Isolation, Identification, and Pathogenicity of *Fusarium* spp. from Guayule in Arizona

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ABSTRACT

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Fusarium equiseti, *F. oxysporum*, and *F. solani* were isolated from necrotic root tissue of diseased guayule plants. *F. solani* was the most prevalent of the three species isolated. The pathogenicity of 22 isolates was tested using the slant board nutrient solution technique. Although isolates of all species caused root rot symptoms, some isolates of *F. oxysporum* were the most virulent, causing root rot symptoms in 60–70% of the roots inoculated. In additional tests for pathogenicity, greenhouse-grown plants were inoculated with spore suspensions of *Fusarium* spp. Isolates of *F. oxysporum* discolored lateral roots and taproots and reduced fresh weights. Isolates of *F. oxysporum* directly penetrated and colonized roots and formed distributive hyphae in the root cortex.

Additional keywords: *Parthenium argentatum*

Guayule (*Parthenium argentatum* A. Gray), a perennial shrub native to regions of Mexico and Texas, was once extensively cultivated in the southwestern United States as a domestic source of rubber (3,12). Because of current world political and economic trends, interest in guayule as a source of natural rubber has been renewed, and guayule is again being produced (3,12). Disease problems previously associated with guayule production were soon encountered (12). Guayule plants with root rot symptoms have been commonly observed. Symptoms associated with these plants include brown cortical necrosis of feeder and main roots, loss of feeder roots, stunted growth, and chlorotic lower leaves. *Fusarium* spp. have been isolated previously from diseased roots (1,9,10), but, with the exception of Norton (9), the *Fusarium* spp. isolated were not tested for pathogenicity.

The objectives of this investigation were to identify the *Fusarium* spp. associated with root rot of guayule and to evaluate their relative pathogenicity to guayule. A preliminary account of this work has been reported (15).

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MATERIALS AND METHODS

Isolation and identification. Isolations were made from 39 guayule plants, collected from several locations in Arizona, that exhibited root rot symptoms. Segments of roots with necrotic lesions were placed in wide-mouth mason jars fitted with several layers of cheesecloth and washed under running water for 10 min (14). The roots were removed from the jars, rinsed twice with distilled water, surface-sterilized for 30 sec with 1% sodium hypochlorite, blotted dry, and then washed with sterile distilled water. Mycelial growth was induced by placing the root pieces on water agar in petri dishes, which were incubated at room temperature. Isolates were single-spored, transferred to V8 juice agar and potato-dextrose agar, and identified from descriptions of Nelson et al (8). Additional isolates from diseased guayule plants were provided by S. M. Alcorn, Department of Plant Pathology, University of Arizona. Identification of most isolates was verified by the Fusarium Research Center at The Pennsylvania State University.

Plant materials. For all experiments, guayule seeds (Variety 593) were placed in distilled aerated water for 24 hr. Seeds were then rinsed with distilled water and planted 1 cm deep in plastic pots (7.5 × 7.5 × 5 cm) containing premoistened, medium grade, autoclaved vermiculite.

Pots were maintained at 25 C under fluorescent lights for 1 wk and transferred before transplanting for an additional week to a greenhouse, where they were maintained at temperatures of 27 C during the day and 16 C at night.

Inoculation and pathogenicity tests. Isolates of *Fusarium* were screened initially for pathogenicity by inoculating 12-wk-old plants grown using the slant board technique (4), a nutrient solution technique of growing plants for ease of root manipulations and observations. Seedlings were transplanted at 2 wk onto slant boards in the greenhouse. Inoculum was prepared by growing mycelium on 0.5-cm^2 polyester cloth squares laid on the surface of V8 juice agar plates in the dark for 1 wk (5). Plant roots were inoculated by placing mycelium-covered cloth squares directly upon individual root tips. Controls consisted of sterile cloth squares that had also been laid on the surface of V8 juice agar without any fungal inoculum. Different individual roots on the same slant board were inoculated with a different fungal isolate, and one root was treated with the control treatment. Inoculated plants were placed into a controlled-environment chamber (30 C day, 22 C night, 14-hr photoperiod, light intensity = 450 $\mu Em^{-2}s^{-1}$) for 1 wk. This experiment was repeated eight times with 12 replicate slant boards each time.

At the end of 1 wk, the plants were removed from the growth chamber, and root rot symptoms and root growth were evaluated. The number of roots with root rot symptoms was recorded, and the incidence (percentage) of roots with root rot symptoms was calculated. The length of root necrosis was measured with a ruler by removing the cloth square and measuring the length of rot (mm) at the inoculation site. New root growth after inoculations was determined by measuring the distance (in millimeters) from the root tip to the lower edge of the inoculation cloth square. Diseased roots were harvested, and reisolations were made using the same techniques employed for the initial isolations.

In addition to the inoculations using the slant board technique, plants grown in a soilless medium in the greenhouse were inoculated with fungal spore suspensions to evaluate the pathogenicity of the isolates of *Fusarium*. When seedlings were 2 wk old, they were transplanted into individual 1-L Styrofoam cups filled with pasteurized, U.C. soilless medium (7). These seedlings were kept in the greenhouse and watered with tap water for 8 wk. Isolates of *Fusarium* spp. were grown on V8 juice agar plates under fluorescent lights at 25 C for 1 wk. Suspensions containing microconidia and macroconidia were prepared, and conidia were counted with a hemacytometer. The final concentration of conidia was adjusted to 2×10^5 spores per milliliter. Plants were inoculated by making three holes, approximately 7–10 cm deep, into the soilless medium with a knife and pouring 40 ml of spore suspension into each hole. The control consisted of pouring 40 ml of distilled water into each hole. For each treatment, 24 plants were inoculated and then placed in a random pattern in the greenhouse. Pots were elevated above the bench surface. The experiment was conducted as a completely randomized design and was repeated once.

After 2 mo the experiment was terminated, and disease severity was assessed. Plants were removed from their pots, and the roots were gently washed in water and evaluated by visual observations for necrosis of secondary lateral feeder roots, necrosis of main lateral roots, and lesions on main taproot. The incidence of plants with each of these symptoms was calculated. Plants were assessed for severity of root rot symptoms on the secondary lateral feeder roots by placing each plant in one of three categories: 0 = healthy, 1 = less than 50% of the feeder roots affected, or 2 = greater than 50% of feeder roots affected. A disease severity index was calculated for each plant by using the following formula: root rot index (maximum = 4) = necrosis of secondary feeder roots (0, 1, or 2) + main lateral root necrosis (0 not present, 1 present) + main taproot lesions (0 not present, 1 present). Fresh weights were then measured. *Fusarium* spp. were reisolated from roots of inoculated guayule plants.

Analysis of data. Analysis of variance of the data was determined by the general linear model procedure according to SAS (11). The Student-Newman-Keuls multiple range test was used to compare treatment means.

Light microscopy. Using the slant board nutrient solution technique, roots were inoculated at the root tip with mycelium of isolates of *F. oxysporum* Schlectend.:Fr. (80-148-4 and 81-17), as previously described, to study the pattern of root penetration and colonization. At 72 hr after inoculation, roots were

Table 1. Source of *Fusarium* spp. isolated from guayule with root rot symptoms and percentage of inoculated roots with root rot symptoms[y]

Isolate identification	Location of host plant	Incidence of root rot symptoms[z] (%)
F. equiseti		
80-141-4	Litchfield Park, Arizona	19 bc
81-23	Litchfield Park, Arizona	17 bc
82-2	Tempe, Arizona	3 c
84-24-A	Tempe, Arizona	40 b
84-24-B	Tempe, Arizona	23 bc
F. oxysporum		
80-148-2	Tempe, Arizona	27 bc
80-148-4	Tempe, Arizona	73 a
80-149-1	Tempe, Arizona	16 bc
80-149-2	Tempe, Arizona	25 bc
81-17	Phoenix, Arizona	68 a
82-16	Litchfield Park, Arizona	15 bc
84-15	Tempe, Arizona	32 bc
84-25	Tempe, Arizona	21 bc
84-26	Tempe, Arizona	19 bc
84-30	Tempe, Arizona	60 a
F. solani		
80-155	Litchfield Park, Arizona	8 c
81-13-3	Phoenix, Arizona	21 bc
81-86	Litchfield Park, Arizona	8 c
81-86-2	Litchfield Park, Arizona	24 bc
82-20	Litchfield Park, Arizona	22 bc
84-10	Tempe, Arizona	32 bc
84-20	Tempe, Arizona	22 bc
Control		5 c

[y] Inoculated plants were grown using the slant-board nutrient solution culture technique, and individual roots were inoculated with fungal mycelium that had been cultured on 0.5-cm^2 polyester cloth lying on V8 juice agar.

[z] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ according to the Student-Newman-Keuls multiple range test.

Table 2. Length of necrotic lesions on roots of guayule plants grown on slant-boards and inoculated with isolates of *Fusarium oxysporum*

Isolate	Mean length of lesions[z] (mm)
F. oxysporum	
80-148-4	5.0 a
81-17	5.4 a
84-30	2.5 ab
Control	1.3 b

[z] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ according to the Student-Newman-Keuls multiple range test.

excised and stained by boiling for 4 min in a 0.05% aniline blue-lactophenol solution (13). The cleared and stained roots were inspected for the presence of fungal mycelium in the root epidermis, cortex, and vascular tissues.

RESULTS AND DISCUSSION

Three species of *Fusarium* were consistently isolated from decayed roots of plants from several locations in Arizona. Based on the morphology of macroconidia, microconidia, chlamydospores, conidiophores, general colony morphology, and taxonomic descriptions, the isolates were identified as *F. equiseti* (Corda) Sacc., *F. oxysporum*, and *F. solani* (Mart.) Sacc. Isolation of *F. solani* from diseased guayule plants with root rot symptoms had previously been reported (9). Of the *Fusarium* spp. isolated, 13% were identified as *F. equiseti*, 26% as *F. oxysporum*, and 61% as *F. solani*. Twenty-two of these isolates were selected for use in the pathogenicity tests (Table 1).

When plants grown on slant boards were inoculated, root rot incidence was shown to vary significantly among the isolates using analysis of variance. Isolates 80-148-4, 81-17, and 84-30 of *F. oxysporum* caused a higher percentage of root rot symptoms than did the other isolates tested (Table 1). These symptoms included reddish brown necrotic lesions extending in both directions from the point of inoculation. The mean lengths of the necrotic lesions ranged from 5.4 to 2.5 mm at 7 days after inoculation (Table 2). Very small necrotic lesions were present on 5% of the roots that had been subjected to the control treatment (Tables 1 and 2). In most cases, these roots had been wounded accidentally at the treatment site. In 31% of roots inoculated with these pathogenic isolates of *F. oxysporum*, the root tip was necrotic, and no new root growth was observed. *F. oxysporum* was reisolated from necrotic lesions on inoculated roots, completing Koch's postulates.

Inoculated greenhouse-grown plants had necrotic, brown lesions on their roots, fewer feeder roots, and chlorotic lower leaves, which are typical of guayule with root rot symptoms in the field. These isolates varied significantly ($P \leq 0.05$) in pathogenicity, as determined by the severity of symptoms and plant weight (Table 3). Isolate 80-148-4 caused discoloration of 75% of the secondary feeder roots versus 96% for isolate 81-17. Discoloration of secondary feeder roots, which occurred in 20% of the control plants, may have been caused by wounding during the treatment. Isolate 81-17 of *F. oxysporum* caused rot of the main lateral roots in 83% of inoculated plants and of the main taproot in 54% of inoculated plants. Both isolates 80-148-4 and 81-17 of *F. oxysporum* had higher root rot index values when compared to the other isolates tested and control plants. Inoculation with isolates 80-148-4 and 82-2 of *Fusarium* reduced the fresh weight of the harvested guayule plants with the greatest reduction in plants inoculated with isolate 80-148-4. Only *F. solani* had previously been shown to cause root rot symptoms in inoculations of greenhouse-grown guayule (9). Both inoculation experiments were repeated with similar results.

When inoculated, stained roots were examined using light microscopy at 72 hr, both isolates 80-148-4 and 81-17 of *F. oxysporum* were observed to directly penetrate root epidermal cells and extensively colonize the root cortex. Both isolates formed long, parallel, distributive hyphae that grew longitudinally in the intercellular spaces of the root cortex toward both the hypocotyl and the root tip. No fungal hyphae were observed within the vascular tissue of guayule roots. This pattern of development is typical of other *Fusarium* cortical root rot pathogens in other hosts (2,6,13).

These investigations show that *F. equiseti, F. oxysporum*, and *F. solani* were associated with root rot in guayule, and that some isolates of *F. oxysporum* were more virulent in causing root rot symptoms. The isolation of several species of *Fusarium* that differ in pathogenicity may complicate efforts to develop control strategies for this problem. An understanding of environmental conditions associated with the development of root rot in guayule would enhance the development of control strategies.

Table 3. Symptom frequency, root rot index, and fresh weights of greenhouse-grown guayule plants inoculated with isolates of *Fusarium*[w]

Isolate	Root rot symptoms[x]			Root rot index[y]	Plant fresh weight (g)
	Feeder roots (%)	Lateral roots (%)	Tap-roots (%)		
F. oxysporum					
80-148-4	75 b[z]	33 b	4 b	1.88 b	38.7 b
81-17	96 a	83 a	54 a	3.29 a	43.1 ab
F. equiseti					
82-2	42 c	38 b	0 b	1.21 c	40.5 b
F. solani					
80-155	31 cd	25 b	0 b	0.88 c	44.9 a
Control	20 d	33 b	0 b	0.75 c	47.5 a

[w] Inoculum consisted of 40 ml of spore suspension (2×10^5 spores per milliliter) that was poured into soilless mix. Controls consisted of guayule plants treated with distilled water.
[x] Percentages of inoculated plants with necrotic discolorations of different parts of the root system.
[y] Root rot index (maximum = 4) = feeder root necrosis (0, not present; 1, less than 50% feeder roots affected; or 2, greater than 50% of feeder roots affected) + main lateral root necrosis (0, not present or 1, present) + main taproot lesions (0, not present or 1, present).
[z] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$ according to the Student-Newman-Keuls multiple range test.

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