Stub Inoculations Do Not Incite Fusarium Wilt of Chrysanthemum Caused by *F. oxysporum* f. sp. *chrysanthemi*

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ABSTRACT

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Fusarium oxysporum f. sp. *chrysanthemi* normally penetrates roots and root hairs, aided by wounding. Inoculations of two chrysanthemum cultivars susceptible to Fusarium wilt were made at aerial stem stub sites but did not initiate the disease. This supports the hypothesis that the Fusarium wilt fungus cannot be spread by workers taking cuttings or pinching plants.

Fusarium wilt of the florists' chrysanthemum (*Chrysanthemum morifolium* (Ramat.) Hemsl.) caused by *F. oxysporum* f. sp. *chrysanthemi* Litt., Armst., & Armst. (1) has increased in geographic distribution and disease incidence in recent years, a fact that has prompted research aimed at understanding this disease. History and symptoms of the disease were discussed by Emberger and Nelson in 1981 (2).

The infection process in many Fusarium wilt diseases is aided by wounding of roots as in banana wilt (7), tomato wilt (9), and melon wilt (6). The development of Fusarium wilt of chrysanthemum is aided by wounding of the roots (2) although wounding is not necessary for infection to take place, as in sweet potato wilt (5). Interactions between *Meloidogyne* spp. and Fusarium wilt have also been observed to increase disease severity (4). Toop (8) investigated root inoculation procedures that included dipping cuttings in broth cultures of the pathogen as well as planting cuttings directly into infested soil. A search of the literature did not reveal any studies, however, on inoculation of stems with *F. oxysporum* f. sp. *chrysanthemi*. A stub of a stem results from pinching plants to promote branching or from harvesting cuttings from stock plants and may provide an infection site for *Fusarium*. This research sought to answer whether the pathogen could be spread by workers taking cuttings or pinching plants in a commercial chrysanthemum greenhouse.

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MATERIALS AND METHODS

Sixty chrysanthemum cuttings were planted individually in a 1:1:1 soil (Hagerstown silt loam:peat:perlite) mixture in 12.5-cm-diameter clay pots. Plants were maintained on greenhouse benches equipped with heating cables to maintain a soil temperature of 27–29 C and under a 24-hr photoperiod to inhibit bud formation. Plants were watered with a Chapin drip-irrigation system (10) to eliminate water splash and were fertilized weekly with a 20:20:20 (NPK) blend containing ammoniacal nitrogen.

Plants of the susceptible cultivars Yellow Delaware and Illini Trophy were inoculated 2 wk after planting by the finger method or the cutting tool method using one of two known virulent isolates of *F. oxysporum* (FRC-0-693 and FRC-0-734) and a water control. In the finger method, the experimenter's fingers were dipped in a spore suspension (60,000 spores/ml distilled H_2O) and plants subsequently pinched. The tool method involved use of an aluminum cutting tool dipped in a spore suspension and used to break off stem cuttings about 6–7 cm long. A cutting tool of this type is used in the chrysanthemum industry as a measuring device and succulent shoots can easily be snapped off against the edge of the tool. A completely random experimental design was used, with five replicates of each treatment for each of the two cultivars.

Plants were observed for symptoms at 4, 6, 8, 12, and 13 wk after inoculation. On each sample date, one replicate of each treatment for both cultivars (a total of 12 plants) was cut off at the soil line and stripped of leaves and small shoots for indexing. Outlines of plants were made on paper and plants were surface-sterilized in a 10% solution of Clorox (5.25% sodium hypochlorite). Plants were indexed for infection with *Fusarium* at 2.5-cm intervals by placing cross sections of stems on carnation leaf agar (CLA) (3). *Fusarium* could be observed from sections in 3–4 days. All cultures isolated were transferred for identification and tested for pathogenicity on susceptible chrysanthemums at the end of experiments.

The study was repeated using only the cultivar Yellow Delaware and isolate FRC-0-693. Plants were inoculated by placing one drop of a suspension containing 70,000 spores per milliliter on the stub site. Half of the plants were covered with a plastic bag for 48 hr to maintain high relative humidity in the infection court.

RESULTS

Symptoms of Fusarium wilt were not evident on plants at any of the five

Table 1. Colonization of 2.5-cm-long stem sections of chrysanthemum cultivars Yellow Delaware and Illini Trophy by *Fusarium oxysporum* f. sp. *chrysanthemi* isolates FRC-0-693 and FRC-0-734 inoculated at a stem stub site

Treatment	Colonization ratings[a]				
	4 Wk	6 Wk	8 Wk	12 Wk	13 Wk
Illini Trophy FRC-0-734 (F)[b]	1[c]	1	1	4	1
Illini Trophy FRC-0-734 (T)	1	0	0	0	0
Yellow Delaware FRC-0-734 (F)	1	1	2	*[d]	0
Yellow Delaware FRC-0-734 (T)	0	3	1	1	1
Illini Trophy FRC-0-693 (F)	1	1	1	2	3
Illini Trophy FRC-0-693 (T)	1	1	1	1	0
Yellow Delaware FRC-0-693 (F)	1	1	0	0	0
Yellow Delaware FRC-0-693 (T)	0	0	1	2	0

[a]Colonization ratings: 0 = no *Fusarium*, 1 = stub section (2.5 cm), 2 = stub section and section below stub (5 cm), 3 = stub section and two sections below stub (7.5 cm), etc. No colonization observed in control plants.
[b]F = Inoculation with spore suspension on fingers; T = inoculation with cutting tool.
[c]Value is for one plant sampled.
[d]* = Plant colonized from base to height of 17.5 cm, probably root-infected cutting.

observations. When plants were indexed, *F. oxysporum* was often found in the stub section but rarely colonized other portions of the plant. The results are presented in Table 1, which indicates the number of 2.5-cm sections (including the stub site) colonized from the stub downward. The fungus was not isolated from branches rising from the main plant stem. Only data for inoculated plants are presented because control plants were never colonized by *F. oxysporum*.

F. oxysporum was found in 65% of the inoculated plants. In 40% of the plants, the fungus only colonized the stub section. In some plants (15%), the fungus did colonize 2.5–5.0 cm further below the stub but the spread of the fungus was not extensive.

Statistical differences were not found nor could correlations be made between the isolates of *Fusarium* used, the cultivar of chrysanthemum, or the inoculation technique used.

Similar results were obtained when the experiment was repeated, with about 40% of inoculated plants colonized by *Fusarium* at the stem stub site only. Pathogenicity tests with isolates from inoculated plants produced symptoms on Yellow Delaware chrysanthemums, indicating recovery of the original virulent isolates used for inoculations.

DISCUSSION

In this study, inoculations of chrysanthemum stem stubs with *F. oxysporum* f. sp. *chrysanthemi* did not result in vascular wilt disease, even after 12 wk of incubation. These results indicated that Fusarium wilt did not result from transmission of the fungus from plant to plant by workers pinching plants or taking cuttings and carrying *F. oxysporum* spores on their fingers or on cutting tools. The fungus was able to survive, however, in the plant tissue at the stub site. It is possible that the fungus is able to colonize the cortex but cannot penetrate the stele to initiate the typical vascular wilt disease. There may be other explanations for this phenomenon that offer possibilities for further research. Plants under environmental stress should be studied to determine whether the fungus may colonize tissue more readily if the plant is stressed.

The pathogenicity tests with cultures isolated from stub sections showed they were typical of the original virulent isolates used for inoculations. If these fungi had penetrated and colonized plant stems in the usual manner by root infection, they would have been capable of inciting Fusarium wilt. Therefore, strict sanitation measures should continue to be maintained in chrysanthemum production. If spores or infected plant material are left in contact with the soil around the plants, normal root infection and vascular wilt could easily occur.

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