

Survival and Host Range of *Phytophthora citrophthora* in Ohio Nurseries

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ABSTRACT

Phytophthora citrophthora survived freezing to -21°C in several nutrient agar media, only if incubated at 4°C during 4 days prior to freezing. It survived -31°C on hemp agar after 4 C conditioning. In the field, it survived in infected leaves from the fall of 1973 until May 1974, both on the soil surface and buried 10 cm deep. Cultures originated from mycelial fragments, and not from other structures. Roots of several woody ornamentals, and/or shoots of others, were

susceptible to infection. Shoots and roots of *Euonymus radicans* var. *argenteo marginatus* and *Rosmarinus officinalis*, in addition to those of *Pieris japonica*, were susceptible. It was concluded that the distribution of *P. citrophthora* is determined largely by the presence of infected susceptible hosts.

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Additional key words: *Phytophthora* spp., *P. citrophthora*, *Pieris japonica*, in vitro and field survival, host range, longevity.

The distribution of *Phytophthora citrophthora* (Sm. & Sm.) Leonian has been limited primarily to citrus orchards in the subtropical and tropical zones where it is active mainly in the cooler seasons (8). Cultures of *P. citrophthora* can survive freezing to -23°C (15). Other isolates have survived 9 days of continuous freezing at -6.5°C , but were killed after 1 day at -20.6°C (10). Survival apparently was not associated with the formation of specialized structures, such as chlamydospores (15). Many plant families are represented in the host range of *P. citrophthora* including some woody ornamentals (5, 13, 14, 16).

Recently, *P. citrophthora* was discovered in Ohio, where it caused shoot blight, stem dieback, and crown rot of *Pieris japonica* (Thunb.) D. Don (7). The purpose of this investigation was to examine survival of *P. citrophthora* in Ohio nurseries, and to establish its host range on woody ornamentals common in this area.

MATERIALS AND METHODS.—*In vitro* survival of *Phytophthora citrophthora*.—Hemp agar (approximately 100 g hemp seed/1,000 ml broth) (11), M1 agar (9), and pea agar plates (15 ml/plate) (2) were inoculated with 0.5 ml of zoospore suspension (40,000/ml) (7) of isolate W26 (obtained from *P. japonica* in Ohio) to examine the effect of temperature and media on survival. Plates were incubated in the dark at 25°C for 0-4 days, and then incubated at 4°C for 0-4 days. Three replicate plates were frozen for 2 days, either in a vertical freezer at $-21 \pm 1^{\circ}\text{C}$, or in a walk-in freezer at $-31 \pm 1^{\circ}\text{C}$. Plates were brought to room temperature after removal from the freezer and 5-mm diameter agar plugs (10 per plate) were transferred to PBNC agar (12). To determine the structural form of *P. citrophthora* that survived, five

thawed plugs from frozen hemp agar cultures of isolates W26 and P574 (obtained from G. A. Zentmyer, Department of Plant Pathology, University of California, Riverside) were blended in 50 ml of salt solution (12) for 15 seconds at maximum speed in a 100-ml Sorvall Omni-Mixer. The resulting suspension was poured on PBNC agar, incubated 12-24 hours at 22°C , and examined microscopically at intervals to determine structures from which regrowth occurred.

Survival in infected tissues.—Mature, colonized leaves were incorporated in soils at two moisture levels and various temperatures. Nonsterile *Phytophthora*-free soil [a mixture of muck, Canadian peat, and sand (1:1:1, v/v)] stored in polyethylene bags (350 g/bag) was brought to field capacity (F.C.) or 40% F.C. Colonized leaves (10 per treatment) were either incorporated into, or placed on the surface of, each soil sample. Samples were incubated at 2, 8, 16, and 24°C , and separate samples were assayed at 1, 2, 5, 11, 25, and 40 weeks by plating leaf pieces on PBNC agar.

To study survival of *P. citrophthora* in the field, W26-colonized leaves were incorporated (30 leaves in 2 liters of soil) in the nonsterile soil mix. The soil-leaf mixture was buried in nylon gauze bags at a depth of 10 cm in Wooster silt loam soil in early November of 1973. Colonized leaves also were left on the soil surface. Leaves were recovered from separate bags and from the soil surface in April, May, and June, and assayed for *P. citrophthora* as described above.

To identify survival structures in infected leaf debris in the field, free-hand sections were made in April and May of 1974. Other samples were blended in a salt solution (12), and plated on PBNC agar. Attempts were made to

identify structures from which colonies developed.

The effect of drying on survival was examined in fully colonized leaves dried for 48 hours at room temperature on premoistened filter paper in a partially open Plexiglas container. Leaves were weighed before drying, and again prior to plating, to determine moisture content, which was expressed as percentage moisture of original fresh weight. Samples were assayed daily (20 leaves) by plating on PBNC agar.

Host range.—Rooted cuttings of various woody ornamentals obtained from nurseries in Ohio were forced 3-4 months in 10-cm diameter pots in a mixture of Canadian peat and muck (1:1, v/v). Inoculum of isolate W26 in hemp broth (50 ml/250-ml flask) was incubated 2 weeks at 22 C, poured on roots of eight plants (one flask per pot), and covered with soil (9). Four plants were not inoculated. Plants were watered and maintained in a greenhouse as described previously (9). Three months after inoculation, discolored roots were plated on PBNC agar to verify the identity of the pathogen.

Actively growing shoots were sprayed with a zoospore suspension (5×10^4 /ml) to run-off to determine shoot susceptibility. Plants (five per treatment) were incubated for 3 days in a mist chamber (22 ± 2 C, 5,000 lux for 10 hours). Symptoms of disease were recorded over a 10-day period, and reisolations were made to confirm pathogenicity. Two control plants were sprayed with water.

RESULTS AND DISCUSSION.—*In vitro survival.*—Cultures frozen at -21 C survived on all media if incubated at 4 C for 4 days prior to freezing. This is in agreement with a previous report (15). However, pea and M1 agar cultures did not survive if incubated at 4 C for less than 2 days prior to freezing. Hemp agar cultures did not survive if incubated less than 1 day at 4 C. In all media, an increase in survival was associated with an increase in time of cold treatment at 4 C prior to freezing in all media. This may explain reported death of *P. citrophthora* exposed to -20.6 C without preconditioning at 4 C (10). Optimum survival occurred if the cultures were incubated 4 days at 25 C prior to 4 C cold treatment and freezing. Survival was best on hemp agar, followed by pea agar and M1 agar. Cultures frozen to -31 C survived only in hemp agar if incubated 4 days at 25 C after inoculation followed by at least 4 days at 4 C before freezing. Pea and M1 agar cultures did not survive if frozen to -31 C using this procedure.

Isolate P574 was used to determine survival structures because it withstood blending after freezing better than W26. Regrowth originated laterally from walled-off cytoplasm in mycelial fragments. No outgrowth occurred from sporangia or hyphal swellings that also were present. No other structures were observed. The results were similar for hemp, M1, and PBNC agars. Survival, therefore, apparently was not associated with the formation of specialized structures which supports a previous report (15).

Survival in infected tissues.—The pathogen survived at least 40 weeks in infected leaves in contact with nonsterile soil. However, survival was affected considerably by soil moisture. Recovery of the fungus from leaves incorporated in soil maintained at field capacity at 8-24 C decreased steadily to 23% during 40 weeks. Recovery from leaves incubated on the surface of the soil in the

same treatments remained at high levels (approximately 80%) over the entire 40-week period. In soil at 40% F.C., the pathogen survived at very high levels for 40 weeks, whether leaves had been incorporated in soil (approximately 90%) or had been placed on the soil surface (approximately 95%). Apparently, no differences in survival existed between samples incubated at 8, 16, or 24 C. Survival at 2 C was reduced to approximately 10% within 40 weeks under all conditions, except when leaves remained on the surface of the drier soil (survival approximately 90%).

Survival of *P. citrophthora* in the field in artificially inoculated leaves in Wooster soil was consistently high. The minimum soil and air temperatures that winter (1973-74) were -0.5 C and -18 C, respectively. In April, recovery was 96% from leaves on the soil surface and 100% from buried leaves. Recovery was 41% from surface and 88% from buried leaves in May, and in June it was 91% from buried leaves, when surface leaves could no longer be found. The recovery rate from skeletonized leaves was high; the condition of the leaves did not affect recovery. In the spring of 1974, leaf debris also was collected from the soil surface in a nursery in which severe losses had occurred on *P. japonica* in 1973. In March, *P. citrophthora* was recovered from 13% of such leaf debris, whereas recovery in May was 35%. These data show that *P. citrophthora* can overwinter in leaf debris of *P. japonica* in Ohio, and probably in other areas which have temperate climates.

Mycelium survived low moisture levels in leaves. *Phytophthora citrophthora* was isolated from leaves with moisture levels higher than 25% of original fresh weight, but not from those with a moisture of 23%, at which level leaves were completely air dry and brittle. Numerous spherical thick-walled spores of various diameter (10-20 μ m) were observed in free-hand sections of inoculated leaves collected in the field, but none could be definitely associated with a specific mycelium. No *P. citrophthora* cultures were recovered from any of these chlamydospores after leaves were blended in a salt solution and plated on PBNC agar. Most yielded species of *Fusarium*, *Alternaria*, and other genera of the Fungi Imperfecti. Colonies of *P. citrophthora* emerged either from fragments of vascular bundles, or occasionally were associated with epidermal fragments. The evidence suggests that mycelium may be the survival structure in the field. Indirect evidence for mycelial survival has also been reported for other *Phytophthora* spp. (1, 2, 3, 8). *Phytophthora drechsleri* Tucker, *P. nicotianae* var. *parasiitica* (Dastur) Waterhouse, and *P. cinnamomi* Rands, however, may form small, dark-walled, spherical chlamydospores in the field, which have not been observed in culture (4). It is possible that, with appropriate techniques, similar spores may be found for *P. citrophthora*.

Host range.—*Phytophthora citrophthora* also has been isolated from naturally infected twigs of *Euonymus* spp., from foliage and twigs of *Potentilla fruticosa* L., and from roots and crowns of several *Taxus* spp. cultivars in Ohio nurseries. Leaves and roots of *Euonymus radicans* var. *argenteo marginatus* and *Rosmarinus officinalis* L. were susceptible to *P. citrophthora* after artificial inoculation. Furthermore, roots (but not leaves) of *Hedera helix* L., *Ilex crenata*

Thunb., and several *Taxus* spp., were susceptible. A number of other common nursery plants were resistant (6).

The foregoing data indicate that *P. citrophthora* is probably widely distributed in nurseries in the temperate zone. This is in contrast with a report which stated that this fungus was limited to subtropical and tropical zones (8).

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