

# Shoot Blight and Stem Dieback of *Pieris japonica* Caused by *Phytophthora citricola*, *P. citrophthora* and *Botryosphaeria dothidea*

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## ABSTRACT

Dieback of *Pieris japonica* in Ohio nurseries can be caused by *Phytophthora citricola*, *P. citrophthora*, and *Botryosphaeria dothidea*. The *Phytophthora* spp. cause leaf and twig blight on young tissues and olivaceous leafspots on leaves of intermediate maturity that turn necrotic brown and are surrounded by a dark-red margin. Infected leaves commonly abscise, but lesions rarely progress into mature wood. Uninjured mature leaves and stems are resistant. Both of these *Phytophthora* spp., as well as *P. cinnamomi*, cause root and crown rot, which is characterized by stunting and

chlorosis followed by leaf epinasty, dieback, and eventual death of plants.

In landscape plantings and nurseries *Botryosphaeria dothidea* causes dieback on injured plants or plants under stress. Defoliation is followed by dieback of mature twigs and branches and the entire plant may be killed. *Pestalotia sydowiana* and a *Phomopsis* spp. may be involved as secondary invaders in the dieback complex.

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*Additional key words:* Pieris stem dieback, Pieris root rot, *Phytophthora cinnamomi*, *Pestalotia sydowiana*, *Phomopsis* sp.

Dieback of *Pieris japonica* (Thunb.) D. Don, an Ericaceae shrub, can be attributed to a number of causes. *Botryosphaeria dothidea* (Moug. ex Fr.) Ces. & De Not (= *B. ribis* Gross. & Dugg.) frequently has been isolated from *P. japonica* with dieback symptoms in Ohio (W. W. P. Gerlach, et al. *unpublished*) and Rhode Island (*personal communication*, N. Jackson, Dept. of Plant Pathology and Entomology, Univ. of Rhode Island, Kingston). Stem splitting at the crown due to severe cold injury leads to dieback the following spring. Severe mite infestations cause chlorosis followed by tip necrosis and dieback.

In Ohio nurseries, a number of *Phytophthora* spp. have been isolated from leaves, twigs, and stems from still another type of dieback following periods of heavy rain and floods. A root rot and leafspot may be associated with this dieback complex. Some of the symptoms are similar to those caused by root inoculations with *Phytophthora cinnamomi* Rands (8).

In this paper, we report *Phytophthora citrophthora* (Sm. & Sm.) Leonian and *P. citricola* Sawada as new pathogens of *P. japonica*, causing leaf spots, blighting of young leaves and twigs, and root rot. In addition, the role of *B. dothidea*, *Pestalotia sydowiana* Bres. and a *Phomopsis* sp. in the Pieris dieback complex is discussed.

**MATERIALS AND METHODS.**—*Isolation and identification of fungi.*—Diseased crowns and twigs of plants collected in the field were washed in tap water and surface-sterilized with 0.5% NaClO for 1 min. Bark was removed and wood chips from lesion margins were plated on Difco potato-dextrose agar (PDA) and a selective medium (ENC) for pythiaceae fungi (5). Infected tissue and leaf and twig debris was thoroughly washed with tap water and plated directly on ENC. Pythiaceae fungi were identified by morphological characters on a synthetic medium (M1) and on ENC (5).

Three types of inoculations were made to determine pathogenicity: (i) spore suspensions on unwounded plants, (ii) agar culture plugs on wounded plants, and (iii) hemp broth cultures for root inoculations.

*Spore inoculations.*—Conidia of *P. sydowiana* and a *Phomopsis* sp. were collected with a needle from fruiting bodies on PDA. Pycnidia of *B. dothidea* formed in 1-2 wk on oatmeal agar cultures at 24 C under continuous fluorescent light (9,000 lx). To induce zoospore release, *Phytophthora* cultures were incubated for 6 days on M1 medium at 24 C under continuous fluorescent light (2,000 lx), flooded with 10 ml sterile distilled water and incubated for 7 days then reflooded with 30 ml cold (8 C) sterile distilled water. For inoculations, spore concns for all fungi were adjusted to 10,000/ml with a haemocytometer. Leaves and twigs of 1- to 2-yr-old healthy potted plants were sprayed to runoff with spore suspensions, kept in moisture chambers for 1-2 days at 20-22 C, then moved to a greenhouse at 20-27 C. Greenhouse plants with twig-tip necrosis caused by a severe mite infestation, were inoculated with spore suspensions of *B. dothidea*, *P. sydowiana*, and the *Phomopsis* isolate. Two plants were used for each pathogen; one-half of each plant was inoculated and the other half served as control. Inflorescences with senescent flowers were inoculated and covered with moistened polyethylene bags for 2 days before moving to a greenhouse at 20-27 C.

*Wound inoculations.*—Wounded, mature leaves and twigs on 1- to 2-yr-old plants were inoculated with agar plugs bearing *P. citricola*, *P. citrophthora*, *B. dothidea*, *P. sydowiana*, and the *Phomopsis* isolate. Sterile agar plugs were used as controls. Leaves were scratched with a scalpel to induce wounds, and an incision was made on twigs into which inoculum was inserted. Following inoculation, leaves were enclosed for 2 days in moistened polyethylene bags and examined after 2 wk. Twig wounds were covered with lanolin and basipetal lesion progress was recorded after 10 wk.

*Root inoculations.*—Ten 6-mo-old plants were used for root inoculations with *P. citrophthora*, *P. citricola*, and *P. cinnamomi*. Inoculum was from 2-wk-old hemp broth cultures (50 ml) in 250-ml Erlenmeyer flasks maintained at 22 C (5). Inoculum was incorporated into the container medium adjacent to the feeder roots and

covered with peat. Plants were placed at 24-28 C in the greenhouse and watered daily to maintain the soil near saturation. Ten control plants were treated with hemp broth. Ten weeks after inoculation, roots of plants were rated as 1=healthy, 2=mild root rot, 3=severe root rot, 4=crown rot, and 5=dead plants.

**RESULTS.—Pathogen identity.**—Several *Phytophthora* spp. were isolated on ENC from necrotic lesions on young twigs and leaves, blighted shoot tips, rotted crowns and roots of *P. japonica* from ten nursery locations in Ohio. A total of 330 *Phytophthora* isolates were cultured from lesions on leaves and twigs and 83 from infected crowns. The predominant *Phytophthora* sp. was isolated 251 times from leaves and twigs and 36 times from crowns. Our identification of the fungus, *P. citrophthora* (9, 12), was confirmed by G. A. Zentmyer, Dept. of Plant Pathology, University of California, Riverside.

A second *Phytophthora* sp. was isolated 52 times from leaves and twigs and 27 from plant crowns. Symptoms were identical to those caused by *P. citrophthora*. This fungus, identified as *P. citricola*, was identical with the isolate previously described from rhododendron (5). *P. cinnamomi* was isolated occasionally from roots and crowns in nurseries, especially where severe root rot problems also occurred on rhododendron. A culture identified as *P. lateralis* Tucker & Milbrath was recovered only twice from diseased leaves. *P. citrophthora* was isolated infrequently from rhododendron even in nurseries where it caused severe losses on *Pieris*. It was also found on several *Euonymus* spp. and on *Potentilla fruticosa* L.

*Pestalotia sydowiana* Bres. (4) (confirmed by E. F. Guba, University of Massachusetts, Waltham, Mass.), *B. dothidea*, and a *Phomopsis* isolate were commonly isolated on PDA. These fungi were normally isolated from plants with severe mite injury, winter injury, pruning wounds, and also from old inflorescences.

**Pathogenicity of isolates.**—Results of the pathogenicity tests are presented in Table 1. All actively growing twigs inoculated with *P. citrophthora* and *P. citricola* zoospore suspensions became infected. Lesions on young leaves appeared after 2-5 days as small olivaceous spots which enlarged rapidly and became necrotic. Entire infected succulent young shoots died within 1 wk, but the dieback rarely extended into older twigs. Lesions did not develop on mature leaves or twigs.

Isolates of *B. dothidea*, *P. sydowiana*, and the *Phomopsis* isolate did not infect unwounded young or mature leaves, or twigs inoculated with spore suspensions. Wounded, mature leaves were susceptible to *B. dothidea*, *P. citrophthora*, *P. citricola*, and *P. sydowiana*, but resistant to the *Phomopsis* isolate. Isolates of *B. dothidea* and *P. sydowiana* caused limited necrotic lesions and leaf abscission before the entire leaf was killed. Occasionally, the *Phytophthora* spp. invaded the petiole and spread into the stem before abscission occurred.

All mature twigs wound-inoculated with the *Phytophthora* spp. were girdled. Basipetal lesion progressed into mature wood 2-3 cm in 10 wk. *B. dothidea* girdled 65% of inoculated twigs, with an average basipetal lesion progress of 4-8 cm in 10 wk. The *P. sydowiana* isolates and the *Phomopsis* sp. caused only small lesions.

Inoculations with spore suspensions of *B. dothidea* of twigs with tip necrosis caused by a severe mite infestation resulted in an average dieback of 15 cm after 6 wk. Similar twigs inoculated with *P. sydowiana* and the *Phomopsis* sp. died back an average of 1.2 cm and 0.6 cm, respectively.

Flowers with senescent corollas inoculated with spore suspensions of *B. dothidea* became infected. The infection progressed into the twig and killed the entire branch. Similar flowers sprayed with spore suspensions of *P. sydowiana* and the *Phomopsis* isolate became infected, but lesions did not expand significantly into twigs.

Mean root rot ratings on 6-mo-old *Pieris* plants 10 wk after inoculation with *P. citricola*, *P. citrophthora*, and *P. cinnamomi* were 4.9, 4.3, and 4.0, respectively (Table 1). Mean root rot rating for controls was 1.0 (all three species resulted in similar symptoms). Roots were rotted and crowns invaded, which resulted in stunting, a general chlorosis, dieback and, often, death of the plant.

**DISCUSSION.**—Three types of dieback occur on *Pieris japonica* in Ohio; (i) *Phytophthora* dieback on young leaves and twigs. (ii) *Botryosphaeria* dieback following various types of injury and (iii), dieback on plants suffering from root rot. *P. citrophthora* is the most important fungus involved in the dieback complex; *P. citricola* occurs less frequently. Only the *Phytophthora* spp. can infect unwounded, vigorous young plants. Wounded, mature leaf and stem tissue are susceptible to the *Phytophthora* spp. but such wound-infections have not been observed in the field. Leaf clippings from pruning of new growth frequently collect in puddles in

TABLE 1. Pathogenicity of fungi isolated from *Pieris japonica*

| Fungus <sup>d</sup>    | Spore inoculations            |        |                                |                                  | Wound inoc. <sup>a</sup> |        | Root inoc. <sup>c</sup> |
|------------------------|-------------------------------|--------|--------------------------------|----------------------------------|--------------------------|--------|-------------------------|
|                        | Leaves and twigs <sup>a</sup> |        | Senescent flowers <sup>a</sup> | Mite-damaged plants <sup>b</sup> | Twigs                    | Leaves |                         |
|                        | young                         | mature |                                |                                  |                          |        |                         |
| <i>B. dothidea</i>     | -                             | -      | +                              | 15.0                             | +                        | +      |                         |
| <i>P. sydowiana</i>    | -                             | -      | -                              | 1.2                              | ±                        | +      |                         |
| <i>Phomopsis</i> sp.   | -                             | -      | -                              | 0.6                              | ±                        | -      |                         |
| <i>P. citrophthora</i> | +                             | -      | -                              |                                  | +                        | +      | 4.3                     |
| <i>P. citricola</i>    | +                             | -      | -                              |                                  | +                        | +      | 4.9                     |
| <i>P. cinnamomi</i>    |                               |        |                                |                                  |                          |        | 4.0                     |

<sup>a</sup>+ = infection; - = no infection; ± = small lesions on few twigs based on 20-40 inoculations.

<sup>b</sup>Average dieback (cm) 10 wk after inoculation; based on 10-20 inoculations.

<sup>c</sup>Mean root rot rating: 1 = healthy; 2, 3, and 4 = various degrees of root rot; and 5 = dead plant.

<sup>d</sup>*Botryosphaeria dothidea*, *Pestalotia sydowiana*, *Phytophthora citrophthora*, *Phytophthora citricola*, and *Phytophthora cinnamomi*.

poorly drained areas of nurseries, and are readily colonized by the *Phytophthora* spp. This may serve as an important source of inoculum.

Dieback symptoms caused by *P. citrophthora* and *P. citricola* on leaves and twigs are identical. Normally, lesions are confined to new growth. In addition to dieback, the *Phytophthora* spp. also cause root rot, even on plants growing in media with excellent drainage. Abscised leaves from diseased plants usually are present on the surface of container media. It is possible that zoospores are produced on lesions of these leaves after heavy rains and serve as inoculum for root infection. *P. cinnamomi* also causes root rot on *P. japonica*. However, it was only isolated from plants growing in containers having inadequate drainage.

This is the first report of pathogenicity of *P. citrophthora* to *P. japonica* in the temperate zone of the world, although it has been described frequently from various plants (including woody ornamentals) in tropical and subtropical areas (1, 2, 11). It causes a dieback on rhododendron (6) but rarely even in nurseries where Pieris dieback occurs. *P. citrophthora* was isolated occasionally from *Euonymus* spp. and *Potentilla fruticosa* L., but the role of these hosts or others in its overwintering and prevalence in Ohio nurseries is not known.

*P. citricola* is a serious pathogen of black walnut (3) and this is the first report of its occurrence on *P. japonica*. It can cause a dieback and root rot on rhododendron in some nurseries, but the disease was more serious on *Pieris*.

A second type of dieback occurs on plants with severe mite infestations, senescent flower parts, or wounds caused by pruning or other causes. *B. dothidea* frequently is isolated from dead branches on such plants. Uninjured, actively growing and mature leaf and stem tissue is not susceptible. This confirms findings for *B. dothidea* on several other hosts (7, 10, 13). Botryosphaeria dieback occurs in both nursery and landscape plantings in Ohio. Symptoms differ from Phytophthora dieback in that infection with this pathogen does not occur on healthy, new growth of vigorous plants. Furthermore, the *Phytophthora* spp. normally only kill terminal growth, whereas *B. dothidea* may kill an entire branch. Finally, Botryosphaeria dieback appears to be restricted to plants under stress. More information is needed to fully substantiate this observation.

A third type of dieback is associated with root rots both in landscape and nursery plantings. Chlorotic twigs and leaves on these plants become necrotic and are invaded by several types of fungi. *Pestalotia* spp. and a *Phomopsis* sp. are most commonly isolated. In this study, *P. sydowiana* and a *Phomopsis* sp. only caused minor lesions on injured tissue and appear to play only a secondary role in the dieback complex.

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