Isolation and Pathogenicity of *Phytophthora cactorum* from Forest and Ginseng Garden Soils in Wisconsin

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ABSTRACT

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Experiments were conducted to determine if *Phytophthora cactorum* is present in forest soils in ginseng (*Panax quinquefolius*) growing areas of Wisconsin and to determine whether the isolates recovered are pathogenic to American ginseng. An apple cotyledon bioassay was used to bait *P. cactorum* from three of 10 forest soils and three of 10 ginseng garden soils. Twenty-two isolates from infected cotyledons were tested for pathogenicity on 3-wk-old ginseng seedlings. All 10 isolates of *P. cactorum* from ginseng garden soils were pathogenic to ginseng, but only one of 12 forest soil isolates was pathogenic, and it was only weakly virulent. The remaining 11 forest soil isolates failed to cause disease. Results of pathogenicity tests were unchanged when forest isolates were tested in the soils from which they originated. *P. cactorum* indigenous to forest soils does not appear to be a significant source of primary inoculum for disease in cultivated ginseng.

American ginseng (*Panax quinquefolius* L.) is a perennial herbaceous plant native to eastern North America. Its highly valued root has been used in Asia for more than 300 yr as a health tonic and stimulant (12). American ginseng has been cultivated in the United States for the past 100 yr; at present, more than 90% of American ginseng is produced in Wisconsin.

One of the most serious threats to ginseng cultivation is *Phytophthora cactorum* (Lebert & Cohn) Schröt., causal agent of ginseng root rot (11,13,15). The pathogen is extremely damaging during wet weather and is capable of destroying entire ginseng gardens within a few weeks. Infected roots become discolored, spongy, and watery before they disintegrate. Severe damage also can occur when leaves become infected with *P. cactorum* (11,15). The primary source of inoculum for these diseases is not known.

P. cactorum is a soilborne plant pathogen of more than 150 hosts worldwide (10). In addition to agricultural soils from which *P. cactorum* is frequently isolated (2,8,9), *P. cactorum* has been found in nonagricultural soils near apple orchards (7) and in forest soils (3,5). This suggests that *P. cactorum* might be present in forest soils where ginseng occurs naturally. Ginseng gardens are often established on recently cleared land, so it is possible that *P. cactorum*, if present in forest soils, could serve as the source of primary inoculum for root rot of cultivated ginseng. Consequently, experiments were conducted to determine if *P. cactorum* is present in forest soils of north central Wisconsin near areas of ginseng cultivation, and if so, to determine the pathogenicity of such isolates to ginseng.

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MATERIALS AND METHODS

Soil. Soil samples from 10 second- or third-growth mixed deciduous forest sites (eight in Marathon County, one in Portage County, and one in Price County, WI) were collected in June and July 1987. From each site, 10 750-cm^3 soil samples were collected randomly to a depth of 15 cm and bulked together in a polyethylene bag to make one composite sample. Soils from cultivated ginseng gardens adjacent to forest sites were sampled for comparison. No symptoms of Phytophthora root rot were apparent in any of the ginseng gardens sampled. Soil samples were kept cool in an ice chest during transportation to the lab. Soils were then air-dried, sieved through 5- and 2-mm mesh screens, and stored in polyethylene bags at 4 C until assayed.

Baiting. An extended baiting procedure using apple cotyledons (6) was employed to detect and recover *P. cactorum* from soil samples. Thirty cubic centimeters of soil from each sample was placed in 100 × 20 mm glass petri plates for 3 days, then remoistened with 10 ml of sterile distilled water. Plates were incubated in a growth chamber at 20 C with a 12-hr photoperiod for 3 days. Sixty milliliters of distilled water was added, four to six apple cotyledons were floated on the water surface of each plate, and plates were incubated for an additional 4–7 days. There were three replicate plates per soil sample. Plates were examined daily, and apple cotyledons showing dark necrotic areas were observed with a dissecting microscope for sporangia typical of *P. cactorum*. Cotyledons bearing sporangia were removed from the plates as soon as they were detected. A baiting plate was scored as positive if any of the baits bore sporangia of *P. cactorum*. Occasionally, sporangia resembling those of *P. citricola* Sawada were observed, although *P. citricola* was not pathogenic to ginseng in preliminary experiments (M. L. Owen, *unpublished*). To obtain isolates of *P. cactorum* that were free from contamination by *P. citricola*, *Pythium* spp., or bacteria, the following single-spore isolation procedure was used.

Single spore isolation. Single zoospore isolations were accomplished by transferring individual baits with sporangia to test tubes containing 1 ml of sterile distilled water. Tubes were chilled for 30 min at 4 C and then returned to room temperature (22–24 C) to enhance zoospore release. Zoospore suspensions were serially diluted from 10^{-1} to 10^{-4}. From each dilution, 0.1 ml was plated on modified VYS-PBNC medium (40 ml of V8 juice, 0.6 g of $CaCO_3$, 0.2 g of Bacto yeast extract, 1 g of sucrose, 0.02 g of Benlate [50% benomyl], 0.027 g of Terraclor [75% pentachloronitrobenzene], 35 ppm of neomycin sulfate, 20 g of Bacto agar, and 960 ml of distilled water) (16). Plates were incubated overnight at room temperature and germinating zoospores selected at random were transferred to V8 agar (200 ml of V8 juice, 20 g of agar, 2 g of $CaCO_3$, and 800 ml of distilled water) for further growth and observation. Isolates were then stored on V8 agar slants at 4 C. Small pieces of agar were taken from all of the slants after 30 days at 4 C and examined to verify species identification, on the basis that *P. cactorum* produces chlamydospores under such conditions but *P. citricola* does not (4).

Pathogenicity tests. Twelve forest isolates and 10 ginseng garden isolates were chosen at random from the collection and tested for pathogenicity to ginseng seedlings. Isolates were grown on V8 agar plates for 6–7 days under continuous light at 22 C. Agar cultures were cut in half, and one half was moved to another sterile petri plate. Each half was cut again into six to eight strips,

distributed evenly within the plate, and flooded with approximately 15 ml of sterile distilled water. This volume was sufficient to barely cover the surface of the agar. Plates were incubated overnight under continuous light to produce additional sporangia. They were then chilled for 30 min at 4 C and returned to room temperature for 30 min. The concentration of zoospores was estimated with a hemacytometer and adjusted to 10^5 zoospores per milliliter by dilution with sterile distilled water. For slow-growing isolates with relatively few sporangia, only sporangium-bearing areas of the colony were used. Isolates producing fewer sporangia were prepared with more plates.

Apparently healthy germinating ginseng seeds with radicles 1–2 cm long were planted in a flat of coarse vermiculite at a density of 1,000–1,200 seeds per square meter. Seedlings were grown in a growth chamber at 24 C with a 12-hr photoperiod and were watered with full-strength Hoagland's solution every other day. After 3 wk, seedlings were transplanted individually into 60-cm^3 containers (Ray Leach Cone-Tainers, Canby, OR) containing a pasteurized (70 C for 60 min) potting medium (soil/muck/sand, 1:1:1, v/v). Three days after transplanting, each seedling was inoculated by applying 1 ml of zoospore suspension to the soil surface. Twenty replicate plants were inoculated with each isolate. Uninoculated seedlings and seedlings inoculated with a known pathogenic isolate (Pc36C1) were included as controls. Seedling mortality was recorded daily for 10 days and the average survival time of seedlings inoculated with each of the 22 isolates of *P. cactorum* was determined. These data were converted to the percentage of the observation period that each seedling survived (10 days = 100%). Significant difference of the means was determined with LSD based on one-way classification analysis of variance (18) after arcsine square root transformation of the percentage data. The experiment, which was conducted twice, was a completely randomized design.

To determine if weakly virulent forest isolates might be more virulent if provided with biotic or abiotic factors present in their native soil, three isolates from different soils (isolates F4.1.1, F7.3.1, and F8.3.5) were further tested for pathogenicity in the soil from which they originated, using the same techniques as before. In addition to these artificially infested treatments, seedlings were also planted in these soils without the addition of *P. cactorum* inoculum. There were 10 replicate plants for each isolate tested, and the experiment was conducted twice.

Comparison of apple cotyledons and ginseng leaflets as bait for *P. cactorum*. To determine if baits derived from host tissue are more sensitive in detecting isolates pathogenic to ginseng, apple cotyledons and ginseng leaflets were compared for baiting 22 isolates of *P. cactorum* originating from forest and ginseng garden soils. These were the same isolates tested previously for pathogenicity on ginseng seedlings, except for isolates G10.3.13 and G10.1.4, which were substituted for G10.3.15 and G.10.1.13, respectively. Isolate F7.3.2 was no longer available and was not tested in the baiting comparison.

Disks (5 mm in diameter) were removed from the periphery of 3-day-old cultures of *P. cactorum* grown on V8 agar. For each isolate, three disks were placed in each of six 60-mm petri plates. Disks were flooded with 7 ml of sterile distilled water, incubated under continuous light for 2 days, and observed for sporangia. An additional 5 ml of water was added and three baits (either 10-day-old apple cotyledons or 17-day-old ginseng leaflets) were floated in each of three plates per isolate. Plates were chilled at 4 C for 30 min, then held at room temperature for 10–30 min when zoospore release was observed. Baits were incubated under continuous light for 7 days and observed daily for the presence of sporangia. Differences in the abundance of sporangia on the two baits were noted according to the following scale: 1 = a few sporangia, 2 = a moderate number of sporangia, and 3 = sporangia prolific. Macroscopic observations of tissue necrosis and discoloration were also made.

RESULTS

Recovery of *P. cactorum* from different soils. Isolates of *P. cactorum* were recovered from three of the 10 forest soils and from three of the 10 ginseng garden soils with the apple cotyledon bioassay. In only one site was *P. cactorum* recovered from both the forest and adjacent garden soils. Cotyledon necrosis can result from infection by microorganisms other than *P. cactorum*, so microscopic observation of each necrotic cotyledon was necessary. Occasionally, *P. citricola* was recovered from the cotyledons, and it was sometimes difficult to distinguish from *P. cactorum* based on sporangium morphology. Induction of zoospore release from sporangia and plating permitted the differentiation of these species based on growth characteristics of the germinating zoospores. Within 12–18 hr after plating, zoospores of *P. citricola* germinated and formed multibranching hyphae up to 5 mm in length, whereas zoospores of *P. cactorum* germinated to form single-branched hyphae less than 2 mm in length. Some colonies were allowed to develop further to verify species designation.

Pathogenicity of garden and forest isolates to ginseng. The percentage of the observation period that each ginseng seedling survived ranged from 41 to 100% (Table 1). All 10 of the *P. cactorum* isolates from ginseng garden soils were pathogenic to ginseng, and, except for two isolates, their virulence was not statistically different ($P = 0.05$) from isolate Pc36C1, known to be highly virulent on ginseng. Of the 12 forest isolates, only one was pathogenic to ginseng, and it was only weakly virulent.

Further experiments conducted in forest soils from which each of the weakly virulent or nonpathogenic isolates originated resulted in outcomes similar to the original pathogenicity tests conducted in pasteurized soil. Further tests conducted in pasteurized potting mix indicated that isolates F4.1.1 and F8.3.5 were not pathogenic to ginseng at any temperature tested (16, 20, or 24 C), whereas 100% mortality occurred when Pc36C1 was used to inoculate ginseng plants at all three temperatures (*data not shown*).

Comparison of apple cotyledons and ginseng leaflets as baits for *P. cactorum*. Both apple cotyledons and ginseng leaflets were colonized by the 22 isolates of *P. cactorum* tested in vitro. After 7 days, sporangia were detected on all but one of the 396 baits. However, at 3 days,

Table 1. Mean survival time of ginseng seedlings after inoculation with 22 isolates of *Phytophthora cactorum* isolated from three forest and three ginseng garden soils

Isolate[w]	Percentage of observation period each seedling survived[x]
G6.3.4	41.0 a[y]
G6.3.2	41.5 a
G10.3.11	43.0 a
G10.3.15	43.0 a
G10.1.13	44.5 a
G10.1.1	47.5 ab
G10.1.6	49.0 ab
Pc 36C1[z]	49.0 ab
G6.1.2	53.5 b
G4.3.16	63.5 c
G4.3.8	81.5 d
F7.3.1	91.0 e
F7.3.2	94.5 ef
F4.1.1	100.0 f
F4.1.2	100.0 f
F4.2.4	100.0 f
F4.3.3	100.0 f
F4.3.6	100.0 f
F8.1.1	100.0 f
F8.1.2	100.0 f
F8.2.9	100.0 f
F8.2.19	100.0 f
F8.3.5	100.0 f
Uninoculated control	100.0 f

[w] Isolates are labeled according to the following code: G = ginseng garden, F = forest, site (1–10), baiting plate (1–3), and colony number.
[x] Mean of 20 replicate seedlings per isolate. Observation period = 10 days.
[y] Values with the same letters are not significantly different. (LSD, $P = 0.05$).
[z] Pathogenic control.

differences were apparent in the abundance of sporangia according to type of bait and isolate source. For the ginseng garden isolates, sporangia were more prolific on ginseng leaflets (mean rating = 2.94) and less abundant on apple cotyledons (mean rating = 1.7). For the forest isolates, sporangia were more abundant on apple cotyledons (mean rating = 2.27) than on ginseng leaflets (mean rating = 1.64). Apple cotyledons colonized by all isolates of *P. cactorum* became dark and necrotic. In contrast, the difference in ginseng leaflets colonized by forest vs. garden isolates was distinct and consistent. Ginseng leaflets colonized by the garden isolates changed little in appearance, whereas ginseng baits colonized by the forest isolates developed small, localized lesions.

DISCUSSION

Our results confirm the findings of Drilias (5) that *P. cactorum* is present in certain forest soils in Wisconsin. The origin of isolates pathogenic to ginseng remains unclear, however. *P. cactorum* was detected in three of 10 forest soils, but only one forest soil isolate was pathogenic to ginseng, and it was only weakly virulent. In contrast, all of the isolates of *P. cactorum* from ginseng garden soils were pathogenic to ginseng seedlings.

There are several possible explanations for the difference in pathogenicity between forest and ginseng garden isolates. It is possible that a subpopulation of *P. cactorum* particularly virulent on ginseng became widespread among different areas of ginseng cultivation as a result of dissemination by agricultural practices. Alternatively, selection against nonpathogenic or weakly virulent isolates of *P. cactorum* may have occurred after soils were used for ginseng gardens for several years.

P. cactorum infects more than 150 plant species (10). In general, the pathogen is considered to exhibit little, if any, pathogenic specialization. For instance, isolates of *P. cactorum* from orchard and hayfield soils were pathogenic to pear, peach, apricot, and cherry seedlings (9), and isolates from 23 host genera were pathogenic on *Pyrus* (10). All isolates of *P. cactorum* baited with apple cotyledons from nonagricultural soils were pathogenic and virulent on excised apple twigs (7). However, differences in virulence among isolates from various host plant materials or soils have been observed (1,17,19,20). For example, crown and collar rot of apple and crown rot of strawberries are believed to be caused by different pathotypes of *P. cactorum* (17). In our study, differences in the abundance of sporangia and in the symptoms of colonized apple cotyledon and ginseng leaflet baits may provide additional evidence for pathogenic specialization within *P. cactorum*.

Both apple cotyledon and ginseng leaflet baits were colonized by pathogenic and nonpathogenic isolates of *P. cactorum* in our tests, but recovery of *P. cactorum* on either bait was not a good predictor of pathogenicity or degree of virulence to ginseng seedlings. Therefore, methods for detection and quantification of this fungus from soil in order to estimate disease potential, including baiting assays or soil plating assays (6,14), should be validated by pathogenicity tests. The use of ginseng seedlings for pathogenicity tests is relatively easy and fast if a supply of healthy, stratified seed is available, although ginseng seed requires a lengthy stratification period (18–22 mo) before it germinates (12). The relationship of seedling pathogenicity to pathogenicity on older plants also needs to be determined.

P. cactorum is present in some forest soils of north central Wisconsin near areas of ginseng cultivation. The low level of pathogenicity of these forest isolates to ginseng suggests, however, that other possible sources of primary inoculum should be investigated.

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