

**Factors Affecting Systemic Infection of Tobacco
by *Peronospora tabacina***

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ABSTRACT

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Economic crop losses in tobacco induced by *Peronospora tabacina* in the United States during 1980 were due in part to systemic plant infection. Controlled environment studies were conducted to assess the effects of site of infection, plant stress, and temperature on development of systemic infection. Results showed that systemic infection of apical meristematic tissue caused severe stunting. Lateral bud and stem infections did not produce dramatic stunting, and many infected plants appeared healthy. Lateral bud infections on nonstressed plants were the most difficult to diagnose. Similar plant growth rates occurred between plants

with lateral or stem infections and healthy plants. Systemic infection occurred more readily at lateral buds than through the apical bud or stem. Plants stressed from transplant shock were more susceptible to systemic infection than plants that were not stressed. Colonization of the pathogen within the plant as measured by vascular necrosis was greater in nonstressed plants. Systemic symptoms developed at all temperatures tested. A significant temperature effect was observed only for rate of plant growth. Infected stem tissue did not support sporulation of the pathogen.

Additional keyword: blue mold.

During 1979 and 1980, widespread field epidemics of tobacco blue mold caused by *Peronospora tabacina* Adam (syn. *P. hyoscyami* d. By. f. sp. *tabacina*) occurred in the United States and Canada. Leaf lesions supporting active sporulation were observed throughout the growing season (4). Additionally, widespread occurrence of systemic blue mold was observed. Systemic infection was characterized by leaf deformation and severe stunting of infected plants and was economically more important than leaf infection in several tobacco-growing states (13). North Carolina suffered a 40% loss of the burley tobacco

crop due to systemic plant infection. Major crop losses due to systemic blue mold have also been recorded in other tobacco-growing areas of the world (1,5,11).

In systemic infection, the fungus grows both intercellularly and intracellularly and spreads throughout the cambium, external phloem, and into the xylem, resulting in severe necrosis (5,8,9). Systemic infection often occurred when the pathogen advanced from leaf lesions into the leaf midrib and then entered the stem via the petiole (3,5). However, systemic infection has been observed in the absence of leaf lesions in the United States. These infections may have occurred through infected leaves that later dropped from the plant either in the plant seedling bed or field (11). It also is possible that ingress into the stem by the pathogen

may occur via other plant parts and may be affected by the condition of the plant or environmental parameters.

The objective of our study was to assess the effect of site of infection, plant stress, and temperature on the development and manifestation of systemic blue mold.

MATERIALS AND METHODS

Experiments were conducted in 9-m² controlled environment chambers at the Southeastern Plant Environmental Laboratory at North Carolina State University. Chamber conditions and plant culture methods were similar to those previously described (10) unless otherwise stated.

Seed of tobacco (*Nicotiana tabacum* L. 'Burley 21') were germinated in 70-mm-diameter styrofoam cups containing a sand:Peat-lite (W. R. Grace Co., Traveler's Rest, SC) mixture (1:1, v/v) at 22 ± 1 C in a growth cabinet (Sherer CEC 38-15, Rheem Mfg. Co., Asheville, NC). Three weeks after germination, plants were thinned to one plant per cup and moved to an air-conditioned greenhouse at day/night temperatures of 26/22 C. Four weeks later, half the plants were transplanted into 25.4-cm-diameter pots containing a gravel:Peat-lite mixture (1:1, v/v). The remaining plants were transplanted after an additional 3 wk. While the plants transplanted last still were in transplant shock, all plants were moved into growth chambers and watered with a standard nutrient solution (2) as needed. Four replicates were conducted over time.

For each replication, 28 plants were placed into each of four growth chambers and their height recorded. Fourteen plants had been transplanted for 3 wk and were not stressed, whereas the other 14 were newly transplanted and had not recovered from transplant shock. Plants were placed equidistantly on metal trucks 0.8 m above the chamber floor and 1.25 m from chamber light barriers, acclimated for 1-2 days at day/night temperatures of 25/20 C, then inoculated.

Two plants of each stress treatment served as uninoculated controls; 12 were inoculated with an isolate of *P. tabacina* (KPT-79) collected from systemically infected tobacco in Kentucky during the 1979 epidemic. Each plant was inoculated at one of three sites: the apical bud, the lateral bud, or directly onto the stem surface, making a total of four plants per inoculation site within each stress treatment. Lateral buds and stems were inoculated at approximately midheight of the plant. Plants were inoculated with a micropipet with 20 µl of spore suspension adjusted to approximately 10⁴ sporangiospores per milliliter with the aid of a hemacytometer.

After inoculation, atomized distilled water was fog-misted onto plants for 12-sec periods, twice per hour for 24 hr, to maintain free moisture for spore germination (3). After infection (24 hr), day/night chamber temperature treatments of 20/9, 24/13, 28/17, or 32/21 C were imposed, according to a randomized complete block design replicated four times, each replicate lasting an average of 26 days.

At the end of each replicate, each plant was rated plus or minus based on the presence or absence of visible symptoms of systemic infection; plant height also was recorded. In addition, the stem of each plant was split longitudinally, the extent of vascular necrosis due to systemic infection was measured, and the percentage of successful infection (as determined by vascular necrosis) was calculated. Vascular necrosis had been previously described as a result of colonization of stem tissue by the pathogen (9). Split stem tissue with necrotic lesions was placed in moist chambers for 48 hr at 21 C to determine whether sporulation would occur.

Data analysis. Data from two trials of the experiment were analyzed and interpreted separately. Trials were conducted and analyzed as a split-split-plot with temperatures as whole plots, stress treatments as subplots, and inoculation site as subsubplots. Each dependent variable was analyzed with the general linear models (GLM) procedure of SAS (12).

Results indicated that differences among treatments were similar between trials. In addition, comparison of the error mean

squares of each trial with an *F*-test showed homogeneous variances. Because the trials were similar, the data were combined for an overall analysis, and these results are reported. Whenever the temperature or temperature interaction term in the ANOVA model was significant, regression analysis was performed.

Some analyses resulted in nonestimability of least square (LS) means if missing data occurred. LS means are the expected value of class or subclass means that would occur with a balanced design. To produce LS means, the highest order nonsignificant interaction term was dropped from the model. LS means then were separated with the PDIFF option under GLM.

RESULTS

Site of infection was an important factor in determining the proportion of successful systemic blue mold infections that occurred. Results indicated that systemic infection resulting from lateral bud inoculation occurred about three times as often as infection from either apical or stem inoculations (Table 1). Even though entry into the plant was easier at the lateral bud, only 30% of the attempts to produce systemic infection at this site were successful. Stem infections often were characterized by visible necrosis of the epidermal layer of the stem, whereas lateral and apical infections rarely produced visible necrotic symptoms on epidermal tissue. However, lateral and apical infections produced considerable necrosis of the vascular cambium. In severe cases where the stem was girdled with necrotic tissue, plants were prone to breakage at the infection site. Sporulation by the pathogen was not observed after incubation of necrotic cambium or epidermal stem tissue containing fungal mycelium in high humidity at 21 C for 48 hr.

Plant stress also was important in the proportion of successful systemic blue mold lesions that occurred and in the length of the vascular necrosis within the stem. Stressed plants over all inoculation sites had about twice the number of infections when compared with nonstressed plants (Table 1). Apical meristem infections were about three times as successful on stressed plants as they were on nonstressed plants.

Necrosis of the vascular cambium was significantly more extensive in nonstressed plants, with an LS mean and standard error of 9.9 ± 0.44 cm compared with 6.03 ± 0.42 cm for stressed plants.

A significant interaction of site of infection and plant stress influenced our ability to visually diagnose a systemically infected plant, i.e., to diagnose without sectioning or splitting the stem. Systemic infection was the most difficult to diagnose on nonstressed plants with lateral bud infections (Table 2). Correct diagnosis occurred only 47% of the time at this site and stress treatment. Diagnosis of systemic infection on stressed plants with lateral bud infections was similar to that of plants with stem infections. Stem infections were somewhat easier to diagnose because most had visible necrosis of the epidermal layer of the

TABLE 1. Effect of plant stress and site of inoculation on the percentage of successful systemic infections of tobacco caused by *Peronospora tabacina*^a

Inoculation	Plant stress ^b		Site marginal means
	Stressed	Nonstressed	
Apical meristem	15.6 ± 3.5 ^c	4.7 ± 3.5	10.2 ± 2.4 a
Lateral bud	35.9 ± 3.5	25.0 ± 3.5	30.5 ± 2.4 b
Stem	16.4 ± 3.5	8.2 ± 3.5	12.3 ± 2.4 a
Stress marginal means	22.6 ± 1.9 a ^d	12.6 ± 1.9 b	

^aEach inoculation consisted of a 20-µl drop of a spore suspension adjusted to approximately 10⁴ spores per milliliter.

^bStressed plants were in transplant shock; nonstressed plants were transplanted 3 wk before inoculation.

^cData represent least square means ± standard errors.

^dMeans within rows and columns followed by a common letter are not significantly different as determined by pairwise *t*-tests at *P* ± 0.01.

stem. Apical infections were diagnosed correctly more than 90% of the time, due mainly to the dramatic stunting symptoms associated with invasion of the apical meristem by the pathogen.

The interaction between site of infection and plant stress also significantly influenced the rate of growth of systemically infected plants. Separation of LS means for the interaction indicated that stressed plants had a smaller rate of growth than nonstressed plants at all infection sites except apical (Table 2). Successful apical infection resulted in dramatic stunting of both stressed and nonstressed plants in comparison with that seen in controls and for other infection sites. Growth rate of stressed plants with lateral bud and stem infections was similar to that of the controls.

A significant interaction between site of infection and temperature affected rate of plant growth. The interaction was due primarily to the severe stunting of plants at the apical infection site at all temperatures (Fig. 1). Analysis of variance and mean separation of the interaction LS means indicated that apical infections at all temperatures were significantly different from other site/temperature combinations at $P < 0.001$. Because temperature is a quantitative variable, regression analysis would be appropriate to determine the effects of temperature on rate of plant growth for each inoculation site. Since the effect on plant growth rate was so drastically different between apical infections and the others, comparison of regressions between them would not be meaningful. Therefore, analysis of variance was rerun deleting the apical site.

TABLE 2. Effect of infection site and plant stress on the visual diagnosis of infection and on the growth rate of tobacco systemically infected with *Peronospora tabacina*^a

Site	Plant stress ^b	Correct visual diagnosis ^c (%)	Rate of plant growth (cm/day)
Control	Stressed	96.9 ± 4.7 ^d ab	1.5 ± 0.09 c
	Nonstressed	100.0 ± 0.0 a	3.0 ± 0.09 a
Apical	Stressed	92.2 ± 4.7 ab	0.4 ± 0.15 d
	Nonstressed	91.7 ± 4.7 ab	0.5 ± 0.24 d
Lateral	Stressed	71.9 ± 4.7 c	1.2 ± 0.10 c
	Nonstressed	46.9 ± 4.7 d	2.6 ± 0.13 b
Stem	Stressed	82.8 ± 4.7 bc	1.1 ± 0.13 c
	Nonstressed	84.4 ± 4.7 abc	2.9 ± 0.15 ab

^aPositive visual diagnosis was made if plants appeared stunted, if leaf malformation was evident, or if necrosis of the stem epidermis was present.

^bStressed plants recently were transplanted and in transplant shock; non-stressed plants had been transplanted 3 wk before inoculation and were not in transplant shock.

^cCorrect diagnosis was confirmed by splitting the stem longitudinally and observing for the presence or absence of vascular necrosis.

^dData represent least square means ± standard error. Means followed by a common letter are not significantly different as determined by pairwise *t*-tests at $P \leq 0.01$.

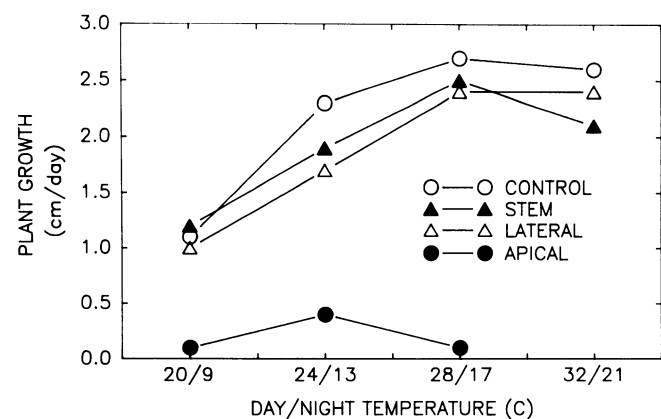


Fig. 1. Effect of temperature and infection site on the rate of growth of tobacco plants systemically infected with *Peronospora tabacina*.

In this analysis, the temperature/site interaction no longer existed, and no differences among lateral, stem, or healthy plants occurred. Temperature effects were significant at $P < 0.05$, so regression analysis was performed on the temperature data for plants infected at the lateral bud or stem infection sites. The quadratic model, $Y = b_0 + b_1X + b_2X^2$, where Y = rate of plant growth in centimeters per day, b_0 , b_1 , and b_2 = parameter coefficients for intercept, X , and X^2 , respectively, and X = daytime temperature, was found to be the most appropriate model to describe the effect of temperature on rate of plant growth (Fig. 2). Rate of growth increased with increasing temperatures for plants infected with systemic blue mold at the lateral bud or stem infection sites.

DISCUSSION

Site of infection and plant stress were found to be important in the development of systemic blue mold and in the losses that subsequently resulted from infection. During the 1980 blue mold epidemic in the United States, severe stunting of tobacco plants systemically infected with *P. tabacina* resulted in significant crop losses. Our studies indicated that severe stunting occurred when infection and necrosis of the apical meristem were observed. Marte and Caporali (7) found that apical infection interfered with cell division and elongation that may lead to disruption of apical dominance of the tobacco plant.

Although apical infections resulted in the most dramatic symptoms of systemic blue mold, this was not the predominant site of infection. Apical meristematic tissue is protected somewhat from direct infection by spores due to its enclosure by leaf primordia. In most cases, a healthy plant would have time for the apical meristem to grow away from the infected area. Systemic infection was more likely to occur via infection through the lateral bud than through the stem or apical meristem. Success of lateral inoculations may be due, in part, to the collection of water drops in the leaf axil, providing free moisture for spore germination. Stem tissue would tend to dry faster than lateral buds after a wetting period and would not offer as conducive an environment for infection.

Although infection was more likely to occur at the lateral bud, visual diagnosis of infection at this site was very difficult. In fact, we correctly identified only 47% of systemic lateral infections that occurred on nonstressed plants 26 days after inoculation. These results indicate that much of the systemic infection that might occur in field situations may go undetected until late into the season. In cases where apical infections occur soon after transplanting, severe stunting symptoms may develop soon enough to allow the grower to disk under infected plants and replant. However, with lateral or stem infections, symptoms occur much later and often too late for the grower to replant. Stem infections were easier to diagnose than lateral infections because epidermal necrosis usually was present. Lateral infections were

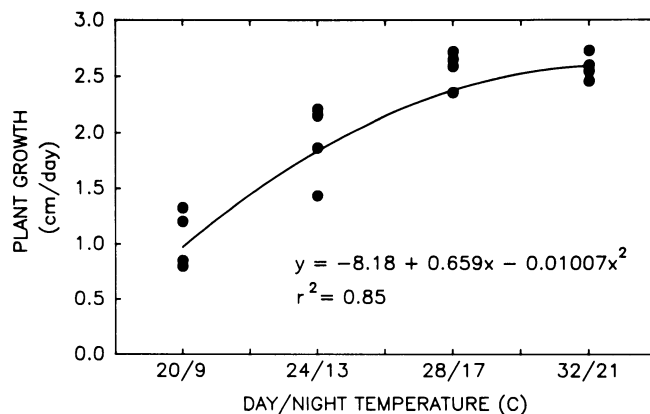


Fig. 2. Relationship between temperature and rate of growth for tobacco systemically infected with stem and lateral bud infections of *Peronospora tabacina*. The X value in the equation represents daytime temperature.

confined mostly to the vascular region of the stem and were not visible unless the epidermal layer was removed.

Stress associated with transplanting significantly increased systemic infection at all infection sites in our studies. The effect of plant stress was most pronounced at the apical meristem site, where successful infection tripled in comparison with the amount in nonstressed plants. In commercial production, tobacco transplants are treated harshly during removal from the plant beds. Tender plants are pulled from the ground, tearing and removing roots in the process. These plants may be held for 1-2 days before transplanting. After planting, growth may not resume for 1 wk or more, depending on environmental conditions due to transplant shock. Our studies indicated that infection at this time may allow the pathogen to colonize and destroy the apical meristematic region of the plant. Conversely, a healthy, growing plant would have time for the apical meristem to grow away from the infected area. Systemic infection still would be present on the stem or lateral bud, but the plant would not exhibit severe stunting.

As with apical infection, lateral bud and stem infections were more likely to occur on stressed plants. Stressed plants grew less than nonstressed plants for all infection sites studied except apical, where no difference in growth rate occurred. This indicated that apical infection can occur on nonstressed plants and that little or no plant growth would occur subsequently. Systemic blue mold development also may be affected by other types of plant stress, including drought or nutritional stress.

Systemic symptoms occurred at all temperatures studied. Although temperature may be important in the establishment of infection, systemic blue mold continued to develop within the plant over the range of temperatures used in this study. Our results agree with the observations of Mandryk (6), who reported that the most outstanding feature of systemic infection in Australia was its destructiveness over very wide temperature regimes. Temperature treatments in this study were imposed 24 hr after inoculation, so spore germination and penetration into the plant occurred during conducive environmental conditions before temperature treatments. Temperature did influence rate of plant growth, as was expected.

Sporulation was observed only when infection proceeded from the tobacco stem through the leaf petiole and into leaf tissue. This process seldom occurred. No sporulation on stem or petiole tissue was observed. Lack of sporulation minimizes the secondary inoculum necessary for epidemic disease development. Thus,

although systemic infection may completely destroy a single plant, it does not contribute significantly to subsequent spatial or temporal disease development and spread.

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