Fusarium Hypocotyl Rot of Sugar Pine in California Forest Nurseries

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ABSTRACT

Brownell, K. H., and Schneider, R. W. 1983. Fusarium hypocotyl rot of sugar pine in California forest nurseries. Plant Disease 67:105-107.

A hypocotyl rot disease of sugar pine is described that caused severe losses in less than 3-mo-old seedlings of *Pinus lambertiana, Abies concolor*, and *A. magnifica* in California forest nurseries. *Fusarium oxysporum* was found to be the causal organism. Greenhouse-grown seedlings developed resistance to infection by the third week after emergence, and roots were resistant at all ages. Hypocotyl rot strains were not pathogenic when tested against 14 agricultural hosts. Nine strains of *F. oxysporum* from agricultural hosts were not pathogenic when tested against *P. lambertiana*. Strains from Oregon ponderosa pine hypocotyl cankers were pathogenic on *P. lambertiana* hypocotyls.

In 1975, a hypocotyl rot disease of unknown etiology began to cause losses greater than 50% in sugar pine (*Pinus lambertiana* Dougl.), red fir (*Abies magnifica* A. Murr.), and white fir (*A. concolor* (Gord. & Glend.) Lindl.) at two California forest nurseries. Observations of disease development revealed hypocotyl lesions and girdling but no root rot. Initial isolations from diseased seedlings implicated a *Fusarium* sp. as a causal agent.

F. oxysporum (Schlecht.) emend. Snyd. & Hans. was isolated from diseased California conifer nursery seedlings in 1960; however, it was not known if the fungus had any role in causing the disease because pathogenicity was not tested (1). This fungus was later found to be a common rhizoplane inhabitant of California nursery-grown sugar pine, but its role as a pathogen was not known (11).

This research was initiated to study the epidemiology of the disease as it occurs on sugar pine, to identify the pathogen and demonstrate pathogenicity, and to determine the partial host range of the pathogen. A preliminary report has been published (4).

MATERIALS AND METHODS

Isolations. Seedlings for observation and isolation were sampled every 2 wk from sowing (May) through September and every 6 wk from October through the second year in California forest nurseries at Magalia and Placerville. Fifty seedlings were dug from randomly selected areas in nursery beds, transported to the laboratory, and inspected for hypocotyl and root rot. To remove iron oxides, which impart a dark brown stain to plant tissues and obscure symptoms, roots were soaked in a solution of 0.23 M sodium dithionite and 0.12 M EDTA disodium salt adjusted to pH 6.5 with sodium hydroxide.

Seedlings used for isolations were refrigerated and processed within 24 hr. Samples were first dipped into 95% ethanol and rubbed gently to remove the cuticle and adhering soil. They were then soaked in 0.5% sodium hypochlorite with 1 drop of Tween 20 per 100 ml for 8 m, removed, sectioned, and plated on potato-dextrose agar (PDA), water agar, King's medium B, and *Fusarium*-selective Komada medium (8). All cultures were maintained at 21 ± 3 C under fluorescent illumination for 12 hr daily.

Pathogenicity tests. Three methods of seedling inoculation were used to screen strains for pathogenicity on sugar pine. Initially, a rapid, severe, mycelial-plug test was used. An infested-soil test was also employed for screening at low inoculum levels representative of the field situation. The use of a "sterile-tube" test verified pathogenicity in the absence of other soil microbes. Seeds for greenhouse studies were from California seed zones; they were stratified and surface-sterilized in 30% H_2O_2 for 1 hr and then kept moist at room temperature to be used for planting when germination began.

Seeds for plug inoculations were planted in autoclaved U.C. mix (9) and grown in a greenhouse at 27 ± 2 C. Six-millimeter plugs were cut with a cork borer from the margin of an actively growing culture on PDA. After the emerging seedling hypocotyl was fully upright, a small amount of soil was carefully pulled away from the top 1 cm adjacent to the hypocotyl. The agar plug was then placed into the cavity next to the hypocotyl and the soil replaced around it. Ten seedlings per strain were inoculated, and after 5 days hypocotyls were inspected for lesion formation. Inoculations were considered positive if two or more seedlings had lesions on hypocotyls adjacent to the inoculum.

Isolates that were pathogenic in the plug inoculations were tested further using a dilute inoculum level in unsterile soil. Inoculum was prepared by first grinding barley straw to pass through a 1-mm screen. The ground straw was then added to flasks and moistened with 2 ml of 0.025 M asparagine per gram of straw. The cotton-plugged flasks were then autoclaved twice for 1 hr on consecutive days. The straw was inoculated with single-spored fungal strains and incubated for 3 wk under fluorescent illumination as described previously. Inoculum was dried and again passed through a 1-mm screen.

Soil to be infested was collected during the spring from a forest nursery that had been fumigated the previous fall and found to be free of the hypocotyl rot organism in greenhouse bioassays. Infested straw was mixed well with field soil at a rate of 1 g/10 L of soil (about 1:10,000, w/w) and put into seven 8-cm pots per strain. Pots were planted with three seeds each and kept in the greenhouse (27 ± 2 C) until they were rated for hypocotyl rot 3 wk after emergence. Isolations were made from two diseased seedlings from each test to confirm pathogenicity. Control plants were grown in soil without added straw and with straw inoculum of two saprophytic *Fusarium* spp. Isolates were rated as pathogenic if six or more of the 21 seedlings developed hypocotyl rot and as weakly pathogenic if two to five showed typical symptoms.

Because secondary invading *Fusarium* spp. are isolated routinely and could be mistaken for the causal agent, a sterile-tube inoculation technique was used to verify the pathogenicity of the suspected pathogens in the absence of other soilborne organisms. Twenty-eight culture tubes 13.5 × 20 cm were one-third filled with moist nursery soil, plugged with cotton, and autoclaved twice for 1 hr on consecutive days. Sugar pine seeds were surface-sterilized, then planted in

Accepted for publication 3 September 1982.

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0191-2917/83/010010503/$03.00/0

the tubes while maintaining sterility. Five milliliters of a macrospore suspension (10^6/ml) of a putative pathogenic strain (M2) was added to 14 of the tubes, and the remainder served as sterile controls. The tubes were kept at room temperature under 12-hr fluorescent light for 20 days. At the end of the incubation period, soil and seedlings were plated on PDA and King's medium B agar to confirm the absence of other microbes in the soil and to verify pathogenicity of the test organism.

Fourteen single-spored *Fusarium* spp. isolates from diseased sugar pine seedlings collected at the Placerville and Magalia nurseries were screened for pathogenicity using both the agar-plug and infested-soil tests. Three pathogenic hypocotyl rot isolates were tested further to determine if the pathogen could cause root rot. Surface-sterilized sugar pine seeds were sown in autoclaved U.C. mix in containers with removable sides, which allowed access to the roots. Preemergent and postemergent seedling roots were inoculated by placing mycelial plugs (as described earlier) beside them. Roots were inoculated at the growing tip and midway between the tip and the hypocotyl. Controls included uninoculated seedlings and seedlings inoculated at the hypocotyl. Plants were inspected weekly for rot or discoloration for 6 wk, at which time the experiment was terminated.

To determine the relationship between plant age and susceptibility, 400 sugar pine seedlings varying in age from 1 to 28 days postemergence were inoculated with the M2 strain by using the plug technique described earlier. Seedling deaths were recorded weekly for 1 mo.

Cross-inoculation and host range. To test ability of pathogenic *Fusarium* spp. from other hosts to cause hypocotyl rot, 12 isolates of *Fusarium* spp. from nonconifer hosts, five Oregon sugar pine root rot isolates of *Fusaium* spp., and five Oregon ponderosa pine seedling hypocotyl canker isolates of *F. oxysporum* (Table 1) were single-spored and tested for pathogenicity on sugar pine. Isolates were first tested using the plug-inoculation method and, if found pathogenic, were tested further using the infested-soil inoculation technique.

The host range of the hypocotyl rot organism was tested on celery (*Apium graveolens* L. '52-70R'), cantaloup (*Cucumis melo* L. var. *reticulatus* Naud. 'Persian Small' and 'Topmark'), squash (*Cucurbita pepo* L. 'Black Zucchini'), cotton (*Gossypium hirsutum* L. 'SJ-2'), flax (*Linum ositatissium* L.), tomato (*Lycopersicon esculentum* Mill. 'Granpac' and 'San Marzano'), bean (*Phaseolus vulgaris* L. 'Kentucky Wonder' and 'Sutter Pink'), radish (*Raphanus sativus* L. 'Scarlet Globe'), and spinach (*Spinacia oleracea* L. 'Marathon' and 'High Pack').

Twenty-five seeds of each cultivar except celery were planted in a naturally infested nursery bed at Magalia in June 1980. Seedlings were observed every 2 wk for 3 mo for wilt or stem rot and sampled twice for root rot by root excavation and visual inspection.

Seeds of each cultivar for greenhouse tests were sown in autoclaved U.C. mix in 10-cm-diameter pots. Within 1 wk after seedlings emerged, they were inoculated using the plug-inoculation technique described earlier.

RESULTS

Disease development in the field. Small, elongated lesions began to form below the soil line on seedling hypocotyls during the second week after emergence. The depth at which the lesions began to form ranged from 1 to 3 cm with a mean of 1.7 cm below the soil line (Fig. 1). The lesions expanded around the hypocotyl, eventually girdling it. At this time, the seedlings began to wilt, and within 2 wk, the tops turned brown. Seedlings began to die about 3 wk after emergence, first appearing as a damping-off; however, mortality continued for 3 mo.

In the youngest seedlings, the fungus macerated the hypocotyl lesion area, leaving only the epidermis and strands of vascular tissue. These younger seedlings then collapsed; however, the ones that died after the first month remained standing because of the development of secondary tissues.

After seedling death, the pathogen continued to colonize the hypocotyl tissue. External white mycelium surrounded the hypocotyl below the soil line, often producing pink sporodochia. Roots below the lesion remained healthy-appearing and turgid for several days after the shoots died.

Seedlings died in an apparent random pattern in the nursery beds without observable correlation with moisture levels (because of an uneven sprinkler pattern), inoculum centers (circular patterns), or seed sources.

Isolations. *F. oxysporum* was consistently isolated from surface-sterilized hypocotyl lesions and up to 2 cm in advance of the lesions. Unidentified bacteria were often isolated from the lesions but not above or below them. Only two small root lesions were found on seedlings 2–3 wk postemergence out of 600 root systems inspected throughout two seasons. Root systems of nursery-grown sugar pine inspected throughout the 2-yr cycle did not have root rot from any cause.

Pathogenicity tests. Four of the nursery *Fusarium* isolates were not pathogenic in either test, but the remaining 10 isolates were pathogenic in both tests. All of the pathogenic strains were *F. oxysporum* and were similar in

Table 1. Strains of *Fusarium* spp. used for cross-inoculation tests on sugar pine

Fungus	Source	Location	Disease	No. of strains
F. oxysporum	Garbanzo bean	California	Wilt	1
F. oxysporum	Cantaloup	California	Wilt	2
F. oxysporum	Cantaloup	California	NP[a]	1
F. oxysporum	Cowpea	California	Wilt	1
F. oxysporum	Tomato	California	Wilt	1
F. oxysporum	Flax	California	Wilt	1
F. oxysporum	Carnation	California	Wilt	1
F. oxysporum	Celery	California	Wilt	1
F. solani	Pumpkin	California	Fruit rot	1
F. solani	Exacum	California	Stem rot	1
F. roseum	Honeydew melon	California	NP	1
F. oxysporum	Ponderosa pine	Oregon	Canker	5
F. oxysporum	Sugar pine	Oregon	Root rot	4
F. roseum	Sugar pine	Oregon	Root rot	1

[a]NP = nonpathogen on source host.

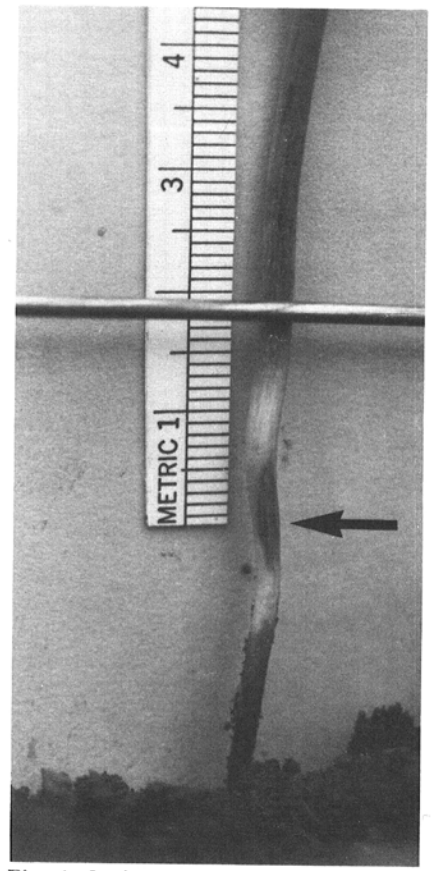

Fig. 1. Lesion on sugar pine hypocotyl excavated from a nursery bed. Bar represents original soil line.

growth rate, colony color, and optimum and maximum growth temperatures. All seedlings grown in the soil tubes developed hypocotyl lesions and died within 10 days after emergence. Seedlings in the sterile control soil remained healthy throughout the experiment. Roots inoculated with the three hypocotyl rot isolates by using the agar-plug test remained healthy. Some roots showed a localized reddening after 6 wk, whereas inoculated hypocotyls were severely rotted.

Hypocotyl tissue remained highly susceptible when inoculated during the first and second weeks after emergence, with 97 and 56% mortality, respectively. The proportion of seedlings killed decreased to 11 and 6% by the third and fourth weeks, respectively.

Cross-inoculation and host range. Of 11 strains of *Fusarium* from nonconifer hosts, only one, *F. roseum* isolated from a diseased honeydew melon root, caused hypocotyl rot on sugar pine. An infested-soil test confirmed that it was a weak pathogen.

All five Oregon ponderosa pine hypocotyl canker strains caused hypocotyl rot on sugar pine in both agar-plug and infested-soil tests. Of the Oregon sugar pine root rot strains, only one strain of *F. roseum* was weakly pathogenic on sugar pine hypocotyls; however, the ability to cause root rot was confirmed in greenhouse root-tip inoculations on sugar pine by using the root inoculation procedure described earlier.

Incense cedar (*Libocedrus decurrens* Torr.), giant sequoia (*Sequoiadendron giganteum* (Lindl.) Buchh), jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), ponderosa pine (*Pinus ponderosa* Laws.), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were grown at the Magalia nursery without experiencing loss to hypocotyl rot, despite severe losses in sugar pine, red fir, and white fir. Therefore, these species appear resistant to the disease. Sugar pine, red fir, and white fir commonly sustained significant losses to hypocotyl rot at the Magalia nursery. Russian olive (*Elaeagnus angustifolia* L.) also was affected, but losses were much less extensive. Agar-plug and infested-soil inoculations confirmed the susceptibility of these species.

No rot or wilt symptoms occurred on any of the 13 agricultural hosts grown in the infested nursery. When inoculated with the M2 isolate by using the agar-plug technique, all the agricultural hosts tested were found to be free of hypocotyl rot symptoms. Two cultivars, Asgrow High Pack spinach and Sutter Pink beans, showed some reddening of the tissue adjacent to the inoculum plug, but no rot developed at these points.

DISCUSSION

The numerous isolations from infected material and successful inoculations and reisolations proved the causal agent to be *F. oxysporum*. Infection in the sterile-tube inoculations showed that the pathogen is capable of causing hypocotyl rot in the absence of other microbes, is an important point in confirming pathogenicity with a common soil inhabitant and aggressive saprophyte. In addition, infested-soil tests demonstrated that the isolated fungus was capable of surviving and causing disease when dispersed in nonsterile field soil in relatively low concentrations.

An apparent contradiction exists in that observations in the nursery indicated that seedlings die up to 3 mo after emergence, yet inoculations showed that plants become highly resistant by the end of the first month. In greenhouse inoculation experiments, it was found that seedlings inoculated during the period of increasing resistance (second and third weeks postemergence), died as much as 3 wk later than those inoculated during the first week postemergence. Seedlings grown in the field generally matured slower than those in the greenhouse, which would extend their age of susceptibility. Seedlings were occasionally found after the period of mortality with lesions that had developed slowly enough so that the plants were able to survive. Thus, it appears that later seedling losses were caused by early infections that developed slowly and became lethal as they eventually girdled the stem. It is unlikely that the inoculum produced on the infected seedlings would be important as secondary inoculum in the current year's crop. The healthy seedlings would have matured beyond the age of susceptibility by the time the inoculum was produced and disseminated.

It appears that the *F. oxysporum* that causes hypocotyl rot of sugar pine is moderately specific and unable to attack many other host species. It is not completely host specific, however, because it infects red and white fir and, to a lesser extent, Russian olive. The situation is confused somewhat because the organism that causes hypocotyl rot of ponderosa pine from Oregon also attacks sugar pine, but the sugar pine organism from California is not pathogenic on ponderosa pine. The hypocotyl rot pathogen appears to differ from the *F. oxysporum* that causes root rot on sugar pine in Oregon because the respective organisms are unable to cross-infect. The hypocotyl rot *Fusarium* also is unable to attack Douglas-fir and is therefore different from the *Fusarium* that causes the widespread damping-off and root rot in the Pacific Northwest.

Hartig (5) first described a *Fusarium* conifer nursery disease causing top, hypocotyl, and root rot in 1892. Since that time, *Fusarium* seedling diseases have been reported from several countries on many conifer hosts (2,5–7,12). The somewhat complex status of the *Fusarium* conifer pathogens described here is representative of the situation worldwide because many species of *Fusarium* infect conifers (7,12,13), and even within the conifer-infecting strains of *F. oxysporum* there are reports of physiological specialization (3,10).

Results from this study suggest several practical considerations in the management of hypocotyl rot. Because older seedlings become resistant to the disease and losses occurred only during the first summer in the nursery, we conclude that hypocotyl rot would not pose a threat to outplanted seedlings. Coniferous species other than sugar pine or true fir planted in infested fields should not succumb to the disease; however, conifer species not discussed here should be tested for resistance before being planted in infested soil. In addition, because the vegetable isolates tested did not cause hypocotyl rot, fields previously planted to vegetable crops with a history of Fusarium wilt would not be a likely hazard if used for conifer seedling production.

ACKNOWLEDGMENTS

We wish to thank D. H. Adams and W. D. Krelle for their assistance. This work was partially funded by the California Department of Forestry.

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