# Phytophthora Root and Crown Rot of Junipers in California

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**ABSTRACT**

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Isolations showed that *Phytophthora cinnamomi* was a predominant cause of root and crown rot of container-grown junipers in California nurseries. In one landscape planting, *P. cryptogea* was isolated from severely diseased junipers. In greenhouse experiments with *P. cinnamomi, Juniperus sabina* 'Tamariscifolia' and *J. chinensis* 'Gold Coast' were the most susceptible of eight cultivars inoculated. *P. cryptogea* caused serious disease on almost all cultivars but only when junipers were periodically exposed to flooded soil conditions, demonstrating the need for careful irrigation.

Root and crown rot of junipers is an important disease in California. It is recognized as a chronic problem in landscape plantings throughout the state and in commercial nurseries where it affects container-grown junipers at all stages of production, sometimes resulting in serious economic losses. Although it is considered an important problem in California, relatively little research has been done on it. In perhaps the only published report, Raabe et al (18) isolated *Phytophthora cinnamomi* Rands, *Rhizoctonia solani* Kühn, *Macrophomina phaseolina* (Tussi) Goid., and several species of *Pythium* from the roots of diseased junipers in a field planting in which the susceptibility of many prostrate cultivars to a "root rot complex" was compared. In Georgia, Gill (8) found that *P. cinnamomi* was an important cause of root and crown rot of container-grown junipers and that shore juniper (*Juniperus conferta* Parl.) was the species most commonly affected. In North Carolina, Fravel and Benson (7) also implicated *P. cinnamomi* in shore juniper decline and suggested that extremes of soil water status might play a role in disease severity.

Many species and cultivars of juniper are grown in California, but it is tam juniper (*J. sabina* L. 'Tamariscifolia') rather than shore juniper that appears to be most severely affected by root and crown rot under California conditions. This paper reports the results of a study undertaken to determine whether species other than *P. cinnamomi* might be involved in disease and the extent to which juniper cultivars might differ in their susceptibility to *Phytophthora* spp. under controlled conditions. Additionally, because irrigation water is applied to landscape plantings and nursery crops in California throughout the growing season, we wanted to study the effect of irrigation practices on disease severity among different cultivars.

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## MATERIALS AND METHODS

**Isolation and identification of *Phytophthora* spp.** Container-grown junipers showing symptoms of root and crown rot were collected from nurseries in the Sacramento Valley, central coast region of California, and southern portion of the Los Angeles Basin. Collection of landscape plants was confined to the area around Davis, CA. Tissue pieces from the margins of crown cankers or diseased roots were cut into 1-cm segments, surface sterilized in 0.5% sodium hypochlorite solution for 1 min, and plated on a selective agar medium (5). A total of 15 to 20 root and crown pieces were plated from each plant, and culture plates were incubated in the dark at 25 C for 5 days. Mycelium from the advancing margin of each colony was then transferred to fresh selective medium. Contaminant-free cultures were transferred to slants of cornmeal agar until further study.

*Phytophthora* spp. were isolated from soils by soil-baiting techniques, using either green, unblemished pear fruit (16) or a modification of the lupine-baiting technique described by Chee and Newhook (3). In this modified method, germinated lupine seeds with radicals 4–6 cm long were placed in glass petri dishes containing 50 $cm^3$ of soil flooded with distilled water. The germinated seeds were laid in the 4–6 mm of water standing on the surface of the flooded soil and were incubated at room temperature for 48 hr, after which they were removed, rinsed in distilled water, and incubated an additional 48 hr at room temperature in humidity chambers on clean, moist blotters. At the end of this incubation period, the radicals were cut into 1-cm segments and plated on the selective medium as described above. Identification of *Phytophthora* spp. isolated from soil or plant material was made using the descriptions of Waterhouse (20,21), Tucker (19), and Leonian (13).

**Pathogenicity.** The pathogenicity of *Phytophthora* isolates obtained from surveys was determined by inoculating junipers in greenhouse tests. Inoculum was prepared by growing each *Phytophthora* isolate for 4 wk at room temperature in 1-L jars containing 500 $cm^3$ of vermiculite and 200 ml of V-8 juice broth (200 ml of V-8 juice, 2 g of calcium carbonate, and 800 ml of distilled water). The inoculum was rinsed repeatedly in tap water to remove remaining nutrients and mixed with steam-pasteurized U.C. soil mix (1) at the rate of one part inoculum to nine parts soil mix.

Liner-size junipers (*J. sabina* 'Tamariscifolia') were obtained from a commercial nursery, removed from their containers, and examined to assure there were no symptoms of disease. The plants then were gently shaken to dislodge the adhering potting medium from their roots and transplanted into 2-L crocks containing the infested U.C. mix. Control plants were transplanted into crocks of U.C. mix blended with uninfested vermiculite. After transplanting, the drainage hole at the base of each crock was plugged and enough water added to completely saturate the soil. Plants were held in this condition for 48 hr after which the soil was allowed to drain and a cycle of twice-weekly irrigations was established. The 48-hr saturation treatments were repeated every 2 wk throughout the 8-wk test period, after which all plants were removed from the soil and their roots washed and examined for symptoms of infection. Decayed root pieces and necrotic crown tissue were plated on the selective medium to confirm the presence and identity of the pathogen.

**Susceptibility of juniper cultivars.** Two species of *Phytophthora* were used in greenhouse experiments to compare the development of root and crown rot on various juniper species and cultivars under moderate and heavy irrigation treatments. The isolates used included an isolate of *P. cinnamomi* Rands of the $A_2$ mating type and an isolate of *P. cryptogea* Pethy. & Laff. The juniper

cultivars used were *J. chinensis* L. 'Gold Coast'; *J. chinensis* 'Plumosa' (= *J. chinensis* 'Shimpaku' of nursery trade [12]); *J. chinensis* 'Prostrata'; *J. conferta* Parl. 'Blue Pacific' (shore juniper); *J. horizontalis* Moench 'Bar Harbor'; *J. horizontalis* 'Prince of Wales'; *J. sabina* 'Tamariscifolia' (tam juniper); and *J. virginiana* L. 'Prostrata.'

Ten plants of each cultivar were transplanted singly into 10-cm-diameter plastic pots containing either pasteurized U.C. mix or mix infested with one of the two *Phytophthora* spp. Five plants in each inoculation treatment were watered twice a week and allowed to drain freely throughout the 8-mo experiment, whereas the other five plants were subjected to 48-hr intervals of flooding every 2 wk. The flooding was done by placing the five pots of one inoculation treatment together in a watertight container and adding water until 0.5 cm of water stood on the soil surface. Between flooding treatments, the plants were irrigated and drained in the same manner as plants in the unflooded treatment. At monthly intervals, a randomly selected pot from each cultivar in each inoculation treatment was assayed for the presence of *Phytophthora* spp. using the lupine-baiting technique. Pots of uninoculated control plants were also assayed to assure that they remained free of *Phytophthora* spp.

At the termination of the experiment, all plants were removed from the pots and their roots washed to remove adhering soil. The roots were separated from the tops by cutting at the soil line and blotted in a standardized manner to remove excess water; fresh weights were then determined. Fresh weights of inoculated and uninoculated plants were compared by means of a *t*-test to determine whether differences in root fresh weight were statistically significant. As an additional index of disease severity, the number of root segments from which *Phytophthora* spp. were successfully cultured was determined for each treatment. Eighteen randomly selected root segments were collected from each root system after weighing, surface sterilized for 30 sec, rinsed in distilled water, and plated on the selective medium.

## RESULTS AND DISCUSSION

**Disease survey.** Symptoms of root and crown rot of junipers, particularly in the warm Sacramento Valley and Los Angeles Basin, were characterized by a stunted, blue gray appearance of the foliage, which became very severe with the onset of high temperatures in the summer months. Eventually, the foliage would turn brown as the plant died. Sometimes, single branches would die first, giving the disease a one-sided appearance. Examination of root and crown tissues of plants exhibiting these foliar symptoms consistently revealed extensive root decay with necrosis of crown tissues that sometimes extended several centimeters above the soil line. In nurseries, severe disease symptoms of both foliage and roots were most commonly encountered in the cultivars *J. sabina* 'Tamariscifolia' and *J. chinensis* 'Gold Coast.' Isolations from diseased tissues consistently gave rise to cultures of *Phytophthora*. At times, particularly during the hot summer months, it was difficult to isolate *Phytophthora* directly from the tissues of diseased plants, but they frequently could be isolated from the rhizosphere of affected plants by either of the soil-bait methods.

Identification of the cultures obtained during this survey clearly implicated *P. cinnamomi* as the major cause of root and crown rot in many large California nurseries. Isolates obtained from different nurseries and different juniper cultivars were paired in culture with $A_1$ and $A_2$ testor strains of *P. cinnamomi* (isolates 433 and 215, respectively, obtained from S. M. Mircetich, USDA/ARS, University of California, Davis) and were all identified as the $A_2$ strain. Although other species of *Phytophthora* may be involved in disease, our failure to detect them in this survey suggests that they play a relatively minor role in nurseries. Indeed, the only other species implicated as a cause of root and crown rot of junipers was *P. cryptogea*, which was isolated from severely diseased plants, believed to be *J. chinensis* 'Gold Coast,' in a landscape at Davis. This is believed to be the first report of a *Phytophthora* sp. other than *P. cinnamomi* attacking junipers (7–9,11,14,18). Also, *Pythium* spp. were sometimes isolated from plants in advanced stages of root rot, but they were not considered to be a significant factor in disease relative to *Phytophthora* spp., and their pathogenicity to juniper was not studied.

The root decay observed in plants collected from nurseries and landscape plantings was readily reproduced in greenhouse inoculations with the isolates of *P. cinnamomi* and *P. cryptogea*. However, over the short term of the pathogenicity tests, crown necrosis was only sometimes evident, and the blue gray discoloration of the foliage observed under hot field conditions was rarely observed in the greenhouse environment. Foliar symptoms of plants maintained in the greenhouse became evident only with extensive (≥50%) decay of the root system; even in the field, it was apparent that junipers generally could tolerate significant amounts of root decay before aboveground symptoms became evident. A similar situation has been observed on pine trees infected with *P. cinnamomi* (17), in which varying degrees of root infection, from a mere darkening of primary and secondary feeder roots to death of an entire portion of the root system, resulted in few aboveground symptoms.

**Susceptibility of juniper cultivars.** Among the juniper cultivars inoculated in the greenhouse experiments, there were significant differences in susceptibility to Phytophthora root and crown rot. Furthermore, the two species of *Phytophthora* used in the inoculations differed significantly in their ability to cause root and crown rot under given sets of environmental conditions. In treatments where plants received only moderate amounts of irrigation, significant decreases in root fresh weights were observed in only a few host-pathogen combinations (Table 1). An isolate of *P. cinnamomi* that originated from *J. sabina* 'Tamariscifolia' from southern California

**Table 1.** Mean root fresh weight of cultivars of *Juniperus* spp. grown in uninfested soil or in soil infested with either *Phytophthora cinnamomi* or *P. cryptogea*

| | Root fresh weight (g)[a] | | | | | |
|---|---|---|---|---|---|---|
| | Not flooded[b] | | | Flooded[b] | | |
| Cultivar | Control | *P. cinnamomi* | *P. cryptogea* | Control | *P. cinnamomi* | *P. cryptogea* |
| *J. chinensis* | | | | | | |
| 'Gold Coast' | 7.53 | 3.09[c] | 6.15 | 5.55 | 1.94[c] | 1.11[c] |
| 'Plumosa' | 5.39 | 7.58 | 4.62 | 3.76 | 2.35 | 0.33[c] |
| 'Prostrata' | 15.04 | 10.89 | 9.46 | 17.20 | 9.45 | 9.75 |
| *J. conferta* | | | | | | |
| 'Blue Pacific' | 12.60 | 8.68 | 3.81[c] | 4.92[d] | 4.24 | 0.88 |
| *J. horizontalis* | | | | | | |
| 'Bar Harbor' | 7.29 | 8.71 | 9.27 | 7.15 | 5.59 | 4.10[c] |
| 'Prince of Wales' | 42.67 | 37.79 | 34.16 | 19.35[d] | 23.31 | 6.22[c] |
| *J. sabina* | | | | | | |
| 'Tamariscifolia' | 11.21 | 4.83[c] | 9.36 | 10.53 | 3.41[c] | 3.69[c] |
| *J. virginiana* | | | | | | |
| 'Prostrata' | 17.18 | 16.20 | 13.92 | 10.15 | 15.71 | 7.95 |

[a] Fresh weights of roots, determined 8 mo after inoculation, are the means for five plants.
[b] Plants not flooded were watered twice weekly and allowed to drain freely; flooded plants were irrigated twice weekly and flooded for 48-hr intervals once every 2 wk.
[c] Means differ significantly from uninoculated controls of the same irrigation treatment (*t*-test, $P = 0.05$).
[d] Means differ significantly from uninoculated controls in the unflooded treatment (*t*-test, $P = 0.05$).

only caused serious root rot on that same cultivar (Fig. 1A) and on *J. chinensis* 'Gold Coast' (Table 1). These results were consistent with our findings in commercial nurseries, where *P. cinnamomi* appeared to be a dominant pathogen and *J. sabina* 'Tamariscifolia' and *J. chinensis* 'Gold Coast' were the most seriously affected cultivars. *P. cinnamomi* also caused noticeable amounts of root rot on *J. conferta* 'Blue Pacific,' but, because of variation among these plants, it was not possible to confirm a significant decrease in root fresh weight (Table 1). With moderate irrigation, all other cultivars appeared fairly resistant or tolerant to *P. cinnamomi*. In contrast to *P. cinnamomi*, the isolate of *P. cryptogea* caused no significant reduction in root fresh weight on *J. sabina* or *J. chinensis* 'Gold Coast' (Fig. 1, Table 1). Indeed, in the absence of flooding, *J. conferta* 'Blue Pacific' was the only cultivar in which a significant reduction in root fresh weight was observed following inoculation with this isolate.

When plants were periodically exposed to flooded soil conditions, the root systems of all cultivars generally appeared less vigorous than those in the moderate irrigation treatment. The cultivars *J. horizontalis* 'Prince of Wales' and *J. conferta* 'Blue Pacific' were particularly sensitive to periodic flooding, as the root fresh weights of uninoculated controls were significantly reduced relative to controls in the unflooded treatment (Table 1). Indeed, when the cultivar *J. conferta* 'Blue Pacific' was flooded, one uninoculated plant died and two others developed badly decayed root systems in the absence of any detectable pathogens.

Under conditions of periodic flooding, *P. cinnamomi* again caused significant reductions in root fresh weight only on *J. sabina* and *J. chinensis* 'Gold Coast' (Table 1). It also caused noticeable amounts of disease in *J. horizontalis*, but there was no statistically significant difference in root fresh weight. In contrast to *P. cinnamomi*, the severity of disease caused by *P. cryptogea* was greatly enhanced by periodic flooding. For example, the cultivars *J. sabina* 'Tamariscifolia' and *J. chinensis* 'Gold Coast,' which were unaffected by *P. cryptogea* in the unflooded treatments, were badly diseased in the flooded treatments (Table 1, Fig. 1A). Most of the other cultivars also showed significant reductions in root fresh weight when inoculated with *P. cryptogea* under flooded conditions (Table 1). Only the cultivars *J. virginiana* and *J. chinensis* 'Prostrata' resisted severe root decay, although symptoms of disease were apparent (Fig. 1B).

Periodic flooding of the soil also increased the percentage of root pieces from which the pathogens could be reisolated. This was particularly true of

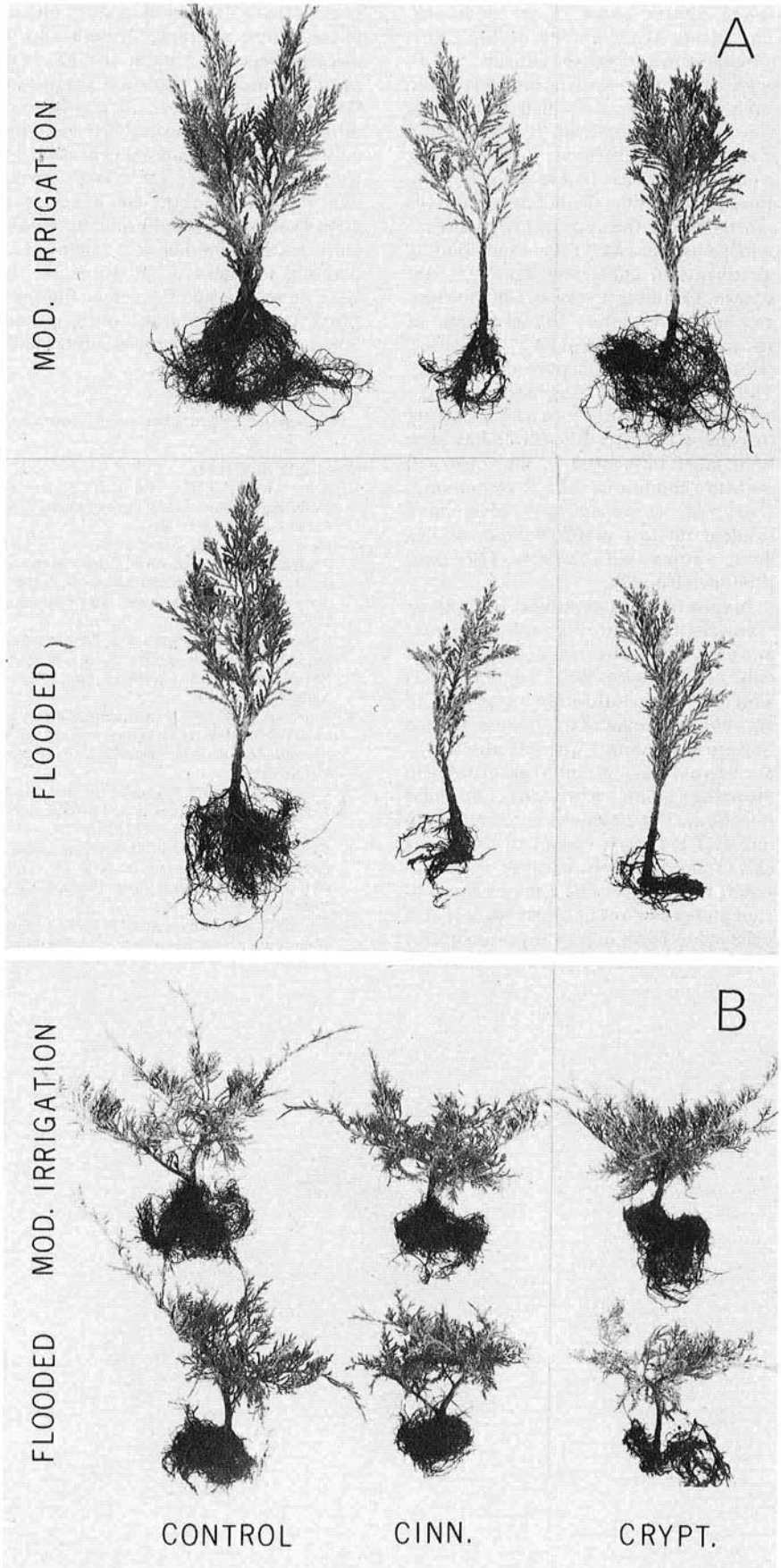


**Fig. 1.** Relative severity of Phytophthora root and crown rot on **(A)** *Juniperus sabina* 'Tamariscifolia' and **(B)** *J. virginiana* 'Prostrata' after 8 mo. Plants were grown in uninfested soil (control) or soil infested with *Phytophthora cinnamomi* (Cinn.) or *P. cryptogea* (Crypt.) and exposed to treatments of moderate irrigation (twice per week) or periodic flooding (48 hr every 2 wk).

*P. cryptogea*, which also caused more severe disease under those conditions. The stimulatory effect of high soil moisture on disease caused by *P. cryptogea* was consistent with the circumstances under which this isolate was originally obtained. It was isolated from severely diseased junipers at a landscape site that had several flooding episodes resulting from heavy rainfalls shortly before the appearance of disease symptoms. The exact role of the flooding treatments in enhancing disease is not known. Certainly, cycles of soil moisture are known to affect the processes of sporangial formation (4,5), zoospore release (15), and zoospore motility (6). The fact that only *P. cryptogea* showed a significant response to the flooding treatments suggests this species may have been more influenced by the high soil moisture conditions than *P. cinnamomi*. However, flooding may also have resulted in host predisposition, as has been shown with alfalfa (10) and rhododendron (2).

In spite of the susceptibility of *J. sabina* 'Tamariscifolia' to Phytophthora root and crown rot, consumer demand for this cultivar has always been high, and it is widely grown in California nurseries. The chronic occurrence of this disease in some nurseries, combined with the absence of aboveground symptoms to aid growers in detecting plants with early or mild infections, has obvious consequences in terms of pathogen spread to new areas (22). Indeed, simply because *P. cinnamomi* was found to be a major cause of root and crown rot in nurseries, it is also believed to be an important cause of this disease in landscape plantings. Although this was a relatively limited study, it is evident that a number of juniper cultivars possess some tolerance or resistance to disease, depending upon the *Phytophthora* sp. and soil moisture conditions. Other workers have also observed differences in the susceptibility of juniper cultivars to *P. cinnamomi* (11) as well as root rot complexes (18). With further evaluation, it might be possible to identify many horticulturally acceptable cultivars that could be recommended for planting in high-risk situations. If this were done, the significance of Phytophthora root rot in both nurseries and landscapes could be reduced substantially.

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