Epidemiology of Cytospora Canker Caused in Colorado Blue Spruce by *Valsa kunzei*

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ABSTRACT

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Epidemiological studies of Cytospora canker of Colorado blue spruce (caused by *Valsa kunzei* [imperfect stage is *Cytospora kunzei*]) were conducted in 1978 and 1979. Laboratory studies revealed 27 C to be the optimum temperature for conidial germination, germ tube elongation, and linear growth of mycelium. Spore trapping studies were carried out at two locations in Michigan during the 2-yr study period. Ascospores and conidia were both waterborne and airborne. Conidia were detected during periods of wetness throughout the season, but the highest number occurred in the spring. The number of waterborne and airborne conidia always exceeded the number of waterborne and airborne ascospores. The latter were common only in the spring. Discharge of both spore types occurred during both daylight and darkness. Dispersal of airborne ascospores and conidia throughout the season was correlated with mean daily temperature, and hours of 100% relative humidity, leaf wetness, and rainfall. Conidia and ascospores isolated throughout the year were viable.

Additional key words: C. kunzei, Douglas fir.

Cytospora canker caused by *Valsa kunzei* Fr. (imperfect stage, *Cytospora kunzei* Sacc.) is a serious disease of Colorado blue spruce (*Picea pungens* Engelm.) (2,3,10,14–16) and other related conifers (4,5,18) in the north temperate zones in the United States. The disease also caused considerable damage to white spruce (*P. glauca* (Moench) Voss) and Norway spruce (*P. abies* (L.) Karst.) plantations in Canada (7,11). The disease usually starts on a lower branch and spreads laterally upward. Blue spruce trees are only rarely killed by the disease, but continued removal of cankered branches destroys the symmetry and consequently the aesthetic value of the tree. However, tree mortality has been reported on Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) (5,18) and Engelmann spruce (*Picea engelmannii* Parry) (4).

Cytospora kunzei overwinters as fruiting bodies (primarily pycnidia) on bark and as vegetative mycelium in cankers. However, the complete life cycle has not been described. Epidemiological information concerning Cytospora canker of Colorado blue spruce is lacking. The literature on *Cytospora* spp. suggests that conidia are released in mass in response to wetting (6,9). Luepschen and Rohrbach (9) indicated that conidia of *Cytospora* sp. were dispersed by windblown rain and localized splashing. Saito (13) reported two mechanisms for ascospore discharge by *Valsa ceratosperma*, the causal agent of Japanese apple canker. Under continuous wet conditions, masses of ascospores oozed from the perithecium the same way that conidia were released. Forcible ascospore discharge was also demonstrated and free water was required to initiate the release. Recently, Bertrand and English (1), found both ascospores and conidia of *V. leucostoma* to be airborne and waterborne.

The purposes of this study were to monitor dispersal of ascospores and conidia of *Valsa kunzei*, to determine the environmental conditions necessary for these processes to occur in

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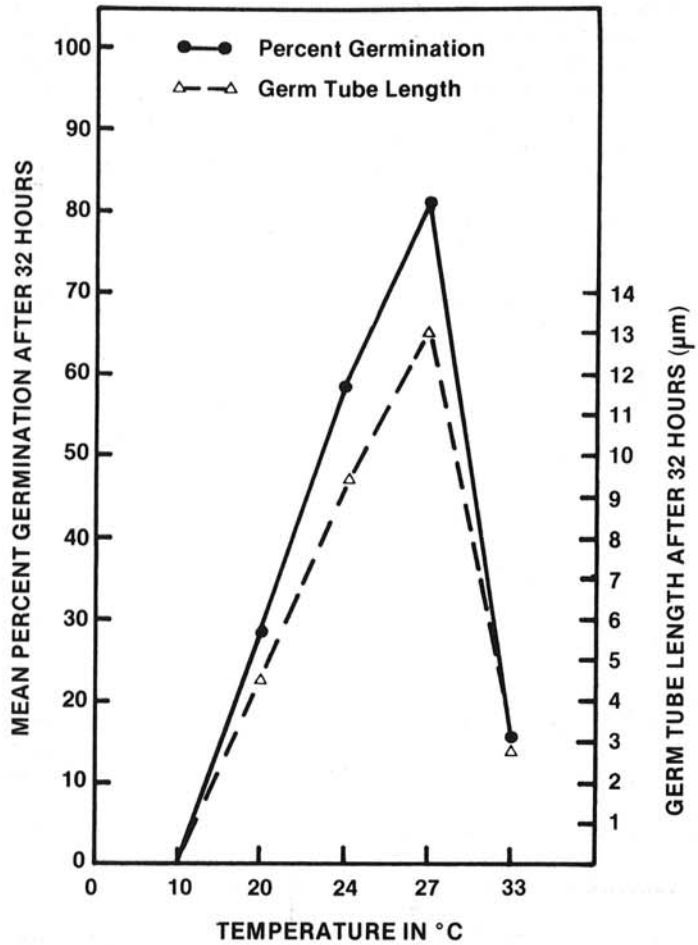

Fig. 1. Conidial germination of *Cytospora kunzei* on double-strength medium B.

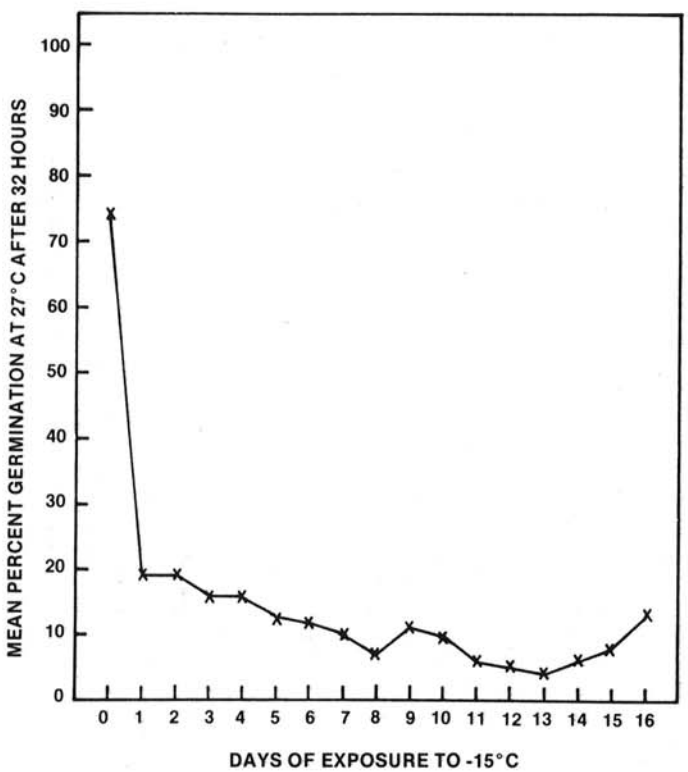

Fig. 2. Effect of preliminary exposure to low temperature on conidial germination of *Cytospora kunzei*.

the field, and to determine the optimum temperature requirements for conidial germination and linear mycelial growth in the laboratory.

MATERIALS AND METHODS

Effect of temperature on conidial germination. Ten blue spruce twigs with cankers were collected for each spore isolation period (replicate) and soaked in a 2.62% sodium hypochlorite solution for 5–10 min with continuous agitation. The twigs were washed in running deionized water for 5 min, soaked submerged in deionized water for 10–30 min, wrapped in a moistened paper towel and held for 3–8 hr room temperature in a moist chamber. The twigs were examined under the microscope for conidial spore masses. Spore tendrils or ooze droplets were removed with a sterile needle and placed in sterile deionized water for preparation of a spore suspension.

Medium A at double strength consisted of (10 g maltose, 20 g agar, 2 g sucrose, 2 g glucose, 2 g yeast extract, and 3.5 g KNO_3 per liter of glass distilled water) was used in all conidial germination studies. Autoclaved medium was poured into 55-mm-diameter plastic petri dishes and allowed to solidify. Plates were incubated overnight at the designated temperatures before the conidia were transferred to them. Two droplets of the spore suspension (containing 2.3×10^6 conidia per milliliter of water) were applied to the culture medium and spread over the surface. Cultures were incubated at 0, 10, 20, 24, 27, and 33 C. Conidia were considered germinated when a germ tube as long as the largest conidial dimension had developed. A total of 100 conidia was counted at random every 8 hr for 40 hr and the number of germinated conidia was recorded as percent germination. Germ tube lengths of 10 randomly selected germinated conidia were measured with an ocular micrometer and an average germ tube length was calculated. These experiments were repeated three times.

To determine the effect of low temperatures on conidial germination, test tubes containing 2 ml of conidial suspension obtained as described above were exposed to −15 C. Each day for 16 days, two test tubes containing conidia were thawed at room temperature for 30 min, placed on culture media, and incubated for 32 hr at 27 C. Incubation periods longer than 32 hr resulted in thick mycelial mats being formed in which individual germ tubes were no longer detectable. Percent germination was determined as explained above. These experiments were repeated two times.

Effect of temperature on linear growth of mycelium. Seven-millimeter-diameter disks of actively growing mycelium from monoconidial and single-ascosporic cultures were placed in the

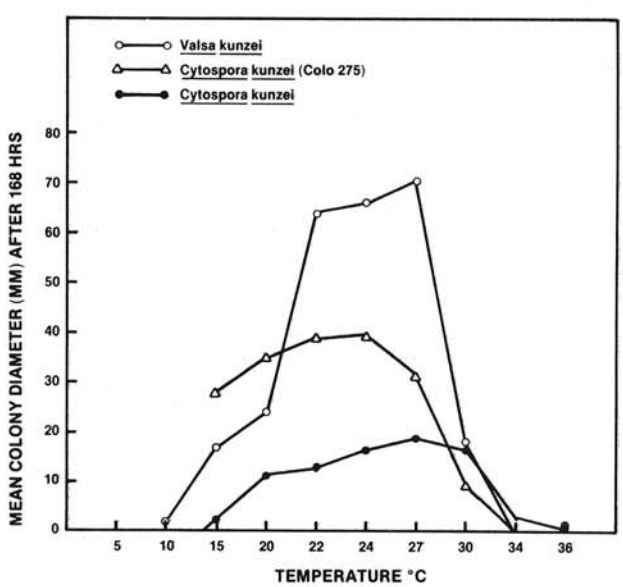

Fig. 3. Effect of temperature on mycelial growth of *Valsa kunzei* cultured on medium B.

center of 90-mm-diameter petri dishes containing medium B agar (40 g cornmeal agar, 20 g Bacto agar, 2 g glucose, 2 g sucrose, 1 g yeast extract per liter of glass distilled water) and incubated at 0, 5, 10, 15, 20, 22, 24, 27, 30, 34, and 36 C. Colony diameters in each of five replicate plates at each temperature were measured to the nearest millimeter at 24 hr intervals. A similar experiment was conducted with isolate Colo. 275 from Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and identified as *Cytospora kunzei* (5). These experiments were repeated twice.

Trapping of ascospores and conidia dispersed by rain. Ascospores and conidia of *V. kunzei* were trapped by placing plastic 13.5-cm-diameter funnels beneath active Cytospora cankers on Colorado blue and Norway spruce at Baker's Natural Wood Lot at the Michigan State University (MSU) Campus, East Lansing, and at Sharp Nursery, Albion, MI, respectively in 1978 and 1979. A length of Tygon® tubing connected the funnel to a 3.79-L (1-gallon) plastic collecting container. Containers were changed weekly. Spore trapping was initiated in either mid-March or early April and terminated at first snowfall.

The collected water from each trap was filtered through six layers of cheesecloth and mixed thoroughly on a commercial Waring® Blendor. A 25-ml aliquot from each trap was centrifuged at 7,000 *g* for 15–20 min on an IEC clinical centrifuge (Damon/IEC Division, Needham Heights, MA 02194). The resulting pellet of spores was returned to 10 ml of suspension in distilled water. This was then mixed on a Vortex mixer (Model S8220, Scientific Products, Evanston, IL 60201) for 5–10 sec and the number of spores in an aliquot was counted in a Levy-Hausser Hemacytometer AO (American Optical, Scientific Instrument Division, Buffalo, NY 14200). The Sharp Nursery trapping station also included 7-day weather-recording instruments: a hygrothermograph (Bendix Corporation, Baltimore, MD 21204), a tipping-bucket rain gauge (Weathermore Corporation, Sacramento, CA 95841), and a DeWitt leaf wetness meter (M. DeWitt Co., Hengelo, The Netherlands). The Baker's Natural Wood Lot site was not instrumented due to lack of security.

Trapping of airborne ascospores and conidia. A Burkard volumetric recording spore trap (Burkhard Scientific [Sales] Ltd., Rickmansworth, England) was used to sample the air for the presence of ascospores and conidia of *V. kunzei* from early April or March until the first snowfall during the 1978 and 1979 study periods, respectively. The trap was placed among a group of four diseased Norway spruce trees bearing both the pycnidial and perithecial stages of *V. kunzei*. The trap was set at ground level and at a distance of 1.67, 1.12, 1.07, and 1.22 m from the four trees. The spore intake orifice was about 5.08 cm above the soil surface. The vacuum pump serving the trap drew air through the trap orifice at a rate of 10 L/min. The suction fan of the trap was driven by a 12-V motor powered by a 12-V car battery. The trap allowed a continuous record of spore catches for intervals of up to 7 days. A continuous record of rainfall, temperature, relative humidity, and leaf wetness was maintained near the spore trap.

For spore counting, the tape was cut at 48-mm intervals, which was equivalent to 24 hr of spore trapping. The sections were mounted on clean microscope slides with two or three drops of water, stained with lactophenol and cotton-blue, and scanned with a Nikon microscope (Nikon, Inc., Instrument Group, Ehrenreich Photo-Optical Industries, Inc., Stewart Ave., Garden City, NY 11530) at ×400. The spore counts were recorded for hourly intervals.

Environmental factors affecting numbers of conidia and ascospores released. The Pearson's correlation coefficient and multiple regression analyses were performed on 1978 and 1979 data to determine what environmental factors correlated with spore release. The environmental parameters considered in calculating the correlation coefficients and in developing the regression equation to account for the spore release variability were: mean daily temperature, hours of 100% relative humidity, hours of rainfall, and hours of leaf wetness.

Spore isolation and spore viability. Colorado blue and Norway spruce twigs with cankers emitting copious amounts of resin were collected biweekly from either March or April until the first snowfall in late November during the study period 1977–1979. Ten randomly selected twigs were soaked in 2.62% sodium hypochlorite solution for 5–10 min with continuous agitation. The twigs were washed in running deionized water for 5 min, soaked submerged in

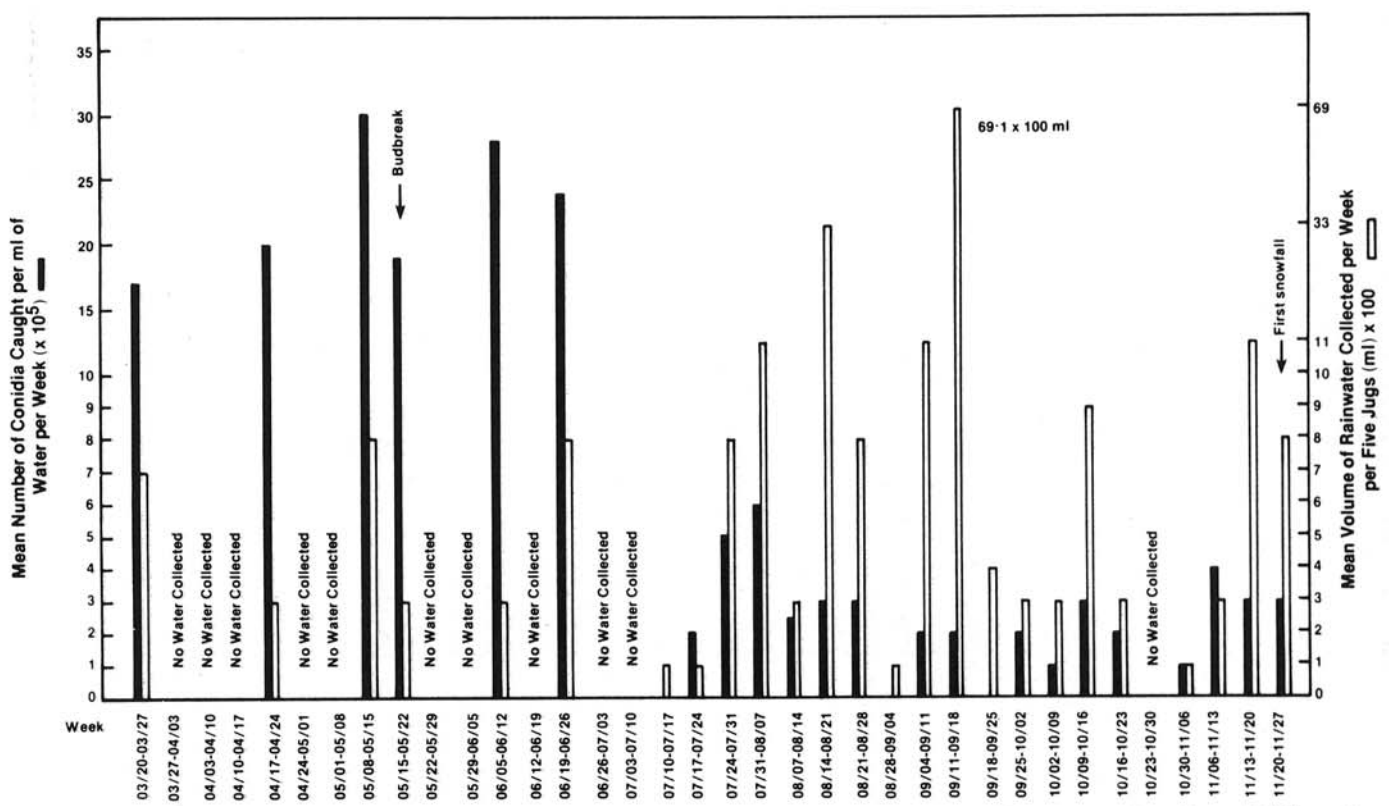

Fig. 4. Number of waterborne conidia of *Cytospora kunzei* caught by funnel traps under cankered Colorado blue spruce branches at Michigan State University, 1978.

deionized water for 30 min, wrapped in a moistened paper towel, and held overnight in a moist chamber. The twigs were examined under the microscope for perithecia, ascospores, pycnidia, and conidia. Spore viability was determined by placing spores on medium B agar in a petri dish and incubating at room temperature for 4–7 days. The resulting colonies were compared with each other and with those of several isolates obtained previously.

RESULTS

Effect of temperature on conidial germination. No germination of *C. kunzei* conidia occurred at either 0 or 10 C (Fig. 1). Slow germination occurred at 20 and 33 C. The germ tubes were extremely short at these temperatures. Germination was highest at 24 and 27 C. Maximum germination and germ tube length was reached at the optimum temperature of 27 C in 32 hr. One or two germ tubes were usually formed at one end or one on each end of most spores. Considerable hyphal branching and the formation of mycelial mats occurred at 27 C after incubation for 32 hr. There was reduction and delay in germination when conidia were exposed to various low temperatures at various time intervals and then transferred to 27 C. Preliminary exposure of conidia to −15 C followed by incubation at 27 C for 32 hr decreased germination by 49–69% (Fig. 2).

Effect of temperature on linear growth of mycelium. The two *C. kunzei* isolates did not grow at 0, 5, 10, and 36 C. Good linear growth of mycelium occurred at 27 C (Fig. 3). The two Michigan isolates of *V. kunzei* tested in this study did not grow at 0, 5, and 36 C. Maximum growth rate for both was at 27 C. Like the Michigan isolates, the Douglas fir isolate of *C. kunzei* (Colo. 275) did not grow at 0, 5, 10, and 36 C. Its maximum mycelial growth, however, occurred at 24 C.

Both conidia and ascospores gave rise to dull white or cream-colored, appressed mycelium, either roughly circular or with fans, and irregular margins. Faint concentric zones of lighter and darker shades of mycelium were also formed. The growth and appearance of Colo. 275 was nearly identical to that of the *Cytospora* and *V. kunzei* isolates from Michigan. However, the mycelium was dark brown with prominent fans.

When mycelia from monoconidial and single-ascospore cultures were grown on medium B containing steam-sterilized or propylene oxide-sterilized blue spruce twigs and incubated at room temperature, they were identical in growth and appearance. Thirty days after inoculation, the monoascospore culture developed globose grayish fruiting bodies but produced no spores. Sixty-nine days after inoculation, the globose fruiting bodies turned brown and bore allantoid conidia, 3 × 4–6 μm. Three months after inoculation, mycelia from monoconidial isolates developed pycnidia that produced typical conidia. Mycelia on medium B without sterilized twigs produced tiny fruiting bodies which did not mature, even after 90 days. No perithecia were formed on any medium.

Relative populations of waterborne ascospores and conida. Only conidia were caught at the MSU trapping site during the 2-yr study period. During 1978, waterborne conidia were counted in greater numbers throughout spring except during the week without rain or with the lowest volume of rain water collected (Fig. 4). No conidia were trapped during the winter months. A similar trend was observed in 1979.

On the other hand, both ascospores and conidia were collected in funnel traps located at the Sharp Nursery site (Fig. 5). While waterborne conidia were trapped during rain throughout the year, waterborne ascospores were present during spring and early summer. The number of waterborne conidia always exceeded the number of waterborne ascospores trapped during the 2-yr study period.

Airborne ascospores and conidia. Both ascospores and conidia were airborne. Conidia were trapped throughout the year, whereas ascospores were trapped during spring. Peak ascospores and conidia catches occurred in April, several weeks before budbreak. Again, the number of airborne conidia always exceeded the number of airborne ascospores when tabulated on an hourly

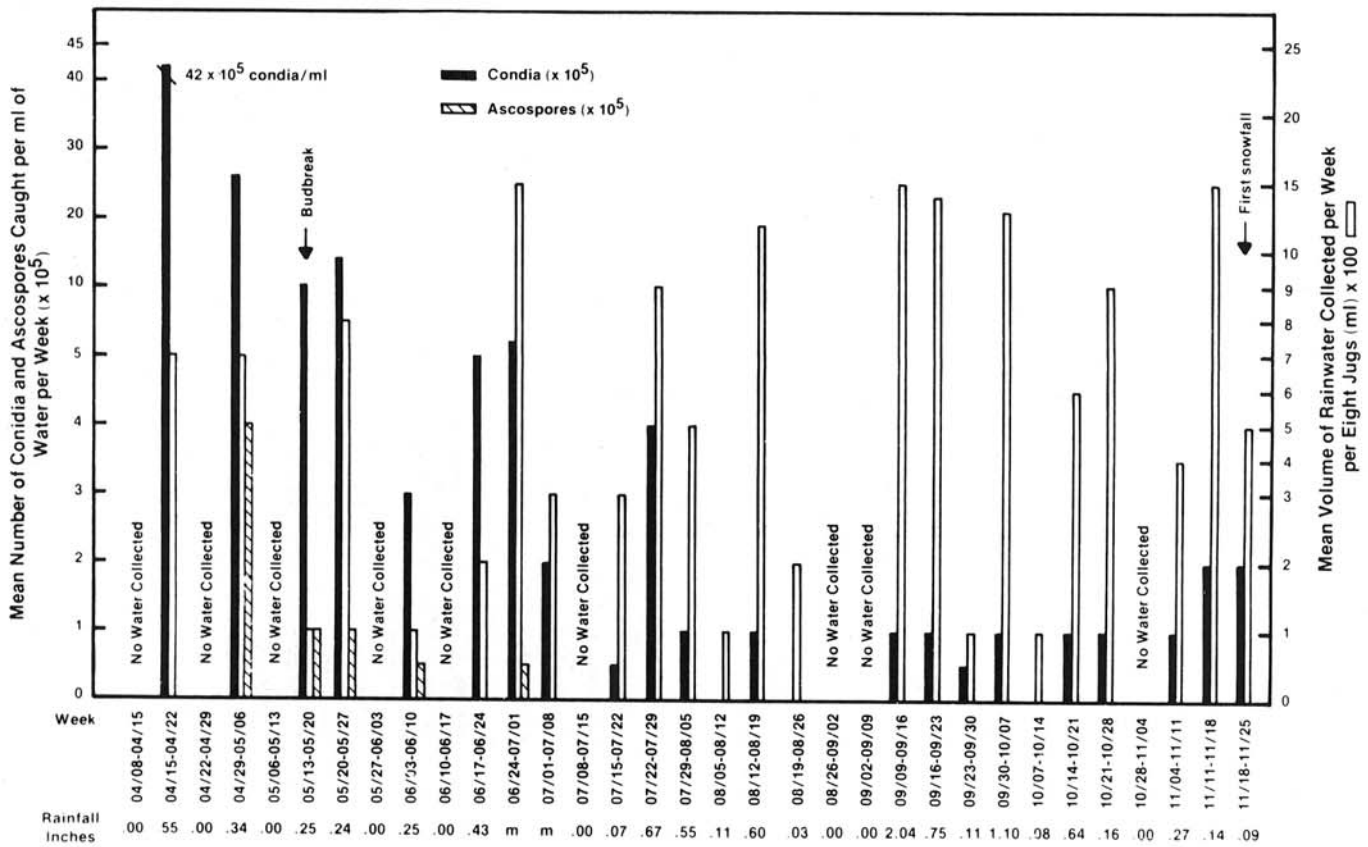

Fig. 5. Number of waterborne ascospores and conidia of *Valsa kunzei* caught by funnel traps under cankered Norway spruce branches at Albion, MI, 1978. (m = missing or unavailable data).

and/or daily basis (Figs. 6 and 7). Discharge of both spore types occurred during both daylight and darkness. Peak conidia discharge occurred at 0300 hours (Fig. 8). Secondary afternoon and evening peaks occurred at 1400 and 2000 hours, respectively. Peak ascospore discharge occurred at 1800 hours with secondary peaks at 1700, 1500, and 2000 hours (Fig. 9). Similar discharge peaks were obtained in 1979 (Figs. 10 and 11).

Environmental factors affecting numbers of ascospores and conidia. Pearson's correlation coefficients determined from the Burkard spore trap data indicated that numbers of ascospores and conidia are correlated with mean daily temperature, hours of 100% relative humidity, hours of rainfall, and hours of leaf wetness ($P = 0.001$). The coefficient of determination (R^2) in the multiple regression analysis showed that the regression equation accounted for 99.11 and 98.4% of the variation in ascospore and conidia numbers in 1978 and 1979, respectively.

Spore isolation and viability. Pycnidia with viable conidia were collected from infected blue spruce and Norway spruce branches from late March until first snowfall throughout the study period. Pycnidia isolation was greater in spring than in summer or fall. No perithecia or ascospores were collected from blue spruce at the MSU site.

Perithecia, ascospores, pycnidia, and conidia were present on cankered Norway spruce at the Albion nursery site. The number of perithecia with viable ascospores isolated was lower than that of pycnidia and conidia. No perithecia with viable ascospores were observed after 29 June during the 2-yr study period. Pycnidia and conidia by comparison were present throughout the year.

DISCUSSION

Laboratory studies with freshly harvested conidia of *C. kunzei* indicated poor spore germination at temperatures <20 C and progressively more rapid spore germination above 20 C to the optimum of 27 C. Low and very high temperatures also inhibit linear growth of mycelium. Rohrbach and Luepschen (12) reported cardinal temperatures for pycnidiospore germination of *C. leucostoma* to be 4–10, and 27–32 C and maximum germination occurred in 24 hr at 27 C. A saturated atmosphere and a carbon source were necessary for germination and germ tube production, but not for pycnidiospore swelling.

There are few reported studies of spore dispersal in *Cytospora* spp. Luepschen and Rohrbach (9) collected conidia of *C.*

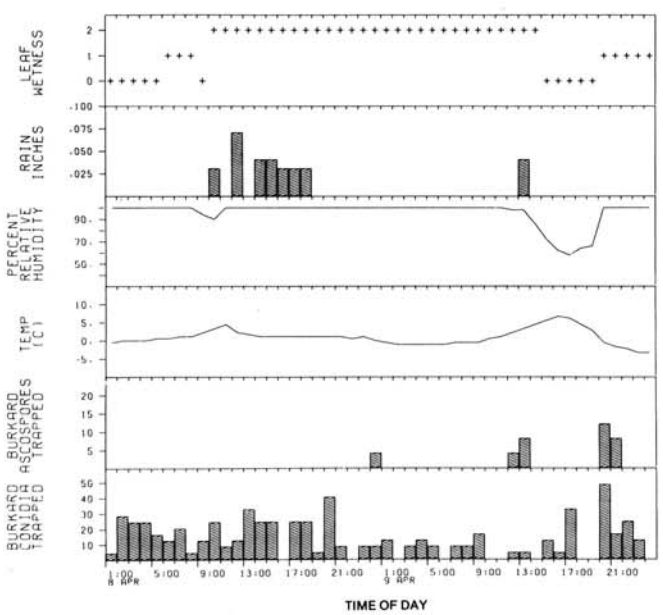

Fig. 6. Hourly total of airborne ascospores and conidia of *Valsa kunzei* compared with hourly temperature, relative humidity, rainfall, and leaf wetness from 8 and 9 April 1979, at Albion, MI. Leaf wetness: 0 = no leaf wetness; 1 = leaf wetness from dew; and 2 = leaf wetness from rain.

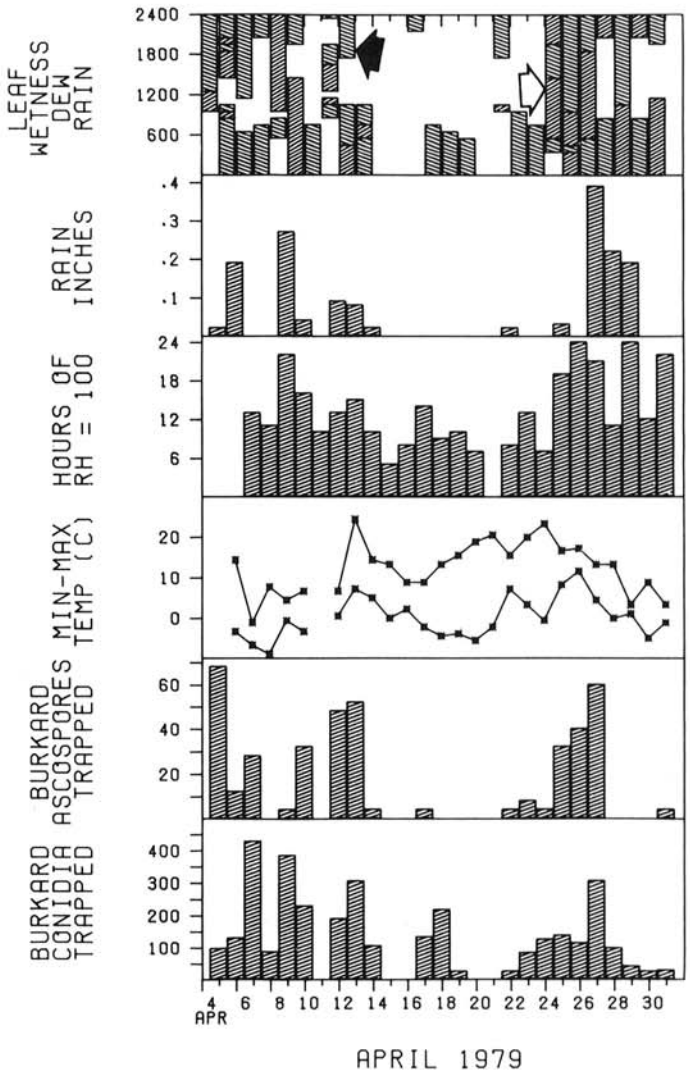

Fig. 7. Daily totals of airborne ascospores and conidia of *Valsa kunzei* compared with daily temperatures (maximum and minimum), hours of 100% relative humidity, rainfall, and leaf wetness (from dew and rain) detected from 4 to 30 April at Albion, MI. Rain (open arrow) and dew (solid arrow).

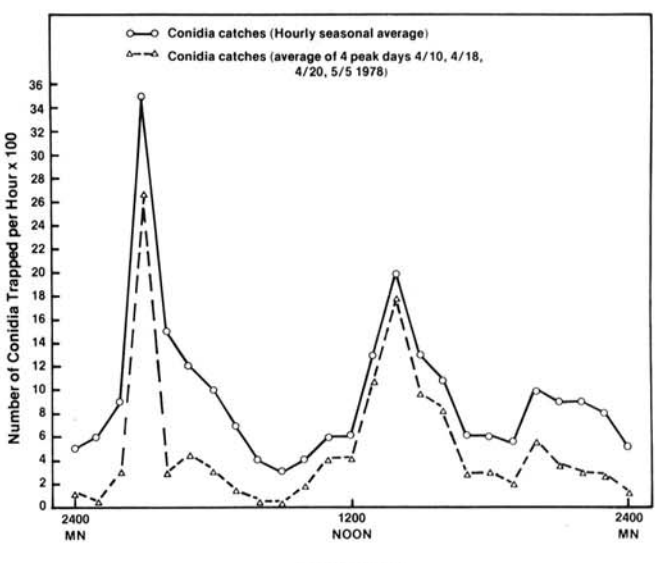

Fig. 8. Number of airborne *Cytospora kunzei* conidia trapped per hour (Burkard spore trap). Albion, MI, 1978.

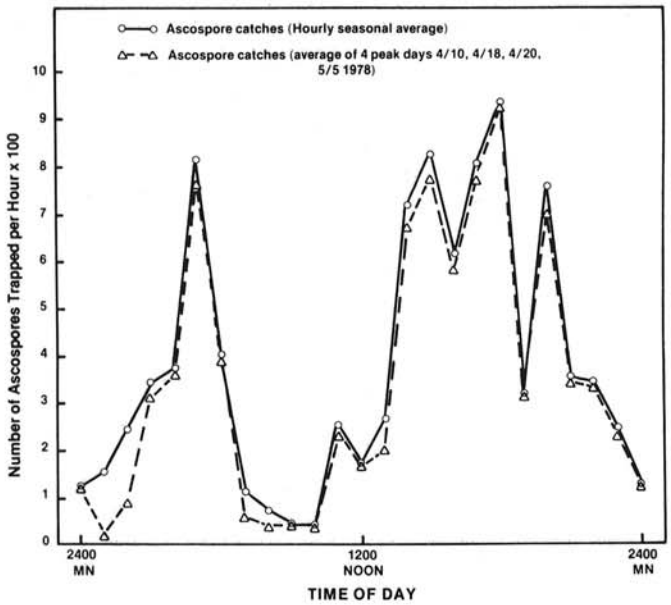

Fig. 9. Number of airborne *Valsa kunzei* ascospores trapped per hour (Burkard spore trap). Albion, MI, 1978.

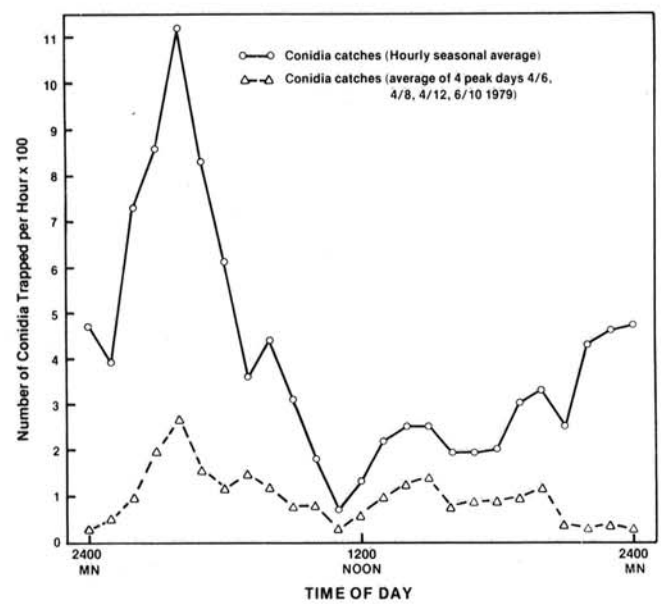

Fig. 10. Number of airborne *Cytospora kunzei* conidia trapped per hour (Burkard spore trap). Albion, MI, 1979.

leucostoma from infected peach trees by washing cankered areas with water. They reported liberation of viable spores throughout the year, with the highest counts occurring in the summer. In their study, spore viability was affected by both temperature and moisture. Bertrand and English (1) reported that conidia of *V. leucostoma* were released in all seasons whereas ascospores were released only in the spring. They concluded that conidia were the dominant spore type throughout the year and in the absence of ascospores may serve as primary inoculum.

Our data regarding liberation of conidia and ascospores of *V. kunzei* are in general agreement with studies of Bertrand and English (1). Under Michigan conditions, conidia were the dominant spore type throughout the year except during December, January, and February. On the other hand, ascospores were common only in the spring and none were trapped during the summer (after 1 July). Conidia were the most commonly observed spore type on cankered branches.

The results of this study indicated that releases of conidia and

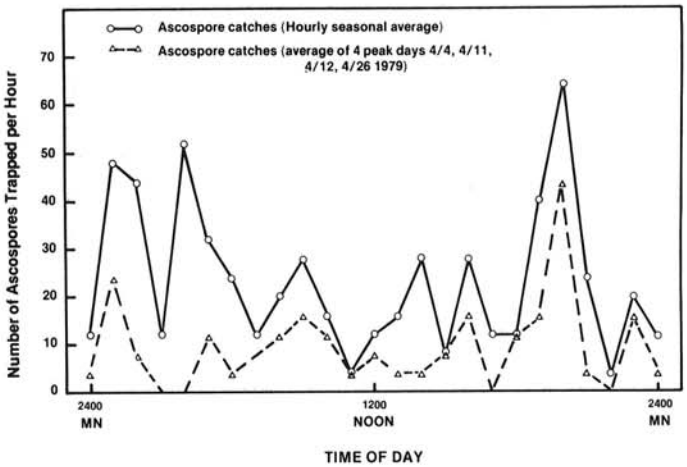

Fig. 11. Number of airborne *Valsa kunzei* ascospores trapped per hour (Burkard spore trap). Albion, MI, 1979.

ascospores were markedly influenced by the environment, but we did not determine the specific mode of ascospore discharge. They may ooze from the perithecium and be splashed up into air currents or they may be forcibly shot from the perithecium into air currents. Although pycnidia are generally assumed to release spores only in response to wetting, windblown rain could result in conidia being trapped in the Burkard spore trap. This phenomenon could be tested in future studies by continuous monitoring of rainfall, wind velocity and direction, and spore entrapment.

In nature, the numbers of perithecia and viable ascospores were small compared to the numbers of pycnidia and conidia produced. This suggests that the *Valsa* (ascosporic) stage may develop several years after a branch becomes infected. Bertrand and English (1) reported that only the *Cytospora* (conidial) stage developed in the 2 yr following the death of a branch from Cytospora canker. The *Valsa* (ascosporic) stage developed 3 yr or more later.

Several aspects of the etiology of the disease are still in question; ie, the major source of primary inoculum and the importance of conidia and ascospores in the natural spread of Cytospora canker. Infection of spruce with conidia of *C. kunzei* has only been reported in two studies (2,17). In both studies, it is not clear whether the inoculum was obtained from pycnidia or perithecia since both types of fruiting bodies exude spores in indistinguishable tendrils on wetting. In our study (8), only the *Valsa* (ascospore stage) were infective in artificially inoculated blue spruce in the greenhouse. Our results agree with those reported by Marsden (10) and Waterman (16). However, in those two studies, the pathogenicity of the species of *Cytospora* that causes the canker disease of spruce was not tested. We suggest that ascospores are the major source of primary inoculum and that infection of blue spruce occurs in spring.

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