Symptom Response and Colonization as Measures of Resistance in Chrysanthemum Cultivars Inoculated with *Fusarium oxysporum* f. sp. *chrysanthemi*

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ABSTRACT

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Sixteen cultivars of *Chrysanthemum morifolium* were tested for susceptibility to wilt caused by *Fusarium oxysporum* f. sp. *chrysanthemi*. Symptomatology was rated according to a scale of 0–5, and variation from resistant to susceptible was noted among cultivars, with variation in symptom type and location on the plant. Colonization of plant stems was a more consistent assay of susceptibility than were symptoms. Results suggest a standardized assay technique to minimize variability in plant responses. The susceptible cultivars Yellow Delaware and Royal Trophy were indexed at regular intervals on the stem to determine the rate and pattern of colonization by *Fusarium*. In single-stemmed and branched plants, colonization increased with time and was discontinuous in 14% of the plants sampled. The fungus was found in the vascular system of symptomless plants, as well as in those expressing symptoms.

Wilt of the florist's chrysanthemum (*Chrysanthemum morifolium* (Ramat.) Hemsl. caused by *Fusarium oxysporum* f. sp. *chrysanthemi* has been observed in Florida for many years (2). A literature review by Emberger and Nelson (3) summarized the history of the disease and clarified confusion concerning *Fusarium* species involved. Recent outbreaks of Fusarium wilt in new geographic areas and increases in disease incidence have alarmed growers and prompted research.

Fusarium wilt of chrysanthemum is often difficult to diagnose because its symptoms are similar to those caused by nutrient deficiencies, improper watering, or diseases such as Pythium root rot or Fusarium stem rot (2,4,5,9). Symptom development is highly dependent on environmental conditions and the nutritional status of the plant. Temperatures above 26 C (80 F), low pH, and ammoniacal nitrogen fertilizers enhance symptom development (3,5–7).

Fusarium wilt is difficult to control because of persistence of *F. oxysporum* in soil and the difficulty of incorporating resistance into breeding programs. Major control, therefore, is prevention through a thorough culture-indexing program for production of clean cuttings (11), followed by strict sanitation.

Preliminary screening of cultivars inoculated with *Fusarium* indicated a range of symptom responses among cultivars. The cultivar Yellow Delaware displayed apical chlorosis and curvature, with progression of symptoms from the top of the plant downward; in contrast, Royal Trophy exhibited symptoms that progressed upward from the base of the plant.

This research combined and repeated earlier trials in an attempt to reduce variability in cultivar response and to develop a technique for inoculation of plants and for symptom observation and rating. The location and progression of symptoms in cultivars currently in commercial use were noted to differentiate symptomatology types, and these cultivars were rated for susceptibility to the disease. Colonization of stem tissue was monitored to discern whether differences in symptom progression could be related to differences in colonization by the fungus.

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MATERIALS AND METHODS

Cultivar trial. Rooted cuttings of 16 chrysanthemum cultivars were obtained (California-Florida Plant Corp., Fremont, CA 94538), and five representative cuttings were indexed for *Fusarium* on carnation leaf agar (CLA) (8). Cuttings were planted in a 1:1:1 soil (Hagerstown silt loam):peat:perlite mix in 12.5-cm clay pots maintained on a greenhouse bench and heated to an average soil temperature of 27–29 C (80–85 F). Plants were grown as single stems under a 24-hr photoperiod to prevent bud formation. A Chapin drip-irrigation system (14) was used to eliminate water splash from pot to pot, and plants were fertilized weekly with a 20:20:20 (NPK) blend containing ammoniacal nitrogen.

The experiment was a completely randomized design with two treatments (inoculated and uninoculated) with five replicates. Plants were inoculated at 2 wk of age with isolate FRC-O-693, a known virulent culture of *F. oxysporum* f. sp. *chrysanthemi* (Fusarium Research Center, The Pennsylvania State University) grown from single spores on potato-dextrose agar (PDA) slants for 14 days under standard conditions (13). Plant roots were wounded by passing a metal spatula through the soil six times around each plant and 100 ml of a suspension of macroconidia and microconidia containing 60,000 conidia per milliliter of H_2O was poured onto the soil. Control plants were wounded and received 100 ml of distilled water.

When symptoms appeared, the plants were rated according to the following scale, which is a modified version of that of Engelhard and Woltz (5): 0 = no symptoms; 1 = chlorosis of leaves (generalized), stunting; 2 = chlorosis, curvature, and/or distortion (puckering) of leaves, stunting; 3 = chlorotic wedges and necrosis of leaves (symptoms often one-sided on plant), stunting of branches or entire plant; 4 = severe chlorosis, necrosis, distortion of leaves, stunting of plant, and wilting of leaves or shoots; and 5 = death of shoots or branches, eventual death of plant, and black streak on stem.

Symptoms were rated 13, 16, 23, 26, 29, 32, 36, and 49 days after inoculation, and plant height was measured 23 and 36 days after inoculation. Seven weeks after inoculation, plant stems were indexed for colonization by *F. oxysporum*. Plants were cut off at the soil line, stripped of leaves, and surface-sterilized in 10% Clorox (5.25% sodium hypochlorite) for 6 min. Cross sections of stems at basal, middle, and subapical portions of each plant were cut with sterile razor blades and placed on CLA plates to assay for *Fusarium*. *F. oxysporum* could be observed growing from sections in several days and could be identified directly from plates. Cultures were single-spored, and pathogenicity tests were performed on susceptible cultivars.

Colonization of single-stemmed plants. Rooted cuttings of cultivars Yellow Delaware and Royal Trophy were obtained, indexed, planted, maintained,

and inoculated as described. The experiment was a randomized complete block design with four replicates and treatments of 15 consecutive sampling dates. Four replicate plants of each treatment were sampled daily to determine the extent of colonization of stems. Plants were cut off at the soil line, stripped of leaves and small shoots, and plant outlines were drawn on paper. Plants were then surface-sterilized in 10% Clorox, with sterilization time increased from 3 to 5 min as plant size increased. Thin cross sections were made at 2.54-cm intervals on the stem and were placed onto CLA plates to index. Outlines of each plant were shaded to show the extent of fungal colonization. The experiment was repeated, with the only difference being a 2-day delay in sampling after inoculation.

Colonization of branched plants. Cuttings of Yellow Delaware and Royal Trophy chrysanthemums were obtained, planted, and maintained as before. At 2 wk of age, however, plants were pinched to promote branching. After 2 wk (to allow for initiation of lateral branches), plants were inoculated. The experiment was a randomized complete block design with four replicates and was repeated once. Treatments consisted of 15 sampling dates as before, although sampling was delayed 10 days to allow colonization by the fungus through the main stem to the branches. Sampling was done as in the previous experiment and plant outlines on paper were shaded to show location and extent of colonization. Height of plants, height to which colonized, number of branches colonized, and total number of branches were determined.

RESULTS

Cultivar trial. About 2 wk after inoculation, plants began to show generalized, apical chlorosis. Symptoms were definitive 23 days postinoculation, with control plants remaining symptomless. Ratings of mean disease severity and corresponding reduction in height of inoculated plants are presented in Table 1. Disease severity for susceptible cultivars usually increased with time but varied significantly among cultivars. Susceptible Royal Trophy was rated 2.2 for disease severity at 23 days postinoculation, and at 36 days, it was severely affected (rating of 3.4). The average rating for Bright Golden Anne, however, decreased from an initial 1.6 to 0.6 at 36 days after inoculation. Cultivars Bravo, Royal Trophy, Illini Trophy, Yellow Delaware, and Orange Bowl were highly susceptible on the basis of symptoms. Giant #4 Indianapolis Pink, Polaris, Puritan, and Torch were resistant. Other cultivars showed moderate susceptibility or resistance. Symptoms progressed downward from the top on Yellow Delaware, Bravo, and Orange Bowl and progressed upward from the bottom on Royal Trophy and Illini Trophy. On Nob Hill, symptoms in the middle of the plant progressed in either direction.

The extent of stunting was determined by a pairwise height comparison of inoculated and uninoculated plants (Table 1). Stunting of plants with tall growth habits, such as Early Golden Hill and Nob Hill, was severe. Stunting often occurred on moderately resistant cultivars before other symptoms appeared.

Isolations from inoculated plants showed that most were colonized by *F. oxysporum*. The extent of colonization could be correlated with susceptibility; plants susceptible on the basis of symptom severity were colonized throughout much of the stem. One plant each of susceptible cultivars Bravo and Orange Bowl were dead and had blackened stems with sporodochia of *Fusarium* produced in abundance. More resistant plants were colonized to a lesser extent than susceptible ones. There were, however, symptomless cultivars such as Puritan and Mountain Snow that were colonized in the basal portions of stems. Giant #4 Indianapolis Pink, Torch, and Polaris (all resistant by symptomatology) were highly resistant to colonization by *Fusarium*.

Colonization of single-stemmed plants. Colonization of plants by *F. oxysporum* was observed consistently 5–6 days after inoculation, frequently with early discontinuous colonization of the stem. The percentage of stem length colonized for each day postinoculation is presented in Table 2. Percent colonization increased with time in Royal Trophy and Yellow Delaware at comparable rates, despite differences in symptomatology. When symptoms first appeared (by 14 days postinoculation) the susceptible plants were colonized throughout their height. Skips in colonization (discontinuous colonization) occurred in 23 of the 248 plants sampled (9.3%). There were 10 skips in Royal Trophy plants

Table 1. Disease severity ratings and stunting of chrysanthemum cultivars inoculated with *F. oxysporum* f. sp. *chrysanthemi*

Cultivar	23 Days postinoculation		38 Days postinoculation	
	Disease severity[w]	Relative stunting[x]	Disease severity[w]	Relative stunting[x]
Bravo	2.4 ab[y]	5.6 bc[y]	3.8 a[y]	12.2 abc[y]
Royal Trophy	2.2 abc	4.6 bc	3.4 ab	11.2 abc
Illini Trophy	2.6 a	4.1 bc	2.4 bcd	6.6 bc
Yellow Delaware	2.0 a	3.6 bc	2.4 bcd	8.1 abc
Orange Bowl	1.4 cd	3.6 bc	3.0 abc	8.1 abc
Mandalay	1.4 cd	6.1 bc	1.2 def	8.1 abc
Nob Hill	1.4 cd	12.7 a	1.8 cde	15.2 ab
Early Golden Hill	1.6 bc	7.6 ab	1.2 def	19.8 a
Bright Golden Anne	1.6 bc	5.6 bc	0.6 ef	9.1 abc
Mountain Snow	0.6 de	5.6 bc	0.4 f	3.6 bc
Mountain Pink	0.4 e	2.5 bc	0.8 ef	4.1 bc
Bluechip #2	0.6 de	0.0 cd	0.8 ef	0.5 c
Giant #4 Indianapolis Pink	0.2 e	−4.1 d[z]	0.2 f	0.0 c
Polaris	0.2 e	2.5 bc	0.0 f	2.5 c
Puritan	0.0 e	0.0 cd	0.2 f	2.5 c
Torch	0.0 e	0.0 cd	0.0 f	0.5 c

[w] Disease severity index: 0 = no symptoms; 1 = generalized chlorosis, stunt; 2 = chlorosis and curvature of leaves, stunt; 3 = chlorosis, necrosis, curvature of leaves, stunt; 4 = severe chlorosis, necrosis, stunt and wilt; 5 = death of shoots or branches, black streak on stem; data are means of five replicates.

[x] Relative stunting = difference in height in centimeters between paired inoculated and uninoculated plants; data are means of five replicates.

[y] Within columns, means with the same letter are not significantly different ($P = 0.05$) according to Duncan's least significant difference test.

[z] Giant #4 Indianapolis Pink plants averaged 4.1 cm taller when inoculated, resulting in negative value.

Table 2. Colonization of single-stemmed plants of two cultivars of chrysanthemum inoculated with *Fusarium oxysporum* f. sp. *chrysanthemi*

Days postinoculation	Percent colonization[y]	
	Yellow Delaware	Royal Trophy
3	0.0 e[z]	8.3 e[z]
4	12.5 cde	11.3 e
5	0.0 e	35.0 cde
6	7.5 de	25.0 de
7	43.8 abc	50.0 bcd
8	32.3 bcde	50.0 bcd
9	38.3 abcd	75.8 ab
10	32.5 bcde	65.4 abc
11	31.3 bcde	44.9 bcd
12	56.3 ab	73.9 ab
13	60.8 ab	59.1 abc
14	50.7 ab	56.9 abcd
15	73.2 a	72.4 ab
16	65.0 ab	85.2 a

[y] Percent colonization = height colonized (cm)/height of plant (cm) × 100; values are means for eight replicates in two experiments.

[z] Within columns, means with the same letter are not significantly different ($P = 0.05$) according to Duncan's least significant difference test.

(8.1%) and 13 skips in Yellow Delaware plants (10.5%). Skips occurred most frequently (43.5%) on days 7, 8, and 9 postinoculation.

Colonization of branched plants. Despite the delay in sampling, colonization increased with time, with no significant differences between cultivars Yellow Delaware and Royal Trophy. The extent of colonization was correlated with the number of branches colonized. The total number of branches per plant varied from three to six (mean 4.6) and the number of branches colonized ranged from zero to five, an important factor that is often indicative of unilateral colonization. About 50% of the branched plants sampled showed unilateral colonization and symptoms (including stunting), whereas the other side was unaffected.

Discontinuous colonization was noted in 19.8% (19 of 96) of the plants sampled. Yellow Delaware plants had 10 of the 19 total skips in colonization, usually with a skip of 2–4 cm. Skips occurred most often at the bases of plants or branches.

DISCUSSION

Chrysanthemum cultivar responses to Fusarium wilt have been difficult to categorize because of symptom variability and similarities to other disorders (2,5). These studies refined and extended the preliminary work of N. L. Fisher (*unpublished*) and other researchers (3,5–7) by describing and rating symptoms on 16 cultivars (including cultivars not previously reported on) under conditions of root wounding that ensured infection with a short incubation period.

Although rating scales and incubation times may need adjustment for conditions other than those used here, results indicated that symptoms can optimally be rated 3 wk after inoculation but should also be rated at 5–6 wk to differentiate those cultivars that may grow out of or tolerate disease.

Stunting is an important symptom, especially on cultivars with a tall growth habit, that occurs early in infection but is often unnoticed. Height of plants was increased for inoculated Giant #4 Indianapolis Pink, but the reason for the stimulation is unknown (1).

Significant differences in cultivar reactions were observed. There is currently no explanation for the extraordinary variation in symptom progression, which would offer possibilities for pathogen/host physiology studies. Some cultivars, such as Bright Golden Anne and Mandalay, showed initial symptoms that diminished as plants aged. Possible explanations for this include walling off of the fungus by tyloses, gums, or gels (1) or perhaps a decrease in mycelial mass or metabolites (10).

Previous colonization studies dealt with histopathology of single-stemmed plants only (3). Our results show that colonization data are important and reliable indicators of cultivar susceptibility or resistance. Rate and extent of colonization and skips in colonization for single-stemmed and branched plants may vary with resistance of cultivars. Susceptible plants were colonized throughout their height by the time symptoms were definitive.

The discontinuous colonization of chrysanthemum stems, usually early in infection, was a significant find. The isolates from sections above skips were tested for pathogenicity and proved to be virulent *F. oxysporum* f. sp. *chrysanthemi*, morphologically identical to the inoculum used. These results indicate that conidia are probably produced in the xylem vessels and carried upward ahead of the advancing mycelium. These spores could germinate and initiate growth in a new location. The open-ended vessels of chrysanthemum would pose little barrier to upward movement of spores. Movement of conidia in vessels has been reported with Fusarium wilt of tomato and banana but does not occur in Fusarium wilt of carnation (12) and was thought not to occur in chrysanthemum on the basis of previous studies (3). In future experiments, sections taken from intervals of less than 2.54 cm should be indexed. Anatomical studies should attempt to observe conidia and initiation of new growth by germination of spores ahead of advancing mycelium.

The occurrence of asymptomatic plants, discontinuous colonization, and unilateral infection of branched plants similar to those grown as stock plants in the chrysanthemum industry (2) raise concern about the effectiveness of culture-indexing (11) as a control measure. Culture-indexing will assist in the production of pathogen-free cuttings for propagation. If there is a possibility of infection above an indexed area, or unilateral infection, extreme care must be taken to ensure clean cuttings. Stock plants should be monitored periodically, infected plants rogued, and strict sanitation measures practiced. Because of the danger to the industry of symptomless, infected plants, the use of controls that mask symptoms is not recommended (6,7). Because of the variability of symptom expression on chrysanthemum cultivars, colonization is a more reliable test for infection and susceptibility. If symptoms are rated, a standardized method as described in this paper should be followed. Resistance to Fusarium wilt does exist in some chrysanthemum cultivars, and should be utilized in breeding programs when possible.

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