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Insect Management

Beneficial fungal endophytes for effective insect management in floriculture crops

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Background: Insect pests are a major constraint to floriculture, costing growers approximately 5% of their direct costs. Traditional approaches remain a challenge to manage existing pests (e.g., mites, western flower thrips, and whiteflies) and new pests continue to appear (e.g., chili thrips, ambrosia beetle, and whitefly biotypes). Providing safe, effective, and economical pest management solutions capable of addressing an ever-changing pest landscape remains a priority for AFE and growers worldwide. In our research, we merged the expertise of Heinz's IPM research in ornamentals and floricultural crops with Sword's research expertise of using fungal insect pathogens (entomopathogens) that can be inoculated to and live naturally within plants as endophytes. While we already have solid evidence that the presence of fungal endophytes can be manipulated to negatively affect the performance, feeding and plant damage caused by both sucking and foliage feeding insect pests across a range of important crop plants, this approach had not been applied to date as a pest management strategy in the floriculture industry. As part of this project, we worked to complete the following: (1) Sample fungal endophyte communities occurring in floricultural plants; (2) Determine the most effective inoculation methods (seed, soil or foliar) for the establishment of promising candidate endophytes; (3) Assess the effects of the candidate endophytes on seed germination, plant growth and development; and (4) Conduct systematic analyses of candidate endophyte efficacy against key target insect pests.

Objective 1: Assess presence of fungal endophyte communities in the field.

Materials and Methods Utilized: We conducted replicated sampling of knockout rose foliar tissues across five geographic locations in Texas. At each site, 10 leaves from 10 randomly-selected asymptomatic (healthy & non-insect-infested) plants were collected, leaves were surface-sterilized, cut into 1cm^2 fragments and plated under sterile conditions on potato dextrose agar (PDA) plates in 9 cm diameter petri dishes.

Resulting endophytic fungi growing from the leaf fragments were subcultured, tentatively identified using morphology, and the identifications confirmed by sequencing the ribosomal DNA internal transcribed sequence (ITS) region as a DNA barcode. DNA sequences were matched to those in the publicly-available GENBANK database for taxonomic identification.

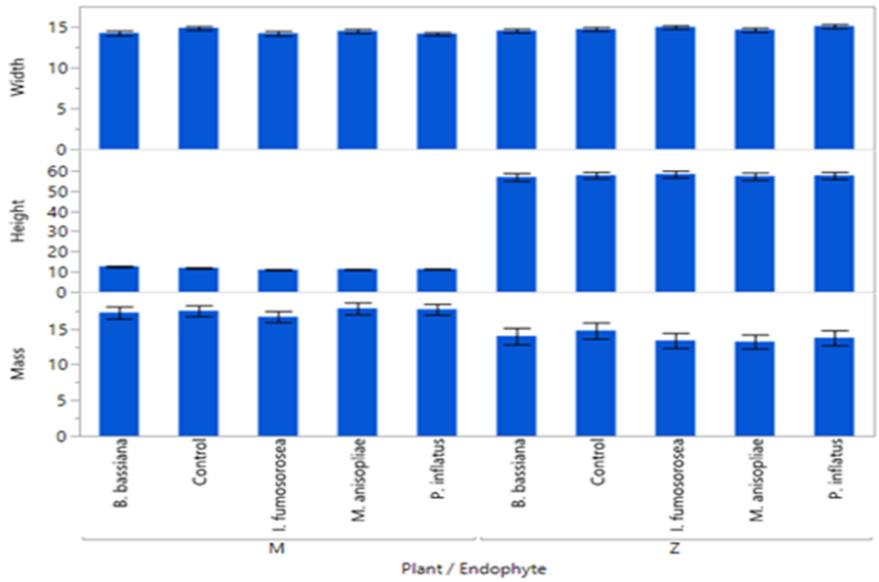
Results: In total, we recovered 61 distinct endophytic isolates representing 28 different fungal genera. DNA sequencing confirmed the identity of 46 isolates. Among the successfully identified isolates were 13 endophytic fungi that are known to be either pathogens or antagonists against a wide range of insects and nematode pests (e.g., *Beauveria bassiana*, *Paecilomyces* sp., *Chaetomium globosum*, *Cladosporium cladosporoides*). These isolates are considered the top candidates for use in downstream evaluations of their efficacy against major insect pests when present as endophytes in key floriculture crops that will comprise a large part of the remainder of this project.

Objective 2: Determine the most effective fungal endophyte inoculation methods.

Objective 3: Determine the effect of inoculation method on plant performance.

Materials and Methods Utilized: We conducted a number of experiments to simultaneously test for the effects of different inoculation methods (Objective 2) and subsequent effects on plant performance (Objective 3) using a number of entomopathogens (*B. bassiana*, *Iseria fumosorosea*, *Metarhizium anisopilae*, and *Paeciloomyces inflatus*). These experiments were conducted using marigold and zinnia as host plants. The inoculation protocols consisted of either (i) soaking surface-sterilized seeds overnight (12h) in aqueous spore solutions (108 spores/ml) or (ii) soil drench treatments at the time of sowing were each pot received 1 mL of treatment suspension pipetted directly to the substrate covering each seed, or (iii) foliar spray treatment where 0.1 mL was pipetted onto the center of each cotyledon. The effects of inoculation method, endophyte species, and plant species (M = marigold, Z = zinnia) on plant mass, height, and width were analyzed using a multi-way ANOVA techniques.

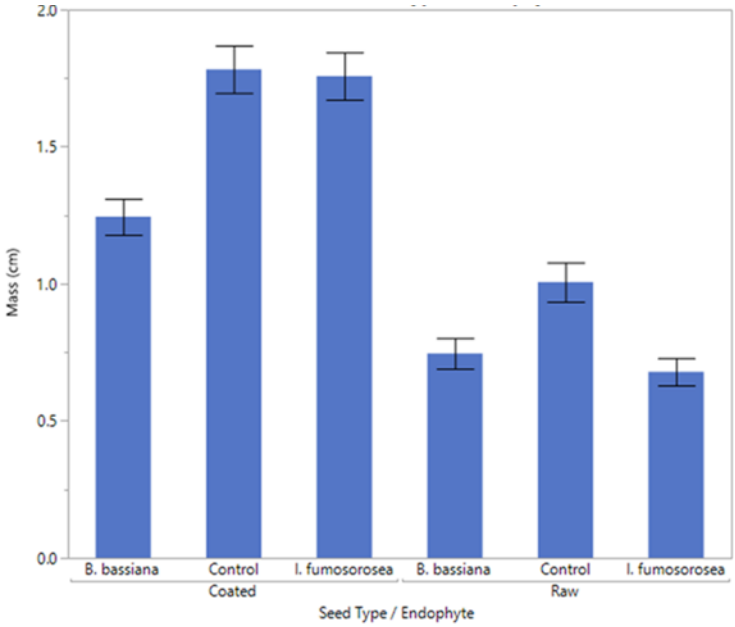
Results: We provided the highlights of our tests in the figure below. We are unable to detect any effects on germination times or the proportion of germinated seeds. A seed was considered germinated when the emerging hypocotyl became visible. Zinnia had greater height ($p < 0.0001$) and width ($p = 0.0045$) than marigold and marigold had larger mass ($p < 0.0001$). Endophytes had no significant impact on the height, width, or mass of inoculated plants (see figure below).



The endophyte candidates appear to have no adverse effects on T. erecta or Z. elegans growth parameters. Similarly, no effects were detected for tomato (not shown here).

Materials and Methods Utilized (Seed Coat Effects): We evaluated the impact of an antimicrobial seed coating, methylisothiazolinone, on the growth of endophyte-inoculated plants. Raw and coated seeds were inoculated with *B. bassiana*, *I. fumosorosea*, or a sterile control, and maintained in the greenhouse for 3 weeks after sowing. Germination was monitored every 12 hours during the first week. After 3 weeks, a random sample of 30 seedlings from each treatment group was collected. The height and width of each plant was recorded, then plants were carefully rinsed in water to remove soil from the roots and the fresh weight was measured. A Chi-square test was used to analyze the germination frequency. Height, width, and mass were analyzed using a multi-way ANOVA techniques.

Results (Seed Coat Effects): Endophytes had no impact on germination frequency ($p = 0.083$). Seed type (coated or raw) and endophyte had a significant effect on all plant size parameters. Coated seeds produced plants that were larger in height ($p < 0.0001$), width ($p < 0.0001$), and mass ($p < 0.0001$) than plants in the raw seed group. Plants that were inoculated with *B. bassiana* or *I. fumosorosea* were smaller than controls in all three size categories. The control group had the highest mass (mean = 1.39 g), followed by *I. fumosorosea* (mean = 1.22 g), and *B. bassiana* (mean = 0.995) (see figure below).



The results of this study suggests that endophyte seed treatments may be useful in regulating plant growth, which presents a new potential function for endophytes in floriculture production. Both raw and coated seeds were affected by B. bassiana treatment, which indicates that this endophyte was still active despite the presence of methylisothiazolinone and it may be compatible with some industry seed coating chemicals.

Objective 4: Efficacy of Candidate Endophytes on Insect Pests

Materials and Methods Utilized (Thrips and Whitefly): These experiments were conducted using marigold and tomato as host plants with whiteflies and thrips as insect pests. Two inoculation methods were tested for each plant/endophyte treatment combination. The inoculation protocols consisted of either (i) soaking surface-sterilized seeds overnight (12h) in aqueous spore solutions (108 spores/ml) or (ii) using a soil drench of 1ml of the spore solution applied directly to the seed and surrounding soil at planting. We used two commercially-available fungal entomopathogens (*B. bassiana* [Botanigard] and *Paecilomyces fumosoroseus* [NoFlyWP]). Seeds from all plant/endophyte treatment combinations were germinated in pots in the greenhouse. Endophyte-mediated effects on thrips were assessed by taking advantage of a naturally-occurring thrips infestation in greenhouse by simply counting the number of thrips per plant across treatment groups. To test for endophyte-mediated resistance to whiteflies, we mass infested caged plants for 72h and counted the number of eggs and emerging red-eye nymphs.

Results (Thrips and Whitefly). Both *B. bassiana* and *P. fumosoroseus* treatments had strong negative effects on average whitefly and thrips abundance per plant independent of inoculation method (ANOVA, $P = 0.007$ for whiteflies; $P < 0.001$ for thrips)(Fig. 1A&B). In tomato, both *B. bassiana* and *P. fumosoroseus* had significant negative effects on the average number of whiteflies (ANOVA, $P < 0.001$)(Fig. 1C). Soil drenching with *P. fumosoroseus* had a stronger effect on whiteflies than seed soaking, whereas the opposite pattern was apparent for *B. bassiana* (ANOVA, Endophyte*Treatment interaction, $P = 0.034$)(Fig. 1C). For thrips on tomato, there was a

non-significant trend for a lower average number of thrips on plants treated with either fungi as a soil drench (Fig. 1D).

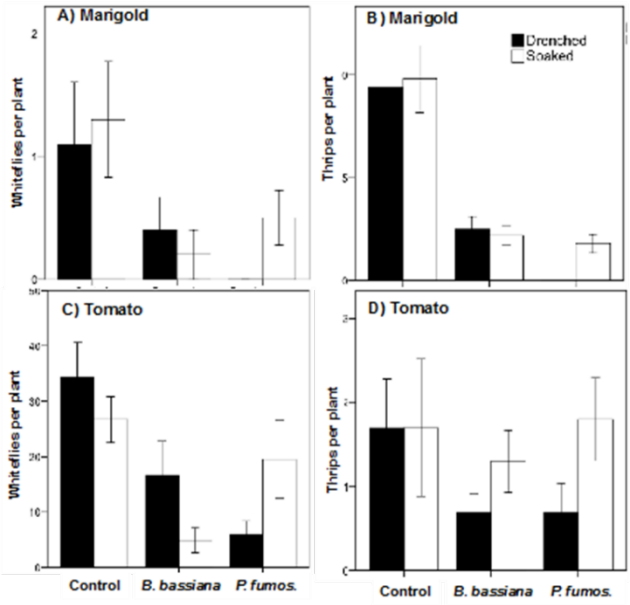


Fig. 1. Fungal endophyte treatments negatively affect whiteflies and thrips in marigold and tomato. All values are mean ± SE insects per plant.

Materials and Methods Utilized (Whitefly): 3.5-inch square pots filled with moist Sunshine® Mix #1 (Sungro Horticulture) were planted with Zinnia seeds per pot and watered with ~100 ml of RO (reverse osmosis)-treated water. One third of the pots were then drenched with an additional 20 ml of RO water, a second third were drenched with 20 ml of a *B. bassiana* (BB) solution diluted to 106 spores per cm^3 with RO water, and the remaining third of the pots were drenched with 20 ml of a *Chaetomium globosum* 520 (CG) solution diluted to 106 spores per cm^3 with RO water. Applications of the drench solutions were repeated on March 20, April 3, and April 17.

On March 31 90 potted plants from each of the three treatments were randomly allocated to 10 cylinder cages, 9 plants per cage, and infested with 18 female and 5 male adult sweetpotato whitefly (SPW) per Lexan™ polycarbonate sheeting cage. Whole-plant destructive whitefly counts were made on each sample date in the lab on one plant from each cage for all three treatments. Number of eggs, crawlers, nymphs, red-eye nymphs and exuvia were counted and recorded using a dissecting microscope for the first two samples. For the third and final sample only red-eye nymphs and exuvia were counted for all replications due to extremely high numbers of eggs and nymphs in all treatments.

In addition to the caged infested plants, a separate set of 20 plants per treatment were left un-caged and un-infested for endophyte assays to determine whether the respective endophytes became established in the plants. Assays were performed for leaves, stems, and roots three days after infestation (April 3) and one week after the third and final SPW sample (May 22).

Results: No presence of endophytes was detected in leaves, stems, or roots of plants from the water control pots or the *Beauveria* treated pots on either sample date. On May 22, however, soil samples taken from five of the *Beauveria* treated pots all tested positive for the presence of *Beauveria*. *C. globosum* was recovered from all plant parts in the *Chaetomium* treated pots on both sample dates. Mean recovery of *Chaetomium* from April 3 samples was 94.17, 35.0, and 30.0 %, respectively, of the leaf, stem, and root samples tested. Leaves, stems, and roots all tested positive on May 22, as well, with mean recoveries rates of 28.75, 25.0, and 100%, respectively, from the leaves, stems, and roots tested.

Whitefly count data were analyzed using 2-way ANOVA (p=0.05) to test for significant differences of both date and treatment effects. As expected, there were statistically significant differences in numbers of whitefly between dates for each SPW stage counted. No significant treatment differences, however, were found in numbers of whitefly at any life stage within each date.

Whitefly counts by stage and treatment on three sample dates (Mean±SE).

Date	Treatment	Eggs	Nymphs	Redeye nymphs	Exuvia
17-Apr	Water	27.10 ± 7.93	43.50 ± 11.19	0	0.20 ± 0.20
	Beauveria	18.90 ± 6.60	49.30 ± 18.42	0	0
	Chaetomium	11.40 ± 2.45	63.90 ± 21.32	1.89 ± 0.59	0.20 ± 0.20
1-May	Water	214.80 ± 100.86	26.80 ± 9.39	7.90 ± 2.44	18.40 ± 6.09
	Beauveria	659.90 ± 239.84	28.30 ± 9.62	7.80 ± 2.75	36.20 ± 11.25
	Chaetomium	383.00 ± 205.49	21.80 ± 8.63	5.30 ± 1.76	31.30 ± 13.59
15-May	Water	*2033.00 ±244.00	*771.50 ± 159.50	22.60 ± 9.48	39.30 ± 15.47
	Beauveria	*3938.00 ± 2375.50	*1754.00 ± 608.00	25.90 ± 8.74	35.60 ± 8.68
	Chaetomium	*819.00 ± 438.50	*699.00 ± 600.00	11.10 ± 4.81	32.80 ± 13.30

* Based on counts of only 2 random plants due to excessive numbers.

Conclusions and Impact: A diverse array of endophytes occur in ornamental plants grown in the landscape. While our results were not consistent, augmentation of select endophytes and their incorporation into plant tissues was not affected by inoculation methodology or endophyte species. In addition, the endophytes tested had no significant impact on the on the plant characteristics tested. Similarly, we were unable to document consistent insect control with the application of endophytes. Environmental conditions, soil mix, and plant species may affect these results. Endophyte applications yield no adverse plant or insect effects; hence, further evaluations by growers might be valuable.

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