective 1

Methods: Field collected adults of June beetles (*Phyllophaga* sp.) and masked chafers (*Cyclocephala borealis*) were collected using black-light traps during May 2014. Beetles were exposed to *Metarhizium* to compare their susceptibility to fungal infection by placing them in individual cups containing greenhouse potting soil treated with varied rates of Mb. Beetles were exposed to Mb conidia (commercial Met 52 G, Novozyme) and granules made with microsclerotia (experimental formulation) applied to potting soil in 1 oz plastic cups for a dosage/response assay. Four fungal dosage rates ranging up to 2.2 x 108 conidia/cup were evaluated against 30 *Phyllophaga* beetles/treatment dosage and 18 *Cyclocephala* beetles/treatment dosage. Beetles in treated cups were incubated in the dark at 25o C. Live and dead insects were counted after 21 days incubation for the larger June beetles and after 14 days for smaller masked chafers.

Results: Adult beetles of common species of white grubs are susceptible to infection by *Metarhizium* fungus and follow an expected dosage response such that higher exposure dosages killed more beetles, Figures 1 and 2. When comparing between species, it is also apparent that the larger June beetles were more difficult to kill (highest mortality at 73%) and time to death was longer (21 days

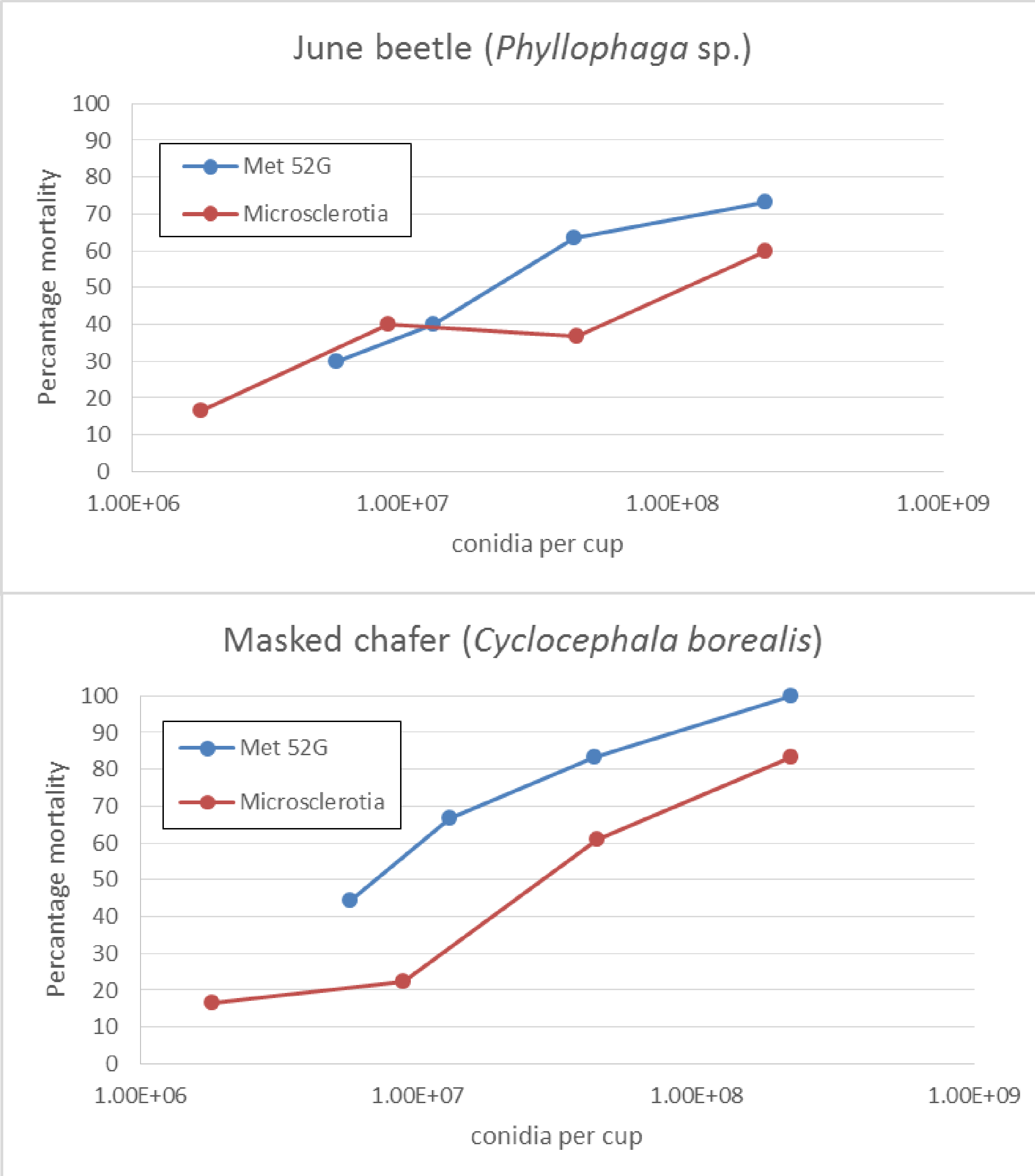

Figure 2. Mortality of field collected adult June beetle and masked chafer beetles when exposed to potting soil treated with varied rates of *Metarhizium* granules (commercial = Met 52G, experimental = Microsclerotia).

after exposure to treatments). By comparison, smaller masked chafer beetles reached 100% mortality in 14 days. Both the commercial and experimental treatments provided insecticidal activity. The commercial granules resulted in slightly higher beetle mortalities when compared with similar rates of the experimental granules.

Objective 2

Methods: Microsclerotia granules are expected to produce the infective conidia after application to the soil. Environmental factors such as the lack of moisture and high temperatures can adversely affect this process. To control white grubs, treatments are typically applied to turf grass during August through September. Soil temperatures may fluctuate greatly and high temperatures may be detrimental to the *Metarhizium* fungus. Soil temperatures monitored in turf plots during the summer of 2013 reached 33° C during the day in late August, but fell to 23° C overnight. We evaluated the ability of the microsclerotia granules to successfully produce conidia when exposed to elevated constant and fluctuating temperatures. Granules were plated on agar plates to

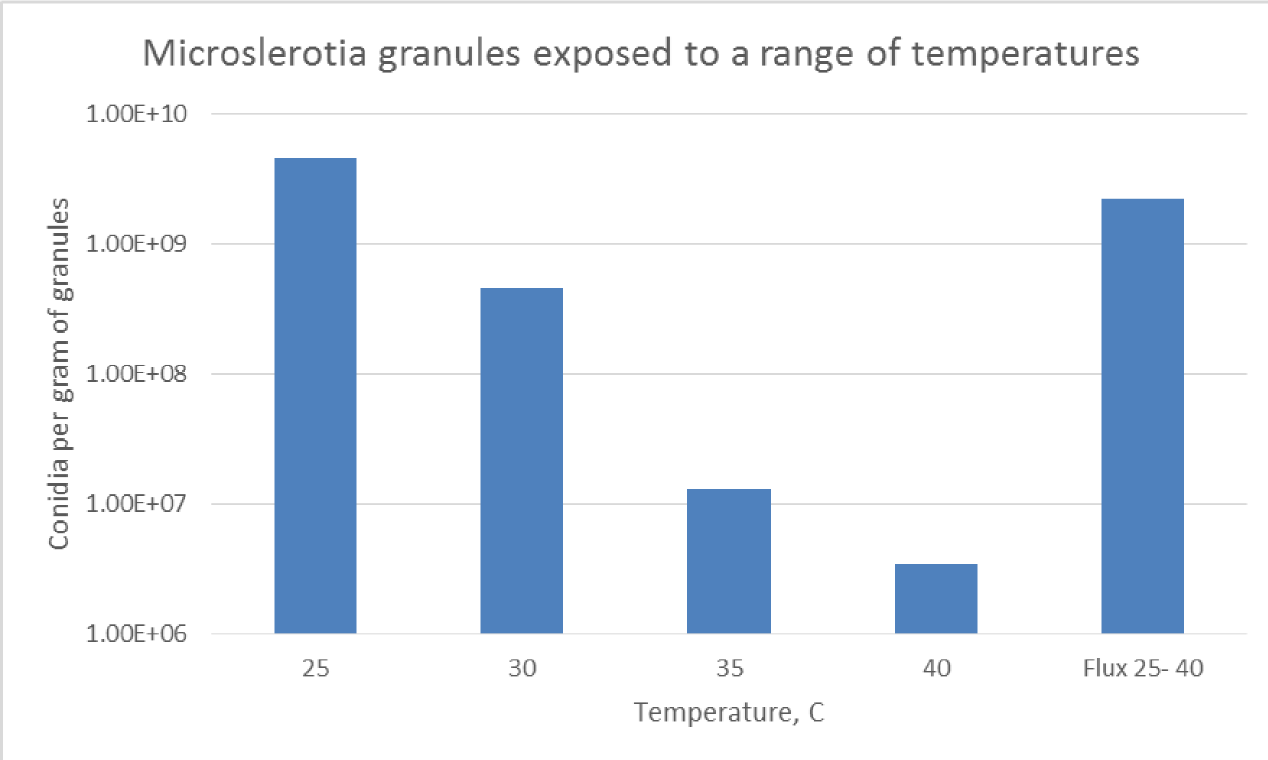

Figure 3. Conidia production by microsclerotia granules placed on agar plates and exposed to 25°, 30°, 35°, and 40° C constant temperatures and a fluctuating 25° to 40° C daily cycle.

supply the needed moisture and placed in incubators at constant temperatures of 25°, 30°, 35°, and 40° C and one fluctuating temperature exposure treatment (40° C for 4.5 hours per day and 25° C for 19.5 hours per day). Samples were exposed for eight days, then the number of spores produced per gram of granules was determined.

Results: High temperatures reduced the ability of microslerotial granules to produce conidia (Figure 3). Based on our experience, we expect granules exposed to favorable growing conditions (25° C temperatures) to produce near 5 x 10^9 conidia/g granules. The number of conidia produced for granules exposed to a constant temperature of 40° C was virtually zero. However, granules exposed to fluctuating temperatures produced conidia at levels similar to lower constant temperatures (2.2 x 10^9 conidia/g granules). These results show that high temperatures are harmful to the fungus, especially for long exposure times. Simply reaching these temperatures as with a daily cycle had minimal impact on fungal growth.

Objective 3

Methods: Formulation samples were applied to field sod plots at Purdue University on different dates to evaluate treatment applications that are timed to target

Figure 4. Evaluating turf grass field plots for white grubs to evaluate the control provided by treatments of *Metarhizium brunneum* fungus, and a fungal infected grub, Purdue University, West Lafayette, IN, 2014.

Table 1. Influence of formulation and application date on efficacy of *Metarhizium brunneum* and Merit 0.2G (standard) against larvae of the Japanese beetle, *Popillia japonica* Newman, in Kentucky bluegrass turf at West Lafayette, IN 2014.

Treatment	Application Date	Target	JB Larvae/ft^2 mean±se	% Control
Untreated	---		17.5±1.3 a	---
Merit 0.2 G	1-Aug	Peak Oviposition	0.0±0.0 f	100.0
Met 52 G	1-Aug	Peak Oviposition	9.5±2.9 abcd	45.7
Microsclerotia G	1-Aug	Peak Oviposition	13.8±4.6 abcd	21.4
Met 52 EC	1-Aug	Peak Oviposition	10.0±1.5 abcd	42.9
Microsclerotia EC	1-Aug	Peak Oviposition	12.3±2.3 abcd	30.0
Merit 0.2 G	21-Aug	1st/2nd Instar	0.8±0.5 ef	95.7
Met 52 G	21-Aug	1st/2nd Instar	11.5±3.2 abcd	34.3
Microsclerotia G	21-Aug	1st/2nd Instar	14.3±3.3 abc	18.6
Met 52 EC	21-Aug	1st/2nd Instar	15.0±5.6 ab	14.3
Microsclerotia EC	21-Aug	1st/2nd Instar	11.3±4.5 abcd	35.7
Merit 0.2 G	10-Sep	3rd Instar	5.5±1.8 def	68.6
Met 52 G	10-Sep	3rd Instar	8.8±2.9 bcde	50.0
Microsclerotia G	10-Sep	3rd Instar	5.5±2.2 def	68.6
Met 52 EC	10-Sep	3rd Instar	5.8±2.8 cdef	67.1
Microsclerotia EC	10-Sep	3rd Instar	9.3±2.5 abcde	47.1

*Values followed by the same letter are not significantly different (α=0.05).

different stages of the insect (Table 1). Five treatments were applied in a randomized complete block design with four replications per treatment. Plots had previously been infested with Japanese beetle (*Popillia japonica* Newman) adults to induce egg laying and increase grub density for treatment evaluation. Treatments were applied on August 1, August 21 and September 10, 2014 to coincide with peak oviposition, 1st/2nd instar, and 3rd instar stages, respectively. Grub densities (number per ft^2) were determined for each treated plot in October 1, 2014.

Results: Not all treatments resulted in significant reductions in Japanese beetle larval densities. The number of grubs found in plots receiving early applications of *Metarhizium* formulations were numerically better, but not significantly different from the untreated plots. In stark contrast, Merit (imidacloprid) provided over 95% control and was significantly better than all other treatments. When targeting 3rd instar, however, all *Metarhizium* formulations (except Microsclerotia EC) provided significant levels of control and were not different from the corresponding Merit treatment. For all the fungal treatments, the average control for this late season application was near 60% compared with the untreated plots.

Summary Points:

- Several species of white grubs that are common pests of turf grass are susceptible to infection and death when exposed to *Metarhizium* fungus treatments as commercial or experimental granules.
- Experimental formulations made with Mb microsclerotia provided similar levels of control for Japanese beetle larvae when compared with applications of commercial Mb formulations applied to field plots of turf grass.
- Control of white grubs with Mb treatments were most effective and comparable with the systemic chemical insecticide Merit when applied later in the season to target 3rd instar grubs.
- The later applications to field plots in 2014 provided similar control of white grubs when compared with corresponding applications during the 2012 and 2013 field season indicating that this biological material may be useful for curative applications.