

Development and Inoculum Potential of *Peronospora tabacina* in the Fall Season

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ABSTRACT

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Development of a *Peronospora tabacina* epidemic on tobacco at season's end in Connecticut was studied from mid-September until mid-November. Plant growth, disease incidence, and production and dispersal of inoculum as affected by weather changing from suboptimal to marginal to submarginal were measured. Under these conditions, the epidemic developed in proportion to inoculum pressure. Although the number of sporangia per lesion decreased as the season advanced, most sporangia in the field appeared during late September and early October, when lesions were most numerous. Shortly after, the amount of inoculum and the number of lesions decreased owing to continuing destruction of leaves by frosts. Inoculum disappeared in mid-November, and no sporangia were left for overwintering. The following phenomena were quantified during the epidemic: Most lesions on older leaves were necrotic, and as the season

progressed, most lesions on all leaves became necrotic. Necrotic lesions yielded fewer sporangia than chlorotic lesions with or without necrotic margins. Also as the season progressed, all lesions, but especially necrotic ones, became infested by *Alternaria* sp. Although abundant after nights with free moisture on leaves, sporangia were also present after dry nights. Mechanical spore traps and potted plants usually trapped sporangia in proportion to the number measured directly on leaves of sporulating plants, but when sporulation was low, mechanical traps did not collect spores. Sporangia caught by potted plants during the afternoon and night accounted for about one-third of the daily infections. The frequency of nighttime infections appeared to be impaired by low temperatures late in the season.

Epidemics caused by *Peronospora tabacina* Adam on tobacco (*Nicotiana tabacum* L.) in regions with severe winters are probably initiated by aerial transport of inoculum from a warmer region (2, 10) or by infected seedlings (9). Laboratory trials in Kentucky suggested that epidemics may also be initiated by overwintering sporangia (6). These different sources of inoculum indicate the need for different control strategies. To overwinter locally and initiate summer epidemics, sporangia must be present at the end of the growing season. Such sporangia must be produced in the fall on sucker growth that appears after the commercial leaves are picked. Blue mold often develops on these suckers in the suboptimal and marginal conditions during early and late fall, respectively. Although sporulation and infection by *P. tabacina* are possible at low temperatures and disease has been observed in seedbeds exposed to low temperatures (9), blue mold epidemics have never been studied under these conditions. Our study helps define such patterns for Connecticut, measures inoculum potential in marginal weather, and leads to conclusions concerning the role of sporangia as an overwintering form of the pathogen.

MATERIALS AND METHODS

A 17 × 20 m plot of the tobacco cultivar Broadleaf was planted with 510 seedlings on 22 July 1982 at the Lockwood Experimental Farm, Hamden, CT. Although late-season epidemics occur only on suckers, we intensified the epidemic by planting the field late in the season and by allowing all leaves to remain on the plants. After sunset on 23 August, three plants on the southwest and three on the northeast edge of the plot were spray-inoculated with a water suspension of sporangia of *P. tabacina*. By the end of August, the inoculated plants showed symptoms of blue mold. From these foci, blue mold spread to other plants.

Host and disease conditions were assessed weekly on 10 plants chosen at random. A key for grading the leaf surface in the field was prepared by examining 180 leaves of various plants. Leaf area was estimated by multiplying the length of the leaf by its maximum width and 0.7 (I. Zelitch, *personal communication*). The grades A,

B, C, D, E, and F corresponded to an average leaf area of 2,062, 1,317, 853, 560, 296, and 98 cm², respectively. Leaves representative of these categories were traced on graph paper, and leaves in the field were graded according to similarity in shape and size to these tracings. Direct measurements of the area of 20 leaves per grade were used for final calibration of the method. Leaves graded A-E differed from measured areas by an average of 1-3.5%, whereas the smallest and youngest leaves graded F differed by 8.1%.

Lesions were counted periodically. According to assumed potentials for sporulation (13), lesions were classified as chlorotic (category A), necrotic with chlorotic margins (category B), and necrotic without chlorotic margins (category C).

The number of sporangia per lesion was counted on 100 lesions. At approximately 0900 hours eastern standard time, before wind induced vigorous dispersal, random leaf pieces with lesions were collected. Routine samples contained mixtures of the various categories of lesions. Leaf pieces were shaken for 60 min in a solution of Formalin, acetic acid, and alcohol. The resulting suspension was filtered on membrane filters, where sporangia were counted (5). Quantities of sporangia were estimated for the whole plot by multiplying the sporangia per lesion by lesions per plant and plants per plot.

On 10 October, more detailed sampling was undertaken to establish the relationships among the position of the leaf on the plant, the category of the lesions on various leaves, and the contribution of each lesion category to the total inoculum produced by the host plant. First, 100 lesions of each category were collected at random to determine the number of sporangia per lesion. Then, 10 plants were chosen at random and the number and areas of leaves were determined. The number of lesions for each category was counted on each leaf.

Airborne spores were trapped with a roto-slide sampler (11) and the results expressed as the average number of sporangia per cubic meter of air per 24 hr. Dissemination, deposition, and survival of sporangia on plants were estimated by exposing healthy potted 6-wk-old tobacco trap plants on selected days from 0900 to 1600 hours and from 1600 to 0900 hours the next morning. Ten replicate plants were used per treatment in this and other trapping trials. The trap plants were transferred to moist chambers (20 ± 1 C) immediately after exposure. After 24 hr in the moist chamber, the plants were incubated for 6 days at 20 C, about 50% relative humidity (RH), and 12-hr photoperiod. Leaf area of the trap plants

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was measured. Results were expressed as the number of lesions on 100 cm² of foliage.

The combined effects of dispersal, deposition, survival, and infection were estimated by exposing plants from 0900 hours until 0900 hours the next day. Some of these plants were then transferred to a growth room without being wetted and others were wetted for 24 hr at 20 C, starting immediately after removal from the field.

Meteorological factors needed to assess the environmental influences on the pathosystem were measured next to the experimental plot with a hygrothermograph housed in a standard weather shelter, a pyrheliograph, and a dew balance, all supplied by Belfort Co., Baltimore, MD.

RESULTS

Weather conditions. For interpretation of epidemiologic phenomena, we divided the weather factors into those expected to affect sporulation and infection (moisture duration, temperature during moist periods, and number of hours of RH \geq 90%) and those expected to inhibit host and disease development and/or sporangia viability (maximum and minimum temperatures, minimum RH, and solar radiation) (12).

Except for one episode of 27 C, temperature maxima from 12 to 30 September ranged from 12 to 24 C, with an average of 18.5 C. In October, daily maxima ranged from 3.5 to 24 C, with an average of 14 C. From 1 to 16 November, the maxima ranged from 6 to 21 C, with an average of 15 C. The solar irradiance ranged from 4 to 21 MJ·m⁻²·day⁻¹ (mean 13.4 MJ·m⁻²·day⁻¹) during the second half of September and from 4 to 17 MJ·m⁻²·day⁻¹ (mean 12.7 MJ·m⁻²·day⁻¹) in October. Because most sporangia were produced on lower and middle leaves, shaded in part by upper leaves, these irradiances were not considered a major factor in disease development in general or in sporangia viability in particular (3).

Leaf moisture duration, indicated by the dew balance, was influenced mostly by dew but occasionally by rain (Fig. 1A). Between 12 and 27 September, moisture duration ranged from 4 to 20 hr per day (mean 12.4 hr) and was considered favorable for sporulation and infection; the last three days of September were dry. During October, 14 of 31 days were moist, with moisture durations ranging from 6 to 24 hr per day (mean 14.6 hr). These were favorable for sporulation and infection, but 17 dry nights made October generally less favorable than September. During 1 to 16 November, four nights were dry, two days had dew periods shorter than 6 hr, and 10 days had moisture durations ranging from 6 to 24 hr (mean 12.3 hr).

The hours with RH \geq 90% were not correlated with moisture duration, exceeding the hours of dew during some nights and falling short other nights. During September and October, the mean temperatures during moist periods ranged from favorable (16.5 C) to marginal (6.5 C) (Fig. 1B). The minima during the three dry nights of September were above marginal (Fig. 1B) but dropped to marginal or submarginal during seven nights in October and the first 2 wk of November. Freezing killed the plants. The upper leaves were frozen on 10 October, but many lower leaves survived until 9 November and some until 16 November.

Plant development. On 13 September, most plants were large and near flowering, but some were small, and these differences caused large standard deviations in sampled leaf area. During the following weeks, size became more uniform. Leaf area per plant increased from 10,735 \pm 3,894 cm² (average \pm standard deviation in a sample of 10 plants) on 13 September to a maximum of 16,874 \pm 3,030 cm² on 6 October. Live leaf tissue area decreased from 14,230 \pm 1,100 on 25 October to 7,021 \pm 2,056 on 1 November, to near zero on 9 November, to zero on 16 November, when the plants were frozen.

Disease development. We consider the number of lesions to be the most sensitive measurement of disease development. The number of lesions increased from 0.25 \pm 0.1 (average and standard deviation of lesions per 100 cm² of foliage of 10 plants) on 20 September to a maximum of 3.4 \pm 0.06 on 1 November. To assess the number of sporangia for the whole plot, we used sample values to calculate total lesions on all 510 plants. This number increased

from 43,400 lesions on 13 September to 286,300 lesions on 6 October, decreasing thereafter (Fig. 1C). The decline in lesion numbers was directly correlated with the decrease in the living leaf area.

Distribution of lesions and sporulation. Table 1 details leaf areas, categories of lesions, and number of sporangia on 10 October. The results are presented for groups of five leaves, ie, first through fifth, sixth through tenth, etc., except for the two upper (youngest) leaves, which are treated together. The number of lesions as well as the number of sporangia produced in these lesions decreased from the oldest to the youngest leaves. Although this apparent decline is partially the result of the larger area of lower leaves, the number of lesions per 100 cm² of leaf also decreased with decreasing age of leaves, except in the two youngest leaves.

The greatest number of lesions belonged to category C (necrotic without chlorotic margins). In absolute numbers as well as in percentages, category C lesions predominated in the five lower (oldest) leaves (82%), but the number quickly decreased to nothing in the younger leaves. Despite their abundance, necrotic lesions produced a low number of sporangia (6,400 per lesion). For instance, 82% of the lesions on the five oldest leaves were necrotic but produced only 38% of the total sporangia (Table 1). Most sporangia were produced by category B lesions (necrotic with chlorotic margins), which were most frequent on leaves 6–10, constituting 42% of the lesions and producing 81% of the total sporangia. Category B lesions were less frequent on younger leaves (scarce or absent above leaf 21) and contributed fewer sporangia. By contrast, category A lesions (chlorotic) increased from the oldest to the youngest leaves and contributed the most sporangia on leaves 16 to 27.

For the whole plant, 9% of the lesions were chlorotic (category A), 27% were necrotic with chlorotic margins (category B), and 64% were necrotic without chlorotic margins (category C), producing, respectively, 15, 65, and 20% of the total sporangia (Table 1). Category A lesions yielded *P. tabacina* sporangia exclusively, category B lesions yielded *P. tabacina* sporangia and some conidia of *Alternaria* sp. (apparently *A. alternata*), and category C lesions yielded *P. tabacina* sporangia and many *Alternaria* sp. conidia.

The number of sporangia per lesion decreased gradually from about 186 \times 10³ on 13 September to zero on 16 November (Fig. 1C). We use the term "actual sporulation" to designate the amount of spores actually counted on lesions in the morning. To check whether the decrease in number of sporangia per lesion derives from adverse environment or from a decrease in the yielding potential of the lesion as it becomes more necrotic, we put some samples in a moist chamber at 15 C for an additional 24 hr. Sporulation obtained from these samples, termed "potential sporulation," showed that both environmental and lesion factors were involved in the seasonal decrease in number of sporangia per lesion. Although additional hours of moisture increased sporulation (Fig. 1C), the trend nevertheless was down and all lesions became necrotic (category C) and stopped producing sporangia at the end of the season. Necrotic lesions produced many conidia of *Alternaria* sp., and this apparently secondary parasite gradually became more abundant as the season progressed. No sporangia were found when the plants were frozen, on 16 November.

Inoculum pressure. Since we define inoculum pressure as total sporangia in the field, inoculum pressure becomes the product of the number of sporangia per lesion and the number of lesions on all plants. We calculated inoculum pressure during four collection periods on four sequential days each and during less frequent samplings when the epidemic declined.

The four periods of daily collection of sporangia showed that inoculum pressure in the field varied daily. These variations were low during 13 through 16 and 20 through 23 September, when inoculum was scarce and the environment relatively uniform (Fig. 1A,B). Large variations occurred during 27 through 30 September and 4 through 7 October, periods that included some dry nights. Inoculum pressure was high during 27 through 30 September but decreased during the next 3 days. Even though these periods were

dewless, the inoculum remained above zero. Inoculum pressure was relatively high on 4 October, despite dry conditions, and decreased only after the second dry night; an increase was noted following two wet nights (Fig. 1D).

Despite the daily variations, a trend in inoculum pressure during the season was obvious. Many sporangia were produced per lesion at the beginning of the epidemic, but the initial inoculum pressure was low because few lesions were present. Inoculum pressure increased, reaching a peak in late September and early October,

when the number of lesions was highest, then decreased, reaching near zero on 1 and 9 November and zero on 16 November.

Dispersal of inoculum. The curves illustrating the number of spores mechanically trapped resembled the curves for inoculum pressure (Fig. 1D), except when inoculum pressure was very low. This phenomenon was most evident at the beginning and at the end of the season, when small amounts of sporangia were found on leaves but few or none were trapped.

Spore trapping by plants. Infection of trap plants exposed in the

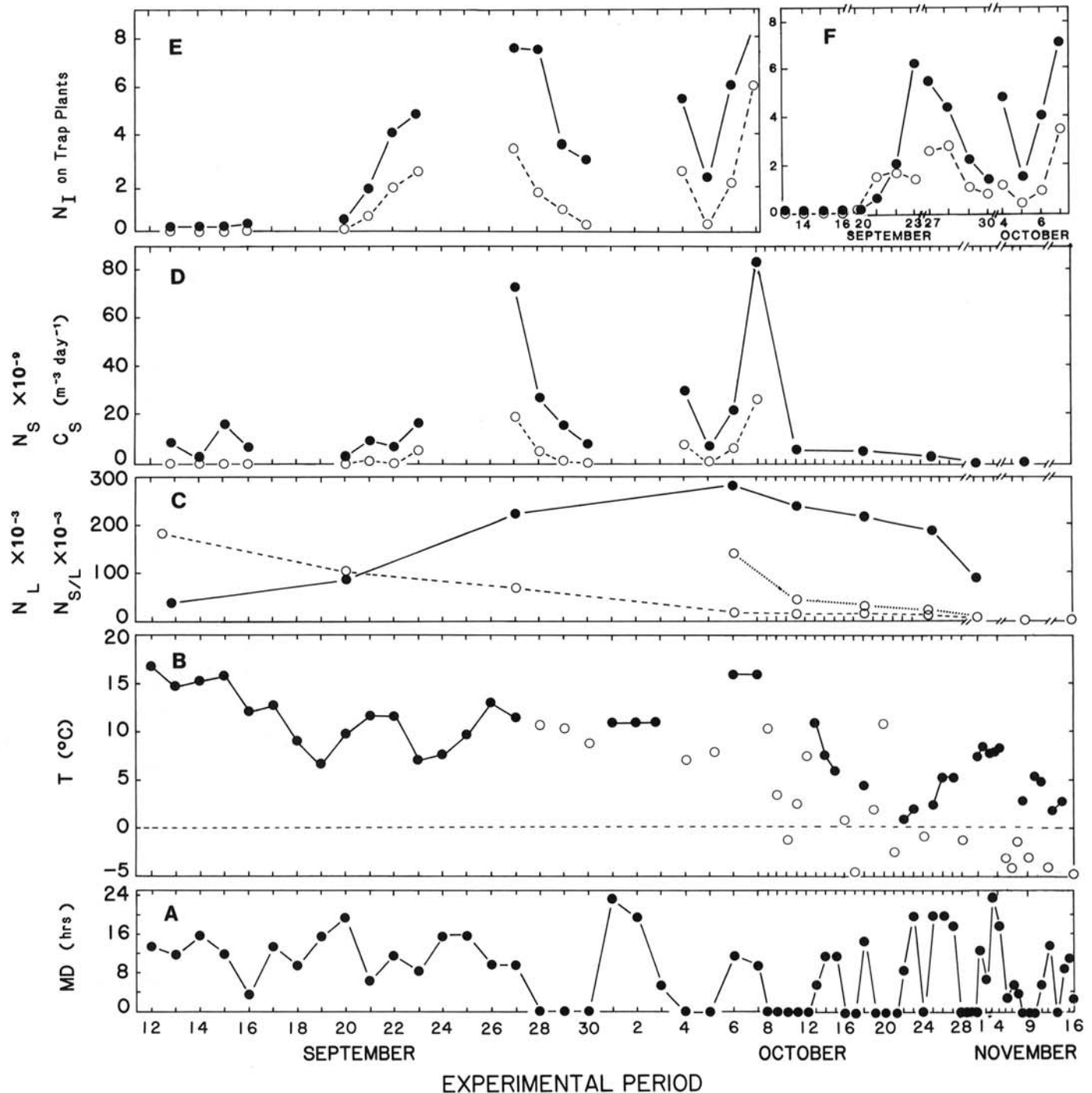


Fig. 1. Selected environmental variables and indicators of epidemic development in tobacco infected by *Peronospora tabacina*: A, Moisture duration (MD). B, Average air temperatures (T) during moist periods (●) and minimum air temperatures during dry nights (○). C, Number of blue mold lesions (N_L) in the field at different times during the epidemic (●); actual sporulation, defined as the number of sporangia per lesion ($N_{S/L}$) present in the morning (○---○), and potential sporulation, defined as sporangia per lesion ($N_{S/L}$) on leaves kept for an additional 24 hr in moist chambers (○.....○). D, Total number of sporangia in the field (N_S) on selected days (●) and in the air (C_S) on selected days (○). E, Number of infections (lesions) (N_I) per 100 cm² of foliage on trap plants exposed in the field for 24 hr and removed to growth chambers without additional wetting (○) and trap plants wetted for 24 hr after removal from the field (●). F, Number of lesions produced on trap plants exposed in the field during 0900–1600 hours (●) and 1600–0900 hours (○). Data are the means of 10 replicate plants. Standard errors ranged from 2.6 to 14% of the mean in 57 cases and from 16 to 50% of the mean in seven cases.

field for 24 hr, then incubated without additional wetting, was controlled by the integrated effects of dispersal, landing, survival of deposited inoculum, plus all the factors affecting infection. Curves illustrating this process (Fig. 1E) follow the patterns of inoculum pressure (Fig. 1D). As with inoculum pressure, numbers of lesions on trap plants were fewer after dry nights, but some developed nevertheless. The limitation of night conditions on infection by deposited sporangia was demonstrated by keeping plants for 24 hr in a moist chamber at 20 C after removal from the field in the morning (Fig. 1E). In general, the additional wetting increased the number of lesions but did not change the shape of the curves illustrating infection without additional wetting.

To enable comparison between plants exposed only for the dry hours (0900–1600) and those in the field during the partly wet hours (1600–0900), both groups of plants were wetted after removal from the field. This trial showed that some infections result from dispersal during the late afternoon and night (Fig. 1F).

DISCUSSION

In summer, epidemics of blue mold in the United States and Canada are associated with temperatures below normal (8). Although disease has been reported in nearly freezing seedbeds (9), blue mold development in suboptimal to marginal temperatures has not been studied. The suggestion that epidemics might be initiated by overwintering sporangia (6) makes quantifying the buildup of inoculum at the end of the growing season important.

The blue mold epidemic described here developed relatively well in the suboptimal conditions of early September. The number of sporangia per lesion was large in early September but decreased with worsening weather late in the month (Fig. 1A–C). Even though the number of sporangia per lesion decreased during this period, the increasing number of lesions induced buildup of inoculum, which peaked in early October, when the environment became marginal (Fig. 1A–C). Despite marginal conditions, the epidemic spread owing to abundant inoculum. Other instances of large amounts of inoculum compensating for unfavorable environments have been reported (13). In our study, the epidemic slowed markedly after the first frost destroyed many upper leaves. Complete destruction of plants and disappearance of inoculum occurred on 16 November, after more frosts (Fig. 1A,B). Therefore, despite the ability of sporangia to withstand freezings and thawings (6), their potential to initiate epidemics in the spring is negated by their absence.

Our study also revealed phenomena important in the main growing season. The apparent absence of free leaf moisture during several nights, as measured by the dew balance, decreased but did not stop sporulation and infection (Fig. 1). *P. tabacina* can sporulate under conditions of high RH (7), but it is also possible that some sporangia produced during previous moist nights are retained on leaves and disperse later, as in other diseases (4), and that our dew balance was not sensitive enough to indicate minute amounts of free leaf moisture. Whatever the reason for sporulation, infection that requires free leaf moisture but occurs at low frequency during relatively dry nights suggests that some free leaf moisture was present.

According to data obtained from mechanical spore traps, most sporangia disperse early in the day (1,14). The occurrence of lesions on trap plants exposed in the field confirmed this; the bulk of the dispersal was confined to 0900–1600 hours, 0900 hours coinciding roughly with disappearance of leaf moisture. Nevertheless, about one-third of the daily total of lesions occurred on trap plants exposed from 1600 to 0900 hours (Fig. 1F). Successful afternoon and night dispersal has been noted in other diseases in which mechanical traps suggested that spores were dispersed mainly during the day, because the traps underestimated the importance of spore dispersal during late afternoon and at night (4).

In trap plants exposed for 24 hr, infection integrated all biotic and environmental factors and was proportional to inoculum pressure during any given day of exposure (Fig. 1D,E). When similarly exposed plants were kept for an additional 24 hr under favorable conditions, 1.4–10.5 times as many lesions formed than on plants without additional wetting (Fig. 1F). This suggests that the night conditions during late fall inhibited infection. The fact that in our trials most lesions were formed on the oldest and lowest leaves (Table 1) suggests the importance of microclimatic effects. Most lesions on old leaves were necrotic (category C), with a low sporulation potential typical in biotrophic pathogens (13). Later in the season, the number of new infections decreased and necrotic lesions also dominated on young leaves. Whereas chlorotic (category A) lesions produced *P. tabacina* sporangia exclusively, lesions that were necrotic with chlorotic margins (category B) also produced conidia of *Alternaria* sp. and necrotic (category C) lesions produced mainly (during the season) or only (at season's end) conidia of *Alternaria* sp. It is possible that, in addition to the adverse effects of weather and of necrotization on sporulation of biotrophic pathogens (13), sporangia of *P. tabacina* disappear because of an antagonistic action of *Alternaria* sp.

TABLE 1. Average distribution of *Peronospora tabacina* lesions on tobacco leaves from 10 plants and average number of sporangia produced on these lesions according to leaf position in the canopy

Leaf position ^a	Total leaf area ^b (cm ²) (percent of total)	Total number of lesions (sporangia × 10 ⁻³)	Percent of lesions (sporangia)	Number of lesions (sporangia × 10 ⁻³) per 100 cm ² of leaf ^c	Number of lesions (sporangia × 10 ⁻³) per category ^d of lesion			Percent ^e of lesions (sporangia) per category ^d of lesion		
					A	B	C	A	B	C
1–5	6,659 (39)	321 (4,451)	57.0 (40)	4.8 (67)	1 (19)	57 (2,751)	263 (1,681)	0.2 (0.4)	18 (62)	82 (38)
6–10	5,259 (31)	168 (4,226)	30.0 (37)	3.2 (80)	8 (241)	70 (3,411)	90 (574)	4 (6)	42 (81)	54 (13)
11–15	2,957 (18)	45 (1,682)	8.0 (15)	1.5 (57)	18 (578)	22 (1,073)	5 (32)	40 (34)	49 (64)	11 (2)
16–20	1,351 (8)	17 (572)	3.0 (5)	1.3 (42)	15 (480)	2 (92)	0	89 (84)	11 (16)	0
21–25	560 (3)	8 (257)	1.4 (2)	0.3 (46)	8 (253)	0.1 (5)	0	99 (98)	1 (2)	0
26–27	88 (1)	2 (70)	0.4 (1)	2.5 (79)	2 (69)	0	0	100 (100)	0	0
Total	16,874	561 (11,258)			52 (1,640)	151.1 (7,332)	358 (2,287)	9 (15)	27 (65)	64 (20)
Average number of sporangia per lesion (×10 ⁻³)					20.1	31.5	48.5	6.4		

^a Position 1 at bottom of plant and 27 at top.

^b Assessed by sorting leaves into six size groups. Ranges of leaf areas and standard deviations were: 1,131–1,540 (SD 213–5,651) for leaf position 1–5; 869–1,148 (SD 227–406) for leaf position 6–10; 375–882 (SD 121–378) for leaf position 11–15; 167–375 (SD 0–121) for leaf position 16–20; 88–148 (SD 29–101) for leaf position 21–25; and 39–49 (SD 48–49) for leaf position 26–27.

^c Calculated from data presented in columns 2 and 3.

^d A = chlorotic lesions, B = necrotic lesions with chlorotic margins, C = necrotic lesions without chlorotic margins. Standard deviations for number of sporangia × 10⁻³ collected from various leaves were 0.8–7.5 for category A, 1.2–49.9 for category B, and 1.3–65.4 for category C.

^e Based on total numbers across various categories in each size group.

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