Isolation, Distribution, Pathogenicity, and Identification of *Phytophthora* spp. on Asparagus in California

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ABSTRACT

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A survey was made in 1982 and 1983 of 57 asparagus fields throughout the main asparagus production areas of California. *Phytophthora* spp. were detected in all fields surveyed in the Sacramento and San Joaquin valleys, five out of six fields surveyed in the Salinas Valley, seven out of eight fields in Orange County, but no fields in the Coachella and Imperial valleys. *Phytophthora* spp. were isolated from spear, crown, or root tissue and baited from soil in fields planted with the asparagus cultivars Mary Washington, U.C. 72, U.C. 800, and U.C. 157. Out of a total of 84 isolates, 74 were identified as *P. megasperma* var. *sojae* and all were pathogenic on asparagus seedlings. Ten isolates of heterothallic *Phytophthora* spp. were isolated. Of these, four were pathogenic on asparagus seedlings and were identified as *P. cryptogea* (A_1 mating type); six that were not pathogenic could not be identified. Some fields in which *Phytophthora* was isolated from asparagus had previously been cropped with alfalfa, but no evidence of cross pathogenicity was found between three isolates of *P. m.* f. sp. *medicaginis* and asparagus (cv. U.C. 157), or *P. m.* var. *sojae* ex asparagus and alfalfa cultivars either resistant (Apollo 2, Washoe) or susceptible (Rere, Wairau) to *P. m.* f. sp. *medicaginis*. However, one isolate of *P. cryptogea* ex asparagus caused mild symptoms on Wairau and may be able to survive on alfalfa in the absence of asparagus. The results of this survey show that *Phytophthora* spp. were widespread in asparagus fields in 1982 and 1983 and could potentially have a major effect on asparagus production in California.

Additional keywords: *Asparagus officinalis* L., Phytophthora rot, resistant cultivars

A *Phytophthora* sp. was first reported as a pathogen of asparagus in California during 1938 when an unidentified species was found in spears before harvest and during shipment to markets (2). Since this first report, *P. richardiae* Buisman and *P. megasperma* Drechsler were found on asparagus in Australia (1,14); *P. cactorum* (Lebert & Cohn) Schroet. was reported to cause a storage rot of asparagus in France (15,16); *P. megasperma* Drechsler var. *sojae* Hildebrand was isolated from asparagus plants and soil throughout New Zealand (3,4,7); *P. m.* var. *sojae* and a second, unidentified species were isolated from asparagus plants and soil in the Sacramento-San Joaquin Delta of California (10); and, most recently, *P. megasperma* was found on asparagus in Switzerland (D. Gindrat, *personal communication*).

There had been no systematic survey of the asparagus growing areas of California to determine the distribution of Phytophthora rot of asparagus in the state. This paper reports the results of such a survey, methods used for isolation of *Phytophthora* from asparagus spears, crowns, roots, and soil, and the identity of the species found. A preliminary report of this work has been published (10).

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MATERIALS AND METHODS

Distribution of *Phytophthora*. A field study of asparagus fields in California was made during the wetter-than-normal winters and springs of 1982 and 1983. Fields were chosen to represent a range of ages of asparagus stand, soil types, and cultivars. Information on field size and previous cropping history was obtained for each field. Diseased spears, crowns, and root tissue were collected from each field and stored with ice in an ice chest or at 4 C in a refrigerator. Spears were surface-sterilized in 1% sodium hypochlorite for 3–5 min, and pieces of tissue (approximately 5 mm square) were excised with a sterile scalpel and plated on antibiotic potato-dextrose agar (PDA) (7). The agar was prepared by adding 50-mg pimaricin and 100-mg vancomycin to each liter of melted PDA. Crowns and roots were washed in tap water. Crowns were longitudinally bisected and tissue was excised from the advancing edge of crown rot and rotten portions of the crown before being surface-sterilized for 3–5 min in 1% sodium hypochlorite and plated on antibiotic PDA. Storage roots were surface-sterilized for 5–10 min, dissected, and plated on antibiotic PDA. Sixty to 90 pieces of root, spear, and crown tissue were plated from each field. The plates were incubated at 22 C in the dark and examined daily for 10 days for development of *Phytophthora* spp. from the plated tissues.

Several soil samples were collected at random from each field to a depth of 150 mm (using a 25-mm-diameter soil corer), bulked, and stored in sealed plastic bags at 4 C until they were assayed for *Phytophthora* spp., using a seedling baiting technique (7).

Pathogenicity tests. Seed of cv. U.C. 157 were surface-sterilized for 1 min in 3% sodium hypochlorite, rinsed in sterile distilled water, planted approximately 20 mm deep in 1.9 L plastic containers filled with a 1:1 (v/v) mixture of U.C mix (13) and steamed river sand (UCM/S), and grown until the seedlings were 100–150 mm high. Each isolate, grown on 20% V-8 juice agar (8), was homogenized in fresh pond water, and 100 ml of inoculum was poured over the surface of the UCM/S in each container. Three replicate containers of each isolate were flooded three times and soil temperature was maintained at 16 ± 2 C during flooding. Severity of root rot was rated using a scale described in an earlier paper and a disease severity index (DSI) was calculated, where 0 = no rot and 100 = all seedlings with >50% of their roots rotten (7,19).

Identification of *Phytophthora* spp. Cardinal temperatures for vegetative growth and colony type were studied on cornmeal agar (CMA). Production, type, size, and method of germination of sporangia were studied on 20% V-8 agar disks flooded with 1.5% nonsterile soil extract. Disks (4-mm-diameter) were cut with a cork borer from the advancing edge of *Phytophthora* colonies that were grown for 5 days at 18 C and an 18-hr photoperiod in 65-mm-diameter petri plates poured with 5 ml of 20% V-8 agar. The disks were placed in 65-mm-diameter petri plates (2 per plate) poured with 10 ml of 1.5% nonsterile soil extract. The disks were incubated at 18 C for 48 hr under continuous light. Soil extract was prepared by suspending 15 g of Yolo loam in 1 L of sterile distilled water, agitating the suspension with a magnetic stirrer for 24 hr at room temperature, centrifuging at 6,000 rpm for 35 min, and collecting the supernatant.

Production of sex organs and compatibility types were studied on clear V-8 juice agar containing V-8 juice cleared by centrifuging 40 ml for 10 min at 6,000 rpm, 16 mg of β-sitosterol dissolved in 20 ml of warm 95% ethyl alcohol, 89 mg of $CaCl_2$, 13.6 g of Bacto

agar (Difco), and 760 ml of sterile distilled water. This medium was adjusted to pH 4.8 with 0.1 N KOH before adding agar and autoclaving. Disks (4-mm-diameter) were cut using a cork borer from the margin of colonies that had been grown on V-8 agar in 65-mm-diameter petri plates at 19–20 C and an 18-hr photoperiod for 33 days. The disks were placed upside down in 65-mm-diameter petri plates (1 disk per plate) containing 10 ml of clear V-8 agar. Cultures were grown for 10 days to 2 wk at 20–21 C in the dark. The asparagus isolates of homothallic *Phytophthora* spp. were identified from descriptions of Waterhouse (22), Newhook et al (17), Tucker (21), and Ribeiro (18). Heterothallic *Phytophthora* spp. were sent to Dr. D. J. Stamps, Commonwealth Mycological Institute, Kew, Surrey, England for identification.

RESULTS

A total of 57 fields were sampled, representing an area of approximately 980 ha, or approximately 7.6% of the total area of asparagus planted in California in 1982 and 1983. *Phytophthora* spp. were isolated from all fields sampled in the Sacramento and San Joaquin valleys, from five out of six fields sampled in the Salinas Valley, from seven out of eight fields sampled in Orange County, but from none of the 13 fields sampled in the desert production areas in Riverside and Imperial counties (Fig. 1). The two fields where *Phytophthora* was not isolated in the Salinas Valley and Orange County had both been planted to asparagus for less than 1 yr. *Phytophthora* spp. were isolated from fields planted with the cultivars Mary Washington, U.C. 72, U.C. 800, U.C. 157, but not Brock's Special, which was planted in several fields in Riverside and Imperial counties. Successful isolations were from spears (77%), soil baiting (15%), crown tissue (6%), and storage root tissue (2%).

Figure 1. Distribution of *Phytophthora* spp. in asparagus production areas of California during 1982 and 1983. • = *Phytophthora* sp. isolated, □ = *Phytophthora* sp. not isolated.

Out of a total of 84 isolates, 74 were identified as *P. m.* var. *sojae*. Cultures were slow-growing and slightly radiate on CMA, but on PDA a moderate to considerable amount of aerial mycelium was produced. Sporangia were not produced on solid agar media, unless these media were flooded with 1.5% nonsterile soil extract. Sporangia were borne on simple or sparingly branched sporangiophores. Internal proliferation was common and sometimes subsporangial elongation was observed. Sporangia were obpyriform in shape, inconspicuously papillate, and no apical thickening of the sporangial wall was observed. Sporangia measured 18.8–62.5 μm long and 9.4–46.9 μm wide, averaging 37.9 × 27.9 μm. Sporangia germinated directly, especially at high temperatures ($\geq$27 C), but usually by production of zoospores.

Oogonia of *P. m.* var. *sojae* were produced readily on clear V-8 agar. They were smooth-walled, globose, colorless or slightly yellow when young, and yellow at maturity. Oogonia measured 15.6–31.3 μm, averaging 27.0 μm. Oospores were smooth-walled, spherical, colorless, and nearly filled the oogonia. Antheridia were predominantly paragynous, and a single antheridium was broadly appressed to each oogonium.

The mycelium growth rate on CMA increased from 9 to 24 C, then decreased to 30 C. No growth occurred at 33 C within 96 hr. All isolates of *P. m.* var. *sojae* caused disease on asparagus seedlings. Mean DSI ranged from 1.85 to 86.93.

Ten heterothallic *Phytophthora* isolates were also found. Four of these isolates (PmACA004, PmACA056, PmACA060, PmACA108) were identified as *P. cryptogea* Pethybr. & Laff. (A_1 mating type), and were highly virulent on asparagus seedlings. Mean DSI's ranged from 30.5 to 77.9. Six heterothallic isolates of unidentified *Phytophthora* spp. (PmACA057, PmACA082, PmACA-100, PmACA102, PmACA103, PmACA-105) did not cause disease on asparagus seedlings under the conditions of the pathogenicity tests. These isolates produced hyphal swellings and few, large sporangia that often proliferated internally when cultures were flooded with water. No oogonia were produced in single cultures or on pairing with A_1 or A_2 mating types.

Information collected during the field survey suggested that there was a relationship between root rot disorders in previous crops, especially alfalfa (*Medicago sativa* L.), and Phytophthora rot of asparagus. To investigate this relationship further, seedlings of two cultivars of alfalfa resistant to *P. m.* f. sp *medicaginis* Kuan & Erwin (Apollo 2, Washoe), two cultivars that are susceptible to *P. m.* f. sp. *medicaginis* (Rere, Wairau), and the asparagus cultivar U.C. 157 were inoculated with a culture of *P. m.* var. *sojae* ex asparagus (PmACA014), *P. cryptogea* ex asparagus (PmACA004), and three isolates of *P. m.* f. sp. *medicaginis* (9-2-7, 30-3-3, 32-1-3) provided by S. M. Mircetich, USDA-ARS, Department of Plant Pathology, University of California, Davis. All isolates of *P. m.* f. sp. *medicaginis* caused severe root rot that resulted in death of seedlings of Rere and Wairau, but did not cause any symptoms on Washoe and Apollo 2. None of the *P. m.* f. sp. *medicaginis* isolates caused disease on U.C. 157. PmACA014 did not cause disease on any of the alfalfa cultivars, but was pathogenic on U.C. 157, whereas PmACA004 was pathogenic on U.C. 157 and also caused mild root rot symptoms on the alfalfa cultivars Rere and Wairau. PmACA004 was reisolated from the roots of Wairau on antibiotic PDA following surface-sterilization in 1% sodium hypochlorite for 3 min.

DISCUSSION

The predominant species of *Phytophthora* that is pathogenic on asparagus in California is *P. m.* var. *sojae*. The morphology, cultural characteristics, and strong pathogenicity toward the original host, asparagus, agree with the earlier description of *P. m.* var. *sojae* on asparagus in New Zealand (3). This species made up 88% of all isolates and was found in all areas of asparagus culture in California, except the desert production areas. *Phytophthora* was not isolated from asparagus in these areas despite field sampling following periods of heavy rainfall when standing water was observed in the furrows between the asparagus beds. A closely related species of *Phytophthora*, *P. m.* f. sp. *medicaginis* is a pathogen of alfalfa in both areas (5,6,12,20). Therefore, it is unlikely that environmental conditions were limiting for the asparagus pathogen. It is possible that the species pathogenic to asparagus has not gained entry to the desert production areas and no evidence of cross pathogenicity between asparagus and alfalfa isolates was found. The apparent association between Phytophthora root rot of alfalfa and asparagus in other areas of the state was probably because environmental conditions favorable to Phytophthora root rot are similar for both hosts. However, *P. cryptogea* (isolate PmACA-004), which caused mild symptoms on alfalfa in greenhouse inoculation studies, may be able to survive on alfalfa roots in the absence of asparagus.

This is the first report of *P. cryptogea* causing disease of asparagus. Although it made up only 5% of the isolates collected in California, all were highly virulent on asparagus seedlings.

Phytophthora was present in all fields,

except two, in the asparagus production areas of northern and coastal southern California. Both fields from which *Phytophthora* was not isolated were 1-yr-old or less and had never been planted to asparagus. In these cases, it is likely that species pathogenic to asparagus had either not gained entry to the fields or inoculum concentration was too low to enable detection.

Phytophthora was isolated from all cultivars of asparagus grown in California, except Brock's Special. Subsequent work (8) has shown that Brock's Special is also susceptible to *P. m.* var. *sojae* and *P. cryptogea*.

The most reliable method for isolating *Phytophthora* from asparagus was from spear tissue, with relatively low success rates from seedling baiting or isolation from crown and storage root tissue. Pure cultures were usually obtained from spear tissue, but bacterial contamination was often found when crown or root tissue was used.

In conclusion, 1) all cultivars of asparagus grown in California, and many of those grown elsewhere (8), are susceptible to Phytophthora rot; 2) the main species pathogenic to asparagus in California was *P. m.* var. *sojae*, although highly virulent isolates of *P. cryptogea* were occasionally found; and 3) Phytophthora rot of asparagus affected production in 1982 and 1983 in soils sampled from an area representing approximately 13,000 ha out of a total planted area of approximately 14,000 ha. Results from fungicide trials show that yield reduction between 30–54% occurred during these seasons (9,11) and Phytophthora rot had a long-lasting, possibly permanent effect on asparagus production. Therefore, two *Phytophthora* spp. are important pathogens of asparagus affecting production in a large proportion of the area planted to asparagus in California.

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LITERATURE CITED

1. Anonymous. 1961. New plant diseases. New South Wales Agric. Gaz. 72:489-491.
2. Ark, P. A., and Barrett, J. T. 1938. Phytophthora rot of asparagus in California. Phytopathology 28:754-756.
3. Boesewinkel, H. J. 1974. *Phytophthora* on asparagus in New Zealand. Plant Dis. Rep. 58:525-529.
4. Bussell, W. T., and Ryan, C. L. J. 1974. Disease tolerance of asparagus varieties. N. Z. J. Agric. 128:27.
5. Erwin, D. C. 1956. Important diseases of alfalfa in southern California. Plant Dis. Rep. 40:380-383.
6. Erwin, D. C. 1966. Varietal reaction of alfalfa to *Phytophthora megasperma* and variation in virulence of the causal fungus. Phytopathology 56:653-657.
7. Falloon, P. G. 1982. Baiting, pathogenicity, and distribution of *Phytophthora megasperma* var. *sojae* in New Zealand asparagus soils. N. Z. J. Agric. Res. 25:425-429.
8. Falloon, P. G. 1986. A method for screening asparagus (*Asparagus officinalis* L.) seedlings for resistance to Phytophthora rot. Proc. Int. Asparagus Symp. 6th. Eucarpia Veg. Sect. Guelph. 220-227.
9. Falloon, P. G., Falloon, L. M., Benson, B. L., and Grogan, R. G. 1986. Effect of *Phytophthora megasperma* var. *sojae* on yield of *Asparagus officinalis*. Plant Dis. 70:15-19.
10. Falloon, P. G., Falloon, L. M., Mullen, R. J., Benson, B. L., and Grogan, R. G. 1983. Effect of Phytophthora spear rot on asparagus yield. Calif. Agric. 27 (7 & 8):16-17.
11. Falloon, P. G., Mullen, R. J., Benson, B. L., and Grogan, R. G. 1985. Control of Phytophthora rot with metalaxyl in established asparagus. Plant Dis. 69:921-923.
12. Flint, M. L., Klonsky, K., and Zalom, F. G. 1984. Use of integrated pest management in alfalfa. Calif. Agric. 38 (1 & 2):28-30.
13. Matkin, O. A., and Chandler, P. A. 1957. The U.C. type soil mixes. Pages 68-85 in: The U.C. System for Producing Healthy Container-grown Plants. K. F. Baker, ed. Calif. Agric. Exp. Stn. Man. 23:33 pp.
14. McGeary, D. J., Ward, P., and Gardner, R. K. 1985. Trends in asparagus production and marketing. Aust. Dep. Agric. Rural Affairs. Tech. Rep. 115. 51 pp.
15. Molot, P. 1962. Les maladies cryptogamiques de l'Asperge. Rev. Zool. Agric. Pathol. Veg. 61:71-76.
16. Moreau, B., and Zuang, H. 1977. Institut National de Vulgarisation pour les fruit, legumes, et champignons. Invuflec Mars, 1977.
17. Newhook, F. J., Waterhouse, G. M., and Stamps, D. J. 1978. Tabular key to the species of *Phytophthora* de Bary. CMI Mycological Papers 143. 20 pp.
18. Ribeiro, O. F. 1978. A source book of the genus *Phytophthora*. J. Cramer. 417 pp.
19. Sherwood, R. T., and Hagedorn, D. J. 1958. Determining common root rot potential of pea fields. Bull. 531. Agric. Exp. Stn., University of Wisconsin, Madison.
20. Summers, C. G., Gilchrist, D. G., and Norris, R. F. 1981. Integrated pest management for alfalfa hay. U. C. Statewide I.P.M. Proj. Div. Agric. Sci. 97 pp.
21. Tucker, C. M. 1931. Taxonomy of the genus *Phytophthora* de Bary. Mo. Res. Bull. 153. 208 pp.
22. Waterhouse, G. M. 1970. The genus *Phytophthora* de Bary. Diagnoses (or descriptions) and figures from the original papers. 2nd ed. CMI Mycological Papers 122. 59 pp.