Epidemiology of Stem Blight of Vinca minor Incited by Phoma exigua var. exigua

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ABSTRACT

The causal organism of stem blight of *Vinca minor* was identified as *Phoma exigua* var. *exigua*. Symptoms appear initially as lesions at the ground line on stems and runners and at the base of new shoots, finally including wilting, blackening, and death of affected stems. Leaf spots caused by the pathogen occur occasionally, but are not associated with stem infections. Production by the pathogen of a toxin, which might account for the rapid blackening and death of affected tissues, could not be demonstrated.

Optimum growth of the fungus in culture occurred at 27 C; there was no growth at 30 C. Maximum infection occurred at 18 C, but dropped off rapidly at higher temperatures. High soil moisture content was necessary for infection. Stem lesions appeared 10 to 15 days after inoculation at 18 C. A high percentage of infection occurred on intact, vigorous stems, and neither stem wounding nor moisture stress increased disease incidence. *Phoma exigua* var. *exigua* should be characterized as a highly virulent, facultative parasite of *V. minor*.

Attempts to control stem blight through the use of various mulches or by foliar applications of fungicides in 1970 were unsuccessful. Phytopathology 61:959-963.

Vinca minor L., also known as periwinkle or ground myrtle, is a semiprostrate, shade-tolerant, perennial evergreen species commonly used as a ground cover in ornamental plantings. In recent years, a dieback or blight has been observed on *V. minor* from many plantings scattered throughout Illinois. Symptoms appear as lesions on shoots and runners and later include wilting and dieback of affected stems. The disease was present in all plantings examined throughout the state in fall 1969 and in spring 1970, and was causing severe damage in many areas.

This paper reports studies undertaken to identify the causal organism and to investigate the factors influencing disease incidence and severity. Isolations made from diseased and dead *V. minor* stems during the summer of 1969 yielded several organisms, but those made from new stem lesions on runners in the fall of 1969 and on new shoots in the spring of 1970 consistently yielded a species of *Phoma*.

A literature survey disclosed several reports of *Phoma*-like fungi occurring on *V. minor* (1, 5, 6, 7, 9, 10, 11, 17, 18, 19, 20). Jansen (11) reported isolating a *Phoma*-like fungus from leaf spots and dying shoots of *V. minor* and *V. major* (big-leaf or variegated vinca) which was morphologically identical to *Phoma exigua* Desm. Boerema & Howeler (4) include this fungus in their monograph on *P. exigua* and its varieties, and place it in *P. exigua* Desm. var. *exigua* Maas.

MATERIALS AND METHODS.—*Vinca minor* plants used as hosts in laboratory tests were obtained as bare-rooted clumps, and grown in the greenhouse in 8.8-cm plastic pots, using a standard pasteurized potting mix composed of equal parts of soil, peat, and vermiculite (1:1:1). Potted plants were watered daily, given light fertilization in irrigation water twice weekly, and sprayed for control of mites as needed.

Isolates of the pathogen were obtained from naturally infected, field-grown plants. Stem sections 1-2 cm long, which included the advancing margin of a lesion, were surface-sterilized in a 10% aqueous dilution of sodium hypochlorite (Clorox) for 2 to 3 min, then placed on potato-dextrose agar (PDA) or Difco malt extract agar (MEA) in petri dishes and incubated at 24 C for 10 to 14 days. The optimum temperature for growth of the fungus on MEA was recorded at 27 C.

Four culture media were used: PDA, MEA, vinca-potato-dextrose agar (VPDA), and vinca agar (VA). VPDA was prepared by autoclaving, for 1 hr, 2- to 3-cm sections of the stems and leaves of *V. minor*, fragmenting the sterile sections in a Waring Blendor, and mixing 200 g of this material with 1,000 ml of PDA. VA was made similarly except that the plant material was added to 1 liter of 1.5% water agar. Both media were autoclaved for an additional 30 min.

A standard inoculation method was developed. Two grooves, 10 mm deep and 2 mm wide, were made with a hot dissecting needle on opposite edges of the bottom section of plastic petri dishes. Each dish was dipped in a 25% Clorox solution, rinsed in sterile water, and filled with 40 g of pasteurized potting mix. Five ml of a conidial suspension of the pathogen (1.5×10^7 conidia/ml) were atomized onto the surface of the potting mix; healthy runners of *V. minor* were laid through the grooves in the edges of the dishes and across the inoculated medium; and the top of each dish was replaced. Sterile water was added as needed to keep the medium moist.

RESULTS.—*Symptoms*.—Disease symptoms were observed on naturally infected plants in the field and on experimentally infected plants in the laboratory. In the field, dieback of *V. minor* begins in late April or early May in Illinois, soon after new growth appears.

Dark brown to black lesions first appear on the stems of old overwintered runners, causing them to die back to the base. These old runners are usually well hidden under the new shoots, and may serve as a source of inoculum for later infections. Girdling lesions appear at the base on new shoots and cause conspicuous wilting and drying. Pathogen spread and symptom development continue for several weeks, often resulting in wilting and death of entire clumps of host plants. As the new shoots begin to form runners, lesions develop on the stems of runners where they come in contact with the soil or other infected plant parts (Fig. 1). As the summer progresses, symptom development becomes less apparent and usually ceases by late June or early July, although resumption of symptom development has been observed during prolonged periods of abnormally cool, wet weather during the summer months. Lesions commonly appear on the stems of runners in the fall.

Dark spots that may develop on the leaves cause browning and death of affected leaves, but diseased leaves usually defoliate and are not associated with stem infection. In contrast, the fungus frequently spreads from stem lesions into the leaf petiole and the base of the leaf (Fig. 1). The stem lesions expand rapidly and cause wilting and dieback of the stem. Some lesions may extend the entire length of the stem within a few days after initial appearance. Pycnidia typical of *Phoma* are apparent in new lesions within a few days. Secondary fungi rapidly invade the dead tissues, and the infected phloem and cortex soon slough off.

Under experimental conditions, lesions developed where stems came in contact with infested soil, often at a node. Symptoms generally appeared on inoculated plants 10 to 15 days after runners were placed in contact with infested soil.

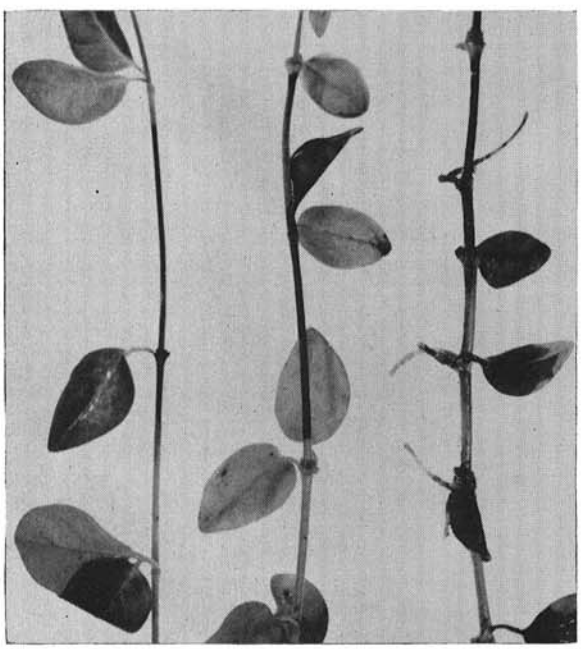

Fig. 1. Stem blight symptoms on *V. minor* caused by *P. exigua* var. *exigua*.

Pathogen morphology and taxonomy.—The species of *Phoma* consistently isolated from lesions on *V. minor* was examined in the laboratory on stem and leaf tissues and in culture. Small brown to black pycnidia with an inconspicuous single ostiole are produced in lesions on the stems and leaves of diseased plants. The inner wall of the pycnidium is lined with hyaline, ovoid, conidiogenous cells which give rise to single-celled, hyaline, biguttulate pycnidioconidia measuring 1.5 to 2.0×4.5 to $5.0\ \mu$. In culture, pycnidia arise from tight knots of mycelia embedded in the medium and are produced singly on VA and MEA but coalesce into irregular fructifications which exude masses of pinkish-colored pycnidioconidia on PDA and VPDA. Pycnidioconidia are most readily produced on VPDA and VA.

Mycelia in culture are irregularly septate and hyaline, but turn dark with age and produce an abundance of oil globules. The growth characteristics in culture are highly variable on different media.

The morphological and cultural characteristics of the fungus are identical to those reported for *P. exigua* Desm. var. *exigua* Maas. (4). According to Boerema & Howeler (4), *P. exigua* var. *exigua* produces a colorless metabolite "E", which can be easily oxidized to a blue-green pigment by the addition of an alkali; e.g., in NaOH. Two-week-old cultures of several isolates of the pathogen, grown in shake culture on malt extract and cherry extract solutions and in petri dishes on malt agar and cherry agar, as described by Boerema & Howeler (4), were tested for the presence of this metabolite. None of the described reactions occurred for any isolate on any of the test media. Aside from this biochemical test, the pathogen is identical to *P. exigua* var. *exigua* and is so designated throughout this paper.

Effect of temperature on infection.—The effect of temperature on infection of *V. minor* stems by the pathogen was studied, using the inoculation method described under MATERIALS AND METHODS. Plants with petri dishes attached were placed in dark incubators at 10, 15, 18, 20, 22, 25, 27, and 30 C. Ten replications were included at each temperature. An additional 5 ml of conidial suspension (2×10^7 conidia/ml) were added to the soil mix in each petri dish 7 days after initiation of the experiment to insure the presence of viable spores. The date and length of lesion development were recorded, and results for each temperature are presented in Fig. 2.

The greatest incidence of infection occurred at 15 and 18 C with seven and eight lesions, respectively. Infection at 10 C was also high, although symptoms were slower to develop than at 15 and 18 C. The incidence of infection dropped off above 18 C, with no infection at 30 C.

Comparison of inoculation methods.—Four inoculation methods were compared to determine how, and under what conditions, infection occurs.

The importance of wounds as an avenue of entrance for the pathogen was studied by sand-blasting host

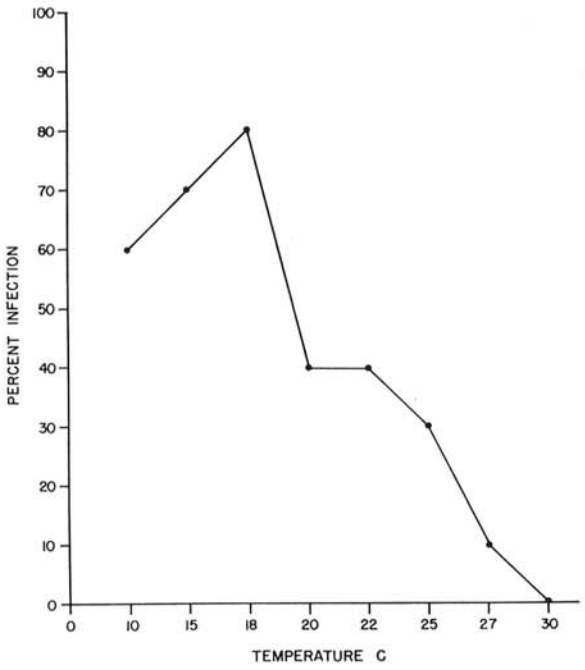

Fig. 2. Percent infection of *V. minor* runners by *P. exigua* var. *exigua* at eight different temperatures.

plants at low pressure with a fine quartz sand. Plants unwounded or wounded by sand-blasting were atomized with a conidial suspension of the pathogen and held under 100% humidity at 18 C for 15 days. Although no stem lesions developed, the pathogen was isolated from dark areas of tissue that formed around wounds on the leaves. Under the conditions of this test, wounding was not effective as a predisposing factor for stem infection.

The inoculation method described under MATERIALS AND METHODS resulted in 90 to 100% infection at 18 C. Lesions developed on stems of runners within 10 to 15 days after runners were placed in contact with soil infested with the pathogen.

Another method utilized the plastic petri dishes as described in MATERIALS AND METHODS except that sterile cotton, covered by a filter paper disc seeded with a conidial suspension, was substituted for the soil mix. From 60 to 75% infection was obtained with this method, and fruiting bodies of the pathogen were produced on the filter paper discs.

A fourth method using mycelia in place of conidia as inoculum was evaluated. Ten ml of a mycelial suspension, prepared by fragmenting five nonsporulating petri dish cultures of the pathogen on VPDA with 500 ml of sterile water in a Waring Blendor for 30 sec, were poured over the soil mix in each petri dish. After 20 days, 30% infection had occurred, but a microscopic examination at this time revealed the presence of small numbers of pycnidia containing spores in the soil mix.

Assay for toxin production.—The pathogen was tested for its ability to produce a toxin which may be involved in the rapid blackening and dying of stems, leaf petioles, and basal leaf tissues following initial stem lesion appearance.

The fungus was grown in shake culture on four liquid media: potato-dextrose solution (250 ml of extract from 200 g of boiled, peeled potatoes and 20 g of dextrose, plus 250 ml of distilled water); potato-dextrose-vinca solution (250 ml of potato-dextrose solution plus 250 ml of vinca extract made by boiling stems and leaves of *V. minor* in 1,000 ml of distilled water for 30 min, then filtering the extract through cheesecloth); malt extract solution (250 ml of malt extract solution, made by dissolving 25 g of malt extract in 500 ml of distilled water, plus 250 ml of distilled water); and malt extract-vinca solution (250 ml of malt extract solution plus 250 ml of vinca extract).

Fifty-ml portions of each medium were placed in 125-ml flasks and autoclaved for 30 min. The flasks were then cooled, and four flasks of each medium were seeded with a 7-mm mycelial plug from the margin of a 6-day-old culture of the pathogen on PDA, and put on a shaker. The remaining flasks were stored at 10 C. After 5 days, the flasks were removed from the shaker and the media filtered through cheesecloth. The extracts were filtered 3 times through filter paper, then through a bacterial filter. Each of the extracts was poured into 10 small vials, and the cut end of a *V. minor* stem, 10 cm long, was submerged in the extract in each vial. Two vials of each of the original sterile media were included as checks. The vials were observed for 6 days for uptake of the extracts, and stems were examined for symptoms of phytotoxicity.

Although the vinca stems readily absorbed the extracts, no phytotoxicity symptoms were observed. These results indicate that no toxin was produced on any of the substrates used, and it is unlikely that a toxin is involved in symptom production.

Effect of moisture stress on disease susceptibility.—Twenty potted clumps of *V. minor* were placed in a growth chamber under constant conditions of ca. 50% relative humidity, 18 C, and 8 hr daily of 800 ft-c of light. Pots were watered daily for 7 days, after which 10 pots were put under moisture stress by the withholding of water for 11 days. The remaining 10 pots were watered daily and used as checks. At the end of this period, all pots were watered and stems of three runners from each pot were inoculated with *P. exigua* var. *exigua* using the method described under MATERIALS AND METHODS. All pots were watered daily for 7 days, after which water was again withheld from the 10 pots in the moisture stress series, whereas daily watering was continued on the check pots. After 25 days, all pots were removed from the chamber, and percentage infection of inoculated stems was recorded.

Disease symptoms appeared on 63% of the inoculated runners on the watered checks, whereas only 33% of the inoculated runners on the plants under moisture stress exhibited disease symptoms.

DISCUSSION.—A *Phoma* species consistently isolated from lesions on stems and leaves of *V. minor* is identical in morphological and cultural characteristics to *P. exigua* Desm. var. *exigua* Maas. Although a bio-

chemical test for metabolite "E" (which Boerema & Howeler (4) consider a distinguishing character of var. *exigua*) was negative for all isolates obtained from *V. minor* in Illinois, regardless of the culture substrate, there is some disagreement on the proper criteria for taxonomic divisions below the species level (3, 12), and the validity of using a biochemical test to identify varieties of *Phoma* sp. is questionable.

The original plant material examined by Weiss (20) and reported as *Phomopsis lirella* Grove on *V. minor* was obtained and examined by the authors. The herbarium sheet lists the specimen as *Phoma* sp., and typical *Phoma* pycnidia were found on the stems. The pycnidia, however, were immature and no spores were present. Desmazieres (8) in 1843 described a species of *Phoma* on *V. minor,* calling it *P. lirella,* which Grove (10) later transferred to the genus *Phomopsis.* Owing to the similarity of the two genera, and the inconsistencies of reports of *Phomopsis* and *Phoma* on *V. minor,* it seems likely that at least some of the reports of *Phomopsis lirella* causing dieback of shoots of *V. minor* in the United States (17, 18, 20) may have been due to incorrect identification, and the pathogen responsible may have actually been *P. exigua* var. *exigua. Phomopsis lirella* was never recovered from any of the isolations made from *V. minor* in Illinois.

Phoma exigua var. *exigua* has been described as a ubiquitous soil-borne fungus and a common inhabitant of dead and dying plant material (4, 7, 11, 12, 13, 14, 15, 16). Pycnidia are produced on dead stems and leaves of *V. minor* in the spring in Illinois, and may provide a source of inoculum for early season infections. The pathogen also grows saprophytically throughout the soil under favorable moisture conditions. Malcolmson (15) showed that moist soil conditions favor the growth of *P. solanicola,* a synonym of *P. exigua* var. *exigua* (2, 4). In the present study, it was found that high soil moisture content (above 50%) is essential for infection.

The fact that cool temperatures and high soil moisture content are prerequisites for infection correlates well with conditions that prevail in spring and fall in Illinois, the periods of maximum disease development. Little or no symptom development was observed during the hot summer months, except following periods of unusually cool, wet weather.

The characterization of *P. exigua* var. *exigua* as a weak, wound parasite of *V. minor* by Jansen (11) is questioned. She obtained infection by injecting conidia of the pathogen into stems and by wounding stems and placing pieces of agar containing mycelia over the wounds. In the present study, a high percentage of infection was achieved by inoculating unwounded stems with conidia in moist soil. Mycelia of the pathogen did not appear to attack unwounded stem tissues, although wounds may offer an infection court for mycelia growing in the soil, as Jansen's results indicate (11). *Phoma exigua* var. *exigua* is better characterized as a facultative parasite of *V. minor.* Under favorable environmental conditions, the fungus is a virulent pathogen which infects vigorous shoots and runners. Jansen (11) suggests that host plants may become more susceptible to infection when under stress conditions such as drought or moisture stress. In the present study, plants under moisture stress were less susceptible to infection.

Phoma exigua Desm. was first described in Europe where *V. minor* is native (8). The pathogen was probably introduced into the United States on imported host plants, and is being spread throughout this country along with plant shipments. During the course of this study, the pathogen was isolated from young *V. minor* plants being shipped into Illinois from nurseries in Indiana, Michigan, and Tennessee.

Since initial infection occurs at or below the soil line where stem tissues are usually well protected by foliage, control of stem blight with surface-active fungicides may not be feasible. Five fungicides, applied in three foliar sprays at 2-week intervals beginning on 5 May 1970, did not significantly reduce disease incidence or severity. Two systemic materials, benomyl and thiabendazole, appear promising, but the method, timing, and rate of application have yet to be evaluated.

Vigorous, established *V. minor* plantings in Illinois produce heavy new growth which masks the damage caused by stem blight. The most severe damage was observed in relatively new plantings. This may be due to unusually high soil moisture resulting from frequent irrigation of new plantings. In 1970, experimental field plantings of *V. minor* were established at Urbana, Ill., to evaluate the influence of various types of mulches on disease development. By the end of the growing season, it was obvious that mulches such as peat moss, corn cobs, and plastic covered with pea gravel, which favor prolonged high soil moisture, created conditions favorable for disease development. Therefore, cultural practices which promote high soil temperatures and/or low soil moisture are recommended for control of vinca stem dieback at the present time.

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