Epidemiology and Chemical Control of Phomopsis Canker of Highbush Blueberry

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ABSTRACT


Rain-dispersed *Phomopsis vaccinii* conidia from highbush blueberry (*Vaccinium corymbosum* L.) stem cankers were caught by the use of a funnel and jug spore trap. The major period of spore dispersal occurred during rains from bloom through petal fall in late May and June, and generally to a lesser extent, during rains in June and August. Populations subsequently declined and no conidia were detected in traps after September. Conidia were trapped from localized leaf lesions for only a short time during late August. Nondamaged bushes were not infected either with conidial or mycelial inoculum. Mechanically-wounded bushes were consistently infected after inoculations with mycelium throughout the growing season (April through October). Bushes experimentally damaged by freezing were infected by inoculation with conidia. Healthy nonwounded plants were not infected as a result of 1-mo exposures to natural field inoculum. In several cases, crowns of heavily diseased bushes were infected beneath the soil surface as evidenced by isolation of *P. vaccinii* onto potato dextrose-streptomycin agar. Difolatan and Benlate spray applications made during the growing season resulted in canker reductions of 36% to 58% ($P = 0.10$).

*Phomopsis vaccinii* is the causal agent of a serious stem canker disease of highbush blueberry (*Vaccinium corymbosum* L.) in the southern growing areas of Michigan and in northern Indiana. The absence of this disease in the more northern areas of Michigan is presumed to be an effect of lower temperatures (6). The pathogen causes elongated, indistinct cankers on stems (Fig. 1); severely affected branches wilt and die during the summer. Pycnidia frequently are found on the grayish portions of dead branches. After a few years of repeated infections, bushes may be killed back to their crowns. The ascomycetous stage of *P. vaccinii* is *Diaporthe vaccinii* Shear. Although perithecia have been induced under artificial conditions (8), the perfect stage has not been reported under field conditions. Raniere (3) suggested that the sudden occurrence of extensive death of blueberry stems caused by *Phomopsis* sp. and *Botryosphaeria* sp. in New Jersey was due to invasion by these weak pathogens following low-temperature damage. In contrast, conidial inoculations of the bushes of nonwounded Katharine and Schammell cultivars were successful (7). The times of spore dispersal and infection periods under field conditions have not been investigated previously and were the objectives of this study.

MATERIALS AND METHODS

Trapping conidia from rain water.—A heavily infected field of a mature planting of highbush blueberry (cultivar Earlblue) near West Olive, Michigan was chosen for field study. Pycnidia of *P. vaccinii* were present on dead, cankered stems and in localized leaf lesions. To determine levels and periods of conidial dispersal from stem cankers during rain periods in the 1974 and 1975 growing seasons, plastic funnels were attached beneath cankers bearing pycnidia. The funnels were connected by Tygon tubing to plastic 3.79-liter (1-gallon) containers which retained the conidia released in water (2). One funnel-jug trap was attached to each of five bushes. Collection of conidia from leaf pycnidia of *P. vaccinii* was accomplished in a similar manner during 1975. In late August after lesions had appeared, leaves bearing pycnidia were detached and placed into 10-cm diameter plastic funnels which were positioned in the upper canopy of the bush. Leaves were replaced weekly. Weekly counts of conidia from both stem cankers and leaves were made with a hemacytometer. Rainfall measurements were recorded with a 7-day recording rain gauge (Weather Measure Corp., Sacramento, CA 95841). Relative humidity (RH) and temperature were measured with a sheltered 7-day hygrothermograph (Bendix Co., Inc., Baltimore, MD 21204), located about 1 m above the ground.

Field exposure of healthy bushes for intervals to determine natural infection periods.—Two-yr-old, healthy, nonwounded, potted blueberry bushes (cultivar Berkeley) were placed beneath heavily diseased bushes in the field for 1-mo periods from mid-April through mid-November to determine periods of natural field infection by *Phomopsis* canker. After 1 mo of field exposure, the

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bushes were put into isolation at an orchard site at Michigan State University for 1 yr prior to being evaluated for disease development. Stems were exam-
ined for evidence of canker development. Suspected areas were surface disinfested with 10% NaOCl and isol-
tions were made onto potato-dextrose agar amended with 100 μg/ml of streptomycin sulfate (PDA-S).

Wounded and nonwounded stems were inoculated with 7-mm diameter mycelial blocks from mono conidial PDA cultures to determine whether wounds were re-
quired for infection. Wounds were made by puncturing the stem epidermis with a sterile dissecting needle in a circular pattern. Mycelial agar blocks were placed on wounded and nonwounded areas and held in place with moistened cheesecloth and aluminum foil. The number of resulting infections was determined the following growing season
by isolating from the inoculated areas onto PDA-S as previously described.

Conidial inoculation studies.—Two-yr-old, healthy, nonwounded, Berkeley blueberry bushes were inoculated with a suspension of \textit{P. vaccinii} conidia each month of the growing season (late April through October). The suspensions contained conidia (10^6/ml) and were applied to bushes with a DeVilbis No. 15 atomizer. Following inoculation, plants were kept in a mist chamber for 48 hr at 20 to 30 C. Plants were maintained in isolation as previously described, until the following year in August, when evaluations were made in the same manner as before.

Effect of environmental stress on \textit{Phomopsis vaccinii} infection.—Twenty-yr-old potted Berkeley blueberry bushes were subjected to a temperature of -3 C for 1 hr in a growth chamber (Sherer-Gillet Co., Marshall, MI 49068); a second group of 20 plants were drought-stressed for a 2-wk period until wilting was evident. Both sets of plants were inoculated by spraying with a conidial suspension containing 10^6 conidia/ml, and incubated for 48 hr under continuous mist at about 25 C. Control plants were: (i) frozen, but noninoculated, and (ii) inoculated, but not frozen nor drought stressed. Infections which developed after 6 to 8 mo of incubation were determined by the previously described isolation techniques.

Determination of the extent of above- and below-
ground infection.—The location of canker infections in the above- and below-ground portions of the crown was determined in a field at West Olive, Michigan, where bushes had been severely diseased for 4 yr. Bushes with se-
vere infection were cut-off at the crown or beneath the soil surface and isolations were made onto PDA-S after the tissues were surface disinfested with 0.5% NaOCl.

Effect of temperature, relative humidity, and free water on germination of conidia.—Freshly collected pycnidia of \textit{P. vaccinii} obtained from cankers were placed into separate drops of sterile glass-distilled water and teased apart with a flamed dissecting needle. To determine whether conidia would germinate at high RH, the spore drops were applied to slivers of glass cover slips held by a small ball of clay, allowed to dry, and then were sealed in Gilson Differential Respirometer vessels (Gilson Medical Electronics, Middleton, WI 53706), containing either a saturated solution of K_2SO_4 or water to effect humidities of 98% and 100%, respectively (5). Germination of conidia in free water was studied by putting the spore drop in depression slides that were held in a sealed container at 100% RH to prevent evaporation. Four flasks (replications) and four depression slides were held at temperatures of 10, 21, and 27 C. Percent germination and germ tube elongation under dark conditions were recorded at 6-hr intervals for a total of 48 hr.

Effect of temperature on mycelial growth.—A 7-mm diameter disk of actively growing mycelium from a mono conidial PDA culture was placed in the center of petri dishes containing PDA. Sets of five plates were main-
tained at six different temperatures: 0, 10, 15, 22, 27, and 32 C. Radial growth was measured after 24 hr.

Fungicidal control experiments in the field.—A field of heavily diseased Rubel blueberry bushes at West Olive, Michigan were sawed off just above the soil surface in a bush rejuvenation test, and the resulting new growth was sprayed with two fungicides at two rates and two timing

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**Fig. 1.** A typical elongated, flattened canker, caused by \textit{Phomopsis vaccinii} on a highbush blueberry stem. Raised pycnidia are indicated by arrow.
schedules. Difolatan 0.49 kg/liter (4 lbs/gal) flowable (captafol) \([\text{cis-N-}((1,1,2,2\text{-tetrachloroethoxy})\text{thio})-4\text{cyclohexene-1,2-dicarboximide}]\) was used at 4.7 liter/ha (2 qts/acre) and 9.4 liters/ha (4 qts/acre). Benlate 50% wettable powder \([\text{methyl 1-(butylcarbamoyl)-2-benzimidazolcarbamate}]\) was used at 1.08 kg/ha (1 lb/acre) and 2.16 kg/ha (2 lbs/acre) in 1,266 liters water/ha (150 gal water/acre). Both fungicides were applied with a power sprayer and a handgun at 3- and 6-wk intervals throughout the growing season from beginning of shoot growth through leaf drop in the autumn. The experiment was arranged in a randomized complete block design with five replications per treatment. Amount of infection was determined the following season by counting the number of flagged (dead) stems per bush.

**RESULTS**

**Trapping conidia from rainwater.**—Conidia from stem cankers were trapped during 1974 from late April through mid-September (Fig. 2-A); only a few conidia were collected during the first week of April. The number of trapped conidia increased to a maximum of \(44 \times 10^3/ml\) during the week of 23-30 May, (full bloom). Numbers of conidia then decreased to about 20% of maximum and no conidia were caught during two rainy periods in late July and early August. A few conidia were trapped during the period 29 August-5 September, but none was trapped thereafter. In 1975, conidia were first caught during the period 25 April-2 May (blossom bud swell stage) (Fig 2-B). A maximum catch of \(8.7 \times 10^3\) conidia/ml occurred during 23-30 May (full bloom). Numbers of conidia declined for a few weeks after petal fall. Many spores were caught during a rainy 5-wk period beginning 11 July, and the largest number was caught during the period of 16-22 August. No conidia were caught after this time, even though there was considerable rain after that date. Spores from leaf pycnidia were trapped only during the week of 16-22 August when \(5.9 \times 10^3\) conidia/ml were trapped. No conidia were trapped after 22 August, even though rainfall was abundant. The total number of conidia caught in 1974 far exceeded that of 1975.

**Field exposure of healthy bushes for intervals to determine natural infection periods.**—Healthy potted blueberry plants subjected to natural field inoculum of *P. vaccinii* during 1973 or 1974 remained free of Phomopsis canker symptoms and *P. vaccinii* was not isolated from randomly selected apparently healthy stem areas.

**Mycelial inoculation studies.**—All stems wounded prior to inoculation with *P. vaccinii* mycelium were infected; wilted and dead branches were observed 1 yr after inoculation and *P. vaccinii* was consistently isolated from wilted stem sections. Nonwounded, inoculated plants remained healthy except for one canker which developed as a result of inoculation in June of 1974.

**Determination of the extent of above- and below-ground crown infection.**—Isolations from three of 16 below-ground crown samples taken from heavily-infected bushes resulted in *P. vaccinii* growth on PDA-S. Ten of 16 above-ground crown samples were infected with *P. vaccinii* as confirmed by isolation of the pathogen.

**Effect of environmental stress on *P. vaccinii* infection.**—All plants subjected to freezing conditions suffered severe injury of succulent shoots and leaves. *Phomopsis vaccinii* infections occurred on two of 20 (10%) freeze-injured inoculated plants. None of the non-frozen, inoculated control plants was infected. In contrast, comparable plants which were drought-stressed prior to inoculation were not infected with *P. vaccinii*.

**Fig. 2-(A, B).** Numbers of rain-dispersed conidia of *Phomopsis vaccinii* caught from infected Earlblue highbush blueberry stems at West Olive, Michigan in (A) 1974 and (B) 1975.
Effect of temperature, relative humidity, and free water on conidial germination.—After 48 hr of incubation in free water at 10, 21, and 27°C, the respective percentage germination and germ tube lengths were as follows: 25.7% and 11.3 μm, 89.0% and 120.3 μm, and 87.3% and 57.3 μm, respectively. Conidia incubated in atmospheres of 98% or 100% RH did not germinate.

After 24 hr of incubation on PDA, the colony areas averaged 1,040 mm² at 27°C, and about 900 mm² at 22°C. Growth was less than 200 mm² after 60 hr of incubation at 10 and 15°C. There was no growth on plates incubated at 0 and 32°C. Mean monthly temperatures recorded at the field site were as follows for 1974 and 1975 seasons, respectively: April-8.9 and 5.4; May-12.8 and 16.5; June-18.3 and 20.3; July-22.8 and 22.4; August-21.5 and 22.3; September-15.8, and 14.9; and October-10.5, and 12.4°C.

Fungicidal control experiments in the field.—Heavily diseased bushes which were cut off to 2.4 cm (1-inch) stumps, and sprayed with either captan or benomyl had 36% to 58% fewer wilted dead stems than nontreated plants in 1974, the year following the applications [LSD (P = 0.01) = 2.72] (Table 1). There were no significant differences in control between the two fungicides or the different rates.

DISCUSSION

Conidia of P. vaccinii were trapped from stem cankers first during late April or early May, but only during rains.

TABLE I. Fungicide control of Phomopsis canker of highbush blueberry (cultivar Rubel), West Olive, Michigan, 1974

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Formulated chemical (Rate Per hectare)</th>
<th>Application interval (1973) (wk)</th>
<th>Wilted 1-yr-old stems per bush (1974)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.7 liters 4F</td>
<td>3</td>
<td>9.0*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>4.8*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.4 liters</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>3.8*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.16 kg</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>4.8*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>4.2*</td>
</tr>
</tbody>
</table>

*Approximately 1.266 liters/ha (150 gal/acre) of spray was applied with a power sprayer using a handgrip. English unit rates per acre were 2 and 4 quarts and 1 and 2 lb, respectively.

bThree- and 6-wk interval sprays were applied to bushes cut off to 2.4 cm (1 inch) stumps at the onset of new growth in June.

1974 was the only year all treatments were evaluated for wilt symptoms on 9 September 1974.

LITERATURE CITED

8. WILCOX, M. S. 1940. Diasporhe vaccinii, the ascigerous stage of Phomopsis, causing a twig blight of blueberry. Phytopathology 30:441-443.