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Beauveria bassiana: Endophytic colonization and plant disease control

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ABSTRACT

Seed application of *Beauveria bassiana* 11-98 resulted in endophytic colonization of tomato and cotton seedlings and protection against plant pathogenic *Rhizoctonia solani* and *Pythium myriotylum*. Both pathogens cause damping off of seedlings and root rot of older plants. The degree of disease control achieved depended upon the population density of *B. bassiana* conidia on seed. Using standard plating techniques onto selective medium, endophytic 11-98 was recovered from surface-sterilized roots, stems, and leaves of tomato, cotton, and snap bean seedlings grown from seed treated with *B. bassiana* 11-98. As the rate of conidia applied to seed increased, the proportion of plant tissues from which *B. bassiana* 11-98 was recovered increased. For rapid detection of B. *bassiana* 11-98 in cotton tissues, we developed new ITS primers that produce a PCR product for *B. bassiana* 11-98, but not for cotton. In cotton samples containing DNA from *B. bassiana*11-98, the fungus was detected at DNA ratios of 1:1000; *B. bassiana* 11-98 was detected also in seedlings grown from seed treated with *B. bassiana* 11-98. Using SEM, hyphae of *B. bassiana*11-98 were observed penetrating epithelial cells of cotton and ramifying through palisade parenchyma and mesophyll leaf tissues. *B. bassiana*11-98 induced systemic resistance in cotton against *Xanthomonas axonopodis* pv. *malvacearum* (bacterial blight). In parasitism assays, hyphae of *B. bassiana* 11-98 were observed coiling around hyphae of *Pythium myriotylum*.

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1. Introduction

Beauveria bassiana is well-known as an entomopathogenic fungus, with worldwide distribution. It is the anamorph stage of *Cordyceps bassiana*, a teleomorph in the ascomycetous family Clavicipitaceae (Sung et al., 2007). Members of this family are found in diverse ecological habitats, and include entomopathogens, plant pathogens, parasites of fungi or slime molds, and endophytes of grasses (White et al., 2003). This family of fungi is also well-known for production of secondary metabolites with toxigenic properties (White et al., 2003). Given the diversity of habitats for this group of fungi, it is not surprising that in addition to parasitizing insects, *B. bassiana* has been recovered as an endophytic colonist from several plant species (Vega, 2008), and has been shown to protect plants against plant pathogens.

2. *Beauveria bassiana*—activity against plant pathogens

In recent years, evidence has been presented that indicates *B. bassiana* has potential as a dual purpose microbial control organism, against both insect pests and plant pathogens. Isolates of *B. bassiana* inhibited mycelial growth *in vitro* of an array of soilborne and foliar plant pathogens, including *Gaeumannomyces graminis* var. *tritici* (Renwick et al., 1991); *Armillaria mellea* and *Rosellinia necatrix* (Reisenzein and Tiefenbrunner, 1997); *Fusarium oxysporum* (Reisenzein and Tiefenbrunner, 1997; Bark et al., 1996), *Botrytis cinerea* (Bark et al., 1996), and *Rhizoctonia solani* (Lee et al., 1999). In addition to inhibition of mycelial growth, *B. bassiana* induced cell lysis of plant pathogenic species, such as *Pythium ultimum*, *P. debaryanum*, and *Septoria nodorum* (Vesely and Koubova, 1994).

Isolates of *B. bassiana* have been evaluated for protection of wheat against take-all disease (caused by *G. graminis* var. *tritici*) and basal rot of onion (caused by *F. oxysporum* f. sp. *cepae*). In pot studies, *B. bassiana* was selected among 1800 rhizosphere microorganisms for effective and consistent microbial control of take-all disease (Renwick et al., 1991). *B. bassiana* applied to onion bulbs in greenhouse and field studies, significantly reduced infection by *F. oxysporum* f. sp. *cepae* (Flori and Roberti, 1993).

Beauveria bassiana can also protect tomato against seedling diseases caused by *R. solani* (Bishop, 1999; Seth, 2001; Ownley et al., 2000, 2004) and *Pythium myriotylum* (Clark, 2006; Clark et al., 2006). In these studies, conidia of *B. bassiana* isolate 11-98, mixed

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in methylcellulose solution, were applied to seed, and allowed to dry. Coated seed were planted in soil artificially infested with *Rhizoctonia* or *Pythium*. Percent plant stand and plant growth were increased in soil infested with *P. myriotylum* when seed were treated with 2×10^5 conidia per seed (Clark, 2006; Clark et al., 2006).

Damping off caused by *R. solani* was significantly reduced with conidial seed treatments of *B. bassiana* 11-98 (1×10^6 conidia per seed) in experiments with high pathogen pressure. In 'Mountain Spring' tomato, percent plant stand of the untreated seed + *R. solani* control ranged from 18% to 27%; for the untreated healthy control (no *R. solani* added) plant stand was 77–82%, while *Beauveria*-treated seedlings in *Rhizoctonia*-infested soil had 62–77% plant stand (Ownley et al., 2004). Similarly, in 'Mountain Pride' tomato, there was 47–50% plant stand in the untreated seed + *R. solani* control, 83–97% plant stand in the untreated healthy control, and 73–75% plant stand in seedlings grown from *Beauveria*-treated seed and planted in *Rhizoctonia*-infested soil (Ownley et al., 2004).

In addition to seed treatments with conidia, other formulations of *B. bassiana* 11-98 have been evaluated for protection of tomato seedlings against *R. solani*, including application of mycelia to seed and soil, and mycelia incorporated into alginate bran pellets, then applied to soil (Ownley et al., 2004; Seth, 2001). However, seed treatments with conidia gave the greatest and most consistent reduction in seedling losses (Ownley et al., 2004; Seth, 2001). Rates of 1×10^6 to 1×10^7 conidia per seed consistently resulted in lower *Rhizoctonia* disease ratings and higher percent plant stands than lower rates of *Beauveria* conidia on seed (unpublished data).

Similar to results with tomato, *B. bassiana* can provide protection to cotton seedlings against *R. solani* (Griffin et al., 2005; Griffin, 2007). Height of seedlings (Fig. 1) and percent seedling survival was increased significantly with 1×10^9 conidia per seed in soil infested with *R. solani*. In *Rhizoctonia*-infested soil, percent survival of untreated seedlings was 31% compared to 69% with *B. bassiana* seed treatment. With both tomato and cotton seedlings grown from seed treated with *B. bassiana* 11-98, endophytic colonization of the seedlings by *B. bassiana* was detected (Leckie, 2002; Ownley et al., 2004; Griffin, 2007).

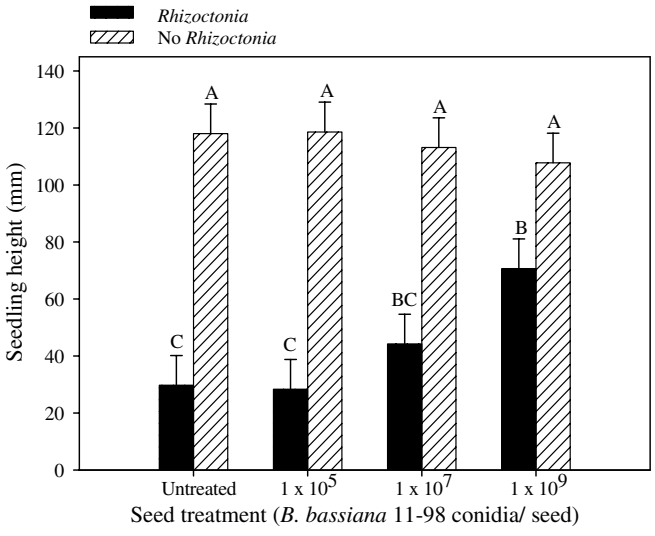

Fig. 1. Effect of different seed treatment rates of *Beauveria bassiana* 11-98 conidia on cotton seedling height in potting soil amended with 1% (w/w) *Rhizoctonia solani*. Seed treatments were: untreated, 1×10^5, 1×10^7, and 1×10^9 *B. bassiana* 11-98 conidia/seed. Data were analyzed for significance with Proc Mixed and F-LSD with PC-SAS (SAS Institute, Cary, NC). Effect of the interaction of seed treatment and pathogen was significant at $P = 0.0379$. Bars with the same letter are not different according to F-LSD at $P = 0.05$. Each bar represents least square means ± SE.

3. *Beauveria bassiana*–endophyte activity

In addition to the aforementioned work in our laboratory with *B. bassiana* 11-98 seed treatments of tomato and cotton, there are several other reports of endophytic colonization by *Beauveria*, either occurring naturally, or as a result of inoculation (Vega, 2008). *B. bassiana* colonizes a variety of cultivated plants, including cocoa (Posada and Vega, 2005), corn (Bing and Lewis, 1991; Lewis and Bing, 1991; Bing and Lewis, 1992a; Bing and Lewis, 1992b; Lewis et al., 2001; Jones, 1994; Wagner and Lewis, 2000), coffee seedlings (Posada and Vega, 2006), pharmaceutical opium poppy (Quesada-Moraga et al., 2006), potato (Jones, 1994), and tissue culture banana (Akello et al., 2007). Endophytic colonization of tree species, such as *Carpinus caroliniana* (Bills and Polishook, 1990), date palm (Gómez-Vidal et al., 2006), elm (Doberski and Tribe, 1980), and western white pine (Ganley and Newcombe, 2006) by *B. bassiana* has also been reported.

In our laboratory, *B. bassiana* 11-98 can be isolated from sections of surface-sterilized tissues of tomato and cotton with traditional plating methods onto selective medium (Doberski and Tribe, 1980). However, the surface-sterilization treatment (95% ethanol for 1 min; 20% bleach for 3 min; 95% ethanol for 1 min) must be done with whole seedlings rather than sections of seedlings for optimal recovery of the endophyte. Otherwise the sterilants are quickly absorbed into the cut surfaces of the seedlings and the endophyte is killed. Although hyphae of isolate 11-98 from tomato and cotton tissues begins to appear after 10–12 days, development of sporulating colonies may take 6–8 weeks at room temperature.

In order to determine the relation between initial population density of *B. bassiana* 11-98 conidia applied to seed and subsequent extent of colonization of tomato seedlings, seed were treated with six rates of *B. bassiana*. An untreated control was included also. Rates of conidia applied to seed ranged from 1×10^2 to 1×10^7 conidia per seed, in log increments. The experiment was designed as a Randomized Complete Block with 10 replicates per block and one seed per replicate. Seedlings were grown in a gnotobiotic system for 21 days, then removed from containers and whole seedlings were surface-sterilized as described above. Seedlings were sectioned into 1-cm increments and sections were plated onto selective medium (Doberski and Tribe, 1980). No *B. bassiana* was detected in plants grown from untreated seed. Percent colonization of seedlings by *B. bassiana* 11-98 was greatest (100%) for plants grown from seed treated with 1×10^7 conidia per seed. However, not all segments from all plants were colonized. For example, *B. bassiana* 11-98 was recovered from 95% of roots, 70% of stems, 85% of cotyledons and 40% of true leaves. For plants from seed treated with 1×10^7 conidia per seed, *B. bassiana* 11-98 was detected down to 4 cm of root. Aerial portions of the plants were colonized up to 8 cm above the soil line.

In a similar experiment with snap bean, two rates (1×10^6 and 1×10^7 conidia per seed) of *B. bassiana* 11-98 were applied to seed. For both rates, endophytic *B. bassiana* was recovered from root, stem, and leaf tissues of 100% of snap bean seedlings. With cotton, seeds were treated with 1×10^5 and 1×10^6 conidia per seed. Colonization of cotton seedlings was less extensive than with tomato and snap bean, and recovery of the fungus was greater from upper portions of the plant (stem and leaf tissues) than roots.

In order to hasten the process of detection of endophytic *B. bassiana* 11-98 from cotton seedling tissues, the fungal-specific PCR primers, ITS1 and ITS4 (White et al., 1990), were used to amplify the internal transcribed spacer (ITS) region of the nuclear rDNA repeat (Griffin, 2007). Although originally developed as fungal primers, detection of fungi in mixtures of plant and fungal DNA is problematic using ITS1 and ITS4 primers because they also exhibit some amplification of plant ITS sequences (Martin and Rygiewicz

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