Decay and Colonization of Gladiolus Corms by the Pine Pitch Canker Fungus

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ABSTRACT

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Gladiolus sp. 'White Friendship' corms were inoculated in the laboratory with strains of *Fusarium moniliforme* var. *subglutinans* (FMS), the causal agent of pitch canker of pines. Isolates were from six species of pines, from pine plantation soil, and from gladiolus corms. The inoculations resulted in slight-to-moderate decay of the corms, and all isolates were recovered from corm tissue beyond the zone of visible decay. In another experiment, corms were inoculated with two strains of FMS from pitch cankers, two other species of *Fusarium*, and several other genera of plant pathogenic fungi. Only the *Fusarium* isolates caused decay and could be recovered from the corms. In a greenhouse experiment, corms were grown in soil infested with two strains of *F. oxysporum* f. sp. *gladioli* and nine strains of FMS. Significant decay was caused by both strains of *F. oxysporum* f. sp. *gladioli* and by two of the nine strains of FMS. Almost all strains were recovered from the corms. The decay of corms and the colonization of asymptomatic tissue by FMS indicate its potential as a pathogen in the storage and production of gladiolus corms.

Pitch canker of southern pines is caused by *Fusarium moniliforme* Sheld. var. *subglutinans* Wr. & Reink (11). Since 1974 the disease has been severe on planted slash pine in Florida (7,18) and on loblolly pine seed orchards in other southern states (4). Isolates of *F. moniliforme* var. *subglutinans* (FMS) from loblolly, slash, shortleaf, longleaf, and Virginia pines cause cankers and shoot dieback on inoculated slash and loblolly pine seedlings regardless of the source (5).

F. moniliforme var. *subglutinans* is a widespread pathogen of many hosts (3). Dwinell and Nelson (6) report that FMS isolates from a wide variety of agronomic and ornamental crops are not pathogenic to slash and loblolly pine with the exception of isolates coming from Florida-grown gladiolus corms.

Gladioli are one of the most important cut-flower crops in Florida. The state produces about two-thirds of the crop sold annually in the United States (12). A serious corm rot of gladiolus is caused by *F. oxysporum* f. sp. *gladioli* (Massey) Snyd. & Hans. (14,20,21); however, Woltz et al (22) demonstrated that other species of *Fusarium* (including FMS) can be isolated from commercially grown corms and that these isolates are potentially important pathogens of gladiolus.

Since some gladiolus isolates of FMS are pathogenic to pine seedlings, pine isolates also may be potential pathogens of gladiolus. Pitch canker has been reported in almost every county throughout Florida (17), so gladiolus-producing areas coincide with those having pine pitch canker disease. If the host range of the pitch canker strain of FMS included gladiolus, it could have an economic impact on gladiolus corm production, storage, and vigor.

This study, therefore, was undertaken to determine whether various strains of FMS from several sources could colonize corms and subsequently cause decay, and whether these strains would be more destructive to corms than several genera of common plant pathogenic fungi. A preliminary report (2) on this research has been published.

MATERIALS AND METHODS

Inoculation of corms with strains of FMS from various sources. A total of 25 cultures of FMS grown from single spores were used to inoculate the corms. Seven cultures were isolated from gladiolus corms. Three were from pine-plantation soils in Volusia County, Florida. These soil isolates were proven to be pathogenic to pines and are considered to be the pitch canker strain of FMS. The other cultures were isolated from active pitch cankers on slash pine (*Pinus elliottii* Engelm. var. *elliottii*), South Florida slash pine (*P. elliottii* var. *densa* Little & Dorman), loblolly pine (*P. taeda* L.), shortleaf pine (*P. echinata* Mill.), longleaf pine (*P. palustris* Mill.), and Virginia pine (*P. virginiana* Mill.).

The inoculum was grown in shake cultures at 25 C for 4 days in Czapek's sucrose nitrate solution in 250-ml Erlenmeyer flasks on a Burell Wrist-Action Shaker. Inoculum density was adjusted to 10^6 conidia per milliliter with a Coulter Particle Counter.

One hundred gladiolus corms (*Gladiolus* sp. L. 'White Friendship') were used as host material. Four corms were inoculated with each fungus isolate, and four others served as controls. Each corm was dehusked, surface sterilized for 5 min in 0.525% sodium hypochlorite, and rinsed in sterile deionized water. There were two inoculation sites per corm, each perpendicular to the row of shoot buds. Holes were punched 1.25 cm deep into the corm with a sterile #3 cork borer. Inoculum (0.1 ml) was dispensed into each hole with a micropipet. Controls received 0.1 ml of sterile deionized water in each hole. The holes were plugged with sterile cotton and the corms were placed individually in polyethylene bags containing a sterile paper towel and 10 ml of sterile deionized water. The corms were then incubated at 25 C for 3 wk under lights (2,691 lux) set for a 12-hr photoperiod.

At the end of the experiment, the corms were split vertically through the inoculation points and evaluated for decay. They were rated on a scale from 1 to 4 (1 = no decay; 2 = slight; 3 = moderate, and 4 = extensive (Fig. 1B to D). To recover the fungus, a total of four plugs (4 × 125 mm) were aseptically removed from tissue areas beyond the zone of visible rotting, one on each side of the two inoculation points in the corm, and plated on PCNB agar (16) amended with 1,000 μg of streptomycin sulfate and 120 μg neomycin sulfate per milliliter.

Data were analyzed by nested analysis of variance procedures (8) and Kramer's extension of Duncan's multiple range test to group means with unequal numbers of replications (10).

Inoculation of corms with FMS and other plant pathogenic fungi. To determine whether pitch canker strains of FMS cause more decay in gladiolus corms than other plant pathogenic fungi, four isolates of FMS and seven isolates of other plant pathogenic

fungi were tested. Two isolates of FMS came from pitch cankers on slash and longleaf pines, and the other two isolates came from gladiolus corms. Two isolates of *F. oxysporum* f. sp. *gladioli* (Massey) Snyd.& Hans. from gladiolus, one virulent (O-700) and the other slightly virulent (O-706), were used. One isolate each of *F. oxysporum* Schlect. Snyd. & Hans. from snapbean, *F. moniliforme* Sheld. from slash pine, *Diplodia* sp. Fr. and *Pestalotia* sp. de Not, both from loblolly pine seed, and *Verticillium albo-atrum* Nees. from cotton were compared against FMS.

Forty-eight gladiolus corms were inoculated and evaluated for decay as described previously. The incubation time of the experiment was 4 wk. To recover the *Fusarium* spp., the corms were plated on specialized PDA for *Fusarium* isolation (1) instead of PCNB (16) to give a better separation of colony color. The *Diplodia, Pestalotia*, and *Verticillium* spp. were plated on noncommercial PDA. Data were analyzed by analysis of variance procedures and by Duncan's multiple range test.

TABLE 1. Decay of gladiolus corms caused by strains of *Fusarium moniliforme* var. *subglutinans*

Source of isolates	No. of isolates	Decay rating (mean)[a]	Recovery of fungus[b] (mean %)
Gladiolus	7	2.79 x	100
Pine plantation soil	3	2.69 x	100
Slash pine	4	2.50 x	84
South Florida slash pine	2	2.39 xy	94
Virginia pine	2	2.25 xy	62
Longleaf pine	1	2.00 xy	94
Loblobby pine	4	1.81 y	75
Shortleaf pine	2	1.75 y	78
Control		1.00	0

[a] Based on four corms per isolate. Decay was rated on a scale of 1 to 4: 1 = none; 2 = slight; 3 = moderate; and 4 = extensive. Means followed by the same letter are not significantly different, $P = 0.05$, according to Kramer's extension of Duncan's multiple range test for grouping means with unequal numbers of replications.

[b] Based on four corms per isolate. A total of four plugs (4 × 125 mm) were aseptically removed from tissue beyond the zone of visible decay and plated on a *Fusarium*-selective medium.

Growth of corms in FMS-infested soil. In a greenhouse experiment, corms were grown in pots of soil infested with FMS and *F. oxysporum* f. sp. *gladioli* to determine whether the fungi would colonize and decay corms planted in the soil. Two isolates of *F. oxysporum* f. sp. *gladioli* (O-700 and O-706) and nine isolates of FMS, three from gladiolus corms, two from pine-plantation soil, two from slash pine pitch cankers, and two from South Florida slash pine pitch cankers, were used as inoculum. The isolates were grown for 7 days on PDA plates. The plates were flooded with 30 ml of sterile deionized water and scraped into 1-L Erlenmeyer flasks containing 200 g of twice-autoclaved wheat grain and 150 ml of sterile deionized water. The flasks were incubated at 24 C for 10 days under a 12 hr photoperiod.

Forty-eight corms were surface sterilized in 0.525% sodium hypochlorite, rinsed in sterile deionized water, and planted in 15.2-cm (6-in.) diameter plastic pots containing a sand:soil:vermiculite (1:1:1, v/v) mix. The corms were planted in a layer of the fungus-wheat mixture 5 cm (2 in.) deep in the pots. Each treatment had four replicate pots each containing one corm. The control consisted of four replicate pots with the corms planted in a layer of sterile wheat grain.

After 4 wk on a greenhouse bench, the corms were dug up and evaluated for decay. They were split vertically and rated for decay as described previously. To recover the fungi, plugs of tissue were aseptically removed from the corms and plated on the *Fusarium*-PDA medium (1).

Data were analyzed by analysis of variance and by Duncan's multiple range test.

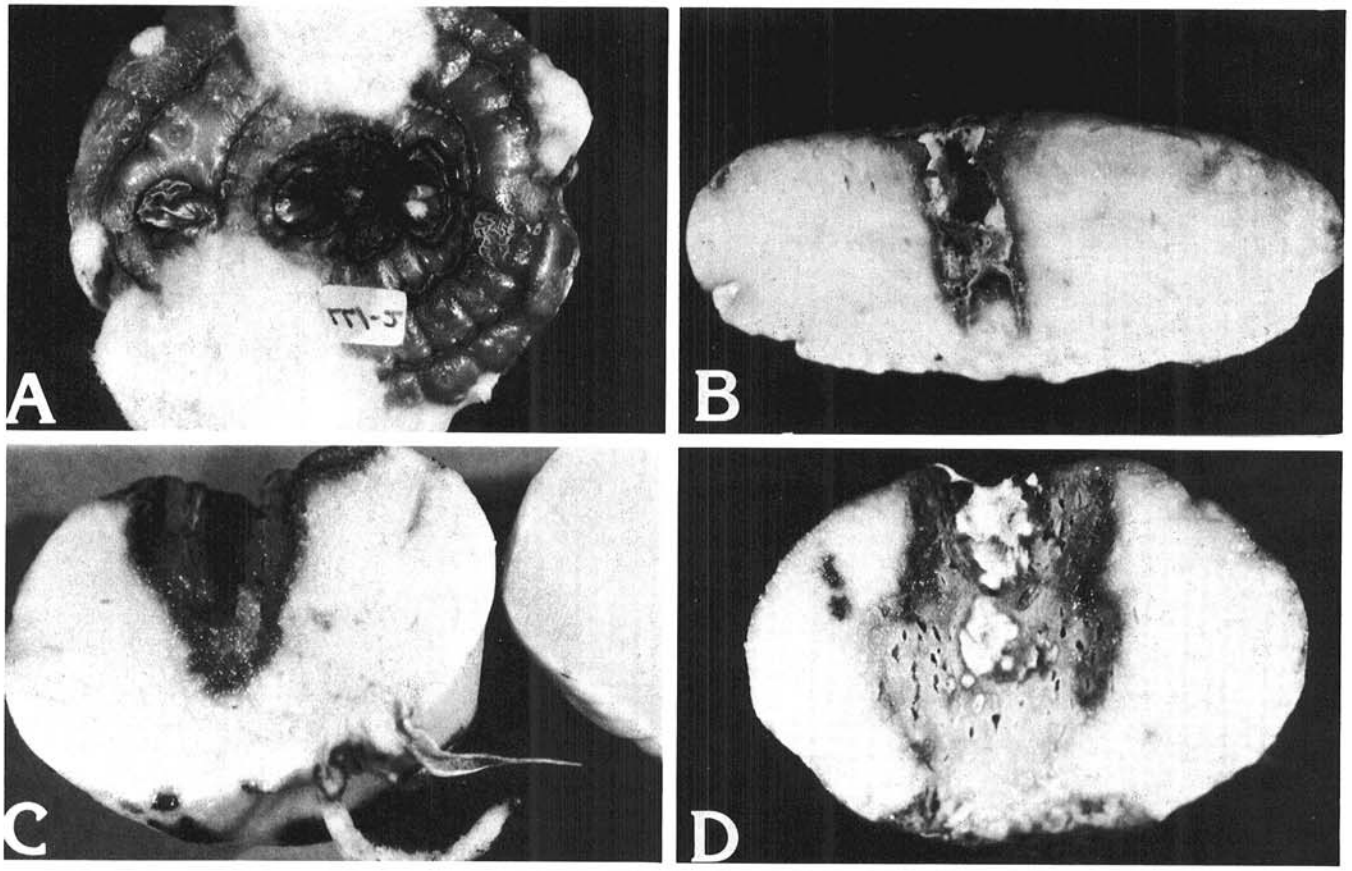

Fig. 1. Signs and progressive symptoms of infection of gladiolus corms by *Fusarium moniliforme* var. *subglutinans*: **A,** mycelium growing from an inoculated gladiolus corm. Decay of inoculated corms was rated on a scale of 1 to 4: No decay = 1; **B,** slight decay = 2; **C,** moderate decay = 3; and **D,** extensive decay = 4.

RESULTS

Inoculation of corms with strains of FMS from various sources. All of the corms inoculated with the 25 isolates of FMS had slight-to-moderate decay depending upon the source of the isolate. Corms inoculated with the isolates from gladiolus, pine-plantation soil, and slash pine had the highest mean decay rating (Table 1), ranging from 2.5 for the slash isolates to 2.79 for the gladiolus isolates. The isolates from longleaf, South Florida slash, and Virginia pines caused slightly less decay. Loblolly and shortleaf pine isolates caused significantly less decay than all other isolates, with ratings of 1.85 and 1.75, respectively. The control corms had no decay and rated 1.0. Statistical analysis revealed that variation was due to isolates within sources.

There was 100% recovery of the fungus from corms inoculated with the gladiolus and pine-plantation soil isolates (Table 1). Recovery of other isolates ranged from 62% for those from Virginia pine to 94% for those from slash pine. No FMS was isolated from the control treatments.

Decayed corms were characterized by growth of fluffy mycelium emerging from the inoculation points (Fig. 1A to D). In severe cases, the entire corm was covered with mycelium. When the decayed corms were split open, a distinct zone of reddish-brown tissue was observed around the inoculation holes. In slightly decayed corms, the zone was confined to an area directly around the holes. In more extensively decayed corms, the zone of rotted tissue extended well into the flesh of the corm. All corms except those with the worst decay sprouted and produced shoots during incubation.

Inoculation of corms with FMS and other plant pathogenic fungi. Of the plant pathogenic fungi tested, only *Fusarium* spp. caused corm decay (Table 2). *F. oxysporum* f. sp. *gladioli* (O-700) and *F. oxysporum* had the highest mean decay ratings: 4.0 and 2.75, respectively. The isolates of FMS from gladiolus averaged 2.37 and the isolates from pitch cankers averaged 1.94. The isolate of *F. moniliforme* had a decay rating of 1.88. The less virulent isolate of *F. oxysporum* f. sp. *gladioli* (O-706), *Diplodia* sp., *Verticillium albo-atrum, Pestalotia* sp., and the control treatments all showed no decay and received a rating of 1.0.

The corms inoculated with FMS isolates showed decay symptoms similar to those observed in the previous experiment. However, in corms decayed by *F. oxysporum* f. sp. *gladioli* (O-700) and *F. oxysporum* large pockets of tissue around the inoculation hole were completely rotted out. The tissue surrounding the decay pockets was yellowish-brown with brown concentric rings. The tissue in the control treatment remained firm and creamy white.

TABLE 2. Reaction of gladiolus corms inoculated with a variety of plant pathogens

Isolate	Mean rot rating[a,b]	Reisolation[c] (mean %)
F. oxysporum f. sp. *gladioli* (O-700)	4.00 w	25
F. moniliforme var. *subglutinans* (gladiolus)	2.75 x	75
F. oxysporum	2.75 x	50
F. moniliforme var. *subglutinans* (pitch canker longleaf)	2.12 y	100
F. moniliforme var. *subglutinans* (gladiolus)	2.00 y	25
F. moniliforme	1.87 y	75
F. moniliforme var. *subglutinans* (pitch canker slash)	1.75 y	75
F. oxysporum f. sp. *gladioli* (O-706)	1.00 z	0
Diplodia sp.	1.00 z	0
Verticillium albo-atrum	1.00 z	0
Pestalotia sp.	1.00 z	0
Control	1.00 z	0

[a] Based on four corms per isolate. Decay was rated on a scale of 1 to 4 (1 = no decay; 2 = slight; 3 = moderate; 4 = extensive).

[b] Means followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

[c] Based on four corms per isolate. A total of four plugs (4 × 125 mm) were aseptically removed from tissue areas beyond the zone of visible decay and plated on appropriate media.

The isolates of the pitch pine canker pathogen were reisolated and recovery averaged 87% (Table 2). Recovery rates for the gladiolus isolates and for *F. moniliforme* averaged 75%. Recovery of the virulent isolate of *F. oxysporum* f. sp. *gladioli* (O-700) from inoculated corms averaged 25%, that of the less virulent isolate (O-706) averaged 75%, and *F. oxysporum* (isolated originally from snapbean) was recovered at 50%. None of the non-*Fusarium* species were recovered from the corm tissue. Only corms inoculated with *F. oxysporum* f. sp. *gladioli* (O-700) failed to sprout and produce normal shoot growth.

Growth of corms in FMS-infested soil. Corms grown in soil infested with *F. oxysporum* f. sp. *gladioli* (O-700) had a high mean decay rating of 4.0 (Table 3). Isolate O-706 and two FMS isolates, one from soil and the other from slash pine canker, had mean decay ratings of 2.75 and 2.00, respectively. The rest of the isolates caused slight decay, but did not differ significantly from the control treatment which caused no visible decay.

Decay in the corms inoculated with *F. oxysporum* f. sp., *gladioli* was characterized by areas of yellowish-brown, water-soaked tissue near the surface of the corm (Fig. 2A). Corms decayed by FMS isolates, regardless of the source, had light brown to black tissue in the center of the corm and an occasional pocket of decay near the surface (Fig. 2B).

All isolates were recovered from the corms at a rate of 50–100% except for the two soil isolates which were not recovered. No *Fusarium* was isolated from the noninoculated controls.

Corms with no visible decay produced vigorous shoot growth

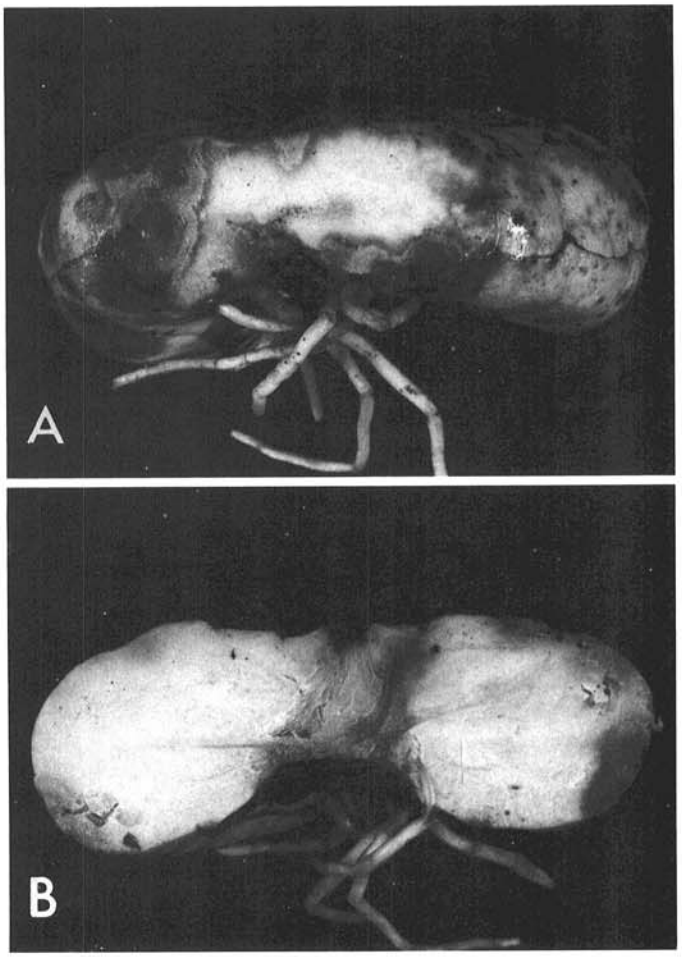

Fig. 2. Symptoms caused in gladiolus corms grown in soil infested by *Fusarium* spp.: **A,** decay of corms infected by *F. oxysporum* f. sp. *gladioli* was characterized by areas of yellowish-brown water-soaked tissue near the surface of the corm; **B,** decay of corms infected by *F. moniliforme* var. *subglutinans* was characterized by light brown to black tissue in the center of the corms with an occasional pocket of decay near the surface (longitudinal section).

during the experiment. However, corms grown in soil infested with *F. oxysporum* f. sp. *gladioli* (O-700) did not grow, and those grown in soil infested with the O-706 isolate and the two FMS isolates which had caused significant decay, had noticeably less shoot growth than did nondecayed corms.

DISCUSSION

F. moniliforme var. *subglutinans* has a wide host range and geographic distribution. Generally, isolates of FMS not originating from pitch cankers, with the exception of some soil isolates, will not cause pitch canker disease symptoms in inoculated pines. However, gladiolus isolates of FMS from Florida were pathogenic to pines in greenhouse tests, indicating that the pitch canker strain of FMS does not come from pines alone. Our results show that the pitch canker strain of FMS can cause decay of corms and colonize asymptomatic tissue. In Florida, the same strain of FMS that causes pitch canker disease of pines also can be isolated from gladiolus corms.

Fusarium spp. other than *F. oxysporum* f. sp. *gladioli* have been implicated in rotting of stored gladiolus corms (19,22). Crop failures due to little or no plant emergence have been associated with FMS and other *Fusarium* species (22). In artificially inoculated corms, isolates of FMS from gladiolus, pine pitch cankers, and pine-plantation soil had approximately the same range of infectivity. However, corms decayed by FMS exhibited different symptomology than those decayed by *F. oxysporum* f. sp. *gladioli*. Most isolates of FMS were recovered from inoculated corms whether they had been decayed or were asymptomatic.

F. moniliforme var. *subglutinans* also has been implicated as a disease agent in storage tissue of other hosts. It was isolated from slash and loblolly pine seed with the gametophyte and embryo tissue rated from healthy to badly deteriorated (15). FMS has also been found in sections of cornstalk tissues contiguous to diseased areas in ear, root, leaf, and crown tissues (9).

Severity of decay may be influenced by environmental conditions. Although the virulent isolate of *F. oxysporum* f. sp. *gladioli* consistently rotted corms in the laboratory and the greenhouse, it was interesting that the less virulent isolate (which caused no decay in the lab experiment) was rated second highest for decay in the greenhouse experiment and also had a high recovery rate in both experiments. Magie and Woltz (13) found that *Fusarium*-infected and susceptible corms grown in red lateritic soil where the previous crop was sugarcane remained healthy. However, when the corms were removed to sandy soils, they rotted, indicating that they continued to harbor *Fusarium* and that the expression of disease was influenced by environmental conditions.

Gladiolus growers should be advised against locating corm production areas near pine stands with a high incidence of pitch canker or on sites previously cleared of diseased trees. Although FMS infects pines only through a wound (7), our results show that the pitch canker strain of FMS can directly colonize gladiolus corms in infested soil. Under the proper conditions, this fungus could pose a problem in the storage and production of gladiolus corms.

TABLE 3. Decay of gladiolus corms grown in soil infested with isolates of *Fusarium moniliforme* var. *subglutinans* and *F. oxysporum* f. sp. *gladioli*

Isolate	Mean rot rating[a,b]	Mean % recovery[c]
F. oxysporum f. sp. *gladioli*		
isolate O-700	4.00 x	50
isolate O-706	2.75 xy	100
F. moniliforme var. *subglutinans* (soil)	2.00 xy	0
(pitch canker slash)	2.00 xy	100
(gladiolus)	1.88 yz	100
(gladiolus)	1.88 yz	100
(pitch canker S. Fla. slash)	1.75 yz	75
(pitch canker loblolly)	1.75 yz	75
(gladiolus)	1.50 yz	100
(soil)	1.25 z	0
(pitch canker S. Fla. slash)	1.25 z	75
Control	1.00 z	0

[a] Based on four corms per isolate. Decay was rated on a scale of 1 to 4 (1 = no decay; 2 = slight; 3 = moderate; 4 = extensive).

[b] Means followed by the same letter are not significantly different according to Duncan's multiple range test, $P = 0.05$.

[c] Based on four corms per isolate. A total of four plugs (4 × 125 mm) were aseptically removed from tissue beyond the zone of visible decay and plated on a *Fusarium*-selective medium.

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