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Crown and Root Rot of Cultivated Wild Rice in California Caused by *Phytophthora erythroseptica* sensu lato

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ABSTRACT

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A species of *Phytophthora*, identified as *P. erythroseptica* sensu lato, was found to be the causal agent of a crown and root rot disease of cultivated wild rice (*Zizania palustris*) in California. In the field, plants ranging in age from the early tillering stage to the grain-filling stage were killed by the pathogen. This is the first report of a species of *Phytophthora* pathogenic on wild rice and the first report of a species of *Phytophthora* causing a serious disease of a mature grass host.

Wild rice (*Zizania palustris* L.) has been recently introduced into northern California and currently there are approximately 8,000 ha in cultivation. Wild rice is seeded into flooded paddies (late February–April), which remain flooded at a depth of 12–30 cm until harvest (July–August). In June of 1985 and in May of 1986, growers reported that large numbers of plants in their fields were dying suddenly. The wild rice plants in affected fields showed drought symptoms even though they were growing under flooded conditions. The leaves of affected plants progressively desiccated and became brittle, turning from green to gray-green and finally to tan. The crown, many of the adventitious roots, the first internode, and portions of the leaf sheaths surrounding the crown were necrotic (Fig. 1A). Typically, the crown showed the most severe necrosis. Plants at different stages of development ranging from the early tillering stage to the grain-filling stage were affected and died. Frequently, the crown became so rotted that the tillers separated from it and floated to the surface of the paddy water, leaving the roots in the soil (Fig. 1B). A species of *Phytophthora* was isolated from the crowns and roots of symptomatic plants and found to be responsible for the syndrome.

MATERIALS AND METHODS

Isolation of the pathogen. Plants showing different stages of symptom development were collected from the field for isolation. To isolate the fungus, pieces of necrotic crowns and roots were placed on water agar or floated in sterile water. Hyphae and or sporangia produced from diseased tissue were transferred to V-8 agar or to PVP agar (5). PVP agar helped to eliminate bacterial contamination, but the fungus grew poorly on PVP agar.

Characterization of the pathogen. To induce sporangia, 3-mm-diameter mycelial plugs of the fungus from V-8 agar (10 ml of agar/100 × 15 mm petri dish) were placed in soil extract and incubated in the dark for 7.5 hr at 24 C. For formation of oospores, the fungus was grown for 1 mo at 24 C in the dark on clear V-8 agar (5) modified to contain 100 ml of clarified V-8 juice, 30 mg of β-sitosterol, 20 mg of tryptophan, 1 mg of thiamine HCl, 100 mg of $CaCl_2$, 17 g of agar, and 900 ml of deionized water. The fungus produced oospores on water agar, but not as abundantly as on the modified clear V-8 media. One hundred sporangia, 100 antheridia, and 100 oospores were measured from each of two isolates, P1 and P3; because organs of P1 and P3 were similar, measurements were combined. Radial growth of the two isolates was measured on V-8 agar plates incubated in the dark at temperatures in three-degree increments from 9 to 42 C and at 4 C. To induce chlamydospore production, mycelial plugs of the two isolates from V-8 agar were placed in 10 ml of sterile water in 19 × 150 mm test tubes at 15 C for 6 wk (2).

Pathogenicity tests. Pathogenicity tests with isolates P1 and P3 were conducted in the greenhouse, where temperatures ranged from 18 to 30 C. Each isolate was inoculated to 10 wild rice plants in separate tests. Each test was repeated once. Wild rice plants were grown individually in 15-cm pots in a sterile, heavy clay soil that was permanently flooded. The plants were inoculated 45 days after planting by placing 12 7-mm-diameter plugs of the fungus from V-8 agar into each of the flooded pots. Sporangia were initiated before inoculation by placing the plugs in sterile water overnight. Control plants in each test received plugs of plain V-8 agar. The previously described procedure was used to reisolate the fungus from symptomatic plants.

RESULTS

Isolation of the pathogen. A single species of *Phytophthora* was consistently isolated from symptomatic plants collected in the field, regardless of the stage of disease development. We observed that the fungus was often located in the xylem of infected tissues, and oospores were observed once in the cortex of a root adjacent to the crown.

Pathogenicity tests. Symptoms like those observed in the field and previously described developed in 7–10 days in 60–70% of the inoculated wild rice plants in each test. All affected plants died. Control plants showed no disease symptoms. The fungus was reisolated from the crown of all symptomatic plants.

Identification of the pathogen. Cultures of the fungus on V-8 agar were uniform and fluffy, with abundant, dense aerial mycelium. Hyphae were 3.5–6 μm in diameter and did not produce hyphal swellings or chlamydospores. Sporangiophores proliferated internally through empty sporangia, sometimes with several sporangia nested one inside the other, or developed sympodially from below each successive sporangium (Fig. 1C). With the latter type of proliferation, the sporangia were generally clustered and not prolaxed. Sporangia were sometimes produced on cleared V-8 agar and water agar. Sporangia were ovoid to ovoid-obpyriform, sometimes ellipsoid, occasionally tapered at the base, nonpapillate, not caducous, 47–84 × 27–45 μm (average 64 × 37 μm) (Fig. 1C). The average length-to-breadth ratio of the sporangia was 1.75. Oogonia formed readily in single culture and were globose and did not taper at the base, although the oogonial stalk was sometimes tapered within the antheridium, 30–53 μm in diameter (average 41 μm). Antheridia were strictly amphigynous, spherical to oval, 10–20 × 13–18 μm (average 15 × 15 μm) (Fig. 1D). Oospores were aplerotic

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to markedly aplerotic, 22–42 μm in diameter (average 33 μm) with very thick walls, 2.5–6 μm (average 4 μm) (Fig. 1D). Both isolates of the fungus grew at temperatures ranging from 9 to 36 C, and the optimum temperature for radial growth was 27 C. Neither isolate grew at 39 or 4 C.

The *Phytophthora* species from wild rice is most similar to *P. erythroseptica* Pethybridge. It differs from *P. erythroseptica* in that the oospore wall is considerably thicker and it does not produce hyphal swellings. At this time, we believe these characteristics do not warrant the exclusion of the fungus from the species and we have placed it in *P. erythroseptica* sensu lato.

DISCUSSION

The pathogenicity tests demonstrated that the fungus was responsible for the disease observed in wild rice fields in California in 1985 and 1986. It is possible that only a portion of inoculated plants became diseased because wild rice is heterogeneous (1) and individual plants could differ significantly in susceptibility to the pathogen. In the field, not all plants in a given area became diseased and healthy plants were observed adjacent to diseased ones. In addition to the genetic variability of wild rice, other factors may influence disease development. In both years, the onset of symptoms in different fields at different stages of growth coincided with a period of hot, windy weather. It is possible that such climatic conditions predisposed the plants to disease.

Initially the disease progressed rapidly in the field, but after 3–4 wk it slowed dramatically and few additional plants showed symptoms. Distribution of the disease was relatively uniform in some fields and patchy in others. Affected fields sometimes looked relatively healthy at the end of the season as plants not killed by the pathogen appeared to compensate in growth for those that had died.

In general, *Phytophthora* spp. are not serious pathogens of the Poaceae, and this is the first report of a *Phytophthora* sp. pathogenic on wild rice and the first case of a *Phytophthora* sp. causing a severe disease of a grass host. There have been several reports of *Phytophthora* spp. causing minor diseases of grasses. *P. erythroseptica* and *P. megasperma* Drechsler have been reported to cause a soft rot of sugarcane seed pieces in the southern United States (8). *P. japonica* Waterhouse (= *Pythiomorpha oryzae* Ito & Nagai) was found to be the cause of a seed rot of rice (*Oryza sativa* L.) in Japan (3,12). Wang and Lu (10) reported a leaf blight of rice seedlings in Japan caused by *P. fragariae* Hickman var. *oryzo-bladis* McMurphy (4) found a *Phytophthora* sp. infecting oat leaves in California, similar to *P. colocasiae* Rac., and Sprague (9) found *P. cactorum* (Lebert & Cohn) Schroeter associated with soft rots of roots and leaves of various grass hosts in the Northwest. *P. cyperi* (Ideta) Ito and *P. cyperi-bulbosi* Seethalakashmi & Ramakrishnan were described from *Cyperus* spp. but have not been grown in culture or observed since first described (6). Waterhouse (11) listed *P. macrospora* (Sacc.) Ito & Tanaka as pathogenic on grasses, but the fungus has been transferred to the genus *Sclerophthora* (7).

The species of *Phytophthora* mentioned above cause mild diseases of grasses, and disease is restricted to the seed or seedling stage or is associated with unusually wet conditions. In contrast, *P. erythroseptica* from wild rice attacks and kills the host at all stages of growth and operates under regimes of moisture normal for host growth.

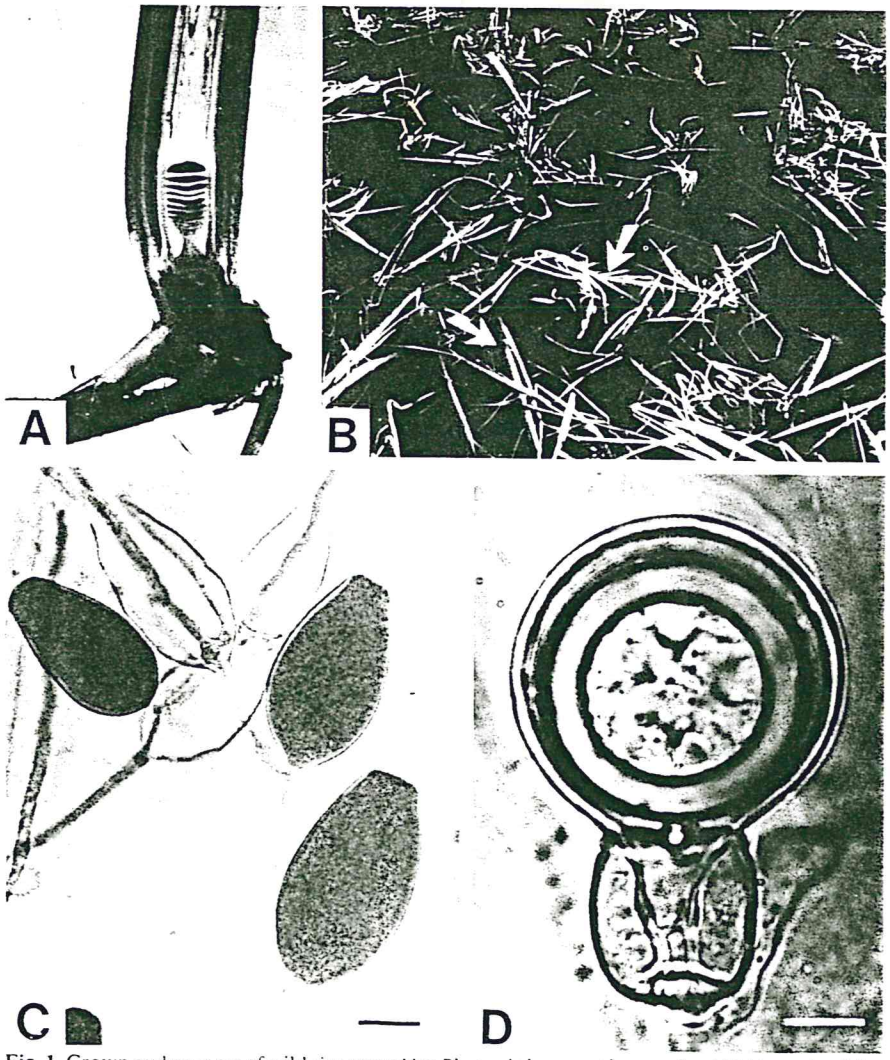

Fig. 1. Crown and root rot of wild rice caused by *Phytophthora erythroseptica*. **(A)** Necrosis of the crown and first internode of an infected wild rice plant. **(B)** Dead and dying infected wild rice plants in the field. Arrows indicate tillers floating on the surface of the paddy water. **(C)** Sporangia of *P. erythroseptica* showing both internal and external proliferation. Scale bar = 20 μm. **(D)** An oospore of *P. erythroseptica* showing the thick oospore wall. Scale bar = 10 μm.

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