Pathogenic Seedborne *Fusarium oxysporum* from Douglas-Fir

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ABSTRACT

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Fusarium oxysporum was isolated from 1–5% of the seed from two of six Douglas-fir seed sources examined. Isolates were recovered both from seed washed but not surface-sterilized and from seed treated with hydrogen peroxide. In addition, isolates were obtained from roots of diseased seedlings where infection occurred on random individual seedlings, some of which were grown in growth chambers, indicating that the pathogen had been introduced with the seed. Soaking Douglas-fir seed in spore suspensions of either seed or root isolates caused significant preemergence damping-off or seed rotting compared with uninoculated controls. Seedling root isolates caused more postemergence damping-off than seed isolates. Two isolates from sugar pine caused preemergence damping-off on Douglas-fir, but only one caused postemergence damping-off. Some preemergence and postemergence damping-off occurred in uninoculated controls from which *F. oxysporum* was recovered; the latter was the result of either seedborne inoculum or cross-contamination. We observed significant sporulation by *F. oxysporum* on aboveground infected stems or cotyledons and seed coats as well as on the medium surface that could readily have been air-blown or water-splashed to other seedlings. Implications regarding seedborne pathogenic *F. oxysporum* on Douglas-fir seed as a means of introduction into fumigated nursery beds or into container nurseries with subsequent spread from sporulation on aboveground plant parts or seed coats are discussed.

Preemergence and postemergence damping-off of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) has often been associated with infection by *Fusarium oxysporum* Schlecht. (2). The disease is thought to initiate from propagules of the fungus in the soil that are associated with dead roots from the previous infected crop (4,5). Soil fumigation gives partial but not complete control of the disease in nurseries (6,12).

Fusarium spp. isolated from conifer seed have been shown to cause both preemergence and postemergence damping-off of seedlings (9,10,14). The possible seedborne origin of *Fusarium* on Douglas-fir has not been reported, although Bloomberg (1) did isolate unidentified *Fusarium* spp. from aseptically grown Douglas-fir. The pathogenicity of these isolates was not tested, however.

Heavy losses from suspected seedborne *Fusarium* spp. have been observed in container-grown longleaf pine (*Pinus palustris* Mill.) in Louisiana (11) and nursery-bed sugar pine (*P. lambertiana* Dougl.) in northern California (R. S. Smith, Jr., *personal communication*). We have observed significant losses in Douglas-fir to *F. oxysporum* in several commercial nurseries as well as in an experimental shelter house, a greenhouse, and a growth chamber where seedlings were grown in fumigated or pasteurized pathogen-free soil media. The fact that most sources of soilborne inoculum were eliminated and air and water sources were reduced under the latter controlled conditions led us to suspect that the *F. oxysporum* was seedborne. This study, therefore, was conducted to determine whether *Fusarium* was carried on seed of Douglas-fir and, if so, to assess the ability of seed *Fusarium* to cause either preemergence or postemergence damping-off of Douglas-fir.

MATERIALS AND METHODS

Douglas-fir seed from six western Oregon seed sources was tested for the presence of *F. oxysporum* in the seed and on the seed coat. Two hundred seeds of each seed source were washed in a Tween 20 solution for 1 hr and then rinsed in running tap water for 30 min. Half of the seeds were plated directly onto Komada's selective agar medium for *Fusarium* (8). The remaining 100 seeds were soaked in 30% hydrogen peroxide for 40 min and rinsed by pouring 2 L of sterile-distilled water (SDW) over the seeds and plating them on Komada's medium. Of the six seed sources examined, two sources yielded one isolate each of *F. oxysporum* from the seed coat (DF-2 and DF-3) (Table 1), and from one of these two sources, we obtained one isolate (DF-1) that escaped the hydrogen peroxide surface-sterilization and was therefore considered an internal seed isolate.

Isolates of *F. oxysporum* were also obtained from roots of diseased seedlings growing under shelter-house (DF-4), greenhouse (DF-8), and growth-chamber conditions (DF-5). We suspected that the latter isolates were carried with the seed because the diseased seedlings occurred individually and were protected from external sources of inoculum. The growth-chamber isolate (DF-5) was obtained from seedlings grown from the same seed source from which a seed-coat isolate was obtained (DF-3).

Fusarium was isolated from the margins of root lesions. Root pieces were surface-sterilized for 2 min in 30% hydrogen peroxide, washed for 1 min in SDW, and plated on Komada's medium. Additional isolates of *F. oxysporum* from nursery-bed sugar pine with a suspected seedborne *Fusarium* disease problem were obtained from M. Srago, U.S. Forest Service, San Francisco, CA. Single-spore cultures of all isolates (Table 1) were maintained on V-8 juice agar (13).

To test the role of seedborne or rootborne isolates of *Fusarium* in preemergence and postemergence damping-off, Douglas-fir seed was surface-sterilized as described earlier to remove *Fusarium* from the seed coat. Seeds were soaked in a suspension of 10^4–10^6 spores per milliliter (macroconidia, microconidia, and chlamydospores) washed from the fungus isolates growing on V-8 juice agar plates. Controls were soaked in SDW decanted from uninoculated V-8 juice agar plates. Treated seeds were sown into 65-cm^3 Leach cells containing steam-pasteurized (70 C for 30 min (1:1:1, v/v) peat-vermiculite-sand mix (pH 6.0). Containers were placed in the greenhouse in five replicate randomized blocks of 20 seeds for each treatment under supplemental light from high-pressure sodium vapor lamps (16-hr photoperiod) and 25/15 C day-night temperatures.

Eight weeks after sowing, counts of

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unemerged seeds (preemergence mortality) and emerged seedlings that damped-off above the soil line (postemergence damping-off) were made. Where possible, unemerged seeds and diseased seedlings were recovered and plated on Komada's medium to confirm the presence of *F. oxysporum*. The colony morphology of reisolates on V-8 juice agar was compared with stock cultures of the isolates used in the inoculation trials.

RESULTS

F. oxysporum was recovered from seeds or seedlings from some but not all seed sources, and the percentage recovery was from 1 to 5%. More isolates originated from seed that was not surface-sterilized than from seed treated with hydrogen peroxide.

All of the isolates of *F. oxysporum* from Douglas-fir seed and seedlings except isolate DF-1 (Table 1) caused significant preemergence damping-off of Douglas-fir seedlings or seed rot (Table 1). Although the internal isolate (DF-1) from the Oakridge seed source did not appear to affect seed emergence, the seed-coat isolate (DF-3) from this seed source was pathogenic. The seed-coat isolate (DF-2) from the Corvallis seed source caused as much preemergence damping-off as seedling isolate (DF-5) of this seed source obtained from Douglas-fir seedling roots growing under controlled growth-chamber conditions. However, although the seedling root isolates from Douglas-fir (DF-4, -5, and -8) caused significant postemergence damping-off, the seed isolates (DF-1, -2, and -3) did not (Table 1). Both sugar pine isolates reduced seedling emergence, but only one caused significant postemergence damping-off. Low levels of both preemergence and postemergence damping-off in the uninoculated control treatment indicated that the seed source tested may also have carried *Fusarium*.

Isolations from ungerminated seed and damped-off seedlings were consistently positive for *F. oxysporum*. The isolates obtained from either diseased seed or plant material within a treatment looked the same on V-8 juice agar. When colony morphology was compared with the original stock culture, however, they were not always similar, indicating that either the original isolate had mutated during the experiment or other *Fusarium* isolates were present as a result of cross-contamination from other treatments. Growth and sporulation of *Fusarium* on aborted seed germinants or cast seed coats and on the soil surface were observed in every treatment including the uninoculated controls. This sporulation was thought to be the source of potential cross-contamination between treatments and would have significant implications for pathogen spread in commercial nurseries.

DISCUSSION

Our study demonstrated that seedborne *F. oxysporum* can cause preemergence damping-off of Douglas-fir when present on the seed coat at the time of sowing into pasteurized soil medium. Seedborne isolates examined did not, however, cause significant postemergence damping-off compared with isolates from diseased roots. Pawuk (10) found that some but not all of the *Fusarium* he isolated from longleaf pine seed caused either preemergence or postemergence damping-off or both when tested on that pine species. Douglas-fir isolates of *F. oxysporum* from different stages of the disease can differ in the types of disease they cause (2). On the other hand, seedling root isolates of suspected seedborne origin may have been those with an additional potential for causing postemergence damping-off. We believe that if a larger number of seed isolates were obtained and tested, more would be found capable of causing postemergence damping-off.

Fusarium isolates from sugar pine also caused preemergence seed rotting or damping-off of Douglas-fir seedlings, indicating that there is a lack of specificity between conifer hosts. The observation that only one of the isolates caused postemergence disease supports the concept of varied disease-producing potential among isolates of *F. oxysporum* (2) or could be explained on the basis of mutation in culture to a less virulent form.

In addition to the fact that pathogenic *Fusarium* can be introduced into fumigated nurseries on seed, our observations that *F. oxysporum* readily sporulated above the soil surface on infected stems or cotyledons as well as on cast or uncast seed coats could be highly significant to the epidemiology of the Fusarium disease in either ground-bed or container nurseries. If confined below the soil surface, *Fusarium* might only kill a low percentage of seedlings from contaminated seed and spread slowly from that point source, but spores produced on the soil surface or on plant parts above the soil surface have much greater potential for being spread some distance by wind or water as demonstrated by Vaartaja (15). If that spread occurred rapidly enough, the disease incidence during the first growing season could be greatly increased and the extent of soil infestation would be much more extensive.

Although the study was confined to greenhouse conditions, it points out the potential for introducing *Fusarium* via seed into fumigated nursery soil. The disease-producing potential of seedborne *F. oxysporum* on Douglas-fir may be significant because the greatest infection occurs within a few weeks after seed is sown (3). Several epidemiological observations of Fusarium disease of Douglas-fir in the nursery support the role of seedborne *Fusarium* as a potential source of the disease (4,5) and might explain in part why soil fumigation often gives incomplete disease control (6,12). In two newly established forest seedling nurseries in Oregon, heavy disease losses of Douglas-fir from *Fusarium* occurred in both fumigated and unfumigated soil (R. G. Linderman, *unpublished*). Thus, treatment of Douglas-fir seed with fungicides or other disinfectants to

Table 1. Identification of *Fusarium oxysporum* isolates from Douglas-fir and sugar pine and their ability to cause preemergence and postemergence damping-off of Douglas-fir

Isolate identification[v]	Seed source	Isolate source	Percentage damping-off		
			Preemergence[w]	Postemergence[x]	Total[y]
Control	...	...	18.9 a[z]	1.5 a	21.7 a
DF-1	Oakridge, OR	Surface-sterilized seed (internal)	25.2 a	0.2 a	26.5 a
DF-2	Corvallis, OR	Non-surface-sterilized seed (seed coat)	51.0 b	8.1 abc	56.2 bc
DF-3	Oakridge, OR	Non-surface-sterilized seed (seed coat)	43.0 b	3.9 ab	48.0 b
DF-4	Reedsport, OR	Seedling roots (shelter house)	59.4 b	25.8 cd	72.5 c
DF-5	Corvallis, OR	Seedling roots (growth chamber)	53.0 b	23.4 cd	62.3 bc
DF-8	Corvallis, OR	Seedling roots (greenhouse)	60.7 b	33.4 d	71.3 c
SP-1	Northern CA	Seedling roots (nursery bed)	49.2 b	2.0 a	53.4 bc
SP-2	Northern CA	Seedling roots (nursery bed)	58.1 b	19.1 bcd	63.3 bc

[v] DF = Douglas-fir (*Pseudotsuga menziesii*); SP = sugar pine (*Pinus lambertiana*).
[w] Percentage of total seeds planted that did not emerge.
[x] Percentage of successfully emerged seedlings that showed symptoms after 6 wk.
[y] Percentage of total seedlings that either did not emerge or emerged and showed symptoms.
[z] Values are the mean of five replicates of 20 observations. Column means followed by the same letter are not significantly different at the 0.05 level according to Duncan's multiple range test of the arc sine transformed values.

control *Fusarium* (7) may be required in conjunction with soil fumigation or use of soil fungicides.

Another effective approach possibly leading to disease control would be to determine and eliminate the source of seedborne *Fusarium*. *Fusarium* spp. have been isolated from loblolly pine (*Pinus taeda*) cones as well as from seed (9). Accordingly, Douglas-fir cones, seed harvesting and extraction equipment, and storage containers and equipment should be examined for the presence of *Fusarium*.

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