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EFFECT OF NATURALLY-OCURRING FUNGAL PATHOGENS FROM A CUT FLOWER PRODUCTION SITE ON FOUR CUT FLOWER SPECIES

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Abstract. **Because of limited land availability for fallowing and rotation, cut flower growers in Florida face the constant challenge of soil-borne pest buildup at their production sites. Soil from a snapdragon (*Antirrhinum majus*) cut flower production site in southeast Florida was found to be naturally infested by *Fusarium* spp. *Pythium aphanidermatum*, and *Rhizoctonia spp.*, with *Fusarium* spp. detected at the highest incidence. The objective of this research was to document the adverse impact of soil-borne pathogens found at this site on four common cut flower species: 'Echo Pink' lisianthus (*Eustoma grandiflorum*, 'Potomac Rose' snapdragon, 'Queen of Africa' white dill (*Ammi majus*), and 'Qis White Cut' larkspur (*Consolida ajacis*). The growth of plants in naturally infested and autoclaved soil was compared in a greenhouse experiment. Final plant height was reduced ($P \leq 0.10$) when snapdragon, lisianthus, and larkspur were grown in infested soil. Root and shoot weight, and number of flower buds, blooms, and total flowers of snapdragon were reduced ($P \leq 0.05$) by growth in the non-autoclaved soil. Shoot weight, number of flower buds, and total number of flowers for lisianthus were lower ($P \leq 0.05$) in the non-autoclaved soil. For larkspur only the total number of flowers was reduced ($P \leq 0.10$) by growth in the non-autoclaved soil. Although the shoot weight of white dill was reduced in the non-autoclaved soil, flower yield was not changed by the soil treatment. Root discoloration was significantly greater ($P \leq 0.01$) on snapdragon and larkspur than on lisianthus and white dill. The lack of impact of soil treatment on flower production suggests that white dill was tolerant to the fungal pathogens in this soil.**

Florida ranks second nationally in floricultural production and sales including cut flowers, flowering potted plants, hanging baskets, potted foliage plants, cut foliage, bedding and garden plants, and woody ornamentals (Florida Agricultural Statistics Service, 2004). Cut flower sales in 2003 totaled $21.9 million. Snapdragon (*Antirrhinum majus* L.) is a high-value crop and one of the most important cut flowers produced in Florida, second only to gladiolus (*Gladiolus* spp.). Of 13 cut flower production firms in Florida, four produce snapdragons. The number of snapdragon spikes sold in 2003 totaled 4.75 million. With each spike selling for a wholesale value of $0.35 per spike, this amount is equivalent to $1.6 million of farm gate.

Snapdragon and other cut flowers are produced in the field, therefore, they are exposed to many pests including pathogens, insects, and nematodes (Greer, 2000). Commercial cut flower producers in Florida routinely start their planting season early in the fall to match the flowering and market seasons. This schedule requires that transplantation will occur during hot and humid weather, an environment conducive to many soil-borne pathogens. Because of the limited availability of land for fallowing and rotation, cut flower producers in Florida, including those that grow snapdragons, face the challenge of soil-borne pathogen buildup. This pathogen buildup has led to yield and quality reduction in cut flower production.

The impetus for this research project was a fungal disease outbreak at a commercial cut flower production site in southeastern Florida (Martin County) after a heavy rain event. McSorley et al. (2004) published a detailed description of the disease outbreak. Infected snapdragon seedlings showed symptoms of basal stem rot, crown rot, and wilt. The disease epidemic destroyed half of the plots in an on-going experiment, and resulted in 67% cut flower yield lost in untreated plots, and 34% in plots receiving solarization treatment, or soil fumigants such as methyl bromide or metam sodium.

Of the soil-borne pathogens listed for snapdragon in Florida (Alfieri et al., 1994), three (*Fusarium*, *Pythium*, and *Rhizoctonia*) are also considered major pathogens of many other crops. These pathogens cause basal stem and root rot, root and crown rot, and root and stem rot, respectively. Although these diseases have been reported (Alfieri et al., 1994), there is very little information regarding their specific impact, such as yield effects including reduction in plant size, flower number, and flower quality, on snapdragons and other cut flowers. If this information were available, it may be possible to improve production methods and decrease losses associated with soil-borne diseases.

Objectives of this study are to identify the causal agents of the above-mentioned snapdragon disease outbreak, and to determine their effect on other commonly grown cut flower species in Florida.

Materials and Methods

Fungal identification. Declining snapdragon seedlings were collected from the production site. Tissue from the rotted basal stem area of the seedling was surfaced sterilized with 0.6% NaOCl (sodium hypochlorite) solution for 30 s, and plated on potato dextrose agar (PDA) (Beever and Bollard, 1970). The pieces of plant stem tissue were approximately 1 mm × 0.5 mm (0.039 in. × 0.019 in.). Plates were incubated for 2-3 days at 23°C (73.4°F). Several fungal isolates in the genera of *Fusarium*, *Pythium*, and *Rhizoctonia* were obtained.

Isolates of *Fusarium* spp. were transferred to carnation leaf agar (CLA) (Nelson et al., 1983) and allowed to grow for 10

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days at 23°C (73.4°F) with exposure to sunlight. Isolates were sent to the Fusarium Research Center (Penn State University, University Park, Pa.) for identification. *Pythium* spp. isolates were transferred to pond water and grass blades medium (Mitchell and Rayside, 1986). Plates were incubated at 23°C (73°F) for 48 hours, and then *Pythium* species were identified using the key from Van der Platts-Niterink (1981).

Multiple approaches were taken to determine the causal agent. Quantification of these fungal population densities in the soil can provide information on which fungus is most abundant. On 13 Jan. 2004, approximately 40 kg (88.2 lbs) of the field soil was collected from the area of the snapdragon production site in Martin Co., Fla. (McSorley et al., 2004) displaying the most severe fungal infection symptoms. This soil consisted of 96% sand, 1% silt, and 3% clay. The soil sample was mixed well, and 10 g (0.35 oz) of soil was sub-sampled and suspended in 100 ml (3.0 fl oz) sterile distilled water followed by 2 series of 10-fold dilutions. One ml (0.03 fl oz) of each soil dilution was plated onto petri dishes containing selective media: Komada's medium (Komada, 1975) for *Fusarium* spp., PARP (Pimaricin Ampicillin Rifampicin Pentachloronitrobenzene) (Mitchell and Kannwischer-Mitchell, 1992) medium for *Pythium* spp., and Flowers' medium (Flowers, 1976) for *Rhizoctonia* spp. Five replicated plates were made for each soil dilution in each medium. An MPN (Most Probable Number)-program was used to quantify the colony forming units per gram (cfu/g) of soil for each fungal genus (Woomer et al., 1990).

Greenhouse Experiment. An experiment was conducted in the greenhouse on the University of Florida campus in Gainesville from January–April 2004, to determine the susceptibility of several cut flower species to the fungal pathogens present in the soil from the cut flower production site.

Half of the field soil was kept at 23°C (73°F), and the other half was autoclaved at 250°C (482°F) for 99 minutes and allowed to air-dry for three days. Both soils were placed in 10-cm (4-in) diameter pots with approximately 0.68 kg (1.5 lbs) soil per pot. Four cut flower species, 'Echo Pink' lisianthus (*Eustoma grandiflorum* (Raf.) Shinn), 'Potomac Rose' snapdragon, 'Qis White Cut' larkspur (*Consolida ajacis* (L.) Schur.), and 'Queen of Africa' white dill (*Ammi majus* L.) were planted into the two soils (non-treated or autoclaved). The experiment was arranged in a randomized complete block design with four replications. Thus, a total of 32 pots were included in the experiment.

Seedlings (2-3 cm tall) of the same age were obtained from Sunshine State Carnations in Martin Co, Florida, on 13 Jan. 2004. Seedlings had been grown in a greenhouse in polystyrene trays (Speedling, Inc., Sun City, FL) using a pathogen-free soilless media. Seedlings were transplanted on 15 Jan. 2004, and plants of similar height for each flower species were placed into the same replication. Plants were watered and fertilized in accordance with commercial nursery practices, and grown under 50% ambient light. Mean maximum and minimum temperatures in the greenhouse were 30°C (86°F) and 13°C (55°F), respectively.

*Data collection and analysis.*Plant heights were measured at 2-week intervals for 12 weeks. At the termination of the experiment when most flowers were at full bloom stage, the fresh weight of roots and shoots, number of blooms, number of flower buds, and total number of flowers were quantified for each plant. Root systems were given a visual rating using a scale from 0-5 based on the percentage of discoloration, where 0 = no discoloration, 1 = 1-20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, and 5 = 81-100% of the root system with discoloration. Data from each date were subjected to a 4×2 (flower × soil treatment) split-plot analysis of variance (ANOVA) using SAS (SAS Institute, Cary, N.C.). Means were separated by a Waller-Duncan *k*-ratio ($k = 100$) *t*-test.

Results and Discussion

Isolates of *Fusarium* spp. were identified as *F. equiseti, F. semitectum,* and *F. oxysporum.* Of these, only *F. oxysporum* is a known plant pathogen (Nelson, et al. 1983). Only one species of *Pythium* was isolated, and it was identified as *P. aphanidermatum*, a known pathogen in Florida soils (Van der Platts-Niterink, 1981). *Rhizoctonia* spp. were also isolated from the diseased tissue, but not identified to species. Soil dilution plating revealed that *Fusarium* spp. were more abundant in the non-autoclaved soil than *Pythium* spp. or *Rhizoctonia* spp. when comparing colony forming units per gram of soil (Table 1).

A significant interaction ($P \leq 0.05$) existed between flower species and soil treatment in the 4×2 split-plot analysis for all of the parameters measured. Therefore data were further analyzed with one-way ANOVA for each cut flower species.

While there were no differences in plant height initially between the autoclaved and fungal infested soils for all the cut flowers tested, plant height for white dill was shorter ($P \leq 0.05$) and for larkspur was taller ($P \leq 0.01$) in the fungal infested soil than in the autoclaved soil on 12 Feb. 2004 (Table 2). On 29 April 2004, all of the cut flowers tested except white dill were shorter ($P \leq 0.10$) in the fungal infested soil than in the autoclaved soil.

Shoot weight, number of buds, and total number of flowers in lisianthus were lower ($P \leq 0.05$) in the fungal infested soil than in the autoclaved soil (Table 3). Lisianthus did not bloom by the end of the experiment in either treatment. For snapdragon, all of the parameters were greatly reduced ($P \leq 0.05$) in plants grown in the fungal infested soil compared to those grown in the autoclaved soil. Although shoot weight of white dill was lower ($P \leq 0.05$) in the fungal infested soil than in the autoclaved soil, flower number and root weight were not affected ($P \geq 0.10$) by the fungal pathogens. For larkspur, the total number of flowers were lower ($P \leq 0.10$) in the fungal infested soil than in the autoclaved soil.

Ratings for percent discoloration in roots were higher ($P \leq 0.10$) in natural soil than in autoclaved soil for snapdragon and larkspur (Table 4). While root discoloration might not be the sole result of fungal infection, we assumed that fungal infection would be the primary cause. When root discoloration was compared among all of the cut flowers grown in the naturally infested field soil, snapdragon and larkspur had higher root discoloration ratings than lisianthus and white dill, indi-

Table 1. Results of quantification of fungal populations from the soil sample taken from snapdragon production site on 13 Jan. 2004.

Fungal isolate	cfu/g soil[z]
Fusarium spp.	23,000
Pythium spp.	108
Rhizoctonia spp.	156

[z]cfu = colony forming units, calculated by Most Probable Number program (Woomer et al., 1990).

Table 2. Plant height of flower species in autoclaved and non-autoclaved soils on six dates during the greenhouse experiment, 2004.

	Plant heights (cm)											
	27 Jan.		12 Feb.		26 Feb.		18 Mar.		30 Mar.		29 Apr.	
Flower species	A[z]	F	A	F	A	F	A	F	A	F	A	F
Lisianthus	0.5[y]	0.4	1.3	0.7	3.4	1.5	11.7	5.4	25.1	24.4	72.0	48.6*
Snapdragon	2.7	2.0	9.5	8.8	19.2	17.5	57.4	42.0	76.2	75.8	131.7	108.0@
White Dill	9.2	6.7	26.6	19.0*	31.2	28.7	44.4	48.4	64.1	61.8	106.2	104.1
Larkspur	5.3	5.6	9.7	11.2**	10.3	14.8	45.6	54.3	79.0	60.8	95.0	61.0**

[z]A = autoclaved soil; F = non-autoclaved soil, contaminated with pathogenic fungi.
[y]Data are means of four replications. Means followed by @, *, and ** indicate a significant difference between corresponding A and F values for each date and flower species at $P \leq 0.10$, 0.05, and 0.01, respectively, according to analysis of variance.

Table 3. Root weight, shoot weight, and number of flower buds, blooms, and total flowers for four flower species in autoclaved and non-autoclaved soils at the termination of the greenhouse experiment in 2004.

	Root weight (g)		Shoot weight (g)		No. buds		No. blooms		Total no. flowers	
Flower species	A[z]	F	A	F	A	F	A	F	A	F
Lisianthus	6.5[y]	6.6	40.2	21.7*	9.2	5.5*	—[x]	—	—	—
Snapdragon	4.2	1.6**	50.5	8.6**	30.2	8.2*	24.2	6.5**	54.5	14.7*
White Dill	15.4	11.3	49.9	40.4*	11.2	11.0	0	2.5	11.2	13.5
Larkspur	4.3	0.1	23.4	7.4	93.0	9.3	70.5	8.6	174.0	19.0@

[z]A= autoclaved soil; F= non-autoclaved soil, contaminated with pathogenic fungi.
[y]Data are means of four replications. Means followed by @, *, and ** indicate a significant difference between corresponding A and F values for each date and flower species at $P \leq 0.10$, 0.05, and 0.01, respectively, according to analysis of variance.
[x]— indicates that plants did not flower prior to the termination of the experiment.

cating that the snapdragon and larkspur cultivars tested were more susceptible to the fungi in this soil.

At the termination of the experiment, the root systems of larkspur and snapdragon were severely affected by the soilborne pathogens identified. Among the cut flowers tested, snapdragon was the most susceptible to the fungal pathogens detected at the cut flower production site. This result is consistent with the report of pathogenicity by Alfieri et al. (1994). Pathogens reported on snapdragon in Florida include *Fusarium* sp. as a basal stem and root rot, *Pythium* sp. as a root and crown rot, and *Rhizoctonia* sp. and *R. solani* as root and stem rots (Alfieri et al., 1994). Species of *Fusarium* and *Pythium* pathogenic to snapdragon have not been reported. All three of these fungal genera were also reported to be pathogenic on larkspur and lisianthus, but no information was reported for white dill (Alfieri et al., 1994). A destructive crown and stem rot caused by *Fusarium avenaceum* (Fr.-Fr.) Sacc. has become widespread for lisianthus production in Florida and California (R. J. McGovern, pers. comm.). *Fusarium solani* (Mart.) Sacc., *Pythium* sp., and *R. solani* (Kuhn) are also important pathogens of lisianthus (Daughtrey, 2000).

Table 4. Root rating[z] of four flower species in autoclaved and non-autoclaved soils at the termination of the greenhouse experiment, 2004.

	Root rating (0-5 scale)[z]	
Flower species	A[y]	F
Lisianthus	0.5 a[x]	0.5 c
Snapdragon	0.2 a	3.0 b**
White Dill	0.0 a	1.0 c
Larkspur	1.5 a	4.8 a@

[z]Root rating on a scale of 0–5 where 0 = no discoloration, 1 = 1–20% discoloration, 2 = 21–40% discoloration, 3 = 41–60% discoloration, 4 = 61–80% discoloration, 5 = 81–100% discoloration of root system.
[y]A = autoclaved soil; F = non-autoclaved soil, contaminated with pathogenic fungi.
[x]Data are means of four replications. Means followed by same letter in a column are not different according to Waller-Duncan k-ratio (k = 100) *t*-test at $P \leq 0.01$. @ and ** indicate a significant difference between A and F for each flower species at $P \leq 0.10$ and 0.01, respectively, according to analysis of variance.

Although *Fusarium* spp. were the most abundant soil fungi among the three fungal genera quantified, it is not clear which genera are pathogenic. A Koch's Postulate test using each of these fungi and their combination will be conducted to further examine the causal agent(s) of this disease epidemic on snapdragon. Disease complexes caused by *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. have been reported in many crops such as bean (Pieczarka and Abawi, 1978), cotton (Lyda and Watkins, 2001), clover, alfalfa (Kucharek, 1997), rapeseed (Barbetti and Roy, 1982), peanut (Csinos et al., 1984), and strawberry (Pscheidt, 2005). It is possible that a disease complex could also be occurring in snapdragon.

Growing larkspur in the non-treated soil reduced the total number of flowers at $P \leq 0.10$ level, but there was no significant impact on shoot and root weights. It is worth noting that larkspur is a shorter-term crop. Many of the larkspur plants began blooming on 18 Mar. and reached senescence at the termination of the experiment. If the experiment had been terminated earlier, we might have seen a greater impact of the fungal pathogens on flower yield of larkspur, similar to that observed in the plant height and root rating data.

The pathogens, *Fusarium*, *Pythium*, and *Rhizoctonia* identified in the naturally infested soil severely affected the plant growth and flower yield of snapdragon, larkspur, and lisianthus. Although shoot weight of white dill was reduced in fun-

gal contaminated soil, plant height and flower yield were not affected by these fungal pathogens. Therefore white dill appears to have tolerance to the fungal pathogens.

Growers could choose to grow white dill at this fungal contaminated site in the future in conjunction with other soil-borne fungal disease management strategies to avoid future disease epidemic outbreaks. However, the long-term effects of these fungi on white dill under field conditions remain untested. Multiple disease management strategies are recommended, as many preventative fungicides do not protect the crop from post-plant infection. Some of the preventative measures include soil solarization (Pscheidt, 2005), use of certified- and fungicide-treated seeds (Kucharek, 1997), and pre-plant soil fumigation. Transplanting should take place after previous crop residues have decomposed, and deep planting should be avoided. Excessive application of fertilizer and water, and especially flooding, could increase fungal proliferation in the soil and predispose the plants to infection.

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