

# Characterization of *Fusarium* Isolates from Gladiolus Corms Pathogenic to Pines

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## ABSTRACT

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*Fusarium subglutinans* is a well-known pathogen of many crops. The fungus causes a serious disease of pines known as pitch canker. A proposal has been made to designate isolates from pines *F. subglutinans* f. sp. *pini* (*F. s. f. sp. pini*). An enigma regarding the nomenclature is that some *F. s. f. sp. pini* isolates have been reported to induce decay of gladiolus corms whereas certain isolates of *F. subglutinans* from gladiolus corms were also found to be weakly to moderately pathogenic to pine seedlings. In this study we re-examined three previously studied isolates from gladiolus in the United States that had been referred to as *F. subglutinans* (= *F. moniliforme* var. *subglutinans*) or *F. moniliforme* in the literature. We found that these isolates were typical of *F. proliferatum*. They were mildly pathogenic on pine seedlings and significantly less virulent than an isolate of *F. s. f. sp. pini*. Inoculation of gladiolus corms with these isolates of *F. proliferatum* from gladiolus, *F. oxysporum* from pine seedlings, and *F. subglutinans* from pines (*F. s. f. sp. pini*), corn, mango, and pineapple indicated that only isolates of *F. proliferatum* were moderately pathogenic to gladiolus corms. This study therefore supports the proposal that isolates of *F. subglutinans* from pines represent a specific forma specialis within the species.

*Fusarium subglutinans* (Wollenweb. & Reinking) P. E. Nelson, T. A. Toussoun, & Marasas is a pathogen of many crops (2). The host range of the fungus includes several hosts of economic importance such as pines, corn, mango, and pineapple (2,6,7,11,20). Some strains of this species are host specific, and apparently do not cause disease of other hosts. This has led to the proposal of formae speciales in *F. subglutinans* (4,25).

Gladiolus (*Gladiolus* L.) is a cut-flower crop widely planted in the United States, especially in Florida (12). A serious disease affecting the culture of gladioli is *Fusarium* corm rot, caused by *F. oxysporum* Schlechtend.:Fr. f. sp. *gladioli* (L. Massey) W. C. Snyder & H. N. Hans. (*F. o. f. sp. gladioli*) (16,27). Other *Fusarium* spp., including *F. subglutinans* and *F. roseum* Link:Fr. emend. W. C. Snyder & H. N. Hans. have also been isolated from commercially grown gladiolus corms; some of these isolates induce crop failure (28).

Pitch canker of pines was first described in 1946 (11), and in 1974 reached epidemic proportions in slash pine (*Pinus elliotii* Engelm. var. *elliotii*) plantations and seed orchards in the southern United

States (9,10,23). Subsequent to 1987, pitch canker has been reported from California (4,17), Mexico (21), and Japan (14,18). In South Africa, the pitch canker fungus has been responsible for a severe root disease of *P. patula* Schlechtend. & Cham. seedlings (26).

In the initial report of the disease, Hepting and Roth (11) suggested that pitch canker was caused by a species of *Fusarium* belonging to the section *Liseola*. In 1947, however, the fungus was placed in the section *Lateritium* and later called *F. lateritium* Nees:Fr. f. sp. *pini* Hepting in W. C. Snyder, Toole, & Hepting (24). Kuhlman et al. (15) characterized the pitch canker fungus and showed that the causal fungus should be called *F. moniliforme* J. Sheld. var. *subglutinans* Wollenweb. & Reinking rather than *F. lateritium* f. sp. *pini*. In 1983, *F. moniliforme* var. *subglutinans* was elevated to species rank as *F. subglutinans* (19). Later, isolates of *F. subglutinans* pathogenic to pines were assigned to a specific forma specialis as *F. subglutinans* f. sp. *pini* Correll et al. (*F. s. f. sp. pini*) (4).

One of the arguments against acceptance of a specific forma specialis for the pitch canker fungus is that isolates of this species from gladiolus corms were weakly to moderately pathogenic on stems of slash and loblolly (*P. taeda* L.) pine seedlings (8). In a subsequent study, Barrows-Broadus and Dwinell (1) also showed that the pitch canker fungus was able to cause decay of gladiolus corms. The occurrence and pathogenicity of the pitch

canker fungus on gladiolus corms therefore continues to confuse the forma specialis concept of this pathogen of growing international importance.

The objective of this study was to re-examine and identify the *Fusarium* isolates from gladiolus corms in Florida, reported to be pathogenic to pines (1,8). We also evaluated the pathogenicity of isolates of *F. subglutinans* from various hosts to gladiolus corms, and re-assessed the virulence of the *Fusarium* isolates from gladiolus corms to pine seedlings.

## MATERIALS AND METHODS

**Isolates used.** Thirteen isolates deposited in culture collections as *F. subglutinans* were used during the study (Table 1). An isolate of *F. oxysporum* (MRC 6212) from pine seedlings in South Africa was included also for comparative purposes. All isolates from single conidia were maintained in 15% glycerol at -70°C prior to use.

In a study by Woltz et al. (28), isolates M-668 and M-685 were referred to as "*F. moniliforme* 'subglutinans'" and M-669 was referred to as "*F. moniliforme*." Dwinell and Nelson (8) did not number six isolates of "*F. moniliforme* var. *subglutinans*" from gladiolus corms reported to be weakly to moderately pathogenic to slash and loblolly pine seedlings. Barrows-Broadus and Dwinell (1) also referred to seven undesigned isolates of "*F. moniliforme* var. *subglutinans*" from gladiolus corms in Florida as causing slight to moderate decay of gladiolus corms. P. E. Nelson (personal communication) indicated that isolates M-668, M-685, and M-669 are the only cultures of *F. subglutinans* isolated from gladiolus corms in Florida available at the culture collection of the *Fusarium* Research Center (FRC) (The Pennsylvania State University, University Park), and suggested that isolates of the study by Dwinell and Nelson (8) and Barrows-Broadus and Dwinell (1) were obtained from the FRC. We therefore believe that at least two of these (M-668 and M-685) are isolates of *F. subglutinans* from gladiolus corms referred to by Dwinell and Nelson (8) and Barrows-Broadus and Dwinell (1).

**Morphological studies.** Isolates from gladiolus corms were compared with an isolate typical of *F. subglutinans* (MRC 6217) from diseased roots of a pine seedling in South Africa. Procedures followed

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were similar to those described by Nelson et al. (19) for the identification of *Fusarium* species. Isolates on potato dextrose agar (PDA) (39 g of Difco potato dextrose agar powder; 1 liter of water) were incubated in the dark at 25°C and observed after 3, 7, and 10 days. Isolates on carnation leaf agar (CLA) were incubated under cool-white and dark-fluorescent lights with a 12-h photoperiod at 25°C, and observed after 4, 7, and 14 days of growth. One isolate from gladiolus corms (M-669) that formed very short chains was transferred to petri dishes containing 0.8% KCl medium to confirm the presence or absence of microconidial chains.

**Inoculation of gladiolus corms.** Gladiolus corms were inoculated using a modification of the method described by Barrows-Broadus and Dwinell (1). Inoculum was grown on PDA at 25°C for 8 days in the dark. Conidial suspensions were prepared by pouring sterile water into petri dishes and rotating them slightly to dislodge conidia. Conidial suspensions were then adjusted to  $10^6$  conidia/ml.

Corms of gladiolus cultivar Peter Pears were used for inoculations. Twelve corms were inoculated per treatment. After dehusking, corms were surface disinfested for 5 min in 3% NaOCl, and rinsed twice in sterile, distilled water. Two holes perpendicular to the row of shoot buds were punched  $\pm 1$  cm deep with a sterile #2 cork borer (diameter 5 mm). Inoculum (0.1 ml) was dispensed into each wound with a micropipet and plugged with sterile cotton wool. Corms used as controls were inoculated with sterile water. Corms were then placed individually on sterile, wet paper towels (5 ml of water) in gamma-irradiated plastic bags. Bags containing gladiolus corms were incubated in a completely randomized block design in a growth room at 24°C for 30 days under cool-white and dark-fluorescent lights set at a 12-h photoperiod. An additional 5 ml of sterile water was added to each plastic bag after 3 weeks to increase the humidity, which is important for disease development (27).

Decay of corms was rated on a scale from 1 to 4, with 1 = no decay, 2 = slight (1 to 25%), 3 = moderate (26 to 50%), and 4 = extensive (>50%) decay (1). Corms were split vertically through the inoculation points and scored. Reisolations were made from the interface of healthy and diseased tissue. The test was repeated once.

**Inoculation of pine seedlings.** Sixty 1-year-old *P. patula* seedlings were inoculated with the three isolates of *Fusarium* from gladiolus corms and one isolate of *F. subglutinans* from diseased *P. patula* seedlings (*F. s. f. sp. pini*). As isolate MRC 6217 was representative in virulence of *F. s. f. sp. pini* (A. Viljoen, unpublished), only this isolate was included in pathogenicity tests. In a preliminary study, none of the *F. subglutinans* isolates from hosts other than pine or the isolate of *F. oxy-*

*sporum* was found to be pathogenic to pine seedlings. Fifteen seedlings were inoculated with each isolate and 15 seedlings were used as controls. Inoculum was grown on CLA, while sterile agar was used for inoculation of controls. Small strips of bark (1 × 10 mm) were first removed from the stems of seedlings and aseptically replaced with similar strips of agar containing the inoculum, and wounds were covered with Parafilm. Seedlings were maintained in a growth room at 24°C under cool-white and dark-fluorescent lights set at a 12-h photoperiod and arranged in a completely randomized block design. Development of stem lesions was assessed and lesions measured distal to the point of inoculation after 15 days. Reisolations were made from margins of necrotic tissue. The test was repeated once.

**Statistical analyses.** Analyses of variance were performed on the SAS/STAT system for personal computers (22). Comparison of virulence was made using Tukey's honestly significant difference (HSD).

## RESULTS

**Morphological studies.** Isolates of *Fusarium* from gladiolus corms showed characteristics that place them in the section *Liseola*. All the isolates produced white aerial mycelium slightly tinged with purple on PDA. When observed from the bottom of the petri dish, the colonies were cream-white with a large purple center. Cream to orange sporodochia developed within 14 days on PDA and CLA. On CLA, microconidia were produced abundantly on both mono- and polyphialides. Macroconidia were less abundant and slightly sickle-shaped with thin, delicate walls. Isolate M-668, however, produced an abundance of long, slender macroconidia.

The gladiolus isolates of *Fusarium* differed from the typical *F. subglutinans* from pines in their microconidial ontogeny. In the former isolates, microconidia were produced in false heads as well as in chains on CLA (Fig. 1A). The microconidial chains became more frequent after 7 days of incubation. Long chains of micro-

Table 1. Isolates of *Fusarium subglutinans* examined for virulence to gladiolus corms

MRC no.	Original no.	Host	Locality	Source of culture <sup>2</sup>
6940	M-668	Gladiolus	Florida, U.S.	FRC
6941	M-685	Gladiolus	Florida, U.S.	FRC
6942	M-669	Gladiolus	Florida, U.S.	FRC
6209		<i>Pinus patula</i> seedlings	South Africa	MRC
6217		<i>Pinus patula</i> seedlings	South Africa	MRC
6226	M-935	Pitch canker	Georgia, U.S.	MRC
6228	M-1290	Pitch canker	Florida, U.S.	MRC
6229	M-3834	Pitch canker	California, U.S.	MRC
2382		Pitch canker	Southern U.S.	MRC
7078	EM-3	Pitch canker	California, U.S.	MRC
1077		Corn	South Africa	MRC
2802		Mango	South Africa	MRC
6784	E-203	Pineapple	Brazil	MRC

<sup>2</sup> FRC = Culture collection of the Fusarium Research Center, The Pennsylvania State University, University Park; MRC = Culture collection of the Medical Research Council, Tygerberg, South Africa.

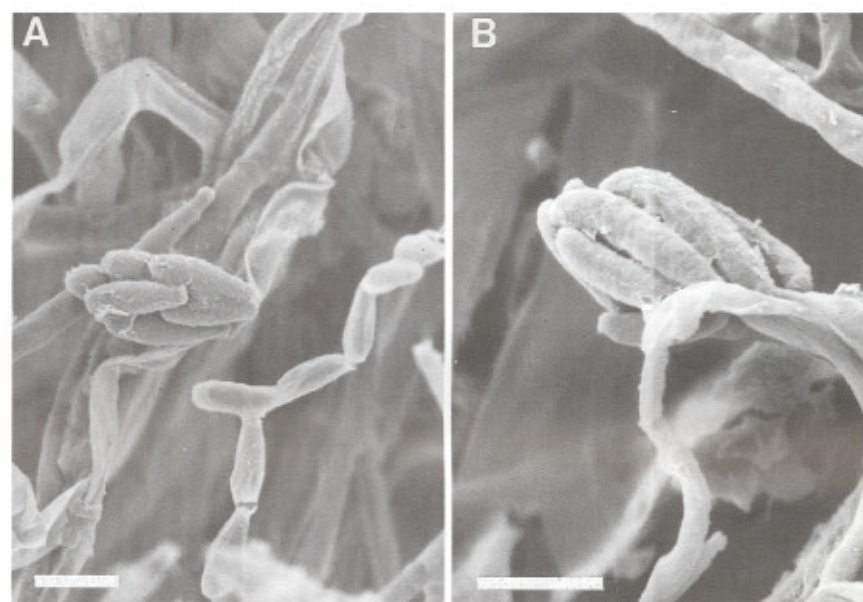


Fig. 1. Scanning electron micrograph of the microconidial ontogeny of (A) *Fusarium proliferatum* (M-668), producing microconidia in false heads and chains, and (B) *F. subglutinans* (MRC 6217), producing microconidia in false heads only. Scale bar = 5  $\mu$ m.



conidia were arranged either side to side or end to end. Isolate M-669 produced short chains of only two to four microconidia on CLA. When plated on 0.8% KCl medium, this isolate also produced long microconidial chains, similar to the others. Isolates of *F. subglutinans* from pine bear microconidia only in false heads on mono- and polyphialides (Fig. 1B). Consequently, the

three isolates of *Fusarium* from gladiolus corms are typical of *F. proliferatum* (T. Matsushima) Nirenberg as defined by Nelson et al. (19), and were previously referred to in the literature as *F. moniliforme* and *F. moniliforme* "subglutinans" (28).

**Inoculation of gladiolus corms.** All three isolates of *F. proliferatum* from

gladiolus corms caused moderate to severe decay of gladiolus corms (Table 2). On most corms, fungal growth on the corm surfaces became visible in the vicinity of the inoculation sites. Where extensive rotting occurred, virtually the entire surface of the corms was covered with mycelium. When split open, the corms revealed various degrees of decay. Slightly affected corms developed lesions only in the vicinity of the inoculation holes while, in most corms, the tissue between inoculation holes became moderately to severely infected (Fig. 2A).

Isolates of *F. subglutinans* from pines (*F. s. f. sp. pini*) and other hosts, as well as *F. oxysporum* from pine, resulted in very little or no decay of gladiolus corms. A thickened zone of reaction tissue developed between the inoculation site and the healthy tissue (Fig. 2A), similar to that in the control inoculations. The isolates of *F. s. f. sp. pini* associated with pitch canker in the United States (MRC 6226, MRC 6228, MRC 6229, MRC 2382, and MRC 7078) and diseased roots of pine seedlings in South Africa (MRC 6209 and MRC 6217) gave decay ratings of 1.21, 1.21, 1.33, 1.42, 1.25, 1.21, and 1.13, respectively (Table 2). These ratings did not differ significantly ( $P = 0.05$ ) from those of *F. subglutinans* from other hosts, *F. oxysporum*, or the control inoculation. They did, however, differ significantly ( $P = 0.05$ ) from decay ratings on gladiolus corms inoculated with *F. proliferatum*. This demonstrates that *F. proliferatum* isolates from gladiolus corms are significantly more virulent than *F. s. f. sp. pini*, *F. subglutinans* isolates from non-pine hosts, as well as *F. oxysporum*, with respect to causing decay of gladiolus corms. Isolates of the respective *Fusarium* spp. used in inoculations were recovered from necrotic margins inside the gladiolus corms.

**Inoculation of pine seedlings.** Isolates of *F. s. f. sp. pini* and *F. proliferatum* resulted in necrotic lesions on all of the inoculated *P. patula* seedlings (Fig. 2B). Stem lesions were slightly sunken with little or no resin flow. The cambium was soaked with resin, and hyphal growth and sporulation could be seen, especially in the cavities, where needles had been stripped before inoculation. Resin soaking and fungal growth were less prominent on pine seedlings inoculated with *F. proliferatum* than on those inoculated with *F. s. f. sp. pini*. No sporodochial formation was observed on the stems. No lesions developed in control inoculations.

The *F. s. f. sp. pini* isolate from pine seedlings (MRC 6217) was significantly ( $P = 0.05$ ) more virulent to *P. patula* seedlings than were the three isolates of *F. proliferatum* from gladiolus corms (Table 2). Two isolates (M-685 and M-669) of *F. proliferatum* were also significantly ( $P = 0.05$ ) more virulent to *P. patula* than the third one (M-668). Fifteen days after in-

**Table 2.** Pathogenicity of *Fusarium* species to gladiolus corms and *Pinus patula* seedlings

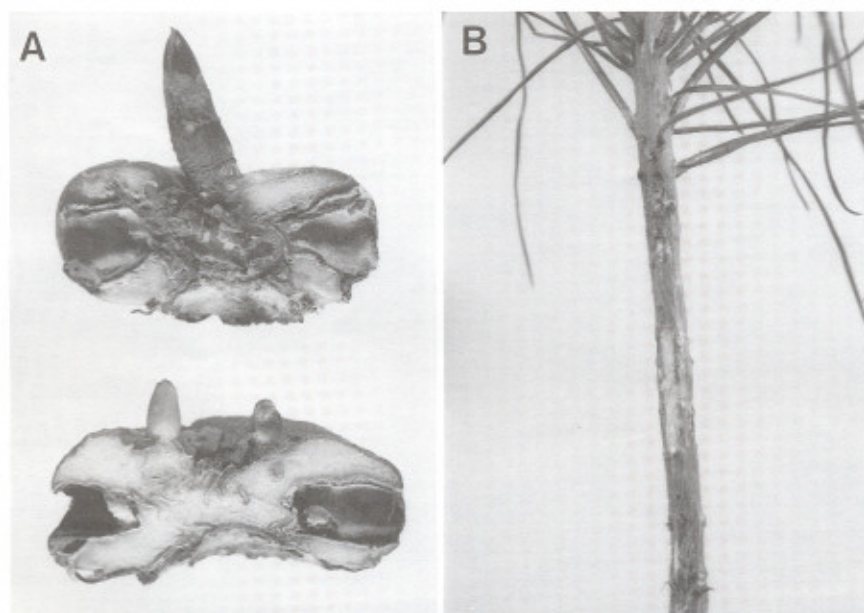
Species isolate*	Host inoculated <sup>x</sup>		
	Gladiolus corms <sup>y</sup>	<i>P. patula</i> seedlings <sup>z</sup>	
	Decay ratings	Lesion length (mm)	Tops killed (%)
<i>F. proliferatum</i>			
M-668	2.29 a	3.27 c	0.00
M-685	2.50 a	9.17 b	16.67
M-669	2.71 a	8.84 b	10.00
<i>F. s. f. sp. pini</i>			
MRC 6209	1.21 b	...	...
MRC 6217	1.13 b	22.16 a	90.00
MRC 6226	1.21 b	...	...
MRC 6228	1.21 b	...	...
MRC 6229	1.33 b	...	...
MRC 2382	1.42 b	...	...
MRC 7078	1.25 b	...	...
<i>F. subglutinans</i>			
MRC 1077	1.25 b	...	...
MRC 2802	1.08 b	...	...
MRC 6784	1.13 b	...	...
<i>F. oxysporum</i>			
MRC 6212	1.25 b	...	...
Control	1.00 b	0.00 d	0.00

\* M- = Culture collection of the Fusarium Research Center, The Pennsylvania State University, University Park; MRC = Culture collection of the Medical Research Council, Tygerberg, South Africa.

<sup>x</sup> Means in the same column followed by the same letter do not differ significantly ( $P = 0.05$ ) according to Tukey's honestly significant difference (HSD).

<sup>y</sup> Means of two runs of 12 corms per isolate. Decay was rated on a scale of 1 to 4, with 1 = no decay, 2 = slight (1 to 25%), 3 = moderate (26 to 50%), and 4 = extensive (>50%) decay.

<sup>z</sup> Means of two runs of 15 1-year-old *P. patula* seedlings inoculated per isolate.



**Fig. 2.** (A) Gladiolus corms inoculated with *Fusarium proliferatum* (M-668) (top) and *F. subglutinans* (MRC 6217) (bottom). (B) Lesion development on the stem of a 1-year-old *Pinus patula* seedling 15 days after inoculation with *F. proliferatum* (M-685).



oculation, most seedlings inoculated with *F. s. f. sp. pini*, but only a few inoculated with *F. proliferatum*, were killed proximal to the inoculation point. Reisolation from necrotic margins on the stems of *P. patula* seedlings was successful in recovering the inoculated *Fusarium* species from the respective treatments.

## DISCUSSION

In this study we showed that some isolates of *Fusarium* from gladiolus corms, previously identified as *F. subglutinans* (1,8) should be called *F. proliferatum*. These isolates from gladiolus corms were identified as *F. proliferatum* based on the presence of microconidia produced in false heads as well as in chains on mono- and polyphialides (19). This is in contrast to the presence of microconidia produced only in false heads, which is typical of *F. subglutinans*.

Confusion leading to the previous identification of *Fusarium* from gladiolus corms as *F. subglutinans* is understandable. *Fusarium subglutinans* was considered a variety of *F. moniliforme* until 1983 (19). Both *F. subglutinans* (*F. moniliforme* var. *subglutinans* at the time) and *F. proliferatum* produce their microconidia on mono- and polyphialides. *Fusarium subglutinans* was, however, included as a variety of *F. moniliforme* that produces microconidial chains only on monophialides. The identity of *F. proliferatum* isolates could easily have been mistaken for *F. moniliforme* var. *subglutinans* (*F. subglutinans*) because of the formation of very short microconidial chains by some isolates on mono- and polyphialides.

In this study, three isolates of *F. proliferatum*, previously referred to as *F. subglutinans*, were pathogenic to gladiolus corms. These results confirm those of Barrows-Broadus and Dwinell (1), who recorded similar decay ratings associated with these isolates. Interestingly, Woltz et al. (28) reported that one isolate of *F. proliferatum* (their *F. moniliforme* "subglutinans") (M-668) contributed to disease development, while another isolate (M-685) apparently protected corms from infection by *F. o. f. sp. gladioli*, and generally increased corm production.

Gladiolus corms in this study were equally susceptible to *F. s. f. sp. pini* (specific to pine), isolates of *F. subglutinans* from other hosts, and *F. oxysporum*. These results are consistent with those of Barrows-Broadus and Dwinell (1), who found that *F. subglutinans* from pines did not differ in virulence from other *Fusarium* spp., such as *F. moniliforme*. It might be argued that other isolates of *F. s. f. sp. pini* would react differently on gladiolus corms. Variation in pathogenicity among isolates of the pitch canker fungus has been demonstrated previously (5). This variation was noticeable in both the present study and that of Barrows-Broadus

and Dwinell (1), but the variation was not significant ( $P = 0.05$ ). Isolates of *F. s. f. sp. pini* in this study are representatives of a number of *F. s. f. sp. pini* isolates from pitch canker in the United States and *P. patula* seedlings (A. Viljoen, unpublished). Additionally, Correll et al. (3) suggested that isolates of *F. s. f. sp. pini* are genetically unified based on mtDNA restriction fragment length polymorphisms, and appear to represent a distinct sub-specific group within the morphological species, *F. subglutinans*. We therefore do not believe that other strains of *F. s. f. sp. pini* from pines would react differently on gladiolus corms.

Various species of *Fusarium* in addition to *F. subglutinans* have been isolated from cankers on the stems of pine trees. These include *F. lateritium*, *F. moniliforme*, and *F. proliferatum*, which were either avirulent or weakly virulent in pathogenicity trials (4,8,15) and are probably not involved in the etiology of pitch canker. Our study found that *F. proliferatum* isolates from gladiolus corms are weakly to moderately pathogenic to *P. patula* seedlings. This is consistent with the results of Dwinell and Nelson (8), except that these authors referred to the pathogen as *F. moniliforme* var. *subglutinans*. *Fusarium proliferatum* can be an aggressive pathogen of container-grown pine seedlings (13). It is, therefore, not unexpected that inoculations of *P. patula* seedlings with *F. s. f. sp. pini* resulted in lesion development on the stems. We doubt, however, if *F. proliferatum* will cause or contribute to pitch canker development under field conditions.

Isolates of *F. subglutinans* from pine were consistently virulent on pines in greenhouse pathogenicity tests (4,8). Isolates from corn, Dracaena, lily, Araucaria, amaryllis, agricultural soil, and deodar weevils were avirulent (8). Correll et al. (4) also reported that 21 of 23 isolates of *F. subglutinans* from non-pine hosts (sorghum, corn, sugarcane, rice, pineapple, and Dracaena) were nonpathogenic on Monterey pine. Two isolates from rice were weakly virulent on Monterey pine seedlings, but it is unclear whether they would be pathogenic in a field situation. Thus, only isolates of *F. subglutinans* with the proven ability to cause pitch canker symptoms on pines are regarded as the pitch canker pathogen (*F. s. f. sp. pini*).

Gladiolus isolates of *Fusarium* causing lesion on the stems of pine seedlings proved to be *F. proliferatum*. Isolates of *F. subglutinans* from various hosts, including pine, were avirulent to gladiolus corms. This counters the supposition that isolates of *F. subglutinans* from pines or gladiolus corms are aggressive pathogens of both hosts. Results of the present study support the view that isolates of *F. subglutinans* pathogenic to pines represent a distinct forma specialis within the species.

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