The Effect of Temperature on Sporulation and Viability of Isolates of *Peronospora Tabacina* Collected in the United States

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ABSTRACT

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Field epidemics of tobacco blue mold recently occurred in the United States during periods of high summer temperature previously considered limiting to disease development. The effects of maximum day/night temperatures on sporulation and spore viability of isolates of *Peronospora tabacina* were determined in growth chamber studies. *P. tabacina* produced viable, infectious sporangiospores when developing lesions were exposed to day/night temperatures up to 36/25 C for 48 hr. Significantly fewer spores were produced at high temperatures compared to optimum conditions; however, such sporangia could be important as secondary inoculum for subsequent disease development should or when optimum field conditions return during the season. Maximum temperature levels reported for North American isolates in the literature before 1979 were 27–32 C. Isolates tested in this study appeared to be more temperature-tolerant.

Blue mold of tobacco caused by the obligate parasite *Peronospora tabacina* Adam (*P. hyoscyami* de Bary f. sp. *tabacina*) has been observed in the United States in most years since 1931. Before 1979, sporadic distribution and severity of the disease depended largely on the extent of cool, wet conditions; therefore, blue mold was largely confined to early-season seed or plant beds. Disease development was occasionally observed in the field, often resulting from the use of infected transplants. On occasions when blue mold did occur in the field, temperatures usually averaged 3–6 C below normal (8). Subsequent disease development was usually halted with the onset of normally high summer temperatures (1).

Dixon et al (4) reported that in the United States, recovery of tobacco plants from the blue mold disease was conditioned by sunny days with maximum daytime temperatures approximating 32 C and night temperatures above 20 C. In Canada (12), daytime temperature above 27 C immediately following disease outbreaks was a critical, limiting factor, even when other environmental variables were favorable for continuing disease development. Clayton and Gaines (1) found that neither infection nor spore germination occurred at temperatures above 29 C. Based on these studies and experiences before 1979, maximum temperatures of 30 C during the summer were generally considered as an ecological constraint preventing large-scale field epidemics of tobacco blue mold in the United States.

During the 1979 and 1980 tobacco growing seasons, field epidemics of blue mold moved northward across the United States and Canada, causing major crop losses estimated at 252 and 95 million dollars, respectively, in those years (13). Sporulating lesions were observed throughout the 1980 growing season, even though temperatures greater than 30 C were frequently observed in May, June, and July (9). Since 1980, field blue mold has been observed in varying locations and severity in years characterized by normal to high summer temperatures. Thus, the ecological temperature constraints on infection in the field that were thought to occur have not prevented recent epidemics in the United States.

Australian (2) and Israeli (11) workers exposed native *P. tabacina* isolates to 27–32 C conditions and significantly reduced sporulation and germination (2,3,10,11). Sporulation of Australian isolates did occur after exposure to day temperatures as high as 35 C when the night temperature was lowered to 20 C (2); germination of sporangia occurred at 35 C (3) when produced at 20 C. Rotem and Cohen (11) observed sporulation up to 40 C when infected plants were exposed for short periods and subsequently incubated at 20 C.

The widespread field epidemics in the United States and the reports of others (2,11) suggest that the temperature tolerance level of North American isolates may have changed from those levels reported in the literature before 1979. Data are also lacking on the viability and quality of sporangia produced under high-temperature conditions. Survival and sporulation following high day/night temperatures would certainly enable the pathogen to better withstand adverse conditions, spread, and become endemic. The objective of this study was to determine the effect of maximum day/night temperatures on the sporulation and viability of U.S. isolates of *P. tabacina*.

MATERIALS AND METHODS

Isolates of *Peronospora tabacina*. Isolates of *P. tabacina* used in this study represented collections of pathogen populations from: 1) Texas on *Nicotiana repanda* Willd collected in 1945 (BPT-45); 2) Oxford, NC, on *N. tabacum* L. collected during the 1979 blue mold epidemic (OPT-79); 3) Clayton, NC, on *N. tabacum* collected during the 1980 epidemic (CPT-80); and 4) Northhampton County, NC, on *N. tabacum* collected during the blue mold outbreaks in 1983 (NPT-83). As far as the authors are aware, BPT-45 is the only viable U.S. isolate of *P. tabacina* still in existence and available that predates the epidemics of 1979 and 1980.

Inoculum of each isolate had been increased previously on 'McNair 944' tobacco (*N. tabacum* L.) using a day/night temperature regime of 25/20 C with relative humidities nearing

saturation during the night. Inoculum was then stored in ice cubes of distilled water at −5 C. Experiments were replicated in time. Inocula for all of the four replications were from the original source of spores frozen in ice cubes.

When inoculum was needed, ice cubes were thawed. The resulting spore suspension was washed with 150 ml of cold distilled water using a millipore funnel with an 8.0-μm filter (Nuclepore Corp., Pleasanton, CA 94566) to remove germination inhibitors. The inoculum density of each isolate was then adjusted to about 500 viable sporangia/20 μl. The sporangial spore suspension was measured using a hemacytometer and was adjusted based on results of germination tests of each isolate conducted on 1% water agar the night before inoculations. The spore suspensions were kept cold in ice baths during inoculation.

Controlled environment studies. Experiments were conducted in 9-m^2 controlled-environment chambers ("A" chambers) at the Southeastern Plant Environmental Laboratory at North Carolina State University. All experiments were conducted under microbiological confinement protocol, policies, and procedures established for exotic pathogens. Twenty-eight T-12, 1,500 mA, cool-white fluorescent and 25 100-W incandescent lamps in each chamber resulted in the spectral energy distribution shown in Figure 1. These lamps yielded a photosynthetic photon flux density (400–700 nm) of 210–240 mol $m^{-2}s^{-1}$ and a photomorphogenic photon flux density (700–800 nm) of 43 mol $m^{-2}s^{-1}$ as measured with a spectraradiometer (LI-1800, LI/COR, Box 4425, Lincoln, NE 68504). Day length was based on a light period of 9 hr/day. Air temperatures were maintained at ±0.25 C of the treatment temperature set point except during misting periods when temperatures were maintained at ±1.5 C of the set point. Temperature was measured with a type "T" (copper-constantan), 24-gauge welded-bead thermocouple in a light-shielded, aspirated housing at canopy level. Relative humidity was measured using a Vaisala HMI 14 humidity instrument (Vaisala, 22 Cummings Park, Woburn, MA 01801). Humidity was maintained below 85% using dehumidifiers, except during infection and sporulation, at which times humidities above 95% were obtained either by misting plants or enclosing the leaves in plastic bags.

Tobacco cultivar 'McNair 944' seeds were germinated in styrofoam cups containing a sand:Peat-lite (W. R. Grace Co., Traveler's Rest, SC 29690) mixture (1:1, v/v) at 22 C in a growth cabinet (Sherer model CEC 38-15, Rheem Mfg. Co., Asheville, NC 28804). Three weeks after germination, plants were thinned to one plant per cup and moved to an air-conditioned greenhouse at 26/22 C day/night temperatures with a long photoperiod provided by a light interruption of 550–600 lux from incandescent-filament lamps from 11 p.m. until 2 a.m. Three weeks later, plants were transplanted in 203-mm diameter pots containing a gravel:Peat-lite mixture (1:1, v/v). Plants were maintained in the greenhouse and watered with a nutrient solution (4) as needed.

At the 7- to 10-leaf stage (3 wk after transplanting), 16 plants were moved into each of four A chambers and placed equidistant from each other on metal trucks 0.8 m above the chamber floor and 1.25 m from chamber light barriers. Plant spacing approximated row and plant spacing of field-grown tobacco. Plants were acclimated for 1–2 days at day/night temperatures of 25/20 C and then inoculated with one of four isolates of *P. tabacina*.

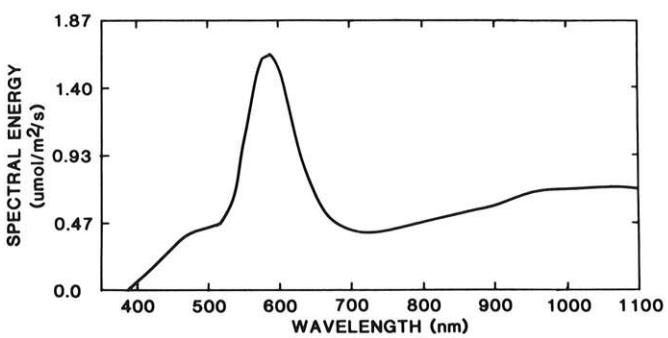

Fig. 1. Spectral energy distribution in controlled-environment growth chambers lighted with cool-white fluorescent and incandescent lamps.

Each isolate was randomly assigned to one plant within each of four blocks in each chamber. All leaves on an individual plant were numbered according to their positions on the stalk and were inoculated with five equidistantly spaced 20-μl drops of spore suspension per leaf. Plants were then misted with a fog of atomized distilled water for 12-sec intervals, four times per hour for 48 hr to provide and maintain free moisture necessary for sporangial germination on the leaf surface (7). Dehumidifiers were used 48 hr following infection to maintain chamber humidity below 85% to prevent sporulation. Nine days after inoculation at uniform conditions, the incubation period was completed and chlorotic lesions were apparent. Day/night temperature treatments of 34/23, 36/25, 38/27, or 40/29 C were then imposed on separate chambers according to randomization procedures in a Latin square design over time. Treatment temperatures beginning with the night treatment were imposed to acclimate plants to the high daytime temperatures. Following 1 and 2 days of treatment and 30–45 min before the night period, plastic bags were placed around each of three randomly selected inoculated leaves on each plant and were secured with twist-ties to petioles. Humidity inside the plastic bags reached saturation within 1 hr after initiation of night conditions at all temperatures studied; therefore, humidity requirements for sporulation were met. Bagged leaves were removed from plants the following morning and kept in plastic bags up to 3 hr at 22 C until evaluation. Replication one of all treatments was evaluated before evaluation of replication two of any treatment.

Infection efficiency, measured as number of lesions produced per five inoculation sites per leaf, number of lesions sporulating, and lesion diameters, were assessed on each leaf. A sporulating lesion was defined as one that had any visible signs of sporulation at 10× magnification. Lesion diameter was used to calculate lesion area for further analysis. Sporangia from all lesions on each leaf were collected by washing lesions with equal volumes of distilled water. Sporangia were counted using a hemacytometer. Number of spores was compared per square centimeter of sporulating lesion area. Sporangial viability was tested by placing 1 ml of sporangial suspension from each leaf into a petri dish containing 1% water agar. Viability tests were made both in the laboratory at 22 C and in the chamber at the temperature at which sporangia were produced. Germination, as a measure of viability, was assessed following incubation of petri dishes for 12–24 hr by counting percentage of sporangia with germ tubes at least one-half the spore length.

Infectiousness of sporangia at high temperatures was determined by inoculating a leaf of a McNair 944 bioassay tobacco plant with a spore suspension from each isolate × temperature treatment. Plants were incubated at temperature treatments in which the sporangia were produced. Five 20-μl drops of sporangial suspension were placed on a leaf which was then enclosed in a plastic bag. The following day, bags were removed from leaves and plants were allowed to incubate in the laboratory at 22 C. Nine days later, infection efficiency and sporulation were assessed after plants were subjected to high humidities during a night period.

Data analyses. Data were collected following both 1 and 2 days of temperature exposure and analyzed using the General Linear Models procedure of SAS (Statistical Analysis System, SAS Institute, Cary, NC). Average ratings over the three individual leaves on each plant were used in data analysis, giving a total of 256 observations for the experiment. Data for each dependent variable were analyzed using a split-plot design where whole plot treatments (temperatures) were randomized according to Latin square design with four replications and with subplot treatments (isolates) randomized according to a randomized complete block design.

Some analyses resulted in nonestimatibility of least square (LS) means for dependent variables where data from an entire block within a chamber were missing. LS means are the expected value of class or subclass means that would occur with a balanced design. To produce LS means, the block term (rep × temp), which was not significant, was dropped from the model.

When the interaction term, temp × isolate, was not significant,

the main effects, temperature and isolate, were evaluated separately. Linear, quadratic, and cubic contrasts for temperature were calculated for each dependent variable. Isolate LS means were compared using pairwise *t* tests. Position of leaves on a plant was used as a covariate in all analyses because younger leaves were found to restrict lesion expansion and were less susceptible to infection than were older leaves. Data collected after 1 and 2 days of treatment were independently analyzed for each dependent variable. A similar analysis was done on the data to detect differences between 2 and 1.

RESULTS

Infection efficiency and lesion area. Isolate and leaf position significantly influenced the number and size of lesions (Table 1). Infection efficiency was similar for all isolates on both days of rating, which indicated that similar numbers of lesions were evaluated after the 1- and 2-day temperature exposure periods for the sporulation and germination variables (Table 2). Inoculum of NPT-83 and OPT-79 retained a greater infection efficiency over the five months of the experiment than did CPT-80 and BPT-45. Those isolates with the greater infection efficiencies also generally had the smaller lesion areas (Table 2). Significant differences in lesion area were not observed between day 1 and day 2 ratings. The significant leaf position covariate indicated a maturity-dependent response for infection and colonization by the pathogen. In general, the younger leaves supported fewer and smaller lesions than did older leaves.

Because plants were inoculated and maintained under similar environmental conditions for the first 9 days following inoculation, temperature treatments did not affect infection or lesion size (Table 1).

Percentage of sporulating lesions and sporangia per square centimeter. Data for the number of sporangia produced were expressed as sporangia + 1/cm^2 of sporulating lesions and were log$_e$-transformed to improve normality and variance homogeneity.

Temperature was the most important factor in determining the percentage of lesions that sporulated and the number of spores produced (Table 1). Following 1 day of high-temperature treatment, both sporulation variables decreased linearly as temperature increased (Table 3). However, sporulation was observed at temperatures as high as 38/29 C. The detrimental effect of high temperatures was more pronounced following the second day of exposure, but abundant sporulation still occurred at 34/23 and 36/25 C.

Isolate differences were observed in the percentage of

TABLE 1. Main and interaction effects on lesion and sporulation variables of tobacco inoculated with four isolates of *Peronospora tabacina* exposed to a range of high temperatures above 30 C for 1 and 2 days[a]

	Main and interaction effects[c]			
Dependent variable[b]	Temperature	Isolate	Temp × isolate	Position
Infection efficiency (%)				
Day 1	NS	***	NS	***
Day 2	NS	***	NS	***
Lesion area (cm^2)				
Day 1	NS	***	NS	**
Day 2	NS	***	NS	***
Sporulating lesions (%)				
Day 1	***	*	NS	NS
Day 2	***	NS	NS	NS
Log$_e$ spores + 1/cm^2 lesion area				
Day 1	***	NS	NS	NS
Day 2	***	NS	NS	NS
Spore germ lab (%)				
Day 1	*	NS	NS	NS
Day 2	NS	**	NS	NS
Spore germ chamber (%)				
Day 1	NS	*	NS	NS
Day 2	NS	NS	NS	*

[a] Temperature treatments include day/night temperatures of 34/23, 36/25, 38/27, and 40/29 C. Plants were exposed to the treatments following inoculation and incubation for 9 days at 25/20 C.
[b] Spore germination was conducted on 1% water agar both in the laboratory at 22 C and in the chamber at the treatment temperature sporangia were produced.
[c] Significance levels: NS = not significant; * = $p \leq 0.05$; ** = $p \leq 0.01$; and *** = $p \leq 0.001$.

TABLE 2. Infection efficiency and lesion development on tobacco for sporangia of four *Peronospora tabacina* isolates stored in ice cubes at −5 C[a]

	Infection efficiency (%)		Lesion area (cm^2)	
Isolate	Day 1[b]	Day 2	Day 1	Day 2
NPT-83	43 ± 2.53[c] a[d]	40 ± 2.59 a	3.53 ± 0.21 c	3.97 ± 0.24 c
CPT-80	25 ± 2.52 b	25 ± 2.58 b	5.01 ± 0.23 a	5.40 ± 0.27 a
OPT-79	38 ± 2.52 a	37 ± 2.58 a	4.06 ± 0.21 bc	4.36 ± 0.23 bc
BPT-45	26 ± 2.53 b	26 ± 2.59 b	4.20 ± 0.23 b	4.93 ± 0.25 ab

[a] Sporangia had been stored 1–5 months, depending on time-replication.
[b] Tobacco plants were inoculated and incubated for 9 days at 25/20 C; then plants were exposed to treatment temperatures for 1 and 2 days.
[c] Data represent least square (LS) means across all temperature treatments ± standard errors of LS means.
[d] Significance levels determined by using LSD for paired means. Means within columns followed by a common letter are not significantly different at $p \leq 0.05$.

TABLE 3. Effect of temperature on sporulation and viability of U.S. isolates of *Peronospora tabacina* on tobacco

	Day/night temperature[a]				Contrasts[b,c]		
Dependent variable[d]	34/23	36/25	38/27	40/29	Linear	Quadratic	Cubic
Sporulating lesions (%)							
Day 1	87 ± 8.12[e]	65 ± 7.98	25 ± 8.69	0 ± 0.00	***	NS	NS
Day 2	85 ± 7.66	64 ± 7.36	0.4 ± 7.74	0 ± 0.00	***	NS	*
Log$_e$ spores + 1/cm^2 lesion area							
Day 1	9.26 ± 0.98	7.34 ± 0.96	2.88 ± 1.05	0 ± 0.00	***	NS	NS
Day 2	9.58 ± 0.76	7.27 ± 0.73	0.07 ± 0.77	0 ± 0.00	***	NS	**
Spore germ—22 C (%)							
Day 1	70 ± 3.91	36 ± 4.26	...	...	...	...	...
Day 2	75 ± 5.88	48 ± 6.80	...	...	...	...	...
Spore germ—treatment temperature							
Day 1	46 ± 10.3	15 ± 11.1	...	...	...	...	...
Day 2	61 ± 12.7	32 ± 14.3	...	...	...	...	...

[a] Plants were exposed to either 1 or 2 days of treatment temperatures 9 days after inoculation and incubation at 25/20 C. Day/night periods lasted 9/15 hr. ... = no data available.
[b] ... = Contrasts were not calculated for germination variables because germination did not occur at the two highest temperatures.
[c] Significance levels: NS = not significant; * = $p \leq 0.05$; ** = $p \leq 0.01$; and *** = $p \leq 0.001$.
[d] Germination tested on 1% water agar either in the lab at 22 C or in the chamber at the treatment temperature at which sporangia were produced.
[e] Data represent LS means across isolates ± standard errors of LS means except for 40/29 in which all data were zero.

sporulating lesions after the first day of high-temperature exposure. BPT-45 had the highest percentage of lesions to sporulate with 52%, whereas NPT-83 had the lowest with 38%. BPT-45 and OPT-79 resulted in similar numbers of sporulating lesions, and no significant differences occurred among OPT-79, CPT-80, and NPT-83. Isolate differences did not occur in percentage of sporulating lesions following 2 days of treatment or in number of sporangia produced.

Spore germination. The viability of sporangia produced under high-temperature conditions was tested in the laboratory at 22 C and in the growth chambers at treatment temperatures. Few spores were produced at the 38/27 and 40/29 C treatments, and as a result, viability data were unbalanced. To produce LS means, only the two lowest temperatures were included in the analyses. When sporangia were produced at the two highest temperatures, germination did not occur.

Viability of sporangia produced after both 1 and 2 days of temperature treatment decreased dramatically as temperature increased (Table 3). However, statistical differences between temperatures were found for only sporangia produced after a 1-day exposure and tested at 22 C in the laboratory (Table 1). Viability of sporangia produced following 2 days of high-temperature treatment was greater than that produced after only a 1-day exposure, although the differences were not significant in this study.

Significant isolate differences were found in the germination of sporangia produced following the 2-day exposure period and tested in the laboratory at 22 C (Table 1). The most recently collected isolates, CPT-80 and NPT-83, germinated significantly better than did OPT-79 and BPT-45 (Table 4).

In the germination tests made in growth chambers, significant isolate effects were found in the sporangia produced after 1 day of high-temperature exposure (Table 1). LS means indicated that CPT-80 produced significantly more viable sporangia than did other isolates (Table 4). The viability advantage of CPT-80 did not persist after 2 days of high-temperature treatment.

Infectiousness of sporangia. Whenever germination of sporangia occurred, spores were also capable of causing lesions. Under conducive conditions, these lesions sporulated and completed the disease cycle.

DISCUSSION

The ability of isolates of *P. tabacina* collected in the United States to survive and produce viable, infectious sporangia, after exposure of mature lesions for 2 days to day/night temperatures up to 36/25 C, is an important component in the development of blue mold epidemics in North America. Although a significant decrease in sporulation may occur at these high temperatures in comparison with optimum conditions, these sporangia can serve as secondary inoculum for subsequent disease development and spread should favorable conditions for epidemic development return during the season. The ability of U.S. isolates to survive high-temperature conditions may help explain the recent field epidemics in North America that were atypical of previous blue mold occurrences. In comparison with maximum temperature levels of 27–32 C reported in early literature for blue mold development in North America (1,4,8,12), isolates collected from recent pathogen populations appear to be more temperature-tolerant.

Studies in Australia and Israel on the independent effects of day or night temperatures on pathogen survival, colonization, sporulation, and germination have been published (2,3,11). Our studies are unique in that both sporulation and sporangial viability were quantified under day/night temperature treatments typical of those that may occur during the growing season in the United States. To detect the maximum temperature for sporulation and sporangial viability, temperature treatments chosen for our study were in 2-C increments. During the 1980 epidemic, the average difference between day and night temperatures in the Southeast during April, May, June, and July was 11 C. Thus, the epidemiological importance of maximum day/night temperatures on secondary inoculum production was assessed.

The maximum temperatures for sporulation for U.S. populations were similar to those reported for Australian and Israeli isolates (2,3,11). Sporulation of Australian isolates was observed after mature lesions were incubated for 8 hr at temperatures as high as 35 C if night temperature was reduced to 20 C (2). Under similar experimental conditions, an Israeli isolate did not sporulate if lesions were incubated for 24 hr at 35 C (11). Cruickshank (2) also found that night temperature was important in limiting sporulation. He found significant reduction in sporulation after a 12-hr incubation at night temperatures above 24 C, with almost no sporulation at 27 C. In our studies, sporulation was observed after a 9/15-hr incubation at temperatures as high as 38/27 C. However, spores produced at this temperature were not viable and would not contribute to disease spread. The sporulation response to temperature in our study followed closely the results of Cruickshank's night temperature studies. Although day temperature may be important if extremely hot, it appears that night temperature may play the most important role in limiting disease development.

Spore viability decreased with increasing temperature and was greater for those spores produced following the exposure of lesions for 2 days, although statistical differences were not found. Lack of significant differences was in part due to the lack of power in testing at the whole plot level, with only two temperatures included in the analysis. Although statistical differences were not found, we feel that the biological differences may be important in the epidemiology of this disease. Only two temperatures were used in the analysis, so contrasts were not calculated.

The viability of spores produced at high temperatures and placed at an optimum temperature for germination was much greater than those sporangia that remained in a high-temperature environment. Therefore, a day of cooler temperature following sporulation at high temperatures enhances the viability of sporangia. Cruickshank (3) found that germination of sporangia produced under optimum conditions could occur at temperatures as high as 35 C. Our studies indicate that sporangia produced under high-temperature conditions do not have the ability to germinate at temperatures above 25 C. Sporangia produced after 2 days of high-temperature exposure appear to be more viable than sporangia produced after 1 day of exposure. This may indicate that lesions exposed to longer intervals of high temperature may produce more heat-tolerant spores. Based on data collected in this study, several days of high temperature of 36/25 C would limit sporulation to 460 viable sporangia per square centimeter of disease tissue, compared to 8,824 viable sporangia at 34/23 C. Thus, a change of two degrees can have a major effect on secondary inoculum production.

Few consistent differences among isolates occurred in this study. BPT-45, the only available isolate that was collected before 1979, had temperature tolerance levels comparable to those of other isolates. The fact that this isolate was not collected from cultivated

TABLE 4. Viability of *Peronospora tabacina* sporangiospores of U.S. isolates exposed to temperatures above 30 C[a]

Isolates	Spore germination lab (%)		Spore germination chamber (%)	
	Day 1	Day 2	Day 1	Day 2
NPT-83	51 ± 3.97[b] a[c]	67 ± 3.64 a	28 ± 3.12 b	44 ± 3.80 a
CPT-80	62 ± 4.62 a	73 ± 4.41 a	40 ± 3.66 a	55 ± 4.61 a
OPT-79	54 ± 3.95 a	54 ± 3.57 b	28 ± 3.06 b	45 ± 3.73 a
BPT-45	45 ± 4.29 a	54 ± 3.86 b	25 ± 3.33 b	42 ± 3.97 a

[a] Plants were inoculated and incubated for 9 days at 25/20 C; then, exposed to either 1 or 2 days of day/night temperature treatments of 34/23, 36/25, 38/27, or 40/29 C. Germination assessed on 1% water agar in the laboratory at 22 C and in the chamber at treatment temperature.

[b] Data represent least square (LS) means across the two lowest temperature treatments ± standard error of LS means.

[c] Significance levels determined by using LSD for paired means. Means within columns followed by a common letter are not significantly different at $p \leq 0.05$.

tobacco but rather from wild tobacco in the typically hot climate of Texas diminishes its suitability as a reference isolate. However, Wolf (14) reported the possibility that initial outbreaks of blue mold in the cultivated tobacco areas of the United States may have resulted from disseminated spores from Texas. It may be reasonable to postulate that the pathogen population now in the cultivated areas may be composed in part of the Texas temperature-tolerant population, which may have been disseminated into cultivated tobacco and initiated recent epidemics. This could explain the lack of differences among isolates in this study.

Infected plants were exposed to temperature treatments 9 days after inoculation. Therefore, infection efficiency and lesion area were independent of temperature during this phase of the study. Our results indicated that significant differences did occur among isolates for infection efficiency and lesion area. Degradation of the inoculum stored in ice cubes at −5 C over time may account for the overall low infection efficiencies, even though sporangial suspensions for each time-replication were adjusted to yield approximately 500 viable spores/20 μl of spore suspension. Increasing the total number of spores to obtain the 500 viable spores may have increased the concentrations of germination inhibitors, resulting in reduced infection. Sporangial germination of BPT-45 and CPT-80 inoculum was 0.7 and $<0.1\%$, respectively, by the end of the experiment, as compared to that of OPT-79 and NPT-83, which were 1.4 and 3.7%, respectively. This indicates that a much higher number of total spores was required for BPT-45 and CPT-80 to obtain comparable viable inoculum densities.

Isolates with the higher infection efficiencies had the smallest total lesion area. Competition may have occurred among infection sites and lesion expansion. When infection was high, lesion development was reduced, and vice versa. Thus, total area available for sporulation may be independent of total lesion numbers on an individual leaf. Infection efficiency and lesion expansion also depended on leaf maturity. The younger the leaf, the fewer the infections and the smaller the lesions.

Chamber uniformity trials were made before this study to identify and correct differences among the four growth chambers used. As a result of our trials, there were no significant chamber effects in our studies. Lee and Rawlings (6) found that in many growth chamber studies, chamber effects were significant both across and within chambers, presumably with common settings. Growth chambers are notoriously variable, and unless the performance of each chamber is thoroughly understood, different environmental regimes may in reality exist among chambers even though they are set alike.

This study confirms the ability of isolates of *P. tabacina* collected in the United States to produce viable, infectious sporangia after exposure of mature lesions to day/night temperatures up to 36/25 C. These sporangia are epidemiologically important as secondary inocula for disease development and spread should favorable environmental conditions for epidemic development return during the season.

LITERATURE CITED

1. Clayton, E. E., and Gaines, J. G. 1945. Temperature in relation to development and control of blue mold (*Peronospora tabacina*) of tobacco. J. Agric. Res. 71:171-182.
2. Cruickshank, I. A. M. 1961. Environment and sporulation in phytopathogenic fungi. II. Conidia formation in *Peronospora tabacina* Adam as a function of temperature. Aust. J. Biol. Sci. 14:198-207.
3. Cruickshank, I. A. M. 1961. Germination of *Peronospora tabacina*: Effect of temperature. Aust. J. Biol. Sci. 14:58-65.
4. Dixon, L. F., McLean, R., and Wolf, F. A. 1936. Relationship of climatological conditions to the tobacco downy mildew. Phytopathology 26:735-759.
5. Downs, R. J., and Thomas, J. F. 1983. Phytotron procedural manual for controlled environment research at the Southeastern Plant Environment Laboratory. N. C. Agric. Res. Ser. Tech. Bull. no. 244.
6. Lee, C., and Rawlings, J. O. 1982. Design of experiments in growth chambers—uniformity trials in the North Carolina State University Phytotron. Crop Sci. 22:551-558.
7. Lucas, G. B. 1975. Diseases of Tobacco. Biological Consulting Associates, Raleigh, NC.
8. McGrath, H., and Miller, P. R. 1958. Blue mold of tobacco. Plant Dis. Rep. Suppl. 250:3-35.
9. National Oceanic and Atmospheric Administration, U.S. Department of Commerce and Economics, Statistics and Cooperative Service. U.S. Dep. Agric. 1980. Weekly Weather and Crop Bull. no. 67.
10. Rider, N. E., Cruickshank, I. A. M., and Bradley, E. F. 1961. Environment and sporulation in phytopathogenic fungi. III. *Peronospora tabacina* Adam: Field environment, sporulation, and forecasting. Aust. J. Agric. Res. 12:1119-1125.
11. Rotem, J., and Cohen, Y. 1970. The effect of temperature on the pathogen and on the development of blue mold disease in tobacco inoculated with *Peronospora tabacina*. Phytopathology 60:54-57.
12. Stover, R. H., and Koch, L. W. 1951. The epidemiology of blue mold of tobacco and its relation to the incidence of the disease in Ontario. Sci. Agric. 31:225-252.
13. Todd, F. A. 1981. The blue mold story. Pages 9-25 in: Blue Mold Symposium II. F. A. Todd, ed. 29th Tobacco Workers Conference, Lexington, KY.
14. Wolf, F. A. 1947. Tobacco downy mildew, endemic to Texas and Mexico. Phytopathology 37:721-729.