Epidemiology and Chemical Control of Godronia (Fusicoccum) Canker of Highbush Blueberry

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ABSTRACT

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A Burkard volumetric spore trap in continuous operation for two seasons in a highbush blueberry field heavily infected by *Godronia cassandrae* captured only negligible numbers of airborne conidia and ascospores of the pathogen. Rain-dispersed conidia were trapped by means of funnels attached beneath cankers and attached via tubing to jugs for collection. Conidia were most abundant from May through mid-June (blossom bud swell to petal fall). The number of conidia trapped ranged up to 1.21×10^5 conidia/ml of trapped rain water. Conidia were fewer in number from June through September and October (late leaf fall) than during April and May. The frequency of natural field infection was greatest from late April through June. Potted bushes inoculated with conidia also had the highest numbers of infections resulting from the April to June inoculation dates. Wounding was not required for infection since nonwounded inoculated plants consistently were infected during the growing season. Bushes exposed to ascospore inocula failed to become infected. In vitro germination of conidia was favored by temperatures of 22 and 30 C. Mycelial growth and infection of blueberry bushes was favored by temperatures of 10 C to 22 C. In fungicide field plot evaluations, captafol (Difolatan®) significantly reduced the numbers of cankers at one location by 82 to 95% [LSD ($P = 0.01$) = 0.12] and at another location by 52 to 65% [LSD ($P = 0.01$) = 0.21].

Additional key words: *Vaccinium corymbosum*, fungicides.

Fusicoccum putrefaciens Shear, the imperfect stage of *Godronia cassandrae* Peck, is one of the main limiting factors of highbush blueberry (*Vaccinium corymbosum* L.) production in the upper peninsula of Michigan and it also causes a serious disease of highbush blueberry in Michigan's lower peninsula. The fungus first was associated with the genus *Vaccinium* by Shear (6). Characteristic concentric ring-shaped (bullseye) cankers appear on stems during the spring (Fig. 1). Death and flagging of individual branches during droughty periods of summer are the most visible expression of the disease (8, 9). Up to 40% of newly planted, 2-yr-old stock were killed in Nova Scotia, and older plants had up to 25% of their stems destroyed (2). According to observations in British Columbia, infection occurred sometime after June (5). Creelman (2) determined that the fungus overwintered as mycelium in cankers on living stems and in the crowns of infected bushes. Ascospore inoculations failed to cause infection. In Massachusetts, sporulating pycnidia were observed from March through mid-July (10). Conidia typical of *F. putrefaciens* were trapped in Michigan with a Hirst spore trap at various time intervals from July through September (1). The only recommended method of control has been field sanitation, whereby dead diseased branches were pruned out and burned. Due to the difficulty of locating all diseased branches, some of which may be deep within the thick crowns, control by sanitation has not been successful. Data concerning spore liberation, natural infection periods,and methods for effective control have been incomplete (2, 10). This report deals with the epidemiology, etiology, and chemical control measures for Fusicoccum canker of highbush blueberry.

MATERIALS AND METHODS

Trapping air-dispersed spores in the field.—Air-borne conidia and ascospore release was determined by the use of a Burkard volumetric seven-day recording spore trap (Burkard Scientific Sales Ltd., Rickmansworth, Herts., England) drawing 8 liters of air per min. Spore tapes were changed weekly and were cut into lengths of 48 mm which is equivalent to 24 hr of spore trapping. Tape sections were floated with trapping surface down in a petri dish of 1% aqueous Safranin O stain for 5 min. The tape sections then were rinsed with distilled water and scanned with a compound microscope at ×400 magnification with wide-angle oculars. Conidia and ascospores were easily distinguishable due to their peculiar morphology. Reference standards were made by applying both types of spores to pieces of trap tape. Periods and amounts of

rainfall were measured with a 7-day recording rain gauge (Weather Measure Corp., Sacramento, CA 95841). Relative humidity and temperature were measured with a sheltered 7-day recording hygrothermograph (Bendix Co. Inc., Baltimore, MD 21204). All instruments were placed about 1 m above ground.

Trapping water-dispersed spores in the field.—Spores dispersed by rainfall were trapped below active cankers with a plastic funnel attached to an infected stem. The funnel was attached to a length of Tygon tubing which connected the funnel to a 3.78-l (1-gal) plastic container (3). Containers were changed at weekly intervals and the number of spores collected were counted on a Levy-Hausser hemacytometer.

The traps were in continuous operation from late April through mid November of 1974 and 1975 in a heavily affected field in Ludington, Michigan. The field, which was planted with cultivars Jersey and Rubel highbush blueberry, had a long history of Fusicoccum canker.

Healthy 'trap' plants used to measure periods of infection in the field.—Two-yr-old potted Jersey blueberry bushes were placed beneath heavily diseased bushes in the field to determine periods of natural infection. Plants were left for periods of 1 mo after which they were returned to East Lansing and kept in isolation from sources of inoculum under field conditions. Nonexposed control plants also were kept in isolation. The following year, the amount of infection which had occurred was assessed by isolation from surface disinfested (0.5% NaOCl) stem sections showing cankers or suspected infections that were plated onto potato-dextrose agar (PDA) amended with 100 μg/ml (100 ppm) streptomycin sulfate (PDA-S). Resultant growth of *F. putrefaciens* was proof of infection. The amount of infection was expressed as the number of cankers per stem.

Artificial inoculation of healthy bushes with conidia.—Conidia from monoconidial cultures of *F. putrefaciens* grown on PDA were harvested by flooding petri dish cultures with sterile glass-distilled water. The spore suspension was filtered through cheesecloth, adjusted to 5×10^5 to 1×10^6 conidia/ml and sprayed onto healthy 2-yr-old Jersey bushes with a DeVilbiss No. 15 atomizer powered by compressed air. Leaves and branches were wetted thoroughly with the inoculum suspension before the plants were placed in a mist chamber for a period of 48 hr at 20 to 25 C. Plants were isolated from any possible sources of inoculum for 1 yr, after which the amount of infection was determined by isolation from infected areas onto PDA-S. Five plants were inoculated each month and one noninoculated control plant was included.

Mycelial inoculation of wounded and nonwounded healthy bushes.—Host injury as a prerequisite for infection by *F. putrefaciens* was studied by inoculating wounded and nonwounded Jersey blueberry stems with 5-mm square blocks of mycelium and agar from monoconidial PDA cultures of the fungus. Inoculation zones were swabbed with a 0.5% NaOCl solution and rinsed with sterile distilled water. Wounding was accomplished by puncturing the epidermis 25 times in a circular zone with a sterile dissecting needle. Inoculated zones were wrapped with moistened cheesecloth and aluminum foil and kept under mist for 48 hr at 20 to 25 C.

Fig. 1-(A, B). **A)** Typical symptoms of Fusicoccum canker of highbush blueberry. Cankers tend to have a concentric configuration (arrow). **B)** A Fusicoccum canker with slightly raised pycnidia near the center (arrow).

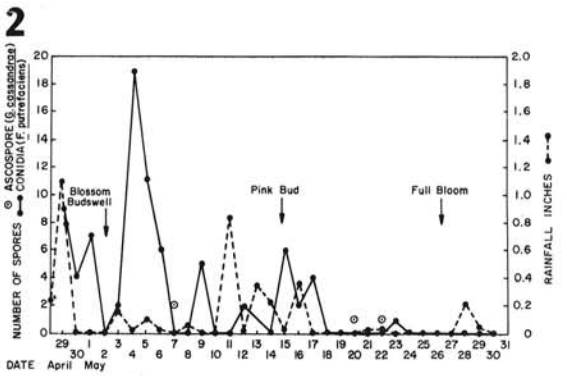

Fig. 2. The number of airborne spores trapped with a Burkard volumetric recording spore trap in a Jersey highbush blueberry field infected by *Fusicoccum putrefaciens*, Ludington, Michigan in 1974.

Evaluations of the amount of disease were made the following year as described previously.

Ascospores as a possible infection source.—The effectiveness of ascospores as inoculum was tested by suspending dead blueberry branches with attached apothecia of *G. cassandrae* over potted 2-yr-old Jersey bushes at East Lansing. Groups of five bushes placed monthly beneath the apothecia were removed to isolation under field conditions following a 1-mo exposure to the ascospore inoculum. Newly collected branches with apothecia were added monthly. The following year, isolations were made from healthy and possible infected stem areas onto PDA-S.

Conidial germination in free water and in high relative humidity.—Freshly collected pycnidia of *F. putrefaciens* were placed into drops of sterile, glass-distilled water and teased apart with a flamed dissecting needle. The resulting spore suspension was placed either into glass depression slides or applied to slivers of glass cover slips. The depression slides were held in closed petri dishes to prevent evaporation. Cover slip slivers with the conidial suspension were held in place with molding clay in the center well of a reaction vessel of a Gilson Differential Respirometer (Gilson Medical Electronics, Middleton, WI 53706). A saturated solution of K_2SO_4 maintained a relative humidity (RH) of 98% and was compared with water (100% RH) in flasks sealed with Parafilm (7). Groups of four flasks and four depression slides held at three different temperatures (10, 21, 30 C) were used to determine the amount of germination and germ tube elongation. One hundred conidia were selected at random to determine the percentage germination. The germ tubes of 10 randomly selected conidia were measured with an ocular micrometer to determine the average germ tube length.

Effect of temperature upon mycelial growth.—To measure the effect of temperature on mycelial growth of *F. putrefaciens*, 7-mm-diam disks of mycelium from the edge of actively growing monoconidial cultures were placed in the center of petri dishes of PDA. The plates were maintained at six different temperatures (0, 10, 15, 22, 27, and 30 C). Measurements of growth were recorded at 8-hr intervals from groups of five plates at each temperature.

Effect of temperature upon infection of stems by conidia.—Two-yr-old potted Jersey blueberry bushes were inoculated by spraying them with a conidial suspension as previously described, before they were enclosed in moistened plastic bags and placed in growth chambers at three different temperatures (10, 22, and 30 C) for a period of 48 hr. Following the 48-hr exposure, they were placed in isolation away from inoculum sources in the field. Test plants were evaluated for infection the following season as described previously.

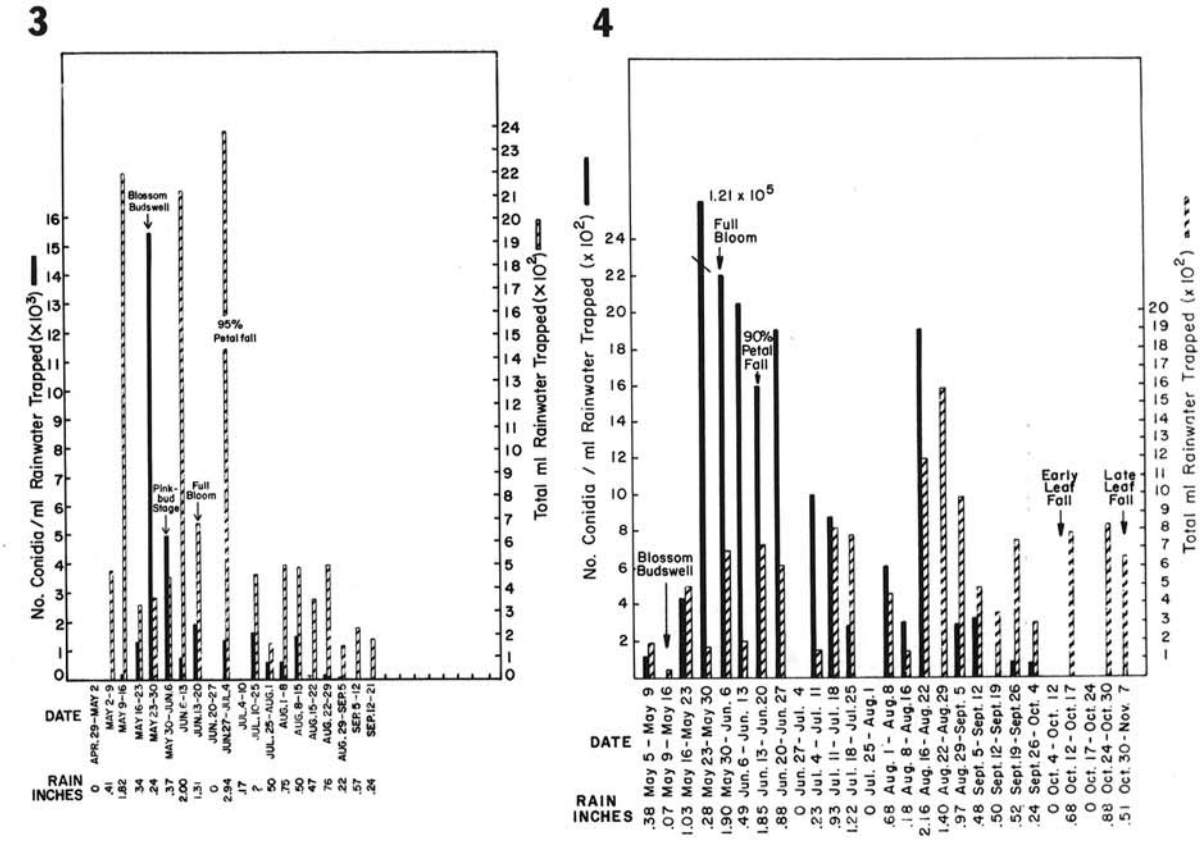

Fig. 3-4. 3) The number of rain-dispersed conidia of *Fusicoccum putrefaciens* caught from cankered Jersey highbush blueberry stems, Ludington, Michigan in 1974. 4) The number of rain dispersed conidia of *F. putrefaciens* caught from cankered Jersey highbush blueberry stems at Ludington, Michigan in 1975.

Field-testing protectant sprays of captafol for canker control.—A randomized complete block design with naturally infected bushes at two locations, Ludington and West Olive, Michigan, was used to evaluate the effectiveness of captafol [Difolatan® 0.45 kg/liter (4 lbs/gal) flowable] [cis-N-((1,1,2,2-tetrachloroethyl)-thio)-4-cyclohexene-1,2-dicarboximide] sprays under field conditions. Our previous in vitro tests (Parker and Ramsdell, *unpublished*) using fungicide-amended agar indicated that captafol was highly active against *F. putrefaciens*. At the Ludington site, five replications per treatment and five bushes per replication were employed. At West Olive, four replications per treatment and five bushes per replication were used. Three different rates of Difolatan 4F were tested at 4- and 6-wk intervals during the growing season. Rates tested were 4.7 liters/ha (2 quarts/acre), 9.4 liters/ha (4 quarts/acre), and 18.8 liters/ha (8 quarts/acre). A hand-held knapsack sprayer was used for all applications. A total of 1,170 liters/ha (125 gal spray/acre) was used at the Ludington plot and 535 liters/ha (62.5 gal spray/acre) was used at the West Olive plot.

RESULTS

Trapping of air- and water-borne spores.—Trapping of airborne spores over two seasons resulted in a negligible number of conidia and ascospores being caught. During 1974 (Fig. 2) only 100 conidia and three ascospores were trapped. All spores were caught before mid-June. There was no apparent correlation between periods or duration of rainfall and catches of conidia or ascospores from the air. However, during the two seasons of trapping for rain-dispersed spores, high numbers of conidia were present in collected rain water the 1st or 2nd wk of May (bud swell) through early September or October (late leaf fall) (Fig. 3, 4). During 1974, (Fig. 3) the first conidia were trapped the 2nd wk of May. Spore catches increased to a peak of 1.55 $\times 10^4$ conidia/ml at blossom bud-swell stage (23-30 May). As the season progressed, numbers of conidia decreased. A few conidia were caught in September, but only trace

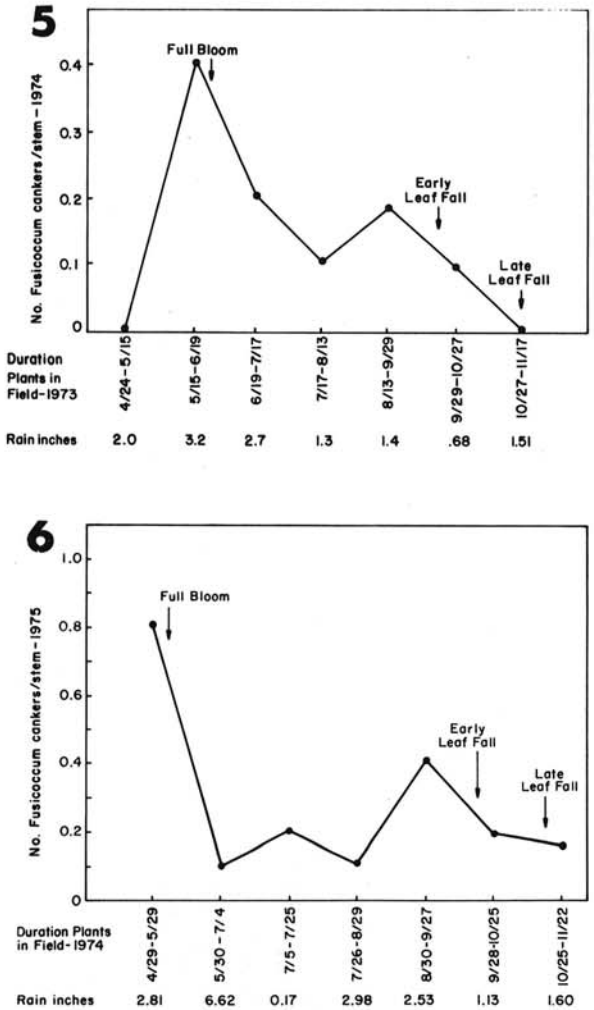

Fig. 5-6. 5) The number of Fusicoccum cankers resulting from monthly exposure of potted healthy Jersey highbush bushes to natural field inoculum at Fruitport, Michigan in 1973. 6) The number of Fusicoccum cankers resulting from monthly exposure of potted healthy Jersey blueberry bushes to natural field inoculum at Ludington, Michigan in 1974.

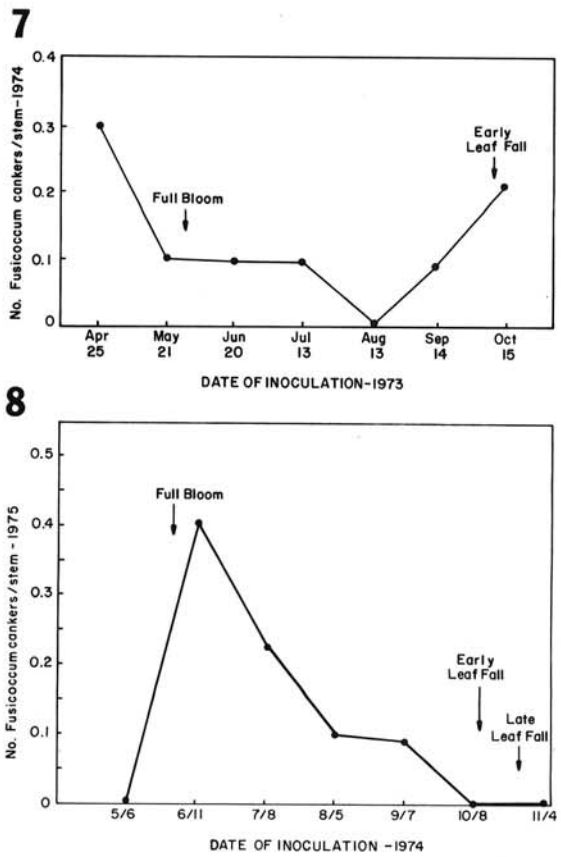

Fig. 7-8. 7) The number of Fusicoccum cankers resulting from monthly conidial inoculations of potted healthy Jersey highbush blueberry bushes at East Lansing, Michigan in 1973. **8)** The number of Fusicoccum cankers resulting from monthly conidial inoculations of potted healthy Jersey highbush blueberry bushes at East Lansing, Michigan in 1974.

numbers in November. Conidia were caught first during the 1975 season from 5-9 May, a week before blossom bud-swell (Fig. 4). The highest number trapped was 1.21 $\times 10^5$ spores/ml during 23-30 May, 1 wk before full bloom. The spore numbers decreased during June. No spores were trapped during several rainless periods, and after mid-August, despite the occurrence of normally heavy rainfall only a few conidia were caught.

Periods of infection.—Natural field infection corresponded closely with times that rain-dispersed conidia were caught, although only one year's infection can be compared directly. The maximum amount of infection occurred from the pre-bloom to petal-fall stages (late April and mid-June) (Fig. 5,6). The amount of infection declined after the initial late-spring peak. However, an increase in infection occurred from mid-August until the end of September during 1973 and an increase occurred during September 1974, just prior to the onset of leaf fall.

Periods of infection resulting from artificial inoculations with conidia.—Plants inoculated by spraying with a conidial suspension in 1973 had the highest amount (0.30 cankers per stem) of infection that resulted from the inoculation on 25 April at bud swell stage (Fig. 7). Fewer infections resulted from inoculations in May, June, and July. The reason for the lack of infection resulting from the 13 August inoculation is not known. The mid-October inoculation at early leaf fall resulted in 0.22 cankers per stem. In 1974, plants inoculated with conidia during June had the highest disease incidence (0.40 cankers per stem) (Fig. 8). For some unknown reason, inoculations in May did not result in any infections. Infections continued to occur but in lesser numbers, through the September inoculation date, prior to early leaf fall (0.10 cankers per stem). No infections resulted from inoculations in October or November.

TABLE 1. Rate and timing evaluation of Difolatan 4F for control of Fusicoccum canker of highbush blueberry (cultivar Jersey) at Ludington, Michigan in 1974[a]

Difolatan 4F rate[b,c] (liters/hectare)	Timing interval (wk)	Fusicoccum cankers (Mean no. cankers/stem, on 20 June 1975)
4.7	4	0.09
	6	0.21
9.4	4	0.12
	6	0.08
18.8	4	0.14
	6	0.06
0 (Control)		1.23
LSD ($P = 0.01$)		0.12

[a]A randomized complete block design was used with five replications per treatment.

[b]A knapsack sprayer was used for all spray applications. A total of 1,270 liters/hectare (125 gal/acre) of water was used per application. English unit rates/acre were 2, 4, and 8 quarts, respectively.

[c]Four-wk treatments were applied at 4-wk intervals on 2 May, 30 May, 27 June, 25 July, 29 August, 26 September, 12 October, and 22 November. Six-wk treatments were made on 2 May, 13 June, 25 July, 12 September, and 17 October. LSD ($P = 0.01$) = 0.12.

Lack of infection by ascospores.—Bushes which were exposed for 1-mo periods beneath apothecia of *G. cassandrae* did not become infected. No cankers developed and isolations from randomly selected apparently healthy stem portions proved to be negative for the presence of *F. putrefaciens*.

Wounding not a prerequisite for infection.—Plants which had been wounded prior to inoculation were consistently cankered, and infections resulted from all inoculation dates. Sporulating pycnidia were sometimes observed near the center of typical cankers, and *F. putrefaciens* was isolated from these cankers and other areas with atypical lesions. Nonwounded, inoculated plants also were consistently infected. Noninoculated plants were free of cankers and the pathogen could not be isolated.

Germination of conidia in free water.—Free water was required for conidial germination. The best germination and germ tube elongation after 40 hr of incubation was 70% with an average germ tube length of 42.2 μm and 63% with an average length of 17.0 μm, at 21 and 30 C, respectively. Only 0.3% germinated at 10 C and there was no measurable germ tube elongation after 40 hr of incubation. A spore with a germ tube as long as the diameter of the spore was considered to be germinated.

Effect of temperature upon mycelial growth.—*Fusicoccum putrefaciens* grew on PDA plates at temperatures of 10, 15, and 22 C, but no growth occurred at 0, 27, or 32 C. Cultures maintained at 10 and 15 C attained expansions of 650 and 750 mm^2, respectively, after 132 hr. Twenty-two C was the optimum and cultures at 22 C had grown to the edge of the petri dishes after 132 hr (>2,000 mm^2).

Bushes inoculated with conidia and exposed to a temperature of 10 C and free water for 48 hr in a growth chamber, eventually developed an average of 0.25 cankers/stem. A slightly lower amount of infection resulted from plants exposed to 22 C (0.20 cankers/stem). Incubations at 30 C resulted in no infection. During 1974, monthly mean field temperatures present at the Ludington, Michigan site, where healthy exposed plant infections occurred, were as follows: May, 11.2 C; June, 17.3 C; July, 21.1 C; August, 20.0 C; September, 14.7 C; October, 8.8 C; and November, 4.3 C.

Protectant fungicide control with captafol.—In fungicide field plot evaluations applied throughout the susceptibility period to infection at 4- and 6-wk intervals at Ludington, Michigan, captafol significantly reduced the numbers of cankers by 82 to 95% [LSD ($P = 0.01$) = 0.12] over untreated controls (Table 1). Reductions at the West Olive, Michigan location ranged from 52 to 65% [LSD ($P = 0.01$) = 0.21].

DISCUSSION

If wind dispersal were a major factor in the disease cycle, one would expect to observe substantial numbers of airborne conidia. However, only relatively few conidia of *F. putrefaciens* were present in the air. Rain dispersal of *F. putrefaciens* conidia is of much more importance in the

disease cycle. The highest incidence of infection of exposed plants corresponded with periods during May when rains occurred and conidia were abundant. During drier months (June and July), total conidial numbers were lower. Despite abundant autumn rains, pycnidia produced fewer conidia earlier in the season. The periods of *Fusicoccum* conidia liberation in Michigan are similar to those reported from Massachusetts (10), but different from Nova Scotia (4) and the Pacific Northwest (5) where pycnidia were not formed until late July.

Because of the paucity of ascospores, their importance in epidemiology is questionable. The presence of heavy infection in areas where apothecia were not present (Fruitport, Michigan), also indicates that ascospores are relatively unimportant. Our experiments support the work of Creelman (2), who failed to get infection using ascospores as inoculum.

The conidia of *F. putrefaciens* germinated well at temperatures from 21 to 30 C. Inoculated bushes incubated at 10 and 22 C for 48 hr in free water became infected. The fairly high level of infection obtained at 10 C in inoculation studies contrasts with the low level of germination of conidia in distilled water at 10 C. Our previous work (Parker and Ramsdell, *unpublished*) showing that leaf decoctions stimulate germination of conidia may cause this difference. Similar temperatures occurred in the field during times when inoculum was present in late spring through autumn, the observed periods of natural field infection.

Experimental germination, growth, and infection studies with the pathogen, have indicated that infection is possible over the time span from bud break in spring through leaf fall in the autumn. Since infection occurs over an extended period of 7 mo, an effective protectant fungicide residue must be present on susceptible tissue at all rainy times during that period. A sharp decrease in the number of cankers resulted from season-long protectant fungicide (captafol) treatments applied at 4- or 6-wk intervals. By tailoring a fungicide protectant program around periods of conidial release and periods of natural infection, significant reductions in the amount of disease have been achieved.

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