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Title: Biological Control of Annual Bluegrass Weevil with novel Formulation Types and Application Systems for Entomopathogenic Fungi: Microsclerotia-based formulations and Hydrogels

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Objectives: The goal is to develop a granular formulation of microsclerotia of *Metarhizium brunneum* F52 as an effective and viable biological control option for ABW. Specifically, we want to determine:

1. Compatibility of formulation with commonly used golf course fungicides.
2. Efficacy of formulation against ABW adults and externally feeding larvae.
3. Effect of hydrogels on efficacy and persistence of formulation and compatibility with golf course turfgrass.

Summary Text:

The annual bluegrass weevil (ABW), *Listronotus maculicollis*, is a major pest of short-mown golf course turf in eastern North America. Its ability to develop resistance to a wide range of insecticides warrants the development of alternative control methods. Products based on the conidial spores of entomopathogenic fungi have thus far given unreliable control of ABW adults and larvae in the field, but the use of fungal microsclerotia may improve economy and efficacy of fungus-based products. Microsclerotia are survival structures naturally formed in soil by fungi. Applied microsclerotia granules produce infective conidial spores over several weeks, thus prolonging the residual effect of the fungus application. Improvements in conidia production by microsclerotia in soil can be made by the addition of hydrogels. Hydrogels have a capacity to hold large volumes of water when moistened and can slowly release this retained water over time, making it available to plants, fungi and other organisms.

The compatibility of *Metarhizium brunneum* F52 microsclerotia with common golf turf fungicides from different classes (Banner Maxx II: 14.3% propiconazole; Chipco 26 GT: 23.3% iprodione; Daconil WeatherStick: 54% chlorothalonil; TwinLine: 12% pyraclostrobin and 7.4% metconazole; Stratego: 11.4% propiconazole and 11.4% trifloxystrobin) was tested in the laboratory. The fungicides were incorporated into 1.2% water agar at rates that included and exceeded typical field rates. Clay-based microsclerotial granules were added to each Petri dish and incubated at 26 °C for 9 days before the number of viable spores produced was determined. Chlorothalonil did not inhibit fungal growth; iprodione was slightly inhibitory at higher concentration; propiconazole, Twinline and Stratego strongly inhibit fungal growth except at the lowest concentration (Fig. 3).

Based on our laboratory findings, three fungicides were applied at two rates (propiconazole: 0.5 and 1.8 kg ai/ha; iprodione: 1.5 and 6 kg ai/ha; chlorothalonil: 2.2 and 8.6 kg ai/ha) to pots with creeping bentgrass in the greenhouse. Microsclerotia granules had been applied to the pots 1 day

earlier. After 10, 20 and 30 days, the number of fungal colony forming units (CFUs) in the top 2.5 cm of soil and the grass was determined. There was no effect of evaluation time and no significant interactions between treatment and evaluation date. Only propiconazole significantly inhibited fungal growth ($F = 8.20$; df = 6, 187; $P < 0.0001$).

In greenhouse experiments to date, different rates of *M. brunneum* microsclerotia had no significant effect on survival of ABW adults and larval population densities. However, in a field experiment, microsclerotia and the insecticide imidacloprid provided additive control, albeit at a low level (44%). Future experiments in greenhouse and field will compare the control efficacy of microsclerotia with that of commercial conidial spore formulations alone and in combinations with hydrogel and/or imidacloprid.

Summary Points:

- *M. brunneum* microsclerotia are compatible with the turf fungicides iprodione and chlorothalonil.
- The fungicide propiconazole has a suppressive effect on *M. brunneum* spore production.
- *M. brunneum* microsclerotia alone did not significantly suppress ABW adult and larval populations.
- Combinations of *M. brunneum* microsclerotia with the insecticide imidacloprid provided additive control of ABW larval populations in the field.

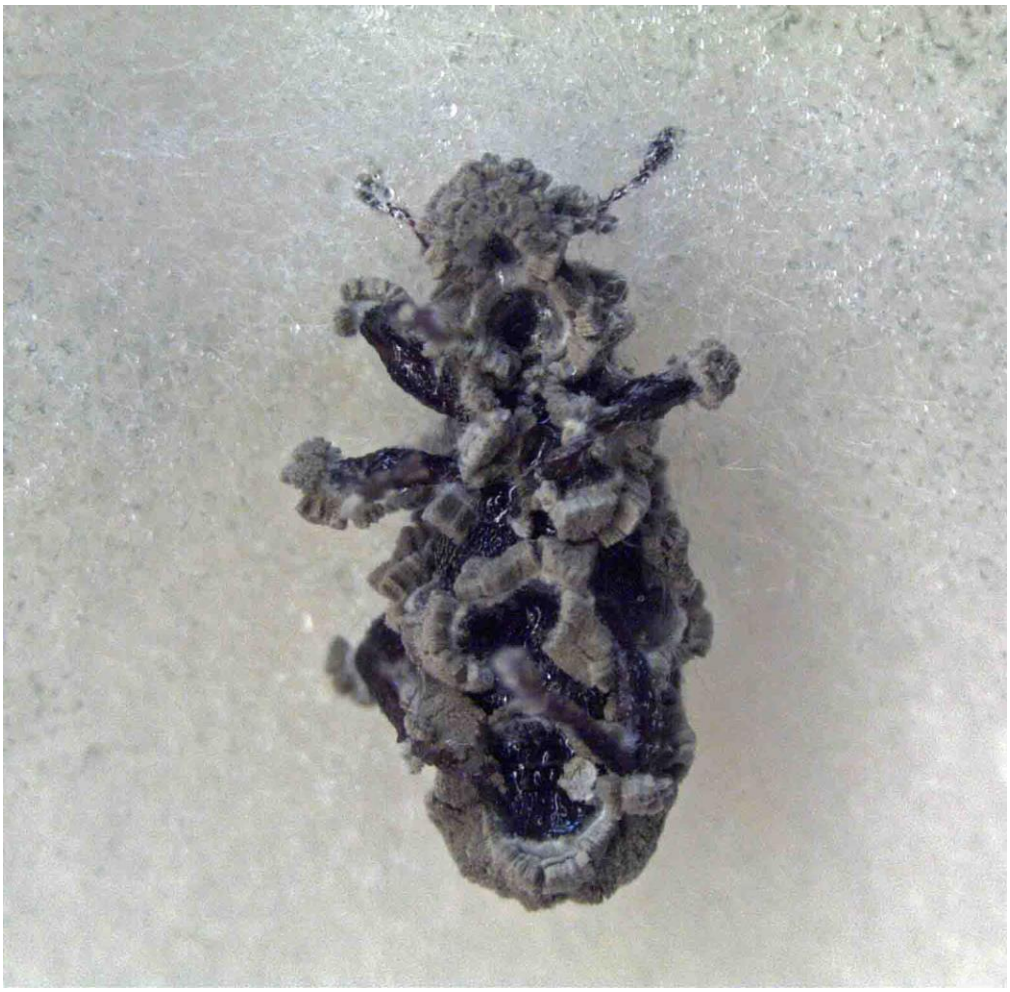

Fig. 1. ABW adult infect with *Metarhizium brunneum* F52.

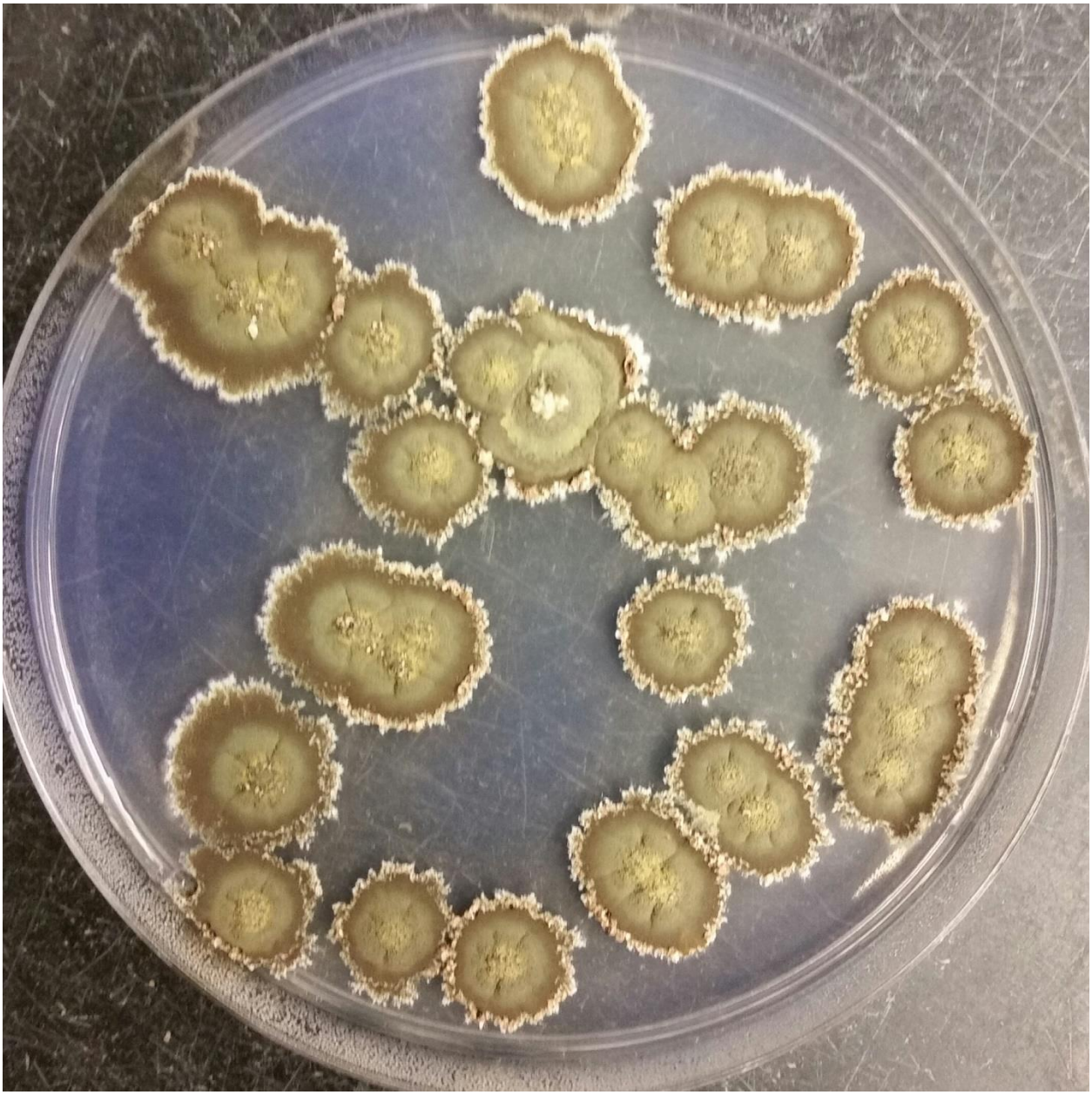

Fig. 2. *Metarhizium brunneum* F52 colonies growing on agar plate.

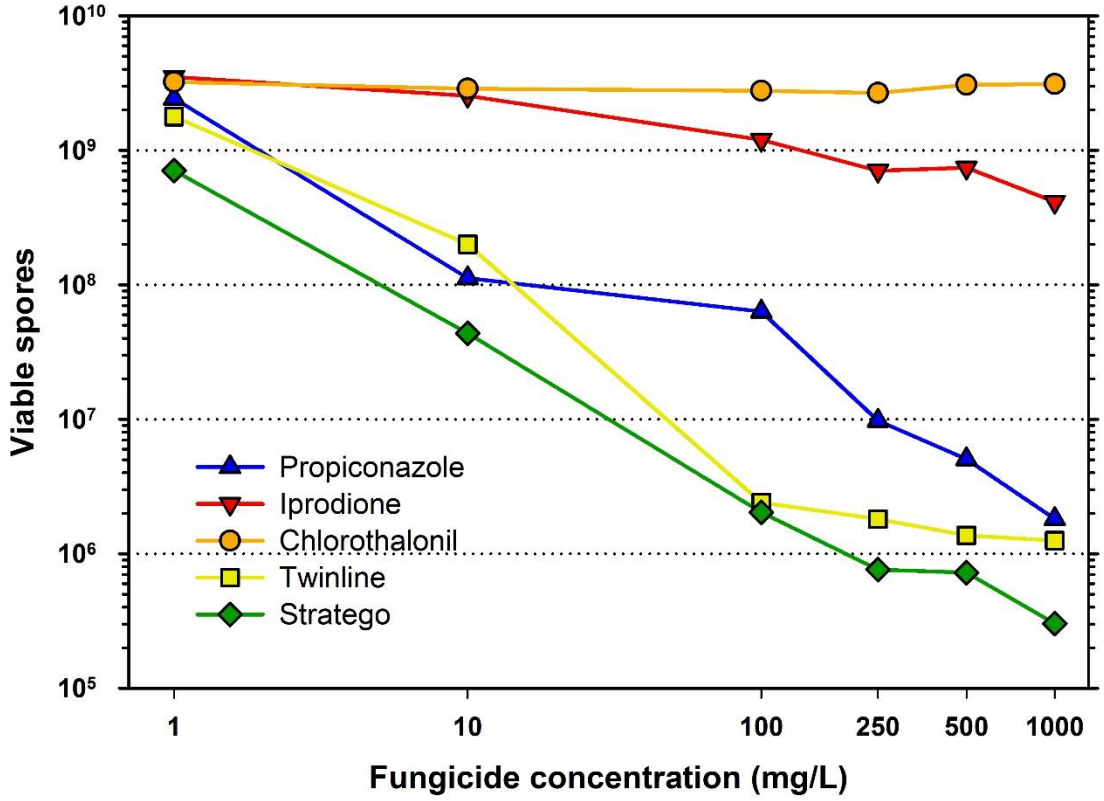

Fig. 3. Number of viable spores per gram of *Metarhizium brunneum* microsclerotia after incubation for 9 days at 26 °C on water agar containing different concentrations of five fungicides.

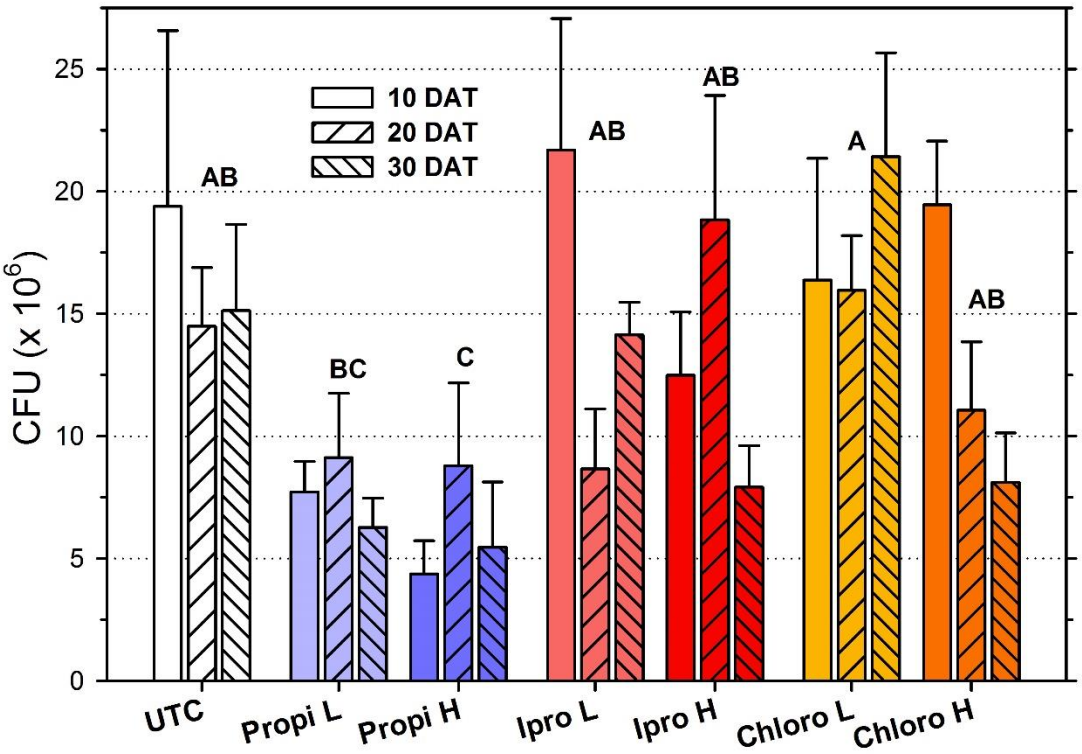

Fig. 4. Number of *Metarhizium brunneum* F52 colony forming units (CFUs) recovered from top 2.5 cm of soil and grass in pots treated with water only (untreated control = UTC), and a low (L) and a high (H) rate of the fungicides propiconazole (Propi), iprodione (Ipro) and chlorothalonil (Chloro) at 10, 20 and 30 days after treatment (DAT). Means (evaluation dates combined by treatment) with same letter did not differ significantly from each other ($P \geq 0.05$).