# Root Rot of Red Raspberry Caused by *Phytophthora citricola* and *P. citrophthora* in Chile

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**ABSTRACT**

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Outbreaks of root rot on red raspberry occur frequently in Chile and often are related to excess soil moisture. Aerial symptoms, including leaf chlorosis, wilting of primocanes and floricanes, poor growth of floricanes, and poor emergence of primocanes, are always associated with root necrosis. Diseased plants eventually die. *Phytophthora citricola* and *P. citrophthora* were consistently recovered from the roots of symptomatic plants of the cultivars Willamette and Glen Clova. These pathogens were identified on the basis of morphological features of sporangia and sex organs and mycelial growth at different temperatures. *P. citricola* has been previously associated with root rots and decline of red raspberry, but this is the first report of *P. citrophthora* as a pathogen on red raspberry and the first confirmed report of any *Phytophthora* species causing root rot of red raspberry in South America.

Root rot, leaf chlorosis and necrosis, wilting, and dieback are common on red raspberry (*Rubus idaeus* L.) plants in central Chile. Severely affected plants produce small floricanes and small, scorched leaves and develop fewer primocanes than healthy plants (Fig. 1). Partially rotted, brown necrotic lesions on roots are always associated with the aerial symptoms. Brown necrotic lesions are often found at the base of wilted primocanes and may extend into the crown. A sharp margin is always observed between diseased and healthy tissue. Symptomatic plants eventually die. Although these symptoms frequently occur in plants growing on fine-textured and wet soils, they also are found as localized foci in apparently well-drained soils where roots have been damaged by intensive disking done to control weeds or where irrigation water accumulates. A similar syndrome has been reported on red raspberry in the Northeast and Pacific Northwest of the United States (2,4,17) and in the United Kingdom (3,16), New Zealand (1), Australia (12), and continental Europe (7,9), where several species of *Phytophthora* have been implicated as causal agents.

*Phytophthora* spp. were identified in isolations from raspberry in a preliminary survey (6), which led us to postulate that these organisms were the causal agent of this newly recognized disease of red raspberry in Chile. We report here the isolation, identification, and proof of pathogenicity of two species of *Phytophthora, P. citricola* Sawada and *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian, commonly associated with diseased red raspberries in central Chile.

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## MATERIALS AND METHODS

**Isolation.** Necrotic roots of red raspberry plants with the aerial symptoms described above were selected in the field, transported in plastic bags to the laboratory, and held at 0 C for 1–3 days before isolation. Pieces of brown necrotic rootlets were carefully rinsed for 10–15 min in tap water, and small segments (2–6 mm long) were placed in petri dishes on a selective medium (ACMA) (5) containing (per liter) 17 g of cornmeal agar (CMA) (Difco), 150 mg of ampicillin (Laboratorio Chile), 10 mg of pimaricin (Delvocid, Gist-Brocades), 16 mg of rifampicin (Rifaldin, Lepetit), 10 mg of benomyl (Benlate 50WP), 100 mg of PCNB (Brassicol 20D, Hoechst), and 30 mg of hymexazol (Tachigaren, Sankyo Co. Ltd.). Cultures were incubated in the dark at 20–22 C, and hyphal tip transfers were made on ACMA from emerging colonies. Pure cultures were kept on unamended CMA at 5 C.

**Identification.** Isolates of *Phytophthora* spp. were identified primarily by colony morphology; mycelial characteristics; production, morphology, and size of sporangia, oogonia, and antheridia; and mycelial growth on CMA after 5 days at 5, 20, 30, and 35 C. The tabular key of Newhook et al (8) and other descriptions (10,13–15,17) were used for identification.

Sporangia were produced by flooding plugs (4–6 mm in diameter) of mycelium, taken from advancing margins of colonies on ACMA, on carrot juice broth (prepared by boiling 500 g of fresh carrots for 10–15 min in 1 L of distilled water) at 20–22 C for 48 hr in plastic petri dishes under continuous light. At least two dishes were used per isolate. The mycelium was then rinsed with chilled sterile distilled water (SDW) and washed for 3–5 min with chilled sterile mineral salt solution containing (per

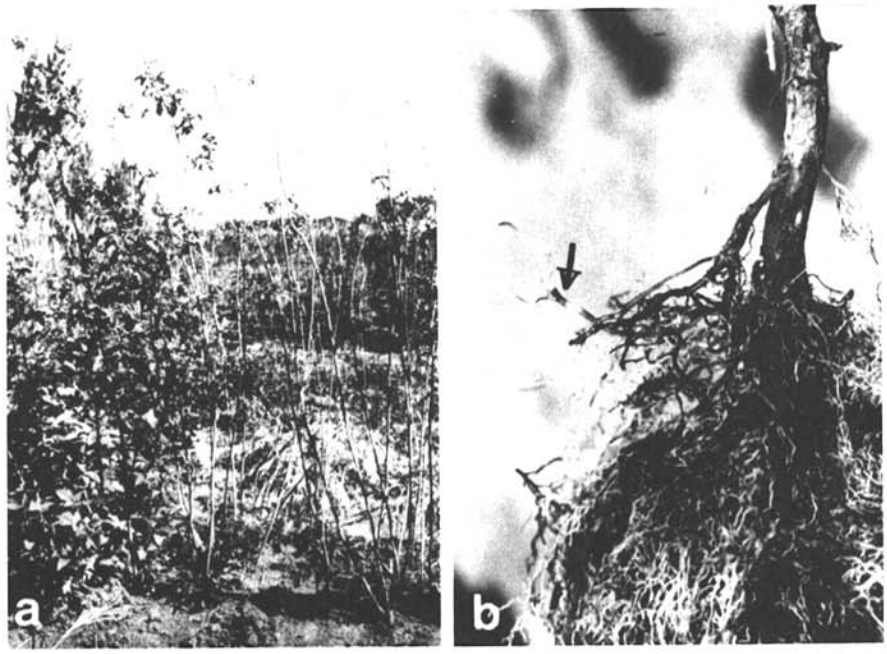


**Fig. 1.** Root rot and decline symptoms caused by *Phytophthora citricola* on red raspberry cultivar Willamette: **(A)** wilting and dieback of floricanes, **(B)** root necrosis (arrow) on naturally infected plants.

liter) 2.36 g of $Ca(NO_3)_2$, 0.5 g of $KNO_3$, 1 g of $MgSO_4 \cdot 7H_2O$, and 1 ml of chelated iron solution (ethylenediaminetetraacetic acid, 13 g/L; KOH, 7.5 g/L; $FeSO_4 \cdot 7H_2O$, 24.9 g/L) (5,10). After incubation for 48 hr, dishes were examined for the presence of sporangia, and those that were negative were treated again with chilled salt solution.

Oospore production was stimulated on unclarified V-8 agar medium (AV-8) amended with 30 μg of β-sitosterol, 20 μg of tryptophan, 1 μg of thiamine, and 100 μg of $CaCl_2 \cdot H_2O$ per milliliter (5,10). Each unknown isolate was placed in triplicate on AV-8 plates either alone or 1 cm from an isolate of *P. cinnamomi* Rands of mating type $A_1$ or $A_2$ to ascertain mating type if heterothallic. Culture dishes were then incubated in the dark at 20 C for at least 21 days.

**Pathogenicity tests.** Inoculum for pathogenicity tests was prepared from 4- to 5-day-old cultures grown in carrot juice broth at 20–25 C. The mycelium was rinsed twice with 50 ml of SDW and ground for 15 min in a blender at high speed. The inoculum was adjusted to $10^6$ fragments per milliliter, as determined with a hemacytometer.

Three experiments were performed, two with cultivar Willamette and one with cultivar Heritage. Rooted crowns were selected from symptomless plants and cultivated under greenhouse conditions in plastic pots (14 × 13.5 cm) containing a soil mixture (organic soil, sand, and loam soil [2:1:2]) that was previously sterilized with methyl bromide (98%) plus chloropicrin (2%). Plants were carefully selected for uniformity when new growth was observed about 45 days after transplanting. Plant roots were cut with a knife to injure them and then inoculated with 100 ml of mycelial suspension per plant. The inoculum was delivered through holes (8–10 cm deep) made around each plant.

In experiment 1, plants were placed in trays with 1 cm of water and were irrigated daily. In experiments 2 and 3, plants were not placed in trays but were irrigated twice a day to ensure very high soil moisture levels. Inoculated and noninoculated plants were distributed on a bench according to a completely randomized design, with three replicate plants per treatment for each experiment.

After 30 days, the pathogenicity and virulence of the isolates were assessed based on the incidence of symptomatic plants and disease severity. Disease severity was rated on the basis of shoot growth rate, visual estimation of the percentage of root mass rotted, root fresh weight, and root volume. The root volume was determined as the volume of water displaced when the root mass of the plant was immersed in water in a 500-ml graduated cylinder. Data were analyzed by analysis of variance, and means were separated by the Waller-Duncan *k*-ratio *t* test with the MULTSTAT program (11).

## RESULTS

*Phytophthora* spp. were isolated from roots of symptomatic plants of the red raspberry cultivars Glen Clova and Willamette in three of eight fields sampled. Based on morphological and cultural characteristics, four of the eight isolates were identified as *P. citricola,* and the other four were identified as *P. citrophthora* (Table 1). Both species were found in each of the three fields.

Colonies of *P. citricola* on CMA were radial or rosette and lacked aerial mycelium. Semipapillate sporangia with one or more apexes were produced abundantly in liquid medium. Ovoid to obpyriform sporangia averaging 49.5 × 35.1 μm with mean length:breadth ratio of 1.46 and a single semipapillate apex were most frequently observed, but multilobed and distorted sporangia with two semipapillate apexes were also found (Fig. 2A–C). No proliferation of sporangia or hyphal swellings were observed. Oogonia were produced abundantly in

**Table 1.** Characteristics of isolates of *Phytophthora citricola* and *P. citrophthora* from red raspberry plants in Chile

| Characteristic | *P. citricola* | *P. citrophthora* |
|---|---|---|
| Colonies on CMA[a] | Radial-rosette | Radiate |
| Hyphal swellings | Not observed | Not observed |
| Sporangia | | |
| Shape | Variable[b] | Variable[b] |
| Papillation | Semipapillate | Papillate |
| Proliferation | Not observed | Not observed |
| Length (L)[c] | 49.50 μm (8.6 μm) | 50.30 μm (8.9 μm) |
| Breadth (B)[c] | 35.10 μm (6.5 μm) | 31.30 μm ((3.8 μm) |
| L:B ratio | 1.46 (0.2) | 1.63 (0.3) |
| Oogonium diameter[c] | 30.50 μm (2.7 μm) | ND[d] |
| Oospore diameter[c] | 28.30 μm (2.3 μm) | ND |
| Antheridia | Paragynous | ND |
| Mycelial growth on CMA[a] after 4 days at | | |
| 5 C | 0.0 mm | 0.0 mm |
| 20 C | 44.3 mm | 40.0 mm |
| 30 C | 33.3 mm | 39.3 mm |
| 35 C | 0.0 mm | 0.0 mm |

[a] Radial mycelial growth determined on cornmeal agar (CMA).
[b] Sporangial shape varied from ovoid or obpyriform to lobated and distorted, with one or more apexes (see also Fig. 2).
[c] Average of at least 50 measurements per isolate of three isolates per species. Standard deviations are given in parentheses.
[d] ND = not determined because they were not produced.

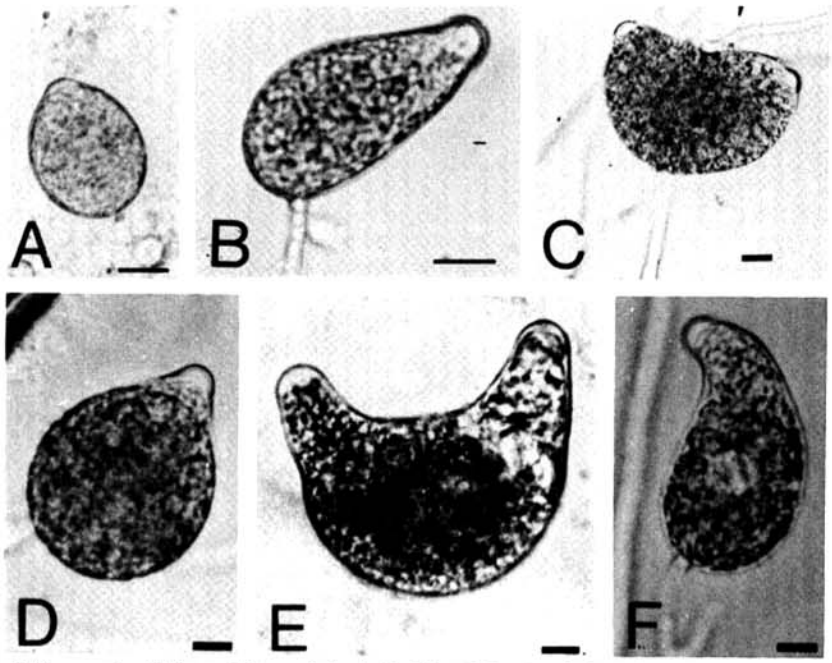


**Fig. 2.** Sporangia of *Phytophthora citricola* **(A–C)** and *P. citrophthora* **(D–F): (A and B)** ovoid and obpyriform semipapillate sporangia with a single apex; **(C)** semipapillate sporangium with two apexes; **(D and F)** ovoid and obpyriform sporangia with one apex; **(E)** sporangium with two apexes. Bars represent 10 μm.

single culture on AV-8; they averaged 30.5 μm in diameter and contained plerotic oospores 28.3 μm in diameter. Only paragynous antheridia were observed.

Colonies of *P. citrophthora* on CMA were radiate with no aerial mycelium. Sporangia varied in shape and size and had one or more papillate apexes (Fig. 2D–F). Sporangia with a single apex averaged 50.3 × 31.3 μm, with mean length:breadth ratio of 1.63. No proliferation of sporangia, hyphal swellings, or chlamydospores were observed. Oogonia were not produced in single culture and were either scarce or not observed when isolates were paired with either a known $A_1$ or a known $A_2$ mating type of *P. cinnamomi*. Growth of *P. citricola* at 30 C was reduced by one-third relative to growth at 20 C, whereas *P. citrophthora* grew equally well at these two temperatures (Table 1). No mycelial growth was observed at 5 or 35 C.

All isolates of *P. citricola* and *P. citrophthora* were pathogenic to red raspberry cultivar Willamette: they induced leaf chlorosis, wilting, and dieback, reduced shoot growth, and produced a moderate to severe brown root rot 30 days after inoculation. Root fresh weight and root volume of inoculated plants were also reduced compared to uninoculated controls, but the differences were statistically significant ($P = 0.05$) only for some isolates (Table 2). The appropriate *Phytophthora* sp. was reisolated on ACMA from diseased roots of at least one plant per isolate, but no *Phytophthora* sp. was isolated from uninoculated control plants.

*P. citricola* and *P. citrophthora* isolates incited only mild symptoms on plants of the cultivar Heritage. Inoculated plants were characterized by leaf chlorosis and scorching, but wilting and dieback were not observed within 30 days. Roots of inoculated plants were only moderately damaged. Differences among isolate means were not statistically significant ($P = 0.05$) for any of the parameters evaluated.

**Table 2.** Pathogenicity of *Phytophthora citricola* and *P. citrophthora* isolates from red raspberry in Chile[w]

| Species Isolate | Foliar symptoms[x] | Shoot growth rate (mm/day) | Root rot[y] (%) | Root volume[z] (ml) | Root fresh weight (g) |
|---|---|---|---|---|---|
| | | **Experiment 1** | | | |
| *P. citricola* | | | | | |
| F-12-0 | 3 | 7.2 ab | 60.0 a | 3.4 a | 3.3 a |
| F-12-1 | 3 | 2.4 a | 60.0 a | 6.0 a | 5.9 a |
| *P. citrophthora* | | | | | |
| F-16-4 | 3 | 6.4 ab | 66.7 a | 3.3 a | 3.3 a |
| F-16-12 | 3 | 9.7 ab | 66.7 a | 5.2 a | 4.4 a |
| Uninoculated checks | 0 | 14.4 b | 10.0 b | 14.6 b | 15.4 b |
| | | **Experiment 2** | | | |
| *P. citricola* | | | | | |
| F-11-0 | 3 | 0.1 a | 80.0 a | 3.1 a | 0.4 a |
| F-12-0 | 3 | 1.1 a | 46.7 a | 9.2 bc | 4.4 b |
| F-12-1 | 3 | 0.7 a | 60.0 a | 5.6 ab | 1.6 ab |
| *P. citrophthora* | | | | | |
| F-16-4 | 3 | 0.6 a | 53.3 a | 8.4 b | 2.5 b |
| F-16-6 | 3 | 0.6 a | 66.7 a | 6.4 ab | 1.3 ab |
| F-16-12 | 3 | 1.4 a | 46.7 a | 10.2 bc | 2.6 b |
| Uninoculated checks | 0 | 2.9 b | 0.0 b | 14.3 c | 4.5 b |

[w] Data are the means of three replicate Willamette plants per isolate. Values in each column for each experiment followed by the same letter do not differ significantly ($P = 0.05$) according to the Waller-Duncan *k*-ratio *t* test.

[x] Number of plants (out of three) that showed aerial symptoms, mainly leaf chlorosis, wilting, scorching, and dieback, within 30 days after inoculation under greenhouse conditions.

[y] Percentage of the root mass that was rotted, estimated visually. Mean separation was based on arcsine-transformed data. The appropriate *Phytophthora* sp. was reisolated from diseased roots of at least one inoculated plant per isolate but not from uninoculated controls.

[z] Measured in a 500-ml graduated cylinder with water.

## DISCUSSION

The cultural and morphological characteristics of all isolates from red raspberry fit the descriptions of either *P. citricola* or *P. citrophthora* (8,10,13–15, 17), and isolates F-11-0 and F-16-12 were confirmed as *P. citricola* and *P. citrophthora*, respectively, by the International Mycological Institute, Commonwealth Agricultural Bureaux, Kew, England. Isolates of both species were pathogenic to red raspberry. The consistent isolation from necrotic roots suggests that these pathogens, previously unrecognized, play a major role in the red raspberry decline syndrome observed in central Chile. The symptoms observed in Chile agree with those previously described for other countries except that the reddish discoloration of the root cortex mentioned in other reports (16,17) was not as evident in either naturally infected or inoculated plants. This is the first report of *P. citrophthora* as a pathogen on red raspberry and the first confirmed report of any *Phytophthora* species causing root rot of red raspberry in South America.

Several species of *Phytophthora* have been implicated as causal agents of root rot of red raspberry elsewhere, including *P. citricola, P. erythroseptica* Pethybr., *P. fragariae* C. J. Hickman, *P. megasperma* Drechs., *P. cryptogea* Pethybr. & Lafferty, *P. cactorum* (Lebert & Cohn) J. Schröt., *P. drechsleri* Tucker, *P. syringae* (Kleb.) Kleb., and *P. cambivora* (Petri) Buisman (2,3,7,12,16,17). However, differences in virulence were reported among the *Phytophthora* spp. implicated in the United States (17) and Great Britain (3). Isolates of *P. citricola* and *P. citrophthora* tested on red raspberry cultivar Willamette in our pathogenicity experiments were equally virulent but were less virulent than isolates previously identified as *P. erythroseptica, P. fragariae,* and *P. megasperma* type 2 (3,17), which appear to be the most important causes of red raspberry root rot in North America and Europe. These isolates were recently shown to be conspecific and have been reclassified as *P. fragariae* C. J. Hickman var. *rubi* Wilcox & Duncan (20). Although we isolated only *P. citricola* and *P. citrophthora* in this study, other species of *Phytophthora* may also be involved in the root rot syndrome of red raspberry in Chile.

Field symptoms were reproduced on artificially inoculated plants of red raspberry cultivars Willamette and Heritage, but only mild symptoms were observed on Heritage, perhaps in part because of the lack of soil flooding in these experiments. Soil flooding appears to predispose plants to infection and favor these and other species of *Phytophthora* (17–19).


### ACKNOWLEDGMENTS

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### LITERATURE CITED


1. Brien, R. M., and Dingley, J. M. 1959. A revised list of plant diseases recorded in New Zealand, fourth supplement, 1957–1958. N. Z. J. Agric. Res. 2:406-413.
2. Converse, R. H., and Schwartze, C. D. 1968. A root rot of red raspberry caused by *Phytophthora erythroseptica.* Phytopathology 58:56-59.
3. Duncan, J. M., Kennedy, D. M., and Seemüller, E. 1987. Identities and pathogenicities of *Phytophthora* spp. causing root rot of red raspberry. Plant Pathol. 36:276-289.
4. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. The American Phytopathological Society, St. Paul, MN.
5. Latorre, B. A., Alvarez, C., and Ribeiro, O. K. 1980. Phytophthora root rot of kiwifruit in Chile. Plant Dis. 75:949-952.
6. Latorre, B. A., Bartollotti, E., Vargas, R., and Alvarez, C. 1989. *Phytophthora* en frutales y hortalizas. Rev. Fruticola (Chile) 10:92-95.
7. Montgomerie, I. G., and Kennedy, D. M. 1991. The pathogenicity of *Phytophthora* species to red raspberry. Acta Hortic. 112:167-176.

8. Newhook, F. J., Waterhouse, G. M., and Stamps, D. J. 1978. Tabular key to the species of *Phytophthora* de Bary. Mycol. Pap. 143. CAB, Commonwealth Mycological Institute, Kew, England.
9. Nourrisseau, J.-G., and Baudry, A. 1987. Un *Phytophthora* cause de dépérissement du framboisier en France. Phytoma 384:39-41.
10. Ribeiro, O. K. 1978. A Source Book of the Genus *Phytophthora*. J. Cramer, Lehre, Germany.
11. Shane, W. W., Joyner, T. G., and Powell, C. C. 1990. APPLICAL, RANDOMA, and MULTSTAT—Three microcomputer utilities for managing field trial experiments. Plant. Dis. 74:333-334.
12. Washington, W. S. 1988. *Phytophthora cryptogea* as a cause of root rot of raspberry in Australia; resistance of raspberry cultivars and control by fungicides. Plant Pathol. 37:225-230.
13. Waterhouse, G. M., Newhook, F. J., and Stamps, D. J. 1983. Present criteria for classification of *Phytophthora*. Pages 139-147 in: *Phytophthora:* Its Biology, Taxonomy, Ecology, and Pathology. D. C. Erwin, S. Bartnicki-Garcia, and P. H. Tsao, eds. The American Phytopathological Society, St. Paul, MN.
14. Waterhouse, G. M., and Waterston, J. M. 1964. *Phytophthora citrophthora*. No. 114 in: Descriptions of Pathogenic Fungi and Bacteria. Commonw. Mycol. Inst., Kew, England.
15. Waterhouse, G. M., and Waterston, J. M. 1966. *Phytophthora citricola*. No. 33 in: Descriptions of Pathogenic Fungi and Bacteria. Commonw. Mycol. Inst., Kew, England.
16. Waterston, J. M. 1937. A note on the association of a species of *Phytophthora* with a die-back disease of the raspberry. Trans. R. Soc. Edinburgh 32:251-259.
17. Wilcox, W. F. 1989. Identity, virulence, and isolation frequency of seven *Phytophthora* spp. causing root rot of raspberry in New York. Phytopathology 79:93-101.
18. Wilcox, W. F. 1991. Phytophthora root rot. Pages 34-36 in: Compendium of Raspberry and Blackberry Diseases and Insects. M. A. Ellis, R. H. Converse, R. N. Williams, and B. Williamson, eds. The American Phytopathological Society, St. Paul, MN.
19. Wilcox, W. F., and Mircetich, S. M. 1985. Effects of flooding duration on the development of Phytophthora root and crown rots of cherry. Phytopathology 75:1451-1455.
20. Wilcox, W. F., Scott, P. M., Hamm, P. B., Kennedy, D. M., Duncan, J. M., Brasier, C. M., and Hansen, E. M. In press. Identity of a *Phytophthora* species attacking raspberry in Europe and North America. Mycol. Res.