N O T E

Beauveria bassiana (Ascomycotina: Hypocreales) Dry Mycelium in Soil Exhibits No Adverse Effects on Bermudagrass and Pinto Bean Plants[1]

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Infective spores, primarily conidia, have been used historically in insect biocontrol efforts employing entomopathogenic fungi as microbial agents (Magalhães and Boucias 2004, J. Orthoptera Res. 13: 155–159). Indeed, Humber (1991, *In* Proc. Aphid-Plant Interactions: Populations to Molecules Oklahoma State Univ. Agric. Exp. Station MP-132. Pg. 45–56) noted that only spores (conidia or blastospores) are capable of successfully invading the host insect body. While fungal mycelium is not infective per se (i.e., cannot invade live insect hosts), the use of mycelia artificially produced as dry granules or pellets has been explored. Such mycelial preparations placed in the field or greenhouse with environmental conditions conducive for fungal growth will grow and eventually produce infective spores that insect pests may contact. Use of dry mycelial preparations may circumvent technical difficulties inherent in the industrial production and the commercial application of aerial fungal spores (Jackson et al. 1997, Mycol. Res. 101: 35–41) while capitalizing on the relatively simple production of filamentous stages (mycelium) using equipment from industrial biotechnology (i.e., fermentation).

Dry mycelium formulations have been tested against insects on foliage and in other environments (Rombach et al. 1986, Environ. Entomol. 15: 1070–1073; Wraight et al. 2003, Biol. Control 28: 60–77). Sanchez-Peña and Thorvilson (1991, Pp. 94–105, *In* Proc. 1991 Imported Fire Ant Conf., Univ. Georgia, Athens) successfully used dry mycelium of *Beauveria bassiana* (Balsamo) Vuillemin sensu lato against red imported fire ant, *Solenopsis invicta* Buren, queens. Krueger et al. (1992, J. Invertebr. Pathol. 59: 54–60) reported shorter lethal times of grubs of the

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Japanese beetle, *Popillia japonica* Newman, and the European chafer, *Rhizotrogus majalis* (Razoumowsky), when placed in soil amended with dry mycelial granules of *Metarhizium anisopliae* (Metschnikoff) Sorokin sensu lato compared to conidia-amended soil under laboratory conditions. Krueger and Roberts (1997, Biol. Control 9: 67–74) also reported significantly improved plant protection and reduced corn rootworm (*Diabrotica* spp.) emergence following incorporation of dry mycelial particles of *B. bassiana* and *M. anisopliae* into soil infested with corn rootworm larvae. Similar approaches have been suggested using *Metarhizium* microsclerotia (pigmented mycelial masses formulated as small, dry, hard granules that produce spores after incorporation to soil) (Jackson and Jaronski 2009, Mycol. Res. 113: 842–850) and artificial colonization of the rhizoplane with entomopathogenic fungi (Bruck 2005, Biol. Control 32: 155–163).

While augmentation of entomopathogenic fungi in agroecosystem soils has merit, there are few reports on the effects of high levels of mycelium in soil (i.e., being in direct contact with growing plant roots) and the impact the amendments may have on plant growth parameters. Of concern are the toxins that are produced by entomopathogenic fungi. For example, *Beauveria* produces several generalistic cytotoxins (Abendstein et al. 2000, Biocontrol Sci. Technol. 10: 789–796). One of those is beauvericin (a cyclodepsipeptide), which is a general ionophoric cytotoxin that can alter the ionic balance of cells and contribute to fungal pathogenicity of plants (Logrieco et al. 2003, Eur. J. Plant Pathol. 109: 645–667). In the present study, the effect of high levels of augmented *Beauveria* mycelium upon developmental parameters of two plants, bermudagrass, *Cynodon dactylon* (L.) Pers., and pinto bean, *Phaseolus vulgaris* L., were measured.

The *B. bassiana* strain used was ARSEF 2484 (Humber 2013, USDA-ARS Collection of Entomopathogenic Fungal Cultures, Catalog of Strains, Ithaca, NY) which was originally isolated from Mexican leaf-cutting ant, *Atta mexicana* (Smith), workers collected in the Mexican state of Sinaloa. The fungal culture used for our study was serially inoculated on harvester ants, *Pogonomyrmex barbatus* (Smith), for 4 yr and reisolated in pure culture on Sabouraud dextrose agar + 1% yeast extract (SDAY) (B-D, Franklin Lakes, NJ). The strain was successively passaged five times through harvester ants attempting to reduce or stabilize virulence changes before its use in tests (Sanchez-Peña and Thorvilson 1995, J. Invertebrate Pathol. 65: 248–252). After the fifth passage on ants, the fungus was reisolated on SDAY. Aerial conidia from those cultures were transferred aseptically into four 300-ml flasks each containing 150 ml of Sabouraud dextrose broth (B-D) plus yeast extract. These were incubated for 6 d on a rotating table (150 rpm) at 25°C. The resultant mycelial biomass was pooled and recovered by filtration through four layers of sterile cotton muslin cloth. The mycelial paste was spread into a 2- to 3-mm-thick layer and air-dried at 20–30% relative humidity (RH) exposed to a pedestal fan operated at high speed. Once the dry mycelium was dried and maintained stable weight (approximately 8 h), it was manually fragmented with a stainless steel blade into 0.3- to 2.0-mm pieces and stored at 4°C for 4 d before use in tests.

Two tests were performed exposing plants to *Beauveria* mycelium to observe the possible effects upon plants. In the first test, the effect of viable dry mycelium amounts upon seedling establishment was observed for both plant species; also, its effect upon the total number of bean leaves produced was recorded. Dry mycelial

fragments were mixed in different percentages (0.001, 0.01, 0.05, 0.1, and 0.4% w/w in grams) with commercial potting soil mix (Baccto, Houston, TX). Forty-five grams of soil with dry mycelial granules in 355-ml plastic cups were moistened with 20 ml of sterile, demineralized water and incubated for 6 weeks at room temperature until sporulation from mycelial growth was visible in the soil. Ten seeds of bermudagrass (Grounds Maintenance, Texas Tech Operations, Lubbock, TX) and three seeds of pinto beans (Great Value pinto beans, Walmart, Bentonville, AR) were added to the surface of the soil–fungus mix in each plastic cup, after which 20 ml of sterile, demineralized water was added to each cup and seeds were covered with soil (2 to 3 mm). Soil without dry mycelium was included in the test as a control. The infective fungal stage (conidia) was present in the pots when the seeds were added. There were eight replicates of each treatment (percentage of dry mycelium in soil). Cups with soil and seeds were incubated at 25–27°C and 100% RH for 3 weeks, and the soil was kept moist to enhance seed germination. Numbers of established seedlings for both plants and total number of bean leaves were compared among treatments.

For the second test, the effect of dry mycelium upon leaf area and biomass was analyzed for bean plants grown in mycelial-amended soil. In this test, 0.5 and 1.0% (w/w) of dry mycelium was added separately to the same commercial potting soil mix, and the soil–fungus mixes were dispensed into 355-ml plastic containers. Control soil with no mycelium was included as a control. Five pinto bean seeds were added in each container. Water and soil were added as previously described, resulting in 5 plants/container. A container represented a replicate, and each treatment was replicated four times. Containers with seeds and fungus were incubated at 25–27°C and 100% RH for 2 weeks. Total plant leaf area and dry aerial biomass were recorded at 14 d. Leaf area was measured with a LI-3100C™ area meter (LI-COR, Lincoln, NE), aerial plant biomass was measured by cutting plants at soil level, drying for 48 h in a drying oven at 80°C, and weighed. Linear regression analysis was performed on seedling establishment and leaf number data (Chang 2000, Statistics Tool Box. The Chinese Univ. Hong Kong. http://department.obg.cuhk.edu.hk/researchsupport/statmenu.asp. Accessed 14 January 2014). Biomass measurements and mean leaf area were square-root transformed (Fowler et al. 1998, Pp. 83–89, Practical Statistics for Field Biology, 2nd ed., Wiley, United Kingdom) and analyzed by analysis of variance procedures (Arsham 2014, Analysis of Variance (ANOVA). Univ. Baltimore. http://home.ubalt.edu/ntsbarsh/STAT-DATA/javastat.htm. Accessed 14 January 2014).

The amount of dry mycelium in soil assessed in this study had no significant effect upon germination and plant establishment for both bermudagrass and pinto bean ($P = 0.1598$ for bermudagrass; $P = 0.4481$ for pinto bean plants). Establishment of bermudagrass plants appeared to be directly proportional to amount of mycelium in soil, but this relationship was nonsignificant ($P = 0.563$) according to the model. The amount of dry mycelium in soil did not have a significant effect on number of bean leaves ($P = 0.7636$) (Table 1). The small size of bermudagrass seedlings and the lack of a clear boundary between leaf and stem of these seedlings prevented measurement of bermudagrass leaf area. The leaf area of bean plants was not significantly influenced by treatment (ANOVA; $F = 0.4797$; df $= 11$; $P = 0.6343$) (Table 2). Similarly, aerial dry biomass of *P. vulgaris* plants was

Table 1. Mean (SE) number of established bermudagrass and pinto bean seedlings and total number of pinto bean leaves in response to levels of *Beauveria bassiana* mycelium added to potting soil.*

Treatment**	No. Grass Seedlings	No. Bean Seedlings	No. Bean Leaves
0.0	6 (0.20)	16 (0.66)	43
0.005	11 (0.13)	17 (0.71)	42
0.01	10 (0.12)	16 (0.66)	39
0.05	15 (0.18)	16 (0.66)	47
0.1	21 (0.26)	13 (0.54)	35
0.4	19 (0.23)	15 (0.62)	44

* Responses of bermudagrass establishment ($P = 0.1598$), pinto bean establishment ($P = 0.4481$, and number of pinto bean leaves ($P = 0.7636$) were not significant as determined by linear regression model.
** Percentage (w/w) of dry mycelium preparation of *B. bassiana* in potting soil.

not significantly influenced by dry mycelium treatments (ANOVA; $F = 0.5554$; df = 11; $P = 0.5927$) (Table 3).

In conclusion, for all the parameters tested, there were no significant deleterious effects to bermudagrass and pinto bean plants grown in soil with high levels (up to 1% w/w) of *B. bassiana* viable dry mycelia in the rhizosphere. The roots of the seedlings were in direct contact with the fungal mycelium and conidia, as well as with any potential exotoxins that could have been released from the actively growing mycelia. None of these factors appeared to have a negative effect on the growing plants. In fact, plant biomass was higher than in the controls for some fungal treatments. Furthermore, establishment of bermudagrass seedlings appeared to be directly proportional to amount of mycelia in soil, although the linear regression model indicated that this relationship was not significant. A positive effect of fungal biomass on plant growth should not be ruled out, in that it is increasingly clear that some fungal entomopathogens, like *Beauveria*, are generalist plant associates and endophytes that can be beneficial to their plant hosts (Brownbridge et al. 2012, Biol. Control 61: 194–200). The results herein indicate that the large amounts of dry

Table 2. Mean (SE) leaf area (in cm^2/pinto bean plant) for plants grown in potting soil with different concentrations of *Beauveria bassiana* dry mycelium.

Treatments*	Leaf Area**
0.5	406.9 (27.148) a
1.0	374.8 (25.767) a
0.0	379.7 (21.39) a

* Percentage (w/w) of viable dry mycelium preparation of *B. bassiana* in potting soil.
** Means followed by the same letter are not significantly different (ANOVA, $P = 0.6343$).

Table 3. Dry weight (g) biomass of aboveground portions of pinto bean plants grown in potting soil amended with different amounts of *Beauveria* dry mycelium.

Treatments*	Replicate Weight (5 Plants/Replicate)				Mean Weight/ 5 Plants (SE)**
	1	2	3	4	
0.5	2.760	2.362	2.779	2.226	2.531 (0.1400) a
1.0	2.626	2.245	2.468	2.805	2.536 (0.1188) a
0.0	2.348	2.574	2.258	2.230	2.352 (0.0779) a

* Percentage (w/w) of viable dry mycelium preparation of *B. bassiana* in potting soil.
** Treatment means followed by the same lowercase letter are not significantly different (ANOVA, $P = 0.5927$).

mycelia and subsequent conidia of *B. bassiana* will not noticeably harm these plant species. Further observations are needed to confirm the lack of adverse effects of dry mycelia for additional plant species.

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