Susceptibility of Forty-six *Lisianthus* Cultivars to Fusarium Crown and Stem Rot

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SUMMARY. Fusarium crown and stem rot, caused by *Fusarium avenaceum* (Fr.: Fr.) Sacc., is a serious disease of lisianthus [*Eustoma grandiflorum* Raf. (Shinn.)]. While more than 80 new cultivars of lisianthus have been released for sale in the United States in the last decade, there is a lack of information on their susceptibility to this pathogen. Forty-six cultivars of lisianthus were evaluated for their response to infection by *F. avenaceum*. Cultivars were grouped according to blue/purple, pink, or white flower colors and evaluated within their color class. Although some plants of all cultivars were susceptible to *F. avenaceum*, partial resistance was observed as indicated by differences in the length of time to symptom expression and in the frequency of diseased plants within each color group. In 21 of the 46 cultivars, 80 to 100% of the plants expressed symptoms within 55 days after inoculation. The lowest frequencies of diseased plants 55 days after inoculation were found in 'Ventura Deep Blue' and 'Hallelujah Purple' (25%), 'Bridal Pink' (23%), and 'Heidi Pure White' (53%) for the blue/purple, pink, and white flower color groups, respectively. Screening cultivars for resistance to *F. avenaceum* is the first step in breeding resistant cultivars. The methods we developed for these studies should be useful in screening for resistance. These results also may help growers select cultivars that are less susceptible to *F. avenaceum*, which should aid in the management of this disease.

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Fusarium crown and stem rot is one of the most damaging diseases of lisianthus. It has been reported as a disease of lisianthus produced as potted plants and cut flowers (Koike et al., 1996; Ozaki, 1992), but has been especially devastating in cut flower production. For example, in a 1997 survey of crown and stem rot incidence in three Florida and four California cut flower production sites, plant mortality was as high as 70% (McGovern et al., 1997).

The causal agent of this disease, *Fusarium avenaceum*, primarily attacks the crown and stems of lisianthus, but may also rot the tap root and large feeder roots near the soil line (McGovern and Harbaugh, 1997). The first above-ground symptom is a gradual loss of green coloration in leaves, which is followed by tan leaf flecks, browning of leaf veins, and a tan discoloration of entire leaves. Wilting and a brown stem rot occurs as the disease progresses, and infected plants rapidly die. Orange spore masses form on the bases of rotted stems and are diagnostically very important.

Chemical and cultural control measures have been improved over the last few years as a result of research on the etiology and biology of *F. avenaceum* (McGovern and Harbaugh, 1997; McGovern and Harbaugh, 1998). However, two approaches to disease management that have received inadequate attention are 1) the evaluation and use of resistance in existing lisianthus cultivars, and 2) the breeding of resistant cultivars. There has been an explosion of new cultivars released within the last decade with over 85 cultivars available in the United States in 1999. However, we are not aware of breeding efforts specifically aimed at developing resistance to *F. avenaceum*. The objective of this research was to evaluate 46 cultivars of lisianthus to determine if resistance exists that could either be used in current control strategies or as a first step toward the breeding of resistant cultivars.

Material and methods

GENERAL. Sixteen blue-purple, 15 pink, and 15 white-flowering cultivars of lisianthus were selected for this study (Tables 1–3). Cultivars were selected from five different breeding programs: American Takii, Inc., Salinas, Calif.; PanAmerican Seed Company, Elburn, Ill; Fukukaen Seed Company, Japan; Sakata Seed America, Inc., Morgan Hill, Calif.; and University of Florida, Bradenton, Fla. Each color group was evaluated separately due to space limitations in the growth chamber and, as a result, there were differences in certain production practices that will be noted for each group.

Plants used in these studies were produced as follows. Seeds were germinated at 72 to 75 °F (22 to 24 °C) with a photosynthetic photon flux (PPF) of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for 12 h from cool-white fluorescent lamps. The soil medium was, by volume, a 3 Canadian peat : 2 vermiculite: 1 perlite mix with an initial pH of 6.7 ± 0.2. Germination trays were moved to a glass greenhouse 2 weeks after sowing. Temperatures were maintained between 60 °F (15.5 °C) and 95 °F (35 °C). Seedlings were fertilized twice per week with a 15N–7P–14K water soluble fertilizer (15–16–17 Peat-Lite Special; Scotts Co., Marysville, Ohio) solution containing N at 250 ppm ($mg \cdot L^{-1}$).

Seedlings were transplanted about 35 to 40 d after sowing into 128-cell plug trays using the same soil medium. We used eight plants of each cultivar per tray, and each tray represented a plot. Seedlings at this stage were fertilized twice per week with a 15N–2P–12.4K water soluble fertilizer (15–5–15 Ca–Mg Excel; Scotts Co., Marysville, Ohio) solution containing N at 500 ppm.

Before inoculation with *F. avenaceum* and for the rest of each study, seedlings were moved to a growth chamber maintained at 66 ± 2 °F (18.9 ± 1 °C) with a PPF of 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by cool-white fluorescent lamps for 16 $h \cdot d^{-1}$. Plug trays were placed in individual 1-inch (2.5-cm) deep trays and plants provided water and fertilizer solution via subirrigation. Seedlings were at the 8

Table 1. Susceptibility of blue/purple-flowering lisianthus cultivars to Fusarium avenaceum at 25, 40, or 55 d after inoculation. Seeds were sown on 4 Dec. 1997 and plants inoculated 25 Feb. 1998. Each value (percentage) represents the mean of five replications with eight plants per replication as the experimental unit.

Cultivar	Seed source[z]	Plants showing symptoms (%)		
		Day 25	Day 40	Day 55
Mermaid Blue	Sakata	90 a[y]	100 a	100 a
Tiara Purple	Takii	55 b	70 b	93 ab
Maurine Blue	U.Fla.	53 bc	73 b	85 ab
Florida Blue	U.Fla.	43 b–d	65 bc	80 bc
Flamenco Purple	Sakata	29 b–e	39 c–e	59 cd
Laguna Deep Blue	PanAm.	48 bc	53 b–d	58 cd
Echo Blue	Sakata	18 de	29 de	56 d
Lisa Blue	PanAm.	28 b–e	43 c–e	50 de
Heidi Deep Blue	Sakata	18 de	28 de	49 de
Tyrol Blue	Sakata	30 b–e	40 c–e	45 d–f
Mariachi Blue	Sakata	25 c–e	35 c–e	40 d–f
Yodel Blue	Sakata	13 e	20 e	38 d–f
Royal Purple	Takii	18 de	30 c–e	33 d–f
Bridal Violet	Takii	20 de	23 e	30 ef
Ventura Deep Blue	PanAm.	10 e	18 e	25 ef
Hallelujah Purple	Fukukaen	18 de	23 e	25 f

[z]Takii = American Takii, Inc.; PanAm. = PanAmerican Seed Company; Fukukaen = Fukukaen Seed Company; Sakata = Sakata Seed America, Inc.; U.Fla. = University of Florida.
[y]Mean separation within columns by Duncan's multiple range test, $P \leq 0.05$. Arcsine square root transformation was performed before data analysis; nontransformed means are presented.

to 12 leaf stage and , if they had bolted, had one or two internodes on the bolted stem at the time of inoculation.

Inoculum was prepared by blending carnation leaf agar plates of 5 to 7-d-old cultures of a highly virulent isolate (96-62B) of *F. avenaceum* in tap water at a ratio of one plate per 100 mL (3.4 fl oz) of water. Ten milliliters of the *F. avenaceum* inoculum was applied to the soil around each plant which saturated the soil medium in each cell.

The frequency of plants expressing characteristic well developed symptoms of *F. avenaceum* (browning of leaf veins, stem lesion and or crown rot) was recorded at 25, 40, and 55 d after inoculation. Although seedlings often have a slight chlorosis before fully developed symptoms are expressed, this chlorosis is similar to that caused by *Pythium* Pringsh. or nutritional imbalances and thus not definitive for signaling infection by *F. avenaceum*. Symptomatic plants were randomly sampled throughout all tests to confirm infection by *F. avenaceum* by reisolation of the pathogen on Komada's medium (Komada, 1975).

The experimental design was a randomized complete block with five blocks, and eight plants per block rep-

Table 2. Susceptibility of pink-flowering lisianthus cultivars to *Fusarium avenaceum* at 25, 40, or 55 d after inoculation. Seeds were sown on 7 Apr. 1998 and plants inoculated 13 May 1998. Each value (percentage) represents the mean of five replications with eight plants per replication as the experimental unit.

Cultivar	Seed source[z]	Plants showing symptoms (%)		
		Day 25	Day 40	Day 55
Maurine Pink	U.Fla.	58 a[y]	90 a	98 a
Florida Pink	U.Fla.	38 a–c	60 bc	90 ab
Lisa Pink	PanAm.	45 ab	65 ab	85 ab
Mermaid Pink	Sakata	25 bc	58 b–d	85 ab
Echo Pink	Sakata	3 e	33 c–e	83 ab
Royal Pink	Takii	18 cd	33 c–e	80 bc
Tiara Pink	Takii	38 a–c	58 b–d	78 bc
Tyrol Rose Pink	Sakata	5 e	25 d–f	73 b–d
Flamenco Rose Pink	Sakata	8 de	23 ef	58 c–e
Hallelujah Pink	Fukukaen	18 c–e	33 c–e	53 d–f
Ventura Rose	PanAm.	3 e	13 ef	50 d–f
Heidi Rose Pink	Sakata	3 e	20 ef	40 ef
Laguna Pink	PanAm.	5 de	13 ef	40 ef
Mariachi Pink	Sakata	5 de	8 f	40 ef
Bridal Pink	Takii	5 de	13 ef	23 f

[z]Takii = American Takii, Inc.; PanAm. = PanAmerican Seed Company; Fukukaen = Fukukaen Seed Company; Sakata = Sakata Seed America, Inc.; U.Fla. = University of Florida.
[y]Mean separation within columns by Duncan's multiple range test, $P \leq 0.05$. Arcsine square root transformation was performed before data analysis; nontransformed means are presented.

Table 3. Susceptibility of white-flowering cultivars to Fusarium avenaceum at 25, 40, or 55 d after inoculation. Seeds were sown on 7 July 1998 and plants inoculated 17 Aug. 1998. Each value (percentage) represents the mean of five replications with eight plants per replication as the experimental unit.

Cultivar	Seed source[z]	Plants showing symptoms (%)		
		Day 25	Day 40	Day 55
Florida White	U.Fla.	4 b[y]	8 bc	100 a
Malibu White	PanAm.	0 b	3 bc	100 a
Maurine White	U.Fla.	0 b	13 bc	100 a
Tiara White	Sakata	0 b	3 bc	100 a
Yodel White	Sakata	5 b	18 ab	100 a
Royal White	Takii	28 a	28 a	98 a
Ballet White	Takii	0 b	10 bc	93 a
Tyrol White	Sakata	0 b	0 c	90 a
Flamenco White	Sakata	0 b	3 c	88 a
Maurine White / Blue	U.Fla.	3 b	33 a	88 a
Mermaid White	Sakata	0 b	3 bc	85 a
Mariachi Pure White	Sakata	0 b	5 c	83 a
Ventura White	PanAm.	5 b	8 bc	60 b
Lisa White	PanAm.	0 b	0 c	55 b
Heidi Pure White	Sakata	0 b	3 bc	53 b

[z]Takii = American Takii, Inc.; PanAm. = PanAmerican Seed Company; Fukukaen = Fukukaen Seed Company; Sakata = Sakata Seed America, Inc.; U.Fla. = University of Florida.
[y]Mean separation within columns by Duncan's multiple range test, $P \leq 0.05$. Arcsine square root transformation was performed before data analysis; nontransformed means are presented.

resented the experimental unit. Statistical analyses were performed on data using analysis of variance, and means separated where appropriate using Duncan's multiple range test, $P \leq 0.05$ (PROC ANOVA, SAS Inst., Cary, N.C.). An arcsine square root transformation for percentage data was performed before data analysis (Little and Hills, 1972).

BLUE CULTIVARS. The blue cultivars were planted 4 Dec. 1997, transplanted into plug trays on 13 Jan. 1998, and moved to the growth chamber and inoculated on 25 Feb. 1998. Seedlings were treated with mefenoxam (Subdue Max, Novartis, Greensboro, N.C.) on 20 Mar. 1998 to suppress *Pythium*.

PINK CULTIVARS. The pink cultivars were sown 7 Apr. 1998, transplanted 13 May 1998, and moved to the growth chamber 28 May. Plants were inoculated on 19 June 1998. Mefenoxam was applied on 17 June to suppress *Pythium* and diflubenzuron (Adept, Uniroyal Chemical, Middleburg, Conn.) On 22 June 1998 to control fungus gnats [*Bradysia Winnertz* (Diptera: Sciaridae)].

WHITE CULTIVARS. The white cultivars were sown 7 July 1998 and transplanted 17 Aug. 1998. Because the greenhouse temperatures were high enough to cause rosetting of some of the cultivars (Harbaugh, 1995; Harbaugh et al., 1992), seedlings were moved to the growth chamber and grown at 75 ± 2 °F (23.9 ± 1 °C) day and 65 ± 2 °F (18.3 ± 1 °C) night. On 22 Sept. 1998, the temperature was set to 50 ± 2 °F (10 ± 1 °C) to limit lisianthus seedling growth until inoculation. Plants were inoculated 14 Oct. 1998 and the temperature finally set to 66 ± 2 °F (18.9 ± 1 °C). Seedlings were treated 11 Sept., 13 Oct., and 19 Nov. 1998 with mefenoxam to prevent *Pythium* development.

Results and discussion

BLUE CULTIVARS. The frequency of diseased plants (i.e., percentage of plants with characteristic symptoms caused by infection with *F. avenaceum*) ranged from a low of 10% for 'Ventura Deep Blue' to a high of 55% for 'Tiara Purple' 25 d after inoculation (Table 1). By day 40, 'Ventura Deep Blue' continued to have the lowest percentage disease (18%), while 100% of 'Mermaid Blue' plants were diseased. The frequency of diseased plants at day 55 ranged from 25% for 'Ventura Deep Blue' and 'Hallelujah Purple' to 85% for 'Maurine Blue', 93% for 'Tiara Purple', and 100% for 'Mermaid Blue'.

PINK CULTIVARS. The frequency of diseased plants ranged from a low of 3% for 'Echo Pink', 'Heidi Rose Pink', and 'Ventura Rose' to a high of 58% for 'Maurine Pink' 25 d after inoculation (Table 2). By day 40, cultivars with the lowest percentage disease were 'Mariachi Pink', 'Bridal Pink', 'Laguna Pink', and 'Ventura Rose' (8 to 13%), while the greatest number of diseased plants occurred with 'Maurine Pink' (90%). The percentage of diseased plants at day 55 ranged from 23% for 'Bridal Pink' to 98% for 'Maurine Pink'.

WHITE CULTIVARS. Only a few cultivars expressed disease symptoms by 25 d after inoculation with the highest frequency at 28% for 'Royal White' (Table 3). By day 40, only 'Lisa White' and 'Tyrol White' did not show symptoms, while 'Maurine White-on-Blue' reached 33% diseased plants. However, by day 55, the lowest percentage of diseased plants was 53% for 'Heidi Pure White' and five cultivars had 100% diseased plants ('Florida White', 'Malibu White', 'Maurine White', 'Tiara White', and 'Yodel White').

The white cultivars appeared to respond differently than the blue / purple or pink-flowering cultivars (evaluated in the other tests) because the disease development was slower. However, direct comparisons can not be made since the plant production conditions were not the same for all three tests. For example, white-flowering cultivars were held at 50 ± 2 °F (10 ± 1 °C) for 22 d while cultivars within the other color groups did not receive this treatment. It is possible the white-flowering cultivar seedlings were less succulent than the blue / purple and pink cultivars at the time of inoculation. Many of the white cultivars were chlorotic 25 and 40 d after inocu-

lation and we suspected plants were infected, but these symptoms are not conclusive evidence of *F. avenaceum* infection (McGovern and Harbaugh, 1997). However, more definitive symptoms of infection by *F. avenaceum* (browning of leaf veins, stem lesion and or crown rot) occurred between day 40 and 55 and then plants rapidly started to die. Thus, if the production practices for the white-flowering cultivars were responsible for the delay in symptom expression, their effects appeared to have diminished toward the end of the test. Additional experiments will be designed to compare the best blue / purple and pink-flowering cultivars with the best white-flowering cultivars in order to determine if the white-flowering cultivars have a genetic basis for delayed disease development.

In summary, all cultivars tested were considered susceptible to *F. avenaceum* since a percentage of all cultivars became infected. However, distinct differences occurred between cultivars within a flower color group in the length of time for symptom development and in the frequency of diseased plants, indicating different degrees of susceptibility. The genetic basis for this delayed symptom development or partial resistance needs to be studied in order to take advantage of these findings for breeding more resistant cultivars. Incorporation of resistance to *F. avenaceum* into breeding efforts could significantly improve control measures and potentially reduce fungicide use.

Reisolation of the pathogen on all symptomatic plants randomly sampled throughout these tests confirmed infection by *F. avenaceum*. We believe the method we developed for screening lisianthus cultivars for their response to infection by *F. avenaceum* would be valuable in a breeding program to develop resistant cultivars. Growers currently experiencing severe losses from *F. avenaceum* may benefit from this research by using cultivars found to be less susceptible to *F. avenaceum* as an aid to management of this disease.

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