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Fungal Endophytes in Knock Out® Rose and Performance Effects of Entomopathogens on Marigold and Zinnia

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Abstract. **Endophytic fungi are increasingly studied for their ability to enhance plant performance in field crops, yet there are few equivalent studies in floricultural crops. Given the economic importance of these crops and pressures faced by growers to produce plants of high aesthetic quality, we surveyed the natural occurrence of foliar fungal endophytes in Knock Out® roses to identify candidate beneficial isolates. We also tested the effects of entomopathogenic fungal inocula on marigold and zinnia plant growth using different application approaches. Our survey of Knock Out® rose foliage collected from five sites within central Texas revealed at least 24 different fungal genera and 30 probable species, including some isolates providing plant stress tolerance and pathogens or antagonists of insects and nematode pests. The effects of entomopathogen inocula on plant growth varied with host plant (marigold vs. zinnia) and inoculation method (soil drench vs. seed soak). Plant responses were complex, but inoculation with *Isaria fumosorosea* Wize tended to have a negative effect on plant performance characteristics whereas *Beauveria bassiana* (Bals.-Criv.) Vuill. tended to have positive effects. When applied to marigold as a seedcoating, *I. fumosorosea* reduced germination, seedling fresh weight, and produced seedlings with a less compact form. By contrast, seeds inoculated with *B. bassiana* required less time to germinate, had higher germination rates, and increased the plant compactness. These results show that the impact of fungal entomopathogens applied as endophytes depends on the specific fungi-plant combination being examined. The effect of plant inoculation with entomopathogenic fungi within a pest management context requires further evaluation.**

Fungi or bacteria causing unapparent or asymptomatic infections entirely within plant tissues are known as endophytes (Stone et al., 2000; Wilson. 2000). Gurulingappa et al. (2010) demonstrated that fungal insect pathogens (entomopathogens) can also be inoculated as endophytes within various monocot and dicot crops, including bean, wheat, corn, pumpkin, tomato, and cotton. Endophytic fungi enhance plant growth in some field crops (Jaber and Enkerli, 2017; Vega et al., 2009); however, few studies have examined how fungal endophytes or entomopathogen inocula may affect floricultural crops.

Floricultural crops are a significant component of U.S. agriculture, with a 2015 wholesale value of $4.37 billion from 15 states (U.S. Department of Agriculture, 2016). Annual bedding plants contribute the greatest proportion (30.4%) of the total wholesale value (United States Department of Agriculture, 2016). Among bedding plants, marigolds (*Tagetes erecta* L.) and zinnia (*Zinnia elegans* L.) are included in the top 15 flowering and foliar annuals in terms of annual sales (U.S. Department of Agriculture, 2014). Marigolds are one of the premiere garden annuals that perform well in dry, hot conditions and are frequently used as a border, pot, or cut flower. Zinnias are popular garden flowers that come in a wide range of flower colors and shapes, can withstand hot summer temperatures, and are easy to grow from seeds.

Babu et al. (2014) report roses are among the most popular flowering shrubs in the United States, with a total wholesale value of U.S. $194 million per year. In Florida and Texas alone, 304,000 potted roses were sold in 2015 (U.S. Department of Agriculture, 2016). The double Knock Out® rose, *Rosa hybrida* L. 'Radtko' is a popular hybrid landscape shrub rose that is shade tolerant, capable of growing in a wide range of soils, is very heat and drought tolerant once established, and is resistant to fungal diseases (black spot, powdery mildew, and rust), and aphids (Harp et al., 2009; Martin, 2010).

Rose, marigold, and zinnia are ideal host plants for endophyte research due to their economic importance, well-known propagation, and finishing guidelines (Ball Seed, 2019; Harp et al., 2009). Investigations into the use of fungal endophytes for enhancing herbaceous ornamentals are primarily limited to mycorrhizal fungi. Marigolds inoculated with an arbuscular mycorrhizal fungus "AMF" (*Glomus constrictum* Trappe) displayed an increase in height, shoot biomass, and flower diameter compared with untreated plants (Asrar and Elhindi, 2011). Greater root biomass has also been documented in petunia, marigold, and aster (Gaur and Adholeya, 2005), and higher shoot biomass in orchids (Hou and Guo, 2009) and geraniums (Biermann and Linderman, 1983) treated with mycorrhizal fungi. Some entomopathogens are also capable of colonizing plants as endophytes and studies have shown they can also enhance plant growth (Jaber and Enkerli, 2017). Cotton plants treated with *Beauveria bassiana* (Bals.-Criv.) Vuill. exhibited increased biomass and median number of flower buds (Lopez and Sword, 2015). A commercial formulation of *B. bassiana* (BotaniGard) was shown to endophytically colonize strawberry and cabbage plants and promote their growth (Dara et al., 2013, 2017).

Further research is needed to determine whether plant growth benefits from inoculation with fungal entomopathogens can be applied to ornamental plants. We first conducted a field survey of fungal endophytes naturally associated with roses to identify candidate beneficial fungal endophytes. We also tested the effects of commercial formulations of entomopathogenic fungal inocula applied as soil drenches and seed soaks on *T. erecta* and *Z. elegans* plant growth. Since commercial *T. erecta* seed is often coated to facilitate sowing in automatic seeders, we

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tested for the effects of seedcoating to mediate marigold performance when treated with entomopathogenic fungi.

Materials and Methods

Survey of Fungal Endophytes in Knock Out® Rose. Foliar fungal endophytes in Knock Out® roses (*Rosa hybrida* L. 'Radtko') were sampled in June 2013 from five geographically distinct locations within the Brazos (Site 1: lat. 30.608685° N, long. 96.288763° W; Site 2: lat. 30.655523° N, long. 96.343588° W; Site 3: lat. 30.596378° W, long. 96.348997° N; and Site 5: lat. 30.634555° W, long. 96.35853° N) and Washington (Site 4: lat. 30.316068° N, long. 96.343081° W) counties of central Texas. At each site, 10 leaves from 10 randomly selected healthy plants 5–7 m apart were collected and returned to the laboratory. Leaves were surface-sterilized, cut into 1cm^2 fragments and plated on potato dextrose agar (PDA) plates in 9 cm diameter petri dishes under sterile conditions. Presumed endophytic fungi growing from the leaf fragments were subcultured, tentatively identified using morphology (Barnett and Hunter, 1998), and the identifications confirmed by sequencing the ribosomal DNA internal transcribed sequence (ITS) region as a DNA barcode (see Ek-Ramos et al., 2013 for methods). Briefly, a 200–300 base pair ITS1 region was PCR amplified using primers ITS1 (5′ TCC GTA GGT GAA CCT GCG G 3′) and ITS2 (5′ GCT GCG TTC ATC GAT GC 3′). Once purified, these sequences were sequenced at Macrogen Corp. facilities (Rockville, MD). GenBank and UNITE databases were used to identify the species level based on a >98% similarity percentage.

Marigold and Zinnia responses to fungal entomopathogen inoculation. The effects of two commercially-available fungal entomopathogens, *B. bassiana* strain GHA (BotaniGard® WP; BioWorks, Inc., Victor, NY) and *Isaria fumosorosea* Wize (NoFly WP; Novozymes BioAg Inc., Houston, TX), were tested on marigold ('Discovery Yellow') and zinnia (Giant Mix) provided by Ernst Benary of America, Inc. (DeKalb, IL). Inocula for tests were obtained by culturing fungi on sabouraud dextrose agar (SDA) and incubated for 5 weeks at 25 °C. Mycelia and conidia were harvested by flooding the media with 6 mL of autoclaved 0.1% Triton X-100, scraping the surface, pipetting the contents, and vortexing for 1 min. The liquid was strained with a steel sieve of mesh size 0.001 inches into a second tube. The suspensions were diluted in 2% methyl cellulose to a concentration of 108 conidia/mL using a haemocytometer. A germination test was performed on each fungal suspension to confirm the viability of conidia before treatment applications as described by Gurulingappa et al. (2010).

Before use, seeds were surface sterilized in 0.5% sodium hypochlorite for 2 min, followed by 2 min in 70% ethanol, and rinsed three times in sterile water and dried on a sterile paper towel (Posada and Vega, 2006). Two inoculation protocols were used for each fungal treatment in a full factorial design: 1) soaking surface-sterilized seeds for 12 h in aqueous spore suspensions (10^8 spores/mL) or the water control, and 2) applying a soil drench (1 mL of the 10^8 spore solution or water control) directly to the seed and surrounding soil at planting. Suspensions of conidia rather than formulated products were used to standardize dosage of a single, pure strain of conidia. Percent conidial germination from seeds soaked for 12 h in suspensions of either of the two fungal isolates was always in excess of 80%.

Seeds from all plant/fungi treatment combinations were planted individually in 8.9 cm wide-by-8.9 cm deep, thinwall square pots (Dillen Products, Middlefield, OH) filled with a commercial mix composed of 75–85 wt% Canadian Sphagnum peatmoss, perlite, dolomite limestone, and a wetting agent (Sunshine Mix #1; Sun Gro Horticulture, Vancouver, BC, Canada). Pots were maintained in a fan-pad cooled glasshouse located on the Texas A&M University, College Station campus for 3 weeks, and irrigated every 2–3 d with reverse-osmosis treated water without fertilizer. Pot locations on benches were randomized every 4 d to reduce inadvertent position effects on treatments. Air temperature was recorded every 30 min using a HOBO Pro Series Temperature/RH data logger (Onset Computer Corporation; Bourne, MA) placed in the middle of the plantings.

The effects of the endophyte inoculation methods were quantified 26 d after planting by measuring aboveground height, belowground root length, and total fresh biomass from 10 randomly selected plants per treatment. Plant material was washed to remove soil debris. Plants were air dried and the roots laid out in alignment with the mainstem of the plant. The aboveground height along the mainstem from the root to the shoot tip and the root length from the mainstem to the root tip were measured to the nearest 0.1 cm. Fresh biomass for each plant was measured using a Mettler Toledo® P1210 balance scale (Mettler Toledo, Columbus, OH).

To assess fungal colonization in plants, the plants were surface sterilized in 0.5% sodium hypochlorite for 2 min, followed by 2 min in 70% ethanol, and rinsed three times in sterile water and air dried on a sterile paper towel (Posada and Vega, 2006). *T. erecta* fragments from each leaflet of the first 5-leaflet leaf or the first fully expanded *Z. elegans* leaf distal from the plant apex were placed on SDA in 55 mm diameter petri dishes. To suppress bacterial growth, 81 mL of penicillin and 81 mL of streptomycin was added to the media after autoclaving. Leaves were incubated in darkness for 2 weeks at 25 °C and visually examined for fungal outgrowth. Colony morphology characteristics were used to make identifications. Mycelia and spores of *I. fumosorosea* appeared dense and grayish in color, and when the plates were turned over, there was intense yellow pigmentation associated with the growth (Hoy et al., 2010). A fungal colony was characterized as *B. bassiana* based on white dense mycelia, becoming cream to pale yellow at the edge (Humber, 1997).

A three-way analysis of variance (ANOVA) was used to test for significant differences in plant performance parameters with host plant, endophyte treatment, and inoculation method as the main effects within the analysis (SigmaPlot Version 11.2, 2008). No transformations were performed before the analysis as the data structure passed Shapiro-Wilk Normality Tests and Equal Variance Tests. When there was a significant effect of endophyte treatment, a Tukey's pairwise multiple comparison procedure was used to test for significant pairwise differences of means.

Inoculation combined with marigold seed coat technology. We investigated *B. bassiana* and *I. fumosorosea* endophytes applied in a commercial antimicrobial seedcoating (Disco Classic Yellow L-072; Incotec, 2017) on 'Discovery Yellow' *T. erecta*. We used a full factorial design to test seed treatment (noncoated and coated) with the fungal treatments used previously, although in this study the Apopka 97 strain of *I. fumosorosea* was used (PreFeRal WG; Biobest USA, McFarland, CA). Fungi were cultured as described previously and *T. erecta* seeds inoculated by submerging in suspensions of *B. bassiana*, *I. fumosorosea*, or a 2 v/v % methyl cellulose sterile control for 1 h and then air-dried.

Seeds of *T. erecta* were individually planted in 8.9 cm wide by 8.9 cm deep, thinwall square pots filled with Sunshine Mix #1. Pots were maintained in a fan-pad cooled glasshouse located on the Texas A&M University, College Station campus for 3 weeks, and irrigated every 2–3 d with reverse-osmosis treated water without fertilizer. Air temperature was recorded hourly using a HOBO Pro Series Temperature/RH data logger placed in the middle of the plantings.

The study was a randomized complete block design with 6 treatments (2 fungal isolates plus a control × 2 forms of marigold seed), 12 blocks per treatment combination, and 10 pots per block, yielding a total of 120 seeds per treatment combination. Germination was recorded every 12 h during the first week after sowing and was defined as the first occurrence of fully expanded cotyledons [defined as Stage II germination by Ernst Benary of America, Inc. Discovery Yellow Marigold Culture guide (Anonymous, 2018)]. After 3 weeks, the height:width ratio was recorded from a random sample of 30 seedlings per treatment group and plant weight assessed as described above.

The median germination time (MGT), when 50% germination was reached, was assessed using probit analysis within treatment combinations (SAS Institute Inc., 2016). Specific effect tests were used to identify significant factors within the model. A two-way ANOVA was performed separately for

percent germination, plant fresh weight, and plant height:width to detect significant differences among the two seed types, three fungal inocula treatments, and significant interaction terms (SAS Institute Inc., 2016). Percent germination data were arcsine-transformed and height:width data were subjected to a log (x + 1) transformation before performing the ANOVA. If the F-statistic from the ANOVA was significant, Tukey's honestly significant difference tests were used to separate treatment responses.

Results and Discussion

Survey of fungal endophytes in Knock Out® roses. Sixty-one pure fungal cultures were identified from 24 Knock Out® rose plants and 43 leaves across the five central Texas samples sites. DNA sequencing confirmed the identity of 44 isolates, with an additional morphological identification from four additional isolates. Identified isolates represented at least 24 different fungal genera, 30 probable species, 2 unidentified, and 11 unknowns (Table 1). Fungal endophyte composition varied among and within sites, with only 2 genera (*Alternaria* and *Epicoccum*) collected from three or more locations and only *B. bassiana* was a commonly recovered endophyte (in 6 of 15 known isolates). The identified isolates include several endophytic fungi known to be either pathogens or antagonists of insects and nematode pests (e.g., *B. bassiana*, *Chaetomium globosum* Kunze ex Fr., and *Cladosporium* sp.) or to contribute to stress tolerance (e.g., *Penicillium janthinellum* Biourge).

At least one million species of plant endophytic fungi are estimated to occur (Ganley et al., 2004); however, Saikkonen et al. (1998) reviewed multiple studies showing that individual plants in the temperate zone may harbor dozens of endophyte species. Our discovery of 30 probable species of fungal endophytes in Knock Out® roses fits well with the conclusion made by Saikkonen et al. (1998). Similarly, the survey conducted by Huang et al. (2007) of *Nerium oleander* L. in Hong Kong, China isolated 42 endophytic fungi from healthy leaves and stems. Márquez et al. (2012) reported an abundant and diverse number of endophytes in grasses. Similar to our Knock Out® rose survey, they reported few endophyte taxa in many grasses and locations and many rare endophyte species were isolated once. Our survey, which only assessed endophytes in leaves, may underestimate the total number of fungal endophytes in rose as endophytes of woody plants are usually highly localized within leaves, petioles, bark, or stems (Saikkonen et al., 1998). Carroll (1988) proposed that endophytes of woody plants provide a defensive role for the host plant because they produce a wide array of mycotoxins and enzymes that can inhibit growth of microbial pathogens and invertebrate herbivores.

Two of the most frequently recovered endophytes in our survey were *Epicoccum nigrum* Link ex. Link and *Epiccocum* sp. collected from three different sites. *E. nigrum* occurs in a wide array of habitats and has broad applications in medicine, industry, and agriculture; we believe, however, that this is the first record of *E. nigrum* and *Epiccocum* sp. as endophytes of rose. In Brazil, *E. nigrum* was found colonizing the surface of sugarcane and was occasionally endophytic. Functionally, this fungus supported sugarcane root growth and helped suppress several sugarcane pathogens (Fávaro et al., 2012). Studies conducted by De Cal et al. (2009) in Spain and Italy with *E. nigrum* Link strain 282 (ATCC number 96794) demonstrated its potential to protect stone fruit (peaches and nectarines) against *Monilinia* spp. brown rot.

Also collected from three locations within our survey were the endophytes *Alternaria* sp. and *Alternaria infectoria* Woudenb. & Crous collected from two sites. *Alternaria* sp. comprise plant pathogens, weak facultative parasites, saprophytes, and endophytes (Thomma, 2003). *Alternaria* sp. metabolites exhibit phytotoxic, cytotoxic, and antimicrobial properties (Lou et al., 2013). Two *Alternaria* spp. were previously recovered as endophytes from floriculture and ornamental plants including wild *Rosa rugosa* Thunb. and *Rosa hybrida* L. in a greenhouse (Zhou et al., 2014). Tobacco seedlings inoculated with both these *Alternaria* spp. isolates exhibited a significant increase in the soluble sugar and chlorophyll levels (Zhou et al., 2014), indicating that these endophytes have potential use as plant growth promoters in floriculture. *Alternaria infectoria* has been collected as an endophyte of grasses growing in areas exposed to ocean spray, mists, and tides along the Oregon coast (Martin and Dombrowski, 2015), and in wheat grain grown in Argentina and barley grain grown in New Zealand (Andersen et al., 2015), but its function(s) is (are) unknown.

Some endophytes may have value in pest management programs. *Chaetomium globosum* Kunze, Mykol. and *Chaetomium cochliodes* Palliser were collected from two survey sites. Zhou et al. (2016) reported that *C. glabosum* strain TAMU 520 inhibited root-knot nematode (*Meloidogyne incognita* (Kofold & White) Chitwood) infection and reduced female reproduction on cotton roots. Aboveground, endophytic *C. globosum* reduced the fecundity of both cotton aphid, *Aphis gossypii* Glover, and beet armyworm, *Spodoptera exigua* (Hübner). In another study leaves of Canada thistle, *Cirsium arvense* (L.), infected with the fungal endophyte *C. cochliodes*, had different effects on two insect herbivores. *C. cochliodes* reduced the growth of the generalist cabbage moth, *Mamestra brassicae* (L.), but increased feeding by a specialist herbivore, the thistle tortoise beetle, *Cassida rubiginosa* Müller (Gange et al., 2012).

Marigold and zinnia responses to endophyte inoculation. The inoculated fungi were re-isolated as endophytes living within surface sterilized leaves treated with *B. bassiana* (20% of the zinnia plants grown

Table 1. Endophytes identified from 61 fungal isolates recovered from Knock Out® rose foliage in central Texas.

Species[z]	Recovery sites[y] (number of isolates per site)	Species[z]	Recovery sites[y] (number of isolates per site)
Acrocalymma vagum	5(1)	*Epicoccum* sp.	3(1)
Alternaria sp.	1(2); 2(1); 5(1)	*Epiccocum nigrum*[x]	4(2); 5(2)
Alternaria infectoria	5(1)	*Fusarium* sp.	5(1)
Aspergillus muricatus	1(1)	*Macrophomina phaseolina*	1(1)
Aspergillus terreus	4(1)	*Mortierella alpina*	3(1)
Beauveria bassiana	1(6); 4(1)	*Penicillium janthinellum*	1(1)
Cephaloteca sulfurea	1(1); 3(1)	*Phialemonium inflaturm*	1(1); 3(1)
Cercospora sp.	2(1)	*Phomopsis* sp.	1(1)
Cercospora chrysanthemi	2(1)	*Preussia* sp.	3(1)
Chaetomium cochliodes	4(1)	*Sordariomycetes* sp.	2(1); 4(1)
Chaetomium globosum	1(1)	*Sporormiella minima*	3(2)
Cladosporium sp.	3(2)	*Stemphyllum* sp.	5(1)
Clonostachys rosea	4(1)	*Xylaria* sp.	2(1)
Colletotricum gloesporiodes	5(1)	*Xylaria cubensis*	3(1)
Geomyces auratus	1(1)	Unidentified[w]	2(1); 1(1)
Elsinoaceae sp.	2 (2)	Unknown[v]	1(2); 2(1); 3(4); 4(2); 5(2)

[z]Have a matching sequence in the publically available GenBank. Accession numbers of the identified sequences are GenBank MH553470-MH553515.
[y]Recovery sites are identified within the text.
[x]Identified by morphology rather than by sequencing.
[w]Although sequence quality was good, isolates did not have a matching sequence in the UNITE or GenBank databases.
[v]Unable to obtain quality sequences from these isolates and the isolates could not be identified morphologically.

from soaked seed, 30% of the zinnia plants treated with a soil drench, 0% of the marigold plants grown from soaked seed, and 20% of the marigold plants treated with a soil drench) but from 0% of the *I. fumosorosea*-treated plants inclusive of inoculation method and 0% of the controls. Re-isolation of endophytic fungi used as seed treatments is often low in seedlings plated onto an agar substrate. Yan et al. (2011) reported re-isolation rates from 10 endophytic fungi as 67% of roots and 39% of leaves when cucumber seedlings grown for 7–10 d were plated on potato dextrose agar (PDA). Additionally, *Paecilomyces* sp. was only isolated from the roots. D'Amico et al. (2008) reported substantial variation in endophyte recovery from lettuce plants after inoculating roots with seven fungal isolates and plating samples on PDA. There is evidence that the reported diversity of endophytes reflects their ease of detection, at least for many species. For example, in surveying endophyte communities along a broad latitudinal gradient (Canadian arctic to the lowland tropical forest of central Panama), Arnold and Lutzoni (2007) found that easily cultivated endophytes have wide host ranges in tropical plants, whereas taxa that are more difficult to culture have much narrower host ranges. In his review of fungal endophytes in biological control programs, Vega (2008) noted that the generalist entomopathogen *B. bassiana* is more frequently reported as an endophyte compared with *Isaria* spp. and *Paecilomyces* spp. Our higher recovery of *B. bassiana* (35% of samples) compared with *I. fumosorosea* (no positive detected samples from inoculated plants) could reflect several factors including 1) ability to re-isolate these fungi on agar, 2) differences in the host range as an endophyte, 3) colonization of root tissues which we did not sample, or 4) environmental conditions under which the studies were conducted. Given that no endophytic colonization of zinnia or marigold by *I. fumosorosea* was detected, the phenotypic effects on treated plants that we observed as a result of the inoculation treatments are assumed most likely to be due to either epiphytic or rhizospheric effects of the fungus, although we cannot rule out root colonization. In the case of *B. bassiana*, we have shown that endophytic colonization of the treated plants is possible, but cannot strictly distinguish between endophytic, epiphytic, or rhizospheric effects as the cause of the plant phenotypic responses we observed. Our results may be constrained by our reliance on PDA culturing and future use of a diagnostic molecular analysis could increase the resolution or positively confirm the presence of the target endophytes in the experimental plants (Lopez et al., 2014).

The average daily maximum temperature (± 1 standard deviation, N = 26 d) was 33.54 °C ± 0.96 °C, the average daily minimum temperature was 27.87 °C ± 2.35 °C, and the average daily temperature was 31.09 °C ± 1.26 °C. While there is variation in life history responses to temperature by *B. bassiana* (Ekesi et al., 1999; Jackson et al., 2010; Svedese et al., 2013), *I. fumosorosea* (Yeo et al., 2003) and *T. erecta* or *Z. elegans* (Anonymous, 2018; Harrington, 1921; Roberts and Struckmeyer, 1939), the average minimum and daily temperatures recorded during the study were within the viability ranges for the entomopathogens used in the experiment. While the entomopathogens and plants used in the study perform well at warm temperatures, the average maximum temperature recorded during the study exceeded the optimum for *I. fumosorosea* by an average of 3 to 4 °C (Ali et al., 2010; Yeo et al., 2003).

There was a significant fungal treatment effect on aboveground vegetation height (F = 6.07; df = 2, 108; P = 0.003), root length (F = 8.57; df = 2, 108; $P < 0.001$), and plant biomass (F = 18.2; df = 2, 108; $P < 0.001$), but the nature of the effect was different between the two plants as indicated by a significant fungal treatment × host plant interaction term for all three plant response parameters (Fig. 1). For marigolds treated with *I. fumosorosea*, the aboveground vegetation height was significantly less than the *B. bassiana* treatment but not significantly different from the control treatment. There was no significant difference in the aboveground vegetation height of marigold between the *B. bassiana* treatment and the control. The aboveground vegetation height for zinnias in the control treatment was significantly greater than the *I. fumosorosea* treatment, but not significantly different from the *B. bassiana* treatment. There was no significant difference in the aboveground vegetation height between the *B. bassiana* and the *I. fumosorosea* treatments. There was no effect of inoculation method on aboveground vegetation height for marigold or zinnia.

The pattern among treatment effects for the root length measurements was more complex. Not only was there a significant effect from the fungal treatment, but there were also significant effects due to fungal treatment × host plant and fungal treatment × inoculation method interactions (Fig. 1). The root length for marigolds treated with *B. bassiana* was not significantly longer than the root length in control treatment, but the *B. bassiana* and control treatments produced marigolds with significantly longer roots than plants from the *I. fumosorosea* treatment. There was no effect of inoculation method on marigold root length. In zinnia, there was no effect of fungal treatment on root length regardless of the inoculation method used. The significant fungal treatment × inoculation method interaction arose from greater differences among fungal treatments in the soil drench treatment vs. the seed soak treatment. The significant fungal treatment × host plant interaction arose from greater differences among fungal treatments in marigold compared with zinnia.

Similar to the root length measurements, the pattern among treatment effects for the plant weight measurements was equally complex. There was a significant effect from the fungal treatment on plant weight, but there were also significant fungal treatment × host plant and fungal treatment × inoculation method interactions (Fig. 1). There was no significant effect of fungal treatment on marigold or zinnia weight when seeds were soaked; however, there was a significant fungal treatment effect on host plants within the soil drench inoculation method. Marigold weight was not significantly different between the *B. bassiana* and water control soil drench treatments, but by comparison marigold weight was significantly lower on plants treated with *I. fumosorosea* compared with controls and those treated with *B. bassiana*. The significant fungal treatment × host plant interaction arose from greater differences among fungal treatments in marigold compared with zinnia.

The complexity in endophytic colonization and associated plant growth responses observed in our study is consistent with other reports. Endophytes influence shoot and root biomass, height, flower diameter, and number of flower buds in other crops (Asrar and Elhindi, 2011; Biermann and Linderman, 1983; Elena et al., 2011; Gaur and Adholeya, 2005; Hou and Guo, 2009; Jaber and Enkerli 2017; Khan et al., 2012; Lopez and Sword, 2015), and these results are also highly variable. Mayerhofer et al. (2013) conducted a meta-analysis of fungal root endophyte inoculation across 21 plant performance parameters. While they reported overall root biomass, shoot biomass, and nitrogen concentration responses to endophyte inoculation to be neutral, total biomass of inoculated plants was 18% less compared with non-inoculated controls. This biomass variation resulted from cases of roots inoculated with *Microdochium* sp. and *Periconia macrospina* Lefebvre and Aar.G. Johnson having increased biomass whereas roots inoculated with *Phialocephala fortinii* s.l. and *Pholocephala subalpina* C.R. Grünig et T.N. Sieber, sp. nov. exhibited a reduced biomass by comparison with the norm. Mayerhofer et al. (2013) concluded variability in host plant responses to fungal endophyte inocula to be the norm rather than the exception.

Inoculation combined with marigold seed coat technology. The overall probit regression model indicated median germination time was significantly influenced by treatments. Seed coating had a significant impact on MGT (df = 1, χ^2 = 592, df = 1, $P < 0.0001$), with a higher MGT in noncoated seeds than coated seeds (Table 2). The fungal inocula also influenced MGT, which was higher in *I. fumosorosea*-treated seeds than *B. bassiana*-treated seeds (χ^2 = 30.5, df = 2, $P < 0.0001$). *B. bassiana* germinated more quickly than the sterile control (χ^2 = 13.5, df = 2, P = 0.0002), while *I. fumosorosea* did not differ from the sterile control (χ^2 = 3.57, df = 2, P = 0.0588). There was no significant interaction between seed and fungal inocula treatments for the MGT (χ^2 = 3.65, df = 2, P = 0.161).

Seed coating significantly increased percent germination (F = 40.746; df = 1, 66; $P < 0.0001$) and fungal inocula also had

an impact on the percent germination (F = 3.925; df = 2, 66; P = 0.0245) (Table 2). *Isaria fumosorosea* treatments were significantly lower than the controls, and *B. bassiana* did not differ from the control or *I. fumosorosea*. There was no significant interaction between seedcoating and fungal inocula effects on percent germination. Seed coating (F = 184.933; df = 1, 174; P < 0.0001) and fungal inocula (F = 16.030; df = 2, 174; P < 0.0001) had significant effects on plant fresh weight (Fig. 2). *B. bassiana* and *I. fumosorosea* treatments decreased fresh weight compared with control groups, and *B. bassiana* treatments resulted in plants with lower fresh weights than plants from the *I. fumosorosea* treatments. Coated and noncoated treatments elicited different fungal inocula effects on plant fresh weight. In noncoated seeds, *I. fumosorose*a treatments had significantly lower fresh weight than the control; and there were no differences between *B. bassiana* and *I. fumosorosea* or between *B. bassiana* and the control. Within the coated seed treatments, *B. bassiana* treatments weighed significantly less than *I. fumosorosea* and control treatments while there was no difference between *I. fumosorosea* and control treatments.

The height:width was affected by seed treatment (F = 13.2367; df = 1, 174; P = 0.0004), and was greater (more elongated) in noncoated seeds compared with coated seeds (Fig. 3). The size/shape parameter was also affected by fungal inocula (F = 7.0891; df = 2, 174; P = 0.0011). Plants from *B. bassiana*-treated seed showed a lower ratio than plants from *I. fumosorosea*-treated seed, but *B. bassiana* and *I. fumosorosea* did not affect plant height:width when compared with the control treatment. A significant seed treatment × fungal inocula interaction was also identified (F = 22.7258; df = 2, 174; P < 0.0001). Within the noncoated treatments, *I. fumosorosea* and *B. bassiana* had increased height:width compared with the control, but there was no difference between *B. bassiana* and *I. fumosorosea*. Within the coated treatments, *I. fumosorosea* showed no difference to the control or *B. bassiana* treatments, while the ratio was significantly lower in *B. bassiana* treatments compared with the controls.

The average daily maximum temperature (± 1 standard deviation, N = 24 d) was 30.94 °C ± 4.51 °C, the average daily minimum temperature was 17.10 °C ± 4.36 °C, and the average daily temperature was 22.57 °C ± 2.59 °C. While there is significant variation in life history responses to temperature by *B. bassiana* (Ekesi et al., 1999; Jackson et al., 2010; Svedese et al., 2013), *I. fumosorosea* (Yeo et al., 2003) and *T. erecta* (Anonymous, 2018; Roberts and Struckmeyer, 1939), the temperatures recorded during the study were within the viability ranges for these entomopathogens and marigold species.

In summary, fungal seed treatments had important effects on *T. erecta* development. *I. fumosorosea* negatively affected germination and seedling fresh weight, and produced seedlings with a less compact form. *B. bassiana* reduced seedling fresh weight. Also, the speed and frequency of *T. erecta* germination, as well as plant biomass and shape, responded differently to each of the fungi. Seeds inoculated with *B. bassiana* performed better than *I. fumosorosea*-inoculated seeds, which required more time to germinate and had lower germination rates. *B. bassiana* treatment also led to smaller plants with

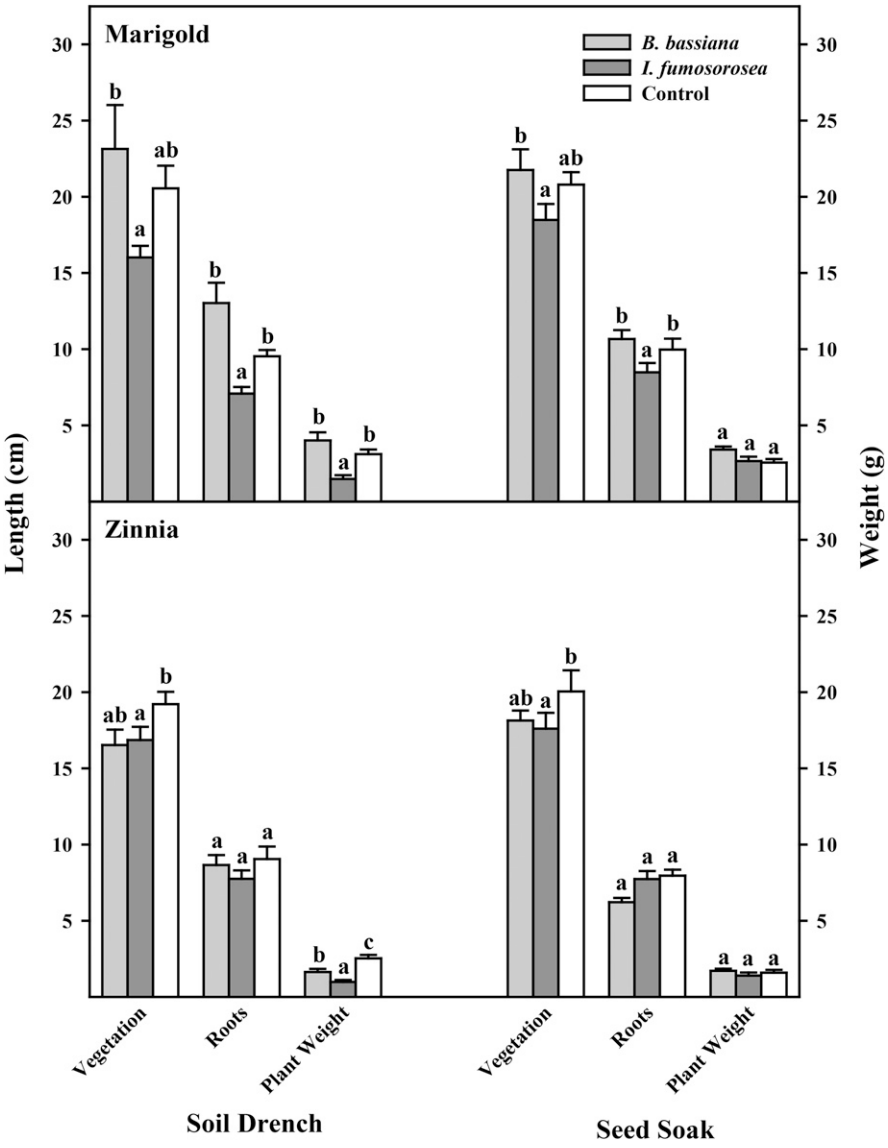

Fig. 1. Mean ±1 SE of three plant response variables (vegetative growth, root growth, and plant weight) associated with two host plants (marigold and zinnia) and three treatments (*B. bassiana* or *I. fumosorosea* conidial preparations, or water only control) and two different inoculation methodologies (soil drench and seed soak). For each host plant and response variable, bars within each of the 6 inoculum × inoculation method clusters followed by the same letter are not significantly different at P = 0.05.

Table 2. Percent germination (mean ± SEM) of noncoated and methylisothiazolinone-coated seeds inoculated with *B. bassiana*, *I. fumosorosea* and controls (water only). Columns with shared letters are not significantly different at P = 0.05.

	Noncoated seed			Coated Seed		
	Control	*B. bassiana*	*I. fumosorosea*	Control	*B. bassiana*	*I. fumosorosea*
Germination (%)[z]	85.4 ± 8.9 abc	81.7 ± 4.5 ab	65.9 ± 9.7 a	97.2 ± 8.6 cd	97.9 ± 7.4 d	94.5 ± 7.4 bcd
MGT (h)[y]	76.1 d (78.6, 73.7)	69.0 c (71.4, 66.7)	77.4 d (80.0, 74.9)	51.5 ab (53.4, 49.6)	49.9 a (51.8, 48.1)	54.0 b (56.0, 52.1)

[z]Data were arcsine transformed for calculation of the mean and standard error for the percent germination and backtransformed for presentation in the table.
[y]The median and upper- and lower-95% confidence limits are presented in parentheses.

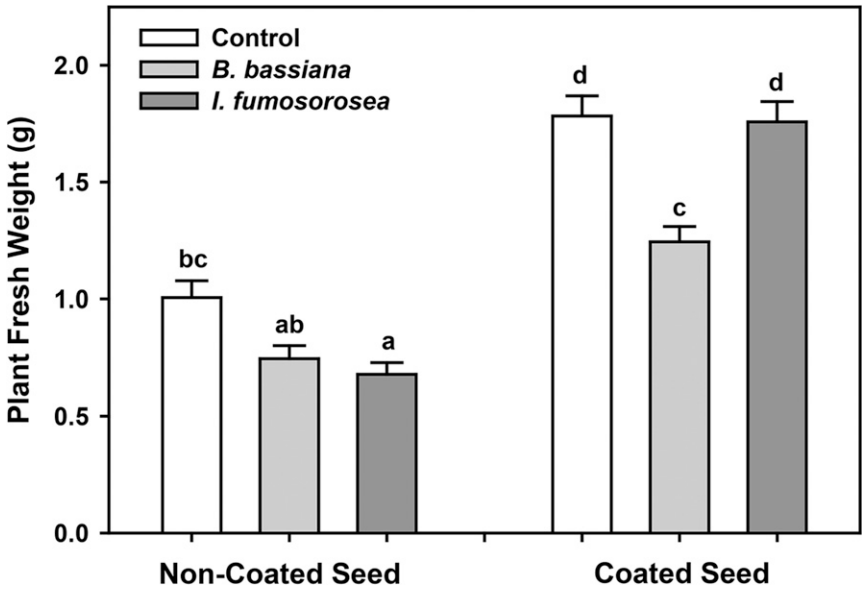

Fig. 2. Mean fresh weight (g) of 3-week old *T. erecta* plants grown from uncoated and methylisothiazolinone-coated seeds inoculated with *B. bassiana*, sterile control, and *I. fumosorosea*. Shared letters indicate no significant difference between the treatments (Tukey's honestly significant difference, $P > 0.05$). Error bars represent 1 standard error.

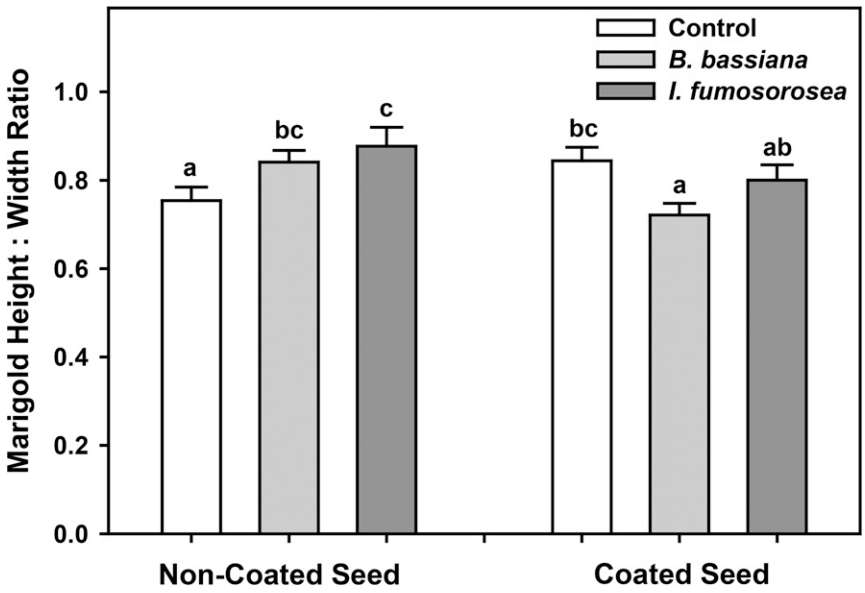

Fig. 3. Mean height:width ratio of 3-week old *T. erecta* plants grown from uncoated and methylisothiazolinone-coated seeds inoculated with *B. bassiana* or *I. fumosorosea* vs. sterile control. Columns with shared letters indicate no significant difference between the treatments (Tukey's honestly significant difference, $P > 0.05$). Error bars represent 1 standard error.

a shorter, wider shape than those treated with *I. fumosorosea*. Seed coating did not affect germination responses to fungal inoculation, but was involved in the fungal effects on plant growth. *I. fumosorosea* effects on plant biomass and form were negated when the seedcoating was present. Alternatively, *B. bassiana* showed more effects on plant growth in the presence of the seedcoating and may be more compatible than *I. fumosorosea* with other industry seedcoating chemicals.

Afzal et al. (2016) described the technique of inoculating seed with beneficial microbes to promote plant performance as biopriming. Biopriming bean seed with spore or bacterial cell suspensions promote improved seedling growth when compared with untreated seed (Junges et al., 2016). Coating winter wheat (*Triticum durum* Desf.) (Cyperales: Poaceae) seed with the arbuscular mycorrhizal fungi *Glomus intraradices* N.C. Schenk & G.S. Sm. (BEG72), *Glomus mossae* (T.H. Nicolson & Gerd) Gerd. & Trappe and *Trichoderma atroviride* P. Karst, Bidrag till Kännedom (MUCL 45632) led to increased seedling establishment, yield, and grain quality (protein content and mineral composition) of the harvested wheat (Colla et al., 2015). While coating of seed with microbes can be beneficial, there are few equivalent studies with floricultural seed. However, Magnitskiy (2004) reported mixed results based on the coatings used, the plant parameters measured and the host plant species evaluated in studies with verbena (*Verbena ×hybrida* Voss., cv. Quartz White), pansy (*Viola wittrockiana*, cv. Bingo Yellow Blotch), salvia (*Salvia splendens*, cv. Vista Red), and marigold (*Tagetes patula* L., cv. Bonanza Gold). While seed biopriming may be a useful tool in disease control and growth promotion (Afzal et al., 2016), additional studies with floricultural seed are needed and the different processes regulating growth and development need to be elucidated.

Conclusion

Endophytic fungi have been shown to enhance plant growth, resistance to pests and pathogens, and tolerance to environmental stress in some field crops; however, very few studies have documented the natural occurrence of fungal endophytes in ornamental plants or if fungal entomopathogen inocula may affect floricultural crops. From a survey of Knock Out® rose foliage collected from five sites we identified at least 23 different fungal genera and 27 probable species, including several isolates of endophytic fungal entomopathogens. While fungal endophyte composition varied greatly among and within sites, we discovered several endophytic fungi of interest for further study as they are known to affect insects and nematode pests or to mediate stress tolerance. Use of fungal inoculants within a pest management program must not detrimentally affect plant performance in groups grown for their aesthetic qualities. While the extent of endophytic colonization and effects on plant growth of the two fungal entomopathogens tested varied with host plant (marigold vs. zinnia) and inoculation method (soil drench vs. seed soak), the use of *I. fumosorosea* tended to have negative effects on plant performance characteristics relative to a water control whereas *B. bassiana* tended to have positive effects on plant performance characteristics relative to a water control. Additionally, we found mixed results from the impacts of seedcoating and entomopathogenic fungi on a number of marigold horticultural characteristics. These results suggest that individual species of fungal used as inoculants might generate very different responses in plant performance parameters.

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