Phytophthora citrophthora on Pieris japonica: Infection, Sporulation, and Dissemination

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ABSTRACT

Young leaves and twigs of *Pieris japonica* are most susceptible to *Phytophthora citrophthora*. Mature leaves are resistant, except when wounded. The minimum time required for penetration of leaves (zoospore inoculum) was 15, 2, and 4 hours at incubation temperatures of 12, 24, and 32 C, respectively. Greatest accumulation of zoospores occurred in the dark at fully developed stomata on the lower surface of young leaves. Abscission of leaves of intermediate maturity was affected by the distance between the lesion and the petiole. Sporangia were produced on wet abscised leaves in light at 8-32 C, but most rapidly at 24 C. Very few sporangia formed on initially wetted leaves that had dried to less than 32% of fresh weight. A negative disease dispersal gradient (b = −8.3) was obtained for the distribution of lesions in the field, indicating that inoculum was splashed up from the ground. Zoospores released from sporangia on abscised leaves also invaded meristematic tissues of axillary buds at the crown and feeder roots, and caused crown and root rot.

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Additional key words: Phytophthora spp., *Phytophthora citrophthora,* sporangia, zoospore production, *Pieris japonica.*

Phytophthora citrophthora (Sm. & Sm.) Leonian causes shoot blight, stem dieback, and crown rot on *Pieris japonica* (Thunb.) D. Don in Ohio nurseries, especially on container-grown plants (6). Three types of symptoms are distinguishable: (i) a blight of young succulent foliage and twigs; (ii) spots on leaves of intermediate maturity; and (iii) root and crown rot associated with twig dieback, eventually leading to plant death. Although *P. citrophthora* has a wide host range (4, 22, 25), most reports on epidemiology and control in tropical and subtropical regions have been limited to citrus brown rot and trunk gummosis (3, 7, 12, 26). Fruit and leaf lesions on citrus are confined to the lower 50 cm of trees (19) unless secondary inoculum is splashed from infected low-hanging fruit lesions to higher tree parts (26). Lemon fruits become infected within 3 hours at 12-30 C, and penetration of young leaves and fruit can occur without wounding (13, 26). Root infection and trunk gummosis occur in waterlogged soils, and cause reduced growth and eventual death of citrus (12).

Because of this apparently unusual occurrence of *P. citrophthora* in a temperate climate, the effects of various factors on its infection, sporulation, and dissemination of *P. japonica* were investigated.

MATERIALS AND METHODS.—*Plants and cultures.*—Cultures of *P. citrophthora* (isolate W26) from *P. japonica* were maintained on a selective medium (PBNC) at 12 C (20). All plants were obtained from Ohio nurseries and kept in a greenhouse at 22-26 C with 12 hours of supplemental light (20,000 lux). Plants were fertilized weekly with Peters Azalea acid special fertilizer (1.0 g/liter 21-7-7).

Inoculum production.—For sporangial production, 4- to 5-day-old M1 medium (8) cultures (incubated at 25 C in 2,000 lux continuous light) were flooded with 10-15 ml of sterile distilled water and incubated for 4-6 additional days. To induce zoospore release, plates were washed twice with a salt solution (9) then flooded 30-60 minutes with approximately 40 ml of chilled (8 C) salt solution. Numbers of zoospores/ml were determined with a hemocytometer and adjusted to the desired inoculum levels by addition of salt solution. Temperature of added solution was the same as that of the zoospore suspension which avoided encystment.

Leaf infection.—Detached leaves were placed on moistened filter paper in petri dishes, inoculated with 0.02-ml drops of a zoospore suspension (1×10^4/ml), and incubated in a growth chamber (Environmental Growth Chambers, Chagrin Falls, Ohio) at 24 ± 0.5 C, 92-95% relative humidity, and approximately 10,000 lux continuous light. Zoospore suspensions were applied to the leaf surface with a 1.02-mm diameter (18-gauge) hypodermic needle. A disease severity rating was calculated from the number and size of lesions that developed 3 days after inoculation. Lesions were classified as 0 = no disease, 1 = 1-3 mm in diameter, 2 = 4-10 mm in diameter, and 3 = larger than 10 mm in diameter. Susceptibility ratings were expressed as the sum

of lesion size classification divided by the number of inoculations per treatment. Thus, disease severity ratings ranged from 0-3.

Three classes of leaves were used in inoculation experiments: (i) very young (less than one-half expanded); (ii) leaves of intermediate maturity (one-half expanded to fully expanded); and (iii) mature leaves. In some tests, leaves were classified by a penetrometer rating. For this a Soiltest Model CL700 penetrometer (Soiltest, Inc., Evanston, Ill.) was modified with a weaker spring. The penetrometer rating consisted of the force (g) required to stamp a 6.25-mm diameter hole adjacent to the mid-vein through the middle of the leaf blade.

The penetration time was determined by surface sterilizing leaves with 0.5% NaClO for 30 seconds and rinsing them with distilled water at varying periods of time after inoculation. The time of penetration was defined as the number of hours following inoculation at which surface sterilization did not prevent lesion development. After surface sterilization, leaves in dishes were incubated 3 days in a growth chamber to allow for lesion development.

The effect of temperature on penetration time was examined on leaves of intermediate maturity inoculated abaxially with 0.02-ml drops of a zoospore suspension (5×10^4 zoospores/ml), and incubated in the dark at 8, 12, 16, 20, 24, 28, 30, and 32 C. Eight leaves (four per dish) were each inoculated with three drops (total of 24 inoculations) at each temperature, then surface sterilized at 2, 3, 4, 5, 7, 10, 15, and 24 hours after inoculation to kill the remainder of the inoculum on the leaf surface.

The effect of inoculum concentration on disease severity was examined on leaves of intermediate maturity. Detached leaves (four dishes with three leaves each) were inoculated in the growth chamber with 0.02-ml droplets (three per leaf) of a zoospore suspension with 0.4×10^2, 4.0×10^2, 2.0×10^3, 1.0×10^4, and 5.0×10^4 zoospores/ml. Disease severity ratings were determined 3 days after inoculation.

Zoospore aggregation at stomata.—Leaves on plants incubated in growth chambers in both light (10,000 lux) and dark for 7 hours at 24 ± 0.5 C were next submerged in a zoospore suspension (5×10^4 zoospores/ml) for 1 hour, detached, stained with lactophenol cotton blue, and gently washed with water. More detailed observations on aggregation of zoospores were made on leaves detached before treatment. These leaves were incubated in the growth chamber for 6-9 hours in petri dishes on moist filter paper, then floated with the abaxial surface on the zoospore suspension. The effect of light (10,000 lux) and darkness prior to inoculation, time of floating, zoospore concentration, and leaf maturity on zoospore aggregation at stomata were examined. Five leaves were used for each treatment, and 10 observations per leaf were made.

Leaf abscission.—Leaves of intermediate maturity on each of 12 flushes were wound-inoculated with agar plugs. On each flush, the basal (fully expanded, but immature) leaf was inoculated near the petiole, the next distal leaf was inoculated in the middle of the blade, and the following leaf near the tip. This inoculation sequence was repeated on the same flush immediately beyond the last inoculated leaf. Inoculated plants were incubated for 24 hours in a mist chamber (22 ± 2 C, 10 hours light, 5,000 lux), and then removed to the greenhouse. At various intervals after inoculation, leaves were touched lightly to test for affects on abscission.

Production of sporangia.—Mature *P. japonica* leaves were wounded with a needle or scalpel, and submerged in a zoospore suspension (10^4-10^5 zoospores/ml) at 25 C. After 24 hours, the liquid was decanted, and the moist leaves were incubated an additional 2-3 days until they were completely colonized, but before sporangia had formed. Squares were cut from these leaves (16 mm^2, 10 per treatment) and floated with the abaxial surface down on 50 ml of salt solution (9) in petri plates exposed to 10,000 lux, at 8, 16, 24, 28, and 32 C for 4, 5, 7, 12, 24, 36, and 48 hours. Next, they were fixed with FAA, and stained with lactophenol cotton blue. The number of intact sporangia, as well as those that had released zoospores, were counted in a microscope field (0.98-mm diameter) along two edges of each leaf piece, and on the abaxial surface.

The effect of tissue dryness on sporulation was studied by slowly drying fully colonized mature leaves at 20-24 C. Leaves, incubated on premoistened filter paper in a partially open Plexiglas container, were removed at daily intervals and immersed in the salt solution in petri plates for 3 days. The moisture level of leaves prior to immersion in the salt solution was expressed as percentage of fresh weight. Under these conditions air-dry leaves were 23% of fresh weight.

Inoculated, fully colonized leaves were incubated in polyethylene bags in a mixture of sharp silica sand, peat and muck (1:1:1, v/v) (50% F.C.) at 8, 18, and 28 C to examine the effect of incubation in nonsterile soil on the formation of sporangia. After 0, 3, 6, 12, 24, and 48 days, leaves were thoroughly washed in tap water. The center 1.5-cm portion of each leaf (10 per treatment) was suspended for 36 hours in 25 ml of salt solution at 24 ± 0.5 C and 10,000 lux, and the number of sporangia in two microscope fields per leaf piece (0.98-mm diameter) was determined.

Vertical distribution of lesions on field plants.—Four days after a heavy thunderstorm, the vertical distribution of leaf lesions was measured at 2.5-cm height intervals on five 2-year-old plants. The base of these plants in 5-liter containers was 17 cm above the soil surrounding the containers. On five similar plants, the vertical distribution of the total number of susceptible leaves (very young and intermediate maturity levels) was determined in each 2.5-cm interval.

In a wind-free staircase (20-22 C) 200 water drops (0.025 ml) were impacted after a fall of 10 m on a 1-cm deep zoospore suspension (5×10^4zoospores/ml). Susceptible leaves were mounted at 17.5, 27.5, 37.5, and 47.5 cm above the suspension at a horizontal distance of 12.5 cm from the point of impact. Leaves were mounted with the lower leaf surface facing the source of the splash droplets, and later were incubated in a dark mist chamber (22 ± 2 C) until lesions developed. The number of lesions per leaf was determined. Both field and laboratory distribution counts were used to determine disease dispersal gradients which were calculated from least-squares-fitted regression lines.

Crown and root inoculations.—Three methods were used to study crown and root infection of 4-month-old

plants in a pasteurized mixture of peat and muck (1:1, v/v) in 15-cm diameter pots. In the first method, seven inoculated and completely colonized leaves were placed on top of the container mix or slightly buried in the soil near the crown of 10 plants each. The second method involved four other groups of 10 plants which were placed in polyethylene-lined trays, and immersed 2.5- or 7.5-cm deep in deionized water for 3 or 9 days. In each tray, 100 sporulating *P. japonica* leaves served as the source of zoospores. The zoospore concentration in the water varied from 10^4/ml at 2 days to 10^2/ml at 9 days after immersion. The third method consisted of spraying foliage of *P. japonica* plants with a zoospore suspension (5×10^4 zoospores/ml), incubating them for 2, 5, and 9 days in the mist chamber (22 ± 2 C, 5,000 lux for 10/24 hours), and growing them in the greenhouse for 30 weeks. Abscised leaves were removed daily to eliminate leaf debris inoculum. This method was used to determine if *P. citrophthora* in shoot infections could grow down into the stem under the prevailing conditions.

RESULTS.—*Factors affecting infection and lesion development*.—Lesions formed in 2-3 days on mature and young leaves incubated in a growth chamber after wound-inoculation of either upper or lower leaf surfaces with zoospores. Lesions were rarely formed after inoculation without wounding of the lower leaf surfaces of mature leaves, and no lesions formed after inoculation of the nonwounded upper surface of mature leaves. More lesions were formed after inoculation without wounding on the lower surface of young leaves than of the upper surface. Similar observations on young and mature leaves were made under natural conditions after heavy rainstorms in the field.

Incubation temperature had a significant effect on time required for penetration. No lesions were formed at 8 C. The minimum time required for penetration was 15, 5, 3, 2, 2, 2, and 4 hours at incubation temperatures of 12, 16, 20, 24, 28, 30, and 32 C, respectively, with an inoculum load of 5×10^4 zoospores/ml. Only a few small lesions had formed at these minimum penetration times at 3 days after inoculation. The maximum number and size of lesions developed 4 days after inoculation at 20, 24, 28, and 30 C with penetration times of at least 10, 7, 7, and 15 hours. With a lower inoculum load of 1×10^4 zoospores/ml, the minimum penetration times were 1-2 hours longer at all temperatures, and even after prolonged incubation, no full lesion development occurred.

Disease severity of leaves was highly dependent on leaf maturity as expressed by penetrometer ratings. Expanding young leaves (with a penetrometer rating of less than 200 g) were completely blighted in 1-2 days under optimal conditions (24 C, and inoculated with 5×10^4 zoospores/ml). Leaves with a penetrometer rating of 350, 515, 670, and 750 grams or more developed disease severities of 0.75, 0.5, 0.33, and 0.08, respectively, when inoculated with 1×10^4 zoospores/ml. These disease severity ratings reflected a decrease in number and size of lesions.

Disease severity ratings of 0.0, 0.0, 0.1, 0.4, and 1.6 were obtained 3 days after inoculation with inoculum concentrations of 0.4×10^2, 4.0×10^2 and 0.2, 1.0, and 5.0 $\times$ 10^4 zoospores/ml, respectively. Higher inoculum concentrations caused more and larger lesions, so that with a concentration of 5×10^4 zoospores/ml about one-half of the maximum disease severity rating was attained (maximum = 3.0).

Zoospore accumulation at stomata.—Small numbers of large, functional stomata were formed on the lower surface during early stages of leaf expansion. These were surrounded by numerous small, not fully differentiated stomata, which did not become functional until leaves were almost mature. Preliminary experiments indicated that a distinct accumulation of zoospores occurred at the large differentiated stomata of young detached leaves when they were incubated in the dark while floating on a zoospore suspension. Zoospores did not accumulate around stomata of detached leaves incubated in light.

TABLE 1. Accumulation of zoospores at stomata of *Pieris japonica* leaves with various penetrometer ratings

Penetrometer rating (g)[a]	Cysts per stomate (no.)	Zoospore clusters[b] (no.)
<200	10.4	34.3
200-300	5.4	15.2
300-600	3.1	2.8
>600	1.6	0.9
LSD ($P = 0.05$)	0.62	2.8

[a]Force (grams) required to stamp a hole 6.25 mm in diameter in a leaf blade with a penetrometer.

[b]Number of clusters with five or more cysts per 2.25 mm^2 of infected leaf tissue.

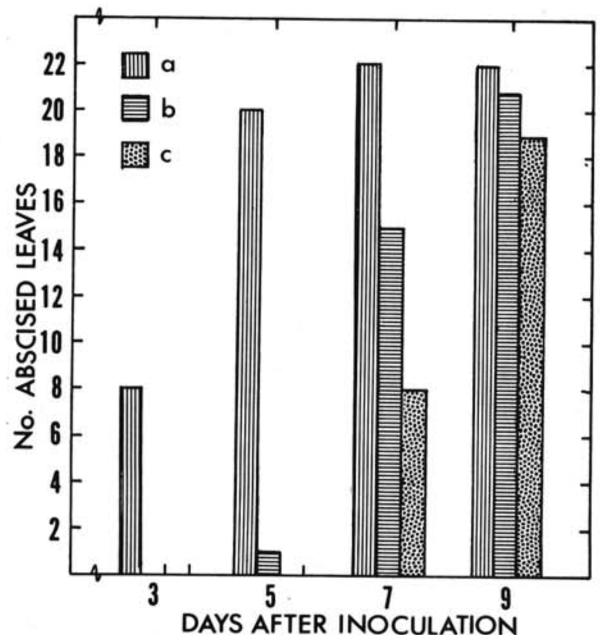

Fig. 1. Effect of lesion position(*Phytophthora citrophthora* on *Pieris japonica*) on absicission. Legend: lesions a) near petiole, b) in middle of leaf blade, and c) near the leaf tip.

Identical results were obtained on leaves attached to plants. A number of factors affecting accumulation of zoospores around stomata were investigated. Because of variability between experiments, each variable was investigated in a separate experiment. An average of 1.4 cysts were found per large stomate if leaves were exposed to light (10,000 lux) for 6 hours prior to inoculation. However, an average of 5.5 cysts were present if leaves were kept in the dark for 6 hours before inoculation. The number of cysts which accumulated at large stomata was also affected by the length of time that leaves floated on the zoospore suspension. The mean number of cysts per stomate was 2.8, 4.1, 5.6, and 10.2 [LSD ($P = 0.05$) = 0.7] for leaves that were floated for 10, 20, 40, and 80 minutes, respectively. At these same time intervals, the mean total number of zoospores attached to the entire lower epidermal surface per microscopic field (0.98-mm diameter) was 37.2, 38.8, 40.0 and 58.3.

The effect of leaf maturity (penetrometer rating) on accumulation of zoospores at stomata is presented in Table 1. More zoospores accumulated at large stomata of young leaves than at those of older leaves. An increase in zoospore concentration in the inoculum resulted in a corresponding increase in accumulation at stomata. The mean number of zoospores accumulated at large stomata was 0.0, 0.0, 4.5, 13.5, and 20.7 with inoculum levels of 0.4 or 4.0×10^2 and 0.2, 1.0, or 5.0×10^4 zoospores/ml, respectively.

Lesion position and leaf abscission.—Very young leaves became blighted 2-3 days after infection, and did not abscise. Mature leaves were rarely infected, and there was no constant correlation between abscission and lesion position on these leaves. On leaves of intermediate maturity, the time between inoculation and abscission was affected by the distance between the lesion and the petiole (Fig. 1). Leaves with lesions near the petiole abscised before those with lesions in the middle of, or at the tip of, the leaf. Abscission of leaves inoculated near the petiole began 3 days after inoculation and was complete at 7 days. Abscission of leaves inoculated at the middle and the tip of the leaf blade started in 5 and 7 days, respectively.

Factors affecting sporangium formation and zoospore release on infected leaves.—The effect of temperature on sporulation is presented in Fig. 2. Sporangia were produced at 8-32 C, but most rapidly at 24 C. The first sporangia were formed 4 hours after leaf immersion in the salt solution at 16-28 C, and zoospore release began 3 hours later. After 24 hours, the maximum number of sporangia had formed and released zoospores. Sporangia were not produced until after 7 hours on leaves incubated at 8 or 32 C, and few zoospores were released. Sporangia that formed at 28 and 32 C were elongate and obclavate in shape, which is atypical for *P. citrophthora*.

After immersion in the salt solution for 3 days under light, sporulation occurred on leaves that had been dried to varying degrees, except on leaves from which *P. citrophthora* could not be isolated. A reduction in sporulation was correlated with decreased leaf moisture levels at immersion time. Leaves weighing more than 45% of fresh weight prior to immersion sporulated profusely (more than 0.5×10^4 sporangia/cm^2). Very few sporangia (less than 10^2/cm^2) formed after leaves had been dried to less than 32% of the original fresh weight.

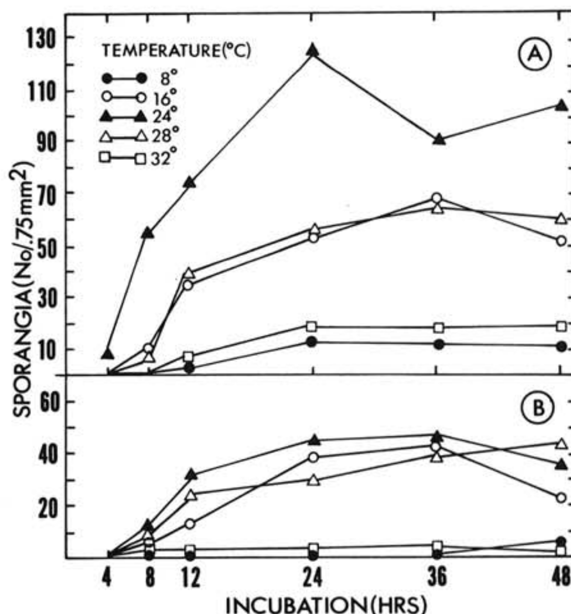

Fig. 2-(A, B). Temperature effects on formation of sporangia by *Phytophthora citrophthora* and zoospore release on infected leaves of *Pieris japonica* after immersion in a salt solution. **A)** mean total number of sporangia/0.775 mm^2; **B)** mean total number of sporangia/0.775 mm^2 that had released zoospores.

The length of time that *P. citrophthora*-colonized-leaves were incubated in nonsterile soil (50% field capacity) affected the number of sporangia produced after leaves were placed in the salt solution (Fig. 3). Sporangia were not produced during incubation at 8, 18, or 28 C in dry soil. The potential for sporangia production decreased with time of incubation in soil. This occurred most rapidly at 28 C, and least rapidly at 8 C.

Vertical distribution of lesions.—The vertical distribution of lesions on container plants in the field is presented in Fig 4 and 5. The maximum number of lesions on 2-year-old plants were found 27.5 cm above the ground. At greater heights, fewer lesions were found, and above 50 cm there were none (Fig. 4-A). The number of susceptible leaves (very young and intermediate maturity levels) increased from 0 at a height of 22.5 cm to a maximum of 70 at 42.5 cm (Fig. 4-B). Lesion distribution was approximately logarithmic, if plotted as number of lesions divided by the number of susceptible leaves at each height against height above the ground (Fig. 5-A). A straight line with the slope of $b = -8.3$ was obtained if the log of the ratio of number of lesions in the field over number of susceptible leaves was plotted against log of height (Fig. 5-B). A slope of $b = -3.7$ was obtained for the distribution of lesions under artificial conditions where inoculum was splashed by water drops out of petri dishes and onto susceptible leaves.

Most probable source of inoculum in the field.—Under conditions of adequate moisture, *P. citrophthora* can completely colonize abscised leaves on the soil surface. Sporangia were observed on such leaves on wet soil. In the spring, summer, and fall, leaves pruned from *P.*

japonica plants became colonized when in contact with infected debris on soil. Leaf spots developed on leaves floated on water containing such debris, and *P. citrophthora* was isolated from these. Leaf spots and shoot blight developed 3-4 days after heavy rainstorms on plants located near puddles with colonized leaf debris. Crown and root rot developed on field-grown container plants with abscised leaves, or colonized leaf clippings on the soil surface.

Crown and root infection.—Infected leaves on the soil surface were the most effective inoculum for crown infection. Plants died sooner (13 of 20 in 8 weeks) if kept in a moisture chamber for 9 days after inoculation, than if kept in the greenhouse continuously (15 of 20 in 18 weeks). Few foliage-inoculated plants died (three of 30 in 30 weeks). There was no evidence that *P. citrophthora* grew from the foliage into the crown of the plants that died. It is presumed that crown infection resulted from zoospores produced on abscised leaves not removed from the soil surface before sporulation occurred. The least effective method of killing plants was immersion of roots in a zoospore suspension either 2.5- or 7.5-cm deep for 3 or 9 days (three of 40 died in all treatments). However *P. citrophthora* was isolated from many rotted feeder roots of these plants. *Phytophthora citrophthora* could not be isolated from uninoculated immersed plants.

DISCUSSION.—The disease cycle of *P. citrophthora* on *P. japonica* in the temperate climate of Ohio appears similar to that on citrus in tropical and subtropical climates (2, 3, 7, 11, 12, 19, 25, 26). Young leaf tissue and inflorescences are the most susceptible parts of the plant. Lesions on leaves generally do not progress very far into mature wood. Sporangia are produced on abscised leaves on wet soil, and zoospores released from these sporangia (probably) are splashed up to the foliage and cause more infection. Crown infection probably originates from zoospores produced on leaf debris invading through dormant buds and leaf axils at the base of the plant. Feeder roots also may become infected, but root rot rarely becomes severe enough to kill plants. It appears that *P. citrophthora* is primarily a leaf spot-dieback pathogen on *P. japonica*, but it may kill plants if crown infection occurs.

Three lines of evidence support the conclusion that inoculum of *P. citrophthora* is at or near the soil surface. Leaf spots were most common on plants along walkways between beds of closely-spaced plants. Secondly, no lesions were found higher than 50 cm above the ground which agrees with findings on citrus (19). The third line of evidence is the negative dispersal gradient with height of diseased tissue in the field. This gradient ($b = -8.3$) is much steeper than that obtained in a wind-free staircase ($b = -3.7$). During a heavy rainstorm the upper foliage (rarely higher than 60 cm) is more exposed to the full force of rain and wind than the lower portions. Therefore, a large proportion of inoculum may be washed and shaken off the upper foliage before the zoospores can become attached to leaf surfaces. This results in a steeper gradient.

Pieris japonica differs from black pepper and potato, which both have foliar Phytophthora problems (14, 23), in that both surfaces of mature *P. japonica* leaves are highly resistant. Lesions developed on mature leaves only if they have been injured. Young leaves are very susceptible and apparently are penetrated largely through

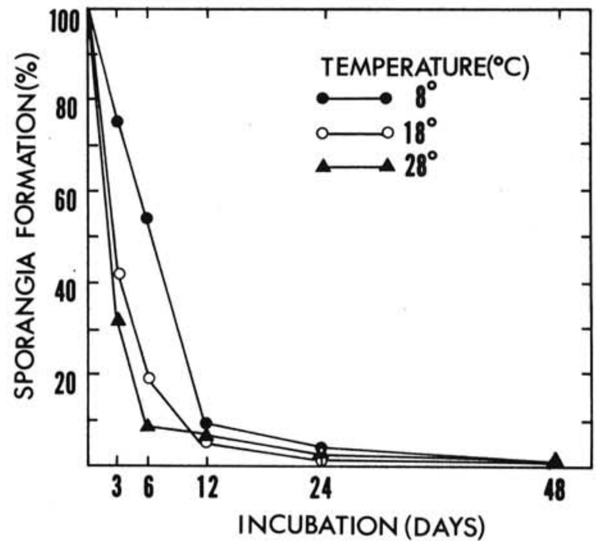

Fig. 3. Sporulation (percent of initial value) of *Phytophthora citrophthora* on the surface of infected leaves of *Pieris japonica* placed in a salt solution after incubation in nonsterile soil (50% field capacity) at various temperatures.

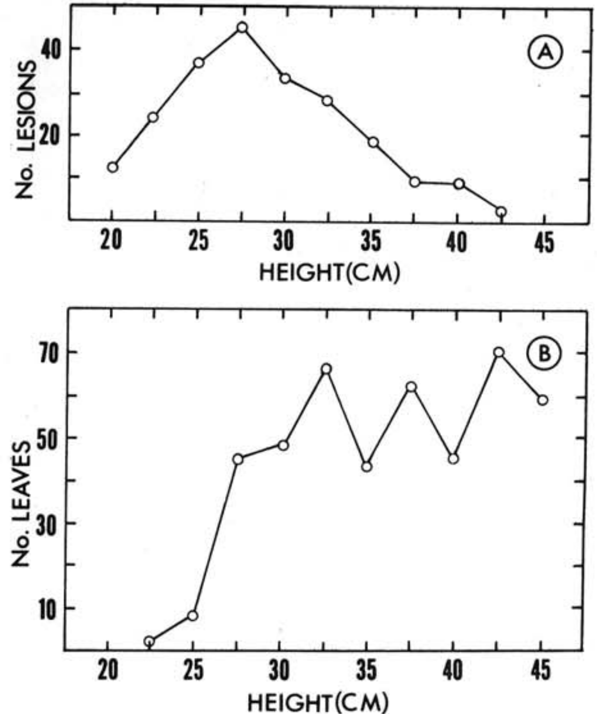

Fig. 4-(A, B). A) Vertical distribution of *Phytophthora citrophthora* lesions on leaves of five 2-year-old *Pieris japonica* plants in the field (5-liter containers, rim 17 cm high); **B)** Vertical distribution of susceptible leaves on five similar plants.

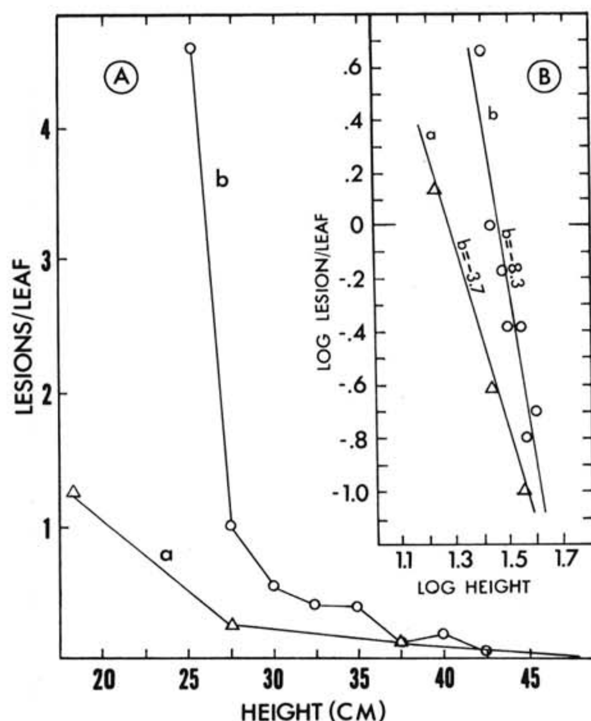

Fig. 5-(A, B). A) Distribution of the ratio of number of *Phytophthora citrophthora* lesions per *Pieris japonica* leaf at various height increments. a) Under artificial conditions (see text); b) on five 2-year-old *P. japonica* plants infected in the field after a heavy rainstorm (derived from Fig. 4-A and 4-B). **B)** Disease dispersal gradients and least-square-fitted regression lines of data in Fig. 5-A.

stomata on the lower leaf surface, which has been reported for other foliar Phytophthora and downy mildew diseases (14, 17, 18). Only a limited number of lesions developed from inoculations on the upper surface of young leaves. This probably was associated with the absence of stomata.

Zoospores accumulated in clusters around large stomata in the dark when they were open, but not in the light when they were closed. This unusual opening of stomata in the dark has also been found on grape (18), *Vicia faba* L., and *Pelargonium zonale* Willd. (27). Accumulation of zoospores around stomata in the dark has been reported for *Plasmopara viticola* (Berk. & Curt. ex de Bary) Berl. & de Toni on grape (18), and no mechanism of accumulation has been put forward. One unexplored possibility may be the electrochemical attraction of zoospores to open stomata; it has been shown that an electrochemical potential exists near stomata (16), and that zoospores of many *Phytophthora* spp. respond to electrical potentials (10).

The large number of zoospores that aggregate in the dark at large stomata of very young *P. japonica* leaves represents a localized increase in inoculum potential. Thus, in the dark, a lower inoculum concentration may be required for disease initiation. This may be most important at low night temperatures, when the infection process requires more time. The lack of zoospore accumulation around stomata of older leaves may explain mature leaf resistance.

At optimal temperatures (24-30 C) penetration occurred within 2 hours on young leaves, but at higher or lower temperatures significantly more time was required (15 hours at 12 C, 4 hours at 32 C). This agrees with the penetration time (3 hours) over a range of 12-30 C reported for *P. citrophthora* on lemon fruit (13).

Zoospore concentration affected not only the time required for penetration, but also disease incidence and severity. Individual zoospores perhaps could infect the leaf and cause small flecks, but 10-20 zoospores were required per inoculum drop to cause large lesions. A synergistic effect may be involved among zoospores in the formation of typical lesions which has been suggested for the formation of lesions by zoospores of *P. infestans* (15). The greater number of zoospores accumulated at stomata in the dark with higher inoculum levels would result in more disease. However, disease did develop following inoculation of leaves with zoospores in light when no accumulation of zoospores around stomata was evident. We should determine if the relation between disease severity and inoculum level differs in light and dark to evaluate the significance of zoospore accumulation around stomata in *P. japonica*.

Infected, young, succulent leaves probably do not contribute substantially to inoculum production in the field because they remain attached and dry out. However, leaves of intermediate maturity abscise after infection and abundant inoculum is produced on them. This has also been observed for *Ilex opaca* Ait. infected with *Phytophthora ilicis* Buddenhagen (1). The rate at which *P. japonica* leaves abscise depends largely on lesion position. This was also reported for coffee leaves infected with *Omphalia flavida* Maubl. and Rang (21). *Pieris japonica* leaves with lesions near the tip abscise later, after the lesions have greatly enlarged. In dry weather, large portions of these lesions on attached leaves become dry. This results in a reduced sporulation capacity, since the pathogen does not survive drying very well (5).

Prolonged exposure of infected leaves to soil, under conditions too dry for sporangium production, reduces the sporulation capacity significantly, when free moisture becomes available again. Microbial lysis and competition may be responsible for this, since low incubation temperatures are less effective in reducing sporulation than high incubation temperatures (24).

The rapid colonization of leaf debris and abundant sporulation on it at 24 C may account for the predominance of *P. citrophthora* over *P. citricola* Sawada, which is also a pathogen of *P. japonica*. *Phytophthora citricola* was only occasionally recovered from debris of *P. japonica* upon which it sporulated less profusely (5). A similar explanation has been proposed for the predominance of *P. citrophthora* over *P. nicotianae* var. *parasitica* (Dastur) Waterhouse on citrus (26).

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