Ecology and Epidemiology

Ascospore Production, Release, Germination, and Infection of *Populus* by *Mycosphaerella populorum*

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Journal Paper No. J-12946 of the Iowa Agriculture and Home Economics Experiment Station, Ames 50011. Project No. 2748.
We thank D. C. McGee for assistance in construction of the ascospore liberation tunnel and L. E. Sweets and M. L. Gleason for support and review of the manuscript.
Accepted for publication 22 May 1989 (submitted for electronic processing).

ABSTRACT

Luley, C. J., and McNabb, H. S., Jr. 1989. Ascospore production, release, germination, and infection of *Populus* by *Mycosphaerella populorum*. Phytopathology 79:1013-1018.

Production and release of ascospores from ascomata and infection of a susceptible clone (NE-1) and a moderately resistant clone (NE-19) of *Populus* by *Mycosphaerella populorum* were monitored in weekly sampling periods in a central Iowa plantation of *Populus*. Ascospores matured at approximately the time of bud swell of *P. deltoides*. Ascospore production, as measured by a liberation tunnel, and ascospore release, as detected by Vaseline-coated slide traps, peaked in mid- to late May and continued for 3–4 mo after initial release. Peak ascospore release in the field corresponded to peak periods of foliar and stem infection of the two clones of *Populus*. Stems of the moderately resistant clone were infected only in May, whereas foliar and stem infection of the susceptible clone occurred periodically throughout the growing season. Hourly ascospore release, monitored with a Burkard spore trap in 1985, was greatest after rains and during daylight hours. Levels of foliar and stem infection were also highly correlated with wetting periods associated with rain and daylight hours. Over 40% of ascospores germinated after 4 hr at 20, 25, and 30 C, and germ tube elongation after 16 hr was greatest at 25 C. These results show a strong relationship between the ascospore inoculum level and the degree of primary foliar and stem infection in a plantation of *Populus*.

Additional keywords: cottonwood, Septoria leaf spot and canker, *Septoria musiva*.

Septoria leaf spot and canker are serious foliar and stem diseases of certain *Populus* species and their hybrids in North America (3,19,27). The prevalence and severity of these diseases have limited the utilization of many fast-growing selections of *Populus* in intensive culture plantations, windbreaks, and shelterbelt plantings (3,19). Most research has focused on the anamorph *Septoria musiva* Peck, which is commonly isolated from foliar and stem lesions during the growing season (3,4,24,27). Initial study of the teleomorph, *Mycosphaerella populorum* G. E.

Thompson, has also indicated an important role for the perfect state in the epidemiology of the diseases (18,26,27).

The disease cycle of Septoria leaf spot and canker is similar to the cycles of other foliage-infecting ascomycetes in that overwintered leaves are the main source of primary inoculum. Infection of stems and leaves of *Populus* in the spring has been related to the flux of ascospores from leaf litter (18,27). Leaf spot symptoms first appear 3–4 wk after leaves open and are most severe in the lower canopy (3,18,27). A high percentage of this early-spring infection has been attributed to ascospores of *M. populorum* released from overwintered leaves (18,27). The role of ascospores in stem infection is less clear. Growth chamber and field observations have suggested ascospores can infect the stems of *Populus* (6,15,16). However, quantitative data linking ascospore release and inoculum levels in the field to foliar and stem infection were not presented in any of these studies.

Several environmental factors, including the duration of foliar and stem wetness, have been related to increased incidence of foliar and stem disease (15,27). Extended periods of free moisture on foliage and stems, occurring in dense stands or in stands with increased density after coppicing of existing stems, have been associated with increased canker and leaf spot disease (15,16,27). Direct study and quantification of environmental effects on ascospore release and infection of *Populus* have been limited. Thompson (26) trapped ascospores on Vaseline-coated slides after rains from May to late August and also found that ascospores germinate in water between 6 and 30 C and at an optimum of 27 C.

The present work was undertaken to determine the role of *M. populorum* in the epidemiology of both foliar and stem disease. Specifically, the objectives were to quantify ascospore production, release, and infection of two clones of *Populus* and to determine the effect of the environment on ascospore release and subsequent infection of foliage and stems.

MATERIALS AND METHODS

Field sampling design. Field trials were conducted in 1984 and 1985 in a plantation of mixed hybrid *Populus* at Ames, Iowa, established in 1977 for evaluating pest resistance in clones (19). Three sites within the plantation proximal to clones susceptible to *M. populorum* were selected for monitoring weekly changes in environmental conditions, ascospore production and release, and foliar and stem infection of two clones of *Populus*. The original test plantation was planted with various clones replicated in three four-tree plots with 3- × 3-m spacing. The clones in the plantation and their growth measurements and survival were previously described (19). The field sites were established in mid-March, before ascospore maturation, and data collection continued until leaf senescence and drop in the fall. The dates of ascospore collection and of foliar and stem infection were expressed as days of the year (23).

Ascospore production and release. An ascospore liberation tunnel, constructed from Plexiglas according to published specifications (9), was used to estimate the time at which ascospores matured and to quantify weekly ascospore production within ascomata present on a standard area of overwintered infected leaves. Five sets of 48 leaf disks each were cut before ascospores matured in the spring. The disks, each 19 mm in diameter, were cut from a random selection of overwintered leaves of *Populus* that contained ascomata of *M. populorum*. The disks were held on the ground in the plantation in nylon mesh bags (with 16-mm^2 holes) and were returned to the laboratory each week to determine the number of ascospores ready for release. Before the weekly collection periods, the leaf disks were tested every 2 days to determine the time of initial ascospore production in the field. The procedure for collecting ascospores with the liberation tunnel described by Hirst and Stedman (9) was used, with the following exceptions. If rain fell in the 24 hr preceding collection, the bags with leaf disks were incubated overnight in a moist chamber. Each set of leaf disks was soaked 3–5 min in tap water before being placed in three rows of 16 disks on a perforated aluminum rack, which was then inserted in the liberation tunnel. To collect ascospores, air was drawn over the leaf disks at an airflow rate of 12 L/min, and the spores were impacted on a Vaseline-coated microscope slide (20) mounted in the tunnel. After 2 hr, the disks were rewetted, and ascospores were collected for an additional hour. The ascospores impacted on the Vaseline-coated slide were stained with lactophenol-acid (cotton) blue. The spore density was estimated by counting the spores that were touching the vertical hash marks of a Leitz 10× eyepiece micrometer in three fields at 400× magnification.

Weekly ascospore release in the field was monitored with Vaseline-coated slide traps (20). At each of the three sites, two slide traps were exposed with the trapping surface facing upward, 30 cm above the leaf litter, and two with the trapping surface facing downward, 10 cm above the leaf litter. The direct impact of raindrops on the upward-facing traps was minimal, because of the overstory of leaves and branches, although some washing and removal of the Vaseline surface was evident in sampling periods with heavy rainfall. The number of ascospores per square centimeter was estimated by making five transects, each 22 mm in length, across the trapping surface at 400× magnification. Ascospores in the larger size range (1) were counted. Conidia matching the description of *S. musiva* (21) were counted in the same transects.

Phenological development of *P. deltoides* Marsh. in the Ames area was observed starting in mid-March in 1984–1986. Growth stages of *P. deltoides* were observed with regard to ascospore collection data to select a phenophase for the stage of development of *P. deltoides* at the time of first ascospore maturity.

In 1985, hourly concentrations of ascospores in the air were monitored with a Burkard spore trap (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, U.K.). The spore trap was placed at one of the sampling sites and continuously sampled the air at a rate of 10 L/min from day 128 to day 191. Ascospores were collected on Melinex tape coated with 10% Gelvatol and an adhesive mixture of 10 g of Vaseline and 10 g of paraffin dissolved in 90 ml of toluene. The number of ascospores trapped each hour was determined by scanning at 400× magnification at appropriate intervals along the tape.

Foliar and stem infection periods. Rooted cuttings of clones NE-1 and NE-19 of *Populus* were used as trap plants to identify infection periods and determine their relative severity. Clone NE-1, a cross of *P. nigra* × *P. laurifolia* 'Strathglass,' is highly susceptible to foliar and stem infection (19). Clone NE-19, a cross of *P. nigra* var. *charkowiensis* × *P. nigra* var. *caudina*, was reported to be moderately resistant to foliar and stem infection by *M. populorum* (19). The clones were exposed 4–6 wk after being rooted under intermittent mist on greenhouse benches, where the rooting medium was maintained at 26.7 ± 1 C with heating coils. After rooting, the cuttings were potted in a 1:1:1 (v/v) mixture of peat, perlite, and vermiculite in pots 10 cm in diameter and were fertilized weekly with 20-19-18 nitrogen-phosphorus-potassium in solution with iron-chelated ethylenediaminetetraacetic acid (FeEDTA, 5 ppm). The propagation bays in the greenhouse where the clones were grown were maintained at 23.9 ± 2.8 C and received 18 hr of light supplemented with incandescent and cool white fluorescent light. Dienochlor (Pentac; Zoecon, Palo Alto, CA) was sprayed weekly for spider mite control. Five rooted, potted cuttings of clone NE-1 and three cuttings of clone NE-19 were exposed for 7-day periods at each of the three sites that contained the spore traps. The potted trap plants were placed in the ground and were watered as needed during the exposure period. After exposure, the trap plants were returned to the greenhouse to allow symptoms to develop and were replaced by a new set of previously unexposed plants. The plants that had been exposed in the field were then held in separate bays of the greenhouse, apart from the unexposed plants, but under the same environmental conditions. Foliar disease was rated 18 days after exposure on a 0–5 scale (0 = no lesions, 1 = 1–10 lesions per leaf, 2 = 11–25, 3 = 26–40, 4 = 41–75, and 5 = more than 75). The first five leaves of clone NE-1 exhibiting symptoms of Septoria leaf spot were rated. Clone NE-19 was not rated for

Septoria leaf spot symptoms, because of the similarity in appearance between leaf spots caused by *M. populorum* and those caused by *Drepanopeziza punctiformis* Gremmen (anamorph *Marssonina brunnea* (Ell. & Ev.) Magn.) in the early stages of disease development on this clone. Stem infection of both clones was rated 25 days after exposure, and the number of lesions per stem and the percentage of stems infected were recorded. Potted, rooted cuttings of the susceptible clone NE-1 maintained in the greenhouse and in an open area near it were used to determine if inoculum was entering the greenhouse from outside sources. The nearest potential source of *M. populorum* was a large ornamental cottonwood approximately 100 m from the greenhouse.

Ascospore germination. Overwintered leaves with ascocarps of *M. populorum* were collected and stored at 8 C until used. Ascospores were discharged for 15 min onto 1.5% water agar from moistened leaf pieces attached to the tops of petri plates. The petri plates with ascospores were placed in plastic crispers in chambers at 5, 10, 15, 20, 25, 30, or 35 ± 0.5 C. The plates were removed after 4, 8, or 16 hr, and the ascospores were immediately stained and killed with lactophenol-acid blue. In each of five replicates, 125 spores were evaluated for germination, the length of the longest germ tube, and the number of germ tubes per spore. An ascospore was considered germinated when a germ tube at least 11.8 μm long, or about three quarters of the average length of ascospores from field collections (13), was evident.

Environmental data. A micrologger (Campbell Scientific, Logan, UT) at the center sampling station monitored and stored data on hourly average temperature, average relative humidity, maximum relative humidity, and the proportion of each hour with free moisture (8). A 7-day recording hygrothermograph was also maintained at the site. Rainfall data were obtained from the National Oceanic and Atmospheric Administration station at the Ames Pollution Control Plant, located approximately 0.8 km north of the plantation.

Data analysis. The SAS GLM and STEPWISE regression procedures (SAS Institute, Raleigh, NC) and correlation analysis were used to relate data on ascospore collection and environmental conditions (collected in the field or developed from field data) to foliar and stem infection of clone NE-1 (see Table 2, below). Regression equations were constructed with data from 22 sampling periods (see Table 3, below) between the time of initial ascospore production and the time at which 98% of the total ascospore production in each year had been monitored by the liberation tunnel. The STEPWISE MAXR procedure, which selects models with the highest R^2, was used initially to select models. Since the best models contained both an environmental component and an ascospore inoculum level component and were improved only slightly by the addition of other environmental variables, the best two-variable equations were retained.

TABLE 1. Phenology of cottonwood (*Populus deltoides*) and detection of the first mature ascospores of *Mycosphaerella populorum* in a central Iowa plantation of *Populus*

	Day of the year		
Developmental stage	1984	1985	1986
Cottonwood flowering[a]	ND[b]	103	90
Bud swell[c]	105	106	97
Ascospore maturation[d]	111[e]	107	93

[a]Male flowers fully expanded.
[b]Not determined.
[c]Outer bud scales breaking away.
[d]Ascospore maturation was determined with an ascospore liberation tunnel.
[e]First sampling date in 1984.

RESULTS

Ascospore production and release. Mature ascospores of *M. populorum* were first detected in early April, approximately at the time of bud swell of *P. deltoides* in central Iowa (Table 1). Ascospore release monitored by the Vaseline-coated slide traps and ascospore production quantified by the liberation tunnel peaked in late May in 1984 and mid-May in 1985 (Fig. 1). The period of peak ascospore production in ascomata occurred 7 days earlier than peak ascospore release in the field (Fig. 1). Ascospores were last detected by the ascospore tunnel on day 218 in 1984 and day 240 in 1985.

Few conidia of *S. musiva* (less than one spore per square centimeter) were trapped by the slide traps during the first 21 days in 1984 and 1985; conidial populations increased after day 146 in 1984 and day 138 in 1985. Conidial densities, in the period when foliar and stem infection was monitored in this study, ranged from 0 to 43.4 spores per square centimeter in 1984 and 0 to 10.1 spores per square centimeter in 1985.

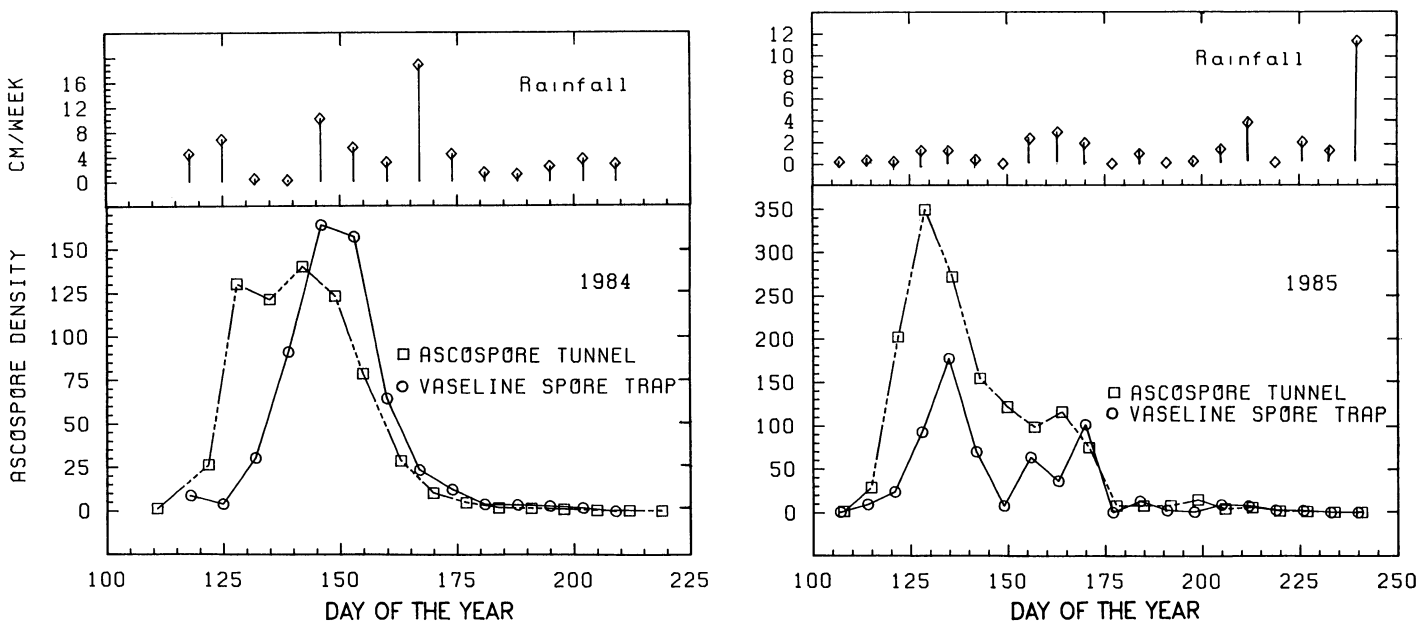

Fig. 1. Rainfall data and ascospore densities of *Mycosphaerella populorum* monitored for 7-day periods in 1984 and 1985 within a plantation of hybrid *Populus* in central Iowa. The ascospore densities for the Vaseline spore traps are an average of four traps at each of three sites and are expressed as the number of spores per square centimeter. Data from the ascospore liberation tunnel are an average of five samples and are expressed as the number of spores touching the vertical hash marks in three fields of a Leitz eyepiece micrometer at 400× magnification.

Ascospores were collected with the slide traps (Fig. 1) and the Burkard spore trap only in weeks with measurable precipitation. Hourly data from the Burkard spore trap showed that dew induced the release of low levels of ascospores only on a few occasions during the period of peak ascospore production. For each hour of the day, the ascospore concentration monitored by the Burkard spore trap in the period from day 128 to day 191 was examined together with the total hours in which moisture was sensed from either dew or rain during that period (Fig. 2). Wetting periods from rain and dew occurred frequently during evening and early morning hours, whereas ascospore release occurred mainly during daylight hours. Most ascospores were collected between 0700 and 1900 hr, and ascospore levels peaked at 1000 hr (Fig. 2).

Foliar and stem infection. Foliar infection of clone NE-1 occurred in all weeks in which ascospore release was detected in the plantation by the slide traps. Sampling periods in which

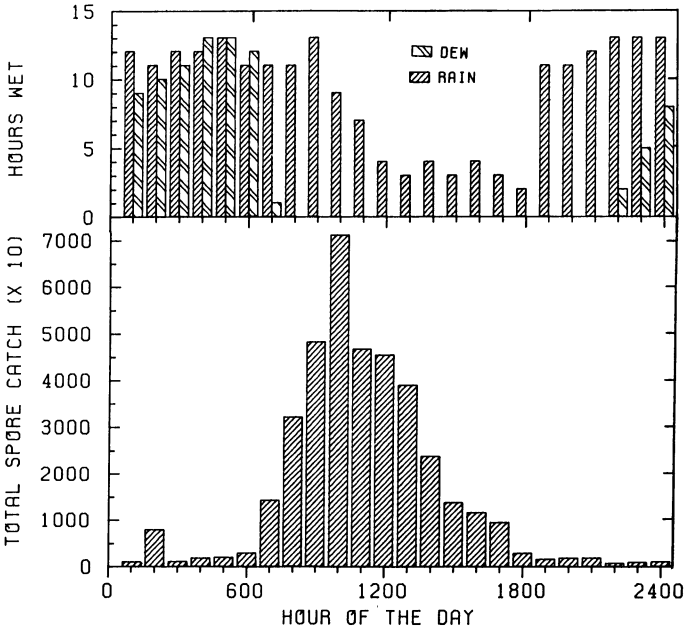

Fig. 2. Concentration of ascospores of *Mycosphaerella populorum* in the air (ascospores per cubic centimeter) collected with a Burkard spore trap in a central Iowa plantation of *Populus* and total hours of moisture from dew or rain. The data are totals for each hour of the day over 63 days (day 128 to day 191 in 1985).

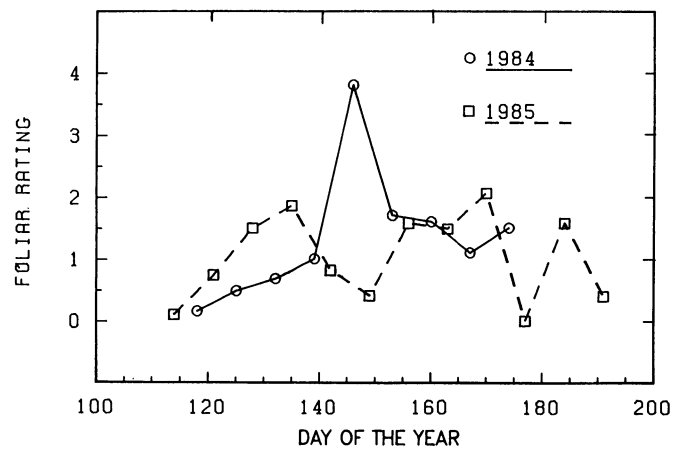

Fig. 3. Foliar disease rating of Septoria leaf spot on clone NE-1 after exposure in the field for 7-day periods in the 1984 and 1985 field seasons. The data are from exposure periods between the time of initial ascospore production and the time at which 98% of the total ascospore production in each year had been monitored by an ascospore liberation tunnel. The foliar ratings were based on a scale of 0–5, with 0 = no infection, 1 = 1–10 lesions per leaf, 2 = 11–25, 3 = 26–40, 4 = 41–75, and 5 = more than 75.

TABLE 2. Correlation coefficients between weekly foliar and stem disease ratings of *Populus* clones NE-1 and NE-19 and ascospore density of *Mycosphaerella populorum* and between disease ratings and selected environmental variables

Variable[a]	Foliar rating (NE-1)[b]	Infections per stem	
		NE-1	NE-19
ASCOSPORE[c]	0.70**	0.69**	0.52**
LONGWET[d]	0.62**	0.32	0.19
TOTWET[e]	0.52**	0.47*	0.29
TOTWET, daytime[f]	0.79**	0.72**	0.46*
TEMPLONGWET[g]	0.49*	0.28	0.14
TEMPTOTWET[h]	0.34	0.18	0.05
TEMP × LONGWET[i]	0.65**	0.35	0.16
TEMP × TOTWET[j]	0.75**	0.59	0.36
TEMP × TOTWET, daytime[k]	0.80**	0.67**	0.35

[a]The correlations are based on combined field data from 1984 and 1985. The variables were measured in 7-day sampling periods during which the clones were exposed in a central Iowa plantation of *Populus,* between the time of initial ascospore production and the time at which 98% of the total ascospore production in each year had been monitored by an ascospore liberation tunnel.

[b]* = Significant at $0.01 < P < 0.05$; ** = significant at $P < 0.01$.

[c]Density of ascospores collected by Vaseline slide traps, in spores per square centimeter.

[d]Longest continuous wetting period, in hours, during the week of exposure. Moisture was detected by gold-plated sensing plates.

[e]Total wetting period, in hours, during the week of exposure.

[f]Total daytime wetting period, in hours, during the week of exposure (i.e., the number of hours in which moisture was detected when wetting was initiated in or extended into the period between 0700 and 1900 hr).

[g]Average temperature during the longest continuous wetting period.

[h]Average temperature during the total wetting period.

[i]Interaction between the length of the longest continuous wetting period and the average temperature during that period.

[j]Interaction between the length of the total wetting period and the average temperature during that period.

[k]Interaction between the length of the total daytime wetting period and the average temperature during that period.

TABLE 3. Stem infection of *Populus* clones NE-1 and NE-19 exposed to *Mycosphaerella populorum* in the field for 7-day periods[a]

Year	End of exposure period (day of the year)	NE-1[b]	NE-19
1984	118	0.00 (0)	0.00 (0)
	125	0.00 (0)	0.00 (0)
	132	0.00 (0)	0.00 (0)
	139	0.00 (0)	0.00 (0)
	146	2.30 (93)	0.22 (22)
	153	0.00 (0)	0.00 (0)
	160	0.00 (0)	0.00 (0)
	167	0.33 (33)	0.00 (0)
	174	0.27 (27)	0.00 (0)
	181	0.00 (0)	0.00 (0)
1985	114	0.00 (0)	0.00 (0)
	121	0.00 (0)	0.00 (0)
	128	0.40 (40)	0.11 (11)
	135	1.30 (60)	0.22 (22)
	142	0.06 (6)	0.00 (0)
	149	0.00 (0)	0.00 (0)
	156	0.20 (20)	0.00 (0)
	163	0.06 (6)	0.00 (0)
	170	0.66 (46)	0.00 (0)
	177	0.00 (0)	0.00 (0)
	184	0.00 (0)	0.00 (0)
	191	0.00 (0)	0.00 (0)

[a]Five trap plants of clone NE-1 and three of NE-19 were exposed at each of three locations within a central Iowa plantation, between the time of initial ascospore production and the time at which 98% of the total ascospore production in each year had been monitored by an ascospore liberation tunnel.

[b]The average number of cankers per stem is given, followed by the percentage of stems infected (in parentheses).

exposure resulted in peak foliar infection corresponded to periods of maximum ascospore release monitored in the plantation (Figs. 1 and 3). Foliar disease ratings were also highly correlated with ascospore release (Table 2).

The number of infections per stem of clones NE-1 and NE-19 was positively correlated with ascospore inoculum levels monitored by the slide traps (Table 2). In both clones, the most infections per stem and the greatest percentage of stems infected occurred during the weeks of peak ascospore collection by the Vaseline traps (Table 3 and Fig. 1). Clone NE-19 was infected only in the week of peak ascospore release in 1984 and in the week before and the week of peak ascospore release in 1985. In contrast, stem infection of NE-1 occurred in six exposure periods after peak ascospore release when mixed ascospore and conidial populations were detected.

Ascospore germination. Over 40% of ascospores were germinated after 4 hr at 20, 25, and 30 C, and germ tube elongation after 16 hr was greatest at 25 C (Fig. 4). No ascospore germination occurred at 5 and 35 C after 16 hr. The average number of germ tubes per spore ranged from 1.0 to 1.5 after 16 hr at temperatures between 10 and 30 C.

Environment and disease. Foliar disease ratings of clone NE-1 and stem infection levels of both clones were correlated with certain environmental variables in the field (Table 2). The variables having the highest correlations with disease ratings were the length of wetting periods associated with daylight hours and the interaction between the length of those periods and the average temperature during them (Table 2). The following regression equations were developed from weekly data on foliar infection (eq. 1) and stem infection (eq. 2) of clone NE-1, environmental conditions, and ascospore density on the Vaseline spore traps:

$$Y = 0.14 + 0.008(\text{ASCOSPORE}) + 0.002(\text{TEMP} \times \text{TOTWET}) \qquad R^2 = 0.86 \qquad (1)$$

$$Y = -0.38 + 0.005(\text{ASCOSPORE}) + 0.024(\text{TOTWET}) \qquad R^2 = 0.69 \qquad (2)$$

For equation 1, Y = foliar rating, ASCOSPORE = density of ascospores collected on the slide traps (in spores per square centimeter), and TEMP × TOTWET = the interaction (product) of the average temperature during wetting periods that were initiated in or extended into the period between 0700 and 1900 hr (TEMP) and the total hours in which moisture was sensed during those wetting periods (TOTWET). For equation 2, Y = the number of infections per stem of clone NE-1, and ASCOSPORE and TOTWET are as described above. All terms in the equations are significant at $P < 0.05$.

DISCUSSION

Ascospore production and release by *M. populorum* exhibited a distinct seasonal periodicity. Both commenced in early April, peaked in mid- to late May, and declined to undetectable levels later in the summer. A similar pattern of ascospore release was found in plantations of *Populus* in Minnesota and Wisconsin (18). Ascospore production peaked before ascospore release, presumably because ascospores produced within ascomata were not released until a later time, when ascomata were wetted by rain. The difference between the time of peak ascospore production and release was one sampling period, or 7 days in this study (Fig. 1).

Bud swell on *P. deltoides* was an observable phenophase for initial ascospore production in Iowa. A relationship between bud swell and the initial release of ascospores has been noted in other studies (3,18), in which the species of *Populus* involved were not reported. Temporal differences between this phenophase and initial ascospore maturation were evident and varied from year to year in this study and a study by Ostry (18). Similar discrepancies have been found in other pathosystems for which phenophases have been established (5,7,14,25). The bud swell phenophase identified here could be used to initiate a model that predicts seasonal ascospore production (12) and to mark the period of primary infection when fungicidal applications should be made (18). Further assessment in multiple years and locations is needed to determine the reliability of bud swell as a phenophase for initial ascospore production.

Monitoring ascospore release in this and other studies has shown that ascospores are present in *Populus* plantations for most of the growing season, or a period of at least 3–4 mo after initial ascospore production (18,26). The duration of ascospore production may be related to the frequency and amount of

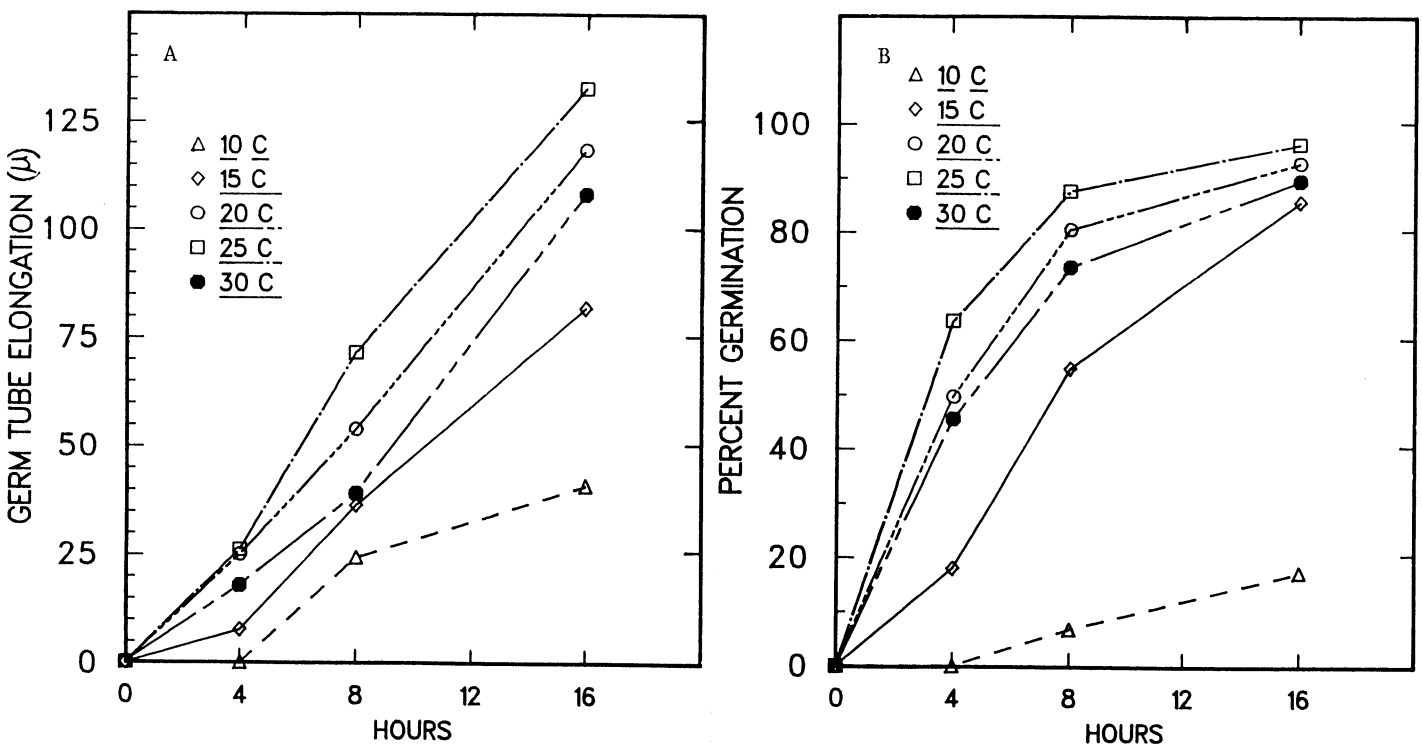

Fig. 4. Germ tube elongation (**A**) and percent germination (**B**) of ascospores of *Mycosphaerella populorum* at various temperatures. Ascospores were discharged from overwintered leaves onto water agar. Each data point is an average of five replications, with 125 spores per replication.

precipitation in a growing season. For example, in 1985 rain was not detected in four weekly sampling periods, and ascospores were collected for an additional month that year (Fig. 1). Thompson (26) also collected ascospores until the end of August in a year with an abnormally dry spring and summer. Further research is required for identifying critical levels of moisture and periods in the growing season when the development of ascospores of *M. populorum* is most affected by dry weather. Methods of monitoring leaf litter moisture should be considered, because moisture levels on living leaves or from rain may not accurately reflect moisture availability in the leaf litter (10).

Most ascospores were present in the air after rains and during daylight hours. Rain-initiated wetting periods associated with daylight hours were also strongly related to levels of foliar and stem infection in the regression and correlation analysis. These results indicate most infection occurred during wetting periods associated with daylight hours when elevated inoculum levels were present in the plantation.

The potential exists for foliar and stem infection with short periods of wetting, because more than 40% of ascospores had germinated after 4 hr at 20, 25, and 30 C. No spores germinated after 16 hr at 5 C, and Thompson (26) found ascospores germinated at 6 C only after 3–4 days. Therefore, under spring weather conditions, with temperatures of 5 C or below, ascospore germination and penetration of stomata (17) or stem tissues probably cause little infection.

The present work demonstrates a strong relationship between ascospore inoculum levels and foliar and stem disease ratings of exposed clones of *Populus*. Foliar infection can occur over a wide range of inoculum levels, because infection was found in all sampling periods during which ascospores were released. Stem infection, however, was more closely related to periods of peak ascospore release. For example, resistance present in clone NE-19 was sufficient to withstand infection except in the week of peak ascospore release in 1984 and 1985 and the week before peak release in 1985. The maximum infection of clone NE-1 also occurred during peak ascospore release, although because of its greater susceptibility to *M. populorum* (19), stem infection of this clone also occurred when lower inoculum levels were present.

The source of conidia found on slide traps early in the season may have been pycnidia in stem cankers (18) or possibly pycnidia that overwintered in fallen leaves. Conidial inoculum from these sources was believed to be too low to cause significant amounts of foliar and stem infection (18) in comparison to ascospore inoculum, which predominates at this time of the year. Conidial populations, however, increased greatly after the period of peak ascospore release and primary infection in the plantation studied, as well as in plantations in Wisconsin and Minnesota (18). Because inoculation experiments have shown that conidia can infect both foliage and stems (28), infection of clone NE-1 after the period of peak ascospore release was probably caused by mixed populations of conidia and ascospores or possibly conidia alone. Infection periods of exposed greenhouse-grown plants may not correspond to infection periods of *Populus* in the field, because differences in morphology (22) and phenology exist between greenhouse- and field-grown plants. However, the ability of ascospores to cause foliar and stem infection on the exposed clones of *Populus* and the strong correlation between ascospore inoculum levels and disease emphasize the importance of primary inoculum in the epidemiology of Septoria leaf spot and canker. Management strategies to reduce primary infection and sources of primary inoculum are useful in controlling apple scab and numerous other diseases (2,11), and similar management strategies may be useful in decreasing losses caused by Septoria leaf spot and canker.

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