# The Effects of Plant Age and Leaf Position on the Susceptibility of Tobacco to Blue Mold Caused by *Peronospora tabacina*

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This work was supported in part by the Burley Tobacco Council of Kentucky, R. J. Reynolds Corporation, and U.S. Department of Agriculture Cooperative Grant 58-7B30-3-540.

Kentucky Agricultural Experiment Station Journal Series Paper 85-11-46.

The authors thank R. S. Ferriss for assistance with the statistical analysis and N. S. Doubrava for technical assistance.

Accepted for publication 7 November 1985 (submitted for electronic processing).

## ABSTRACT

Reuveni, M., Tuzun, S., Cole, J. S., Siegel, M. R., and Kuć, J. 1986. The effects of plant age and leaf position on the susceptibility of tobacco to blue mold caused by *Peronospora tabacina*. Phytopathology 76:455-458.

A leaf disk assay was used to monitor susceptibility of greenhouse and field-grown tobacco plants to blue mold, caused by *Peronospora tabacina*. The susceptibility of leaf disks to the pathogen was influenced by the age of the plants and inoculum concentration. The area of lesions and sporangial production on leaf disks and attached leaves decreased with increasing age of plants. The position of the leaves on plants had less influence on susceptibility than did plant age. These data suggest the presence of a defense mechanism that appears to be dependent on the age of the plant but not the age of the leaves.

*Additional key words*: *Nicotiana tabacum*.

Cultivars of tobacco (*Nicotiana tabacum* L.) commercially grown in the United States are susceptible to blue mold caused by *Peronospora tabacina* Adam (3,9). Hill (6) observed that leaves of field-grown tobacco plants are susceptible to blue mold at all leaf positions on the plant throughout the growing season. However, in later studies (7,8) he reported that the position of leaves of greenhouse-grown tobacco plants influenced their susceptibility to blue mold. Lower leaves inoculated with drops of inoculum suspension containing sporangia of *P. tabacina* produced more lesions of blue mold than did upper leaves. The relationship of age of various host plants to disease susceptibility has been discussed by other workers (1,4,5,10,11,13,14). Warren et al (13) reported that the age of potato plants and position of leaves on stems influenced susceptibility to *Phytophthora infestans*. Leaves of the youngest plants were most susceptible. With older plants, leaf position also was important; upper and lower leaves were more susceptible than those of an intermediate position. More recently Allen et al (2) reported that lower leaves of sunflower plants are more susceptible to infection by *Alternaria helianthi* than are upper leaves.

Our observations on the susceptibility of tobacco plants to blue mold differed from those published by Hill (7,8). This investigation was, therefore, designed to study the effect of plant age and leaf position on the susceptibility of tobacco to blue mold. In addition, a quantitative laboratory assay with leaf disks was developed for monitoring susceptibility to the fungus.

## MATERIALS AND METHODS

**Plants.** Seedlings of burley tobacco, *N. tabacum* 'Ky-14,' were grown in the greenhouse (20–26 C in fall and winter and 22–33 C in spring and summer, 14 hr of light per day). About 4 wk after seeds were sown, seedlings were transplanted to plastic pots (16.5-cm top diameter) containing Pro-Mix Bx (Premier Peat Moss Corp., Marketing, New York, NY), a commercial potting mixture. Five times a week, plants were watered to saturation with a 0.1% 20-20-20 (N-P-K) fertilizer solution.

**Leaf disk assay.** Unless stated otherwise, half leaves were removed from greenhouse-grown plants with a razor blade. The opposite half leaves and midrib were left intact and attached to the plant. Detached half leaves were immediately placed into moistened plastic bags, brought to the laboratory and ten leaf disks (each 18 mm in diameter) were cut from each detached half leaf with a No. 13 cork borer.

Leaf disks were placed, adaxial side up, in 10-cm-diameter plastic petri dishes containing filter paper wetted with 4 ml of an aqueous solution of kinetin, 1 $\mu$g/ml.

**Pathogen and inoculation.** An isolate of *P. tabacina* (Ky-79), obtained in 1979 from plants in a field near Georgetown, KY, was maintained continuously on burley tobacco grown in a growth chamber. Inoculum was obtained from freshly sporulating infected leaves on 7- to 12-wk-old plants 6–7 days after inoculation. Sporangia were gently brushed into a small quantity of distilled water, collected on a filter (3.0 $\mu$m) and resuspended in distilled water to a known concentration of sporangia per milliliter of $H_2O$.

The attached half leaves and the leaf disks in petri dishes were inoculated by applying drops or by spraying. Attached half leaves were each inoculated by placing 10 drops ($\sim$ 3 $\mu$l per drop) of inoculum on the upper surface. One drop of inoculum was placed on the middle of the upper surface of each disk. The leaves, leaf disks, and the inner surface of petri dish covers were sprayed with distilled water after inoculation. When inoculation was by spraying, the upper surface of the attached leaf was uniformly sprayed with sporangial suspensions of a known concentration delivered from a glass chromatography sprayer. The disks were inoculated in the petri dishes with the aid of an air-brush sprayer (Type H 1 W/H-3-OZ, Paasche Airbrush Co., Chicago, IL). Each petri dish was sprayed with 1.5 ml of a known concentration of sporangial suspension for 10 sec. The inner surface of petri dish covers were sprayed with distilled water. Six glass microscope slides (three slides in each group) were sprayed in the same manner as the petri dishes. The sporangia on the slides were counted to calculate the number of sporangia per square centimeter of leaf disk.

After inoculation, plants were covered with plastic bags sprayed lightly on the inside with water and incubated at 19 C for 20 hr in the dark. Plants were then uncovered and kept in a growth chamber (23 C, 60–70 $\mu$Ein$\cdot$m$^{-2}\cdot$sec$^{-1}$, 12 hr of light per day). The petri dishes with leaf disks were incubated under the same conditions.

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Four days after inoculation, leaf disks were transferred from filter papers to sponge rubber pads (9-mm thick and 9-cm diameter) each with ten 14-mm-diameter punched holes. The pads had been previously wetted with an aqueous solution containing 1 $\mu$g of kinetin per milliliter and placed in petri dishes. Disks were placed adaxial side up over the holes to allow sporulation on both sides of the infected leaf disks. Disease severity was recorded 7 days after inoculation; ratings were based on a 0-4 scale, as previously described (12): 0, no lesions; 1, yellow lesions and <25% of the leaf disk area chlorotic; 2, yellow lesions and >24%<51% of the leaf disk area chlorotic; 3, yellow lesions and >51%<75% of the leaf disk are chlorotic; and 4, 75–100% of the leaf disk area chlorotic.

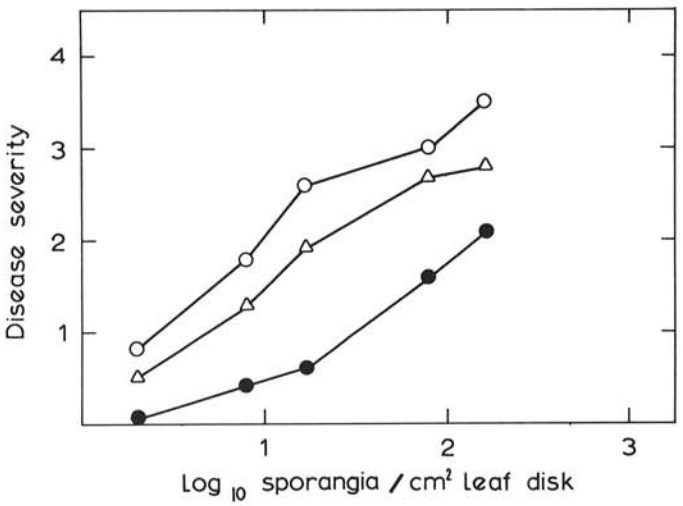


**Fig. 1.** Effect of inoculum concentration and plant age on the susceptibility to blue mold of leaf disks from greenhouse-grown tobacco. Means of 40 disks of each concentration. Symbols: o, disks taken from 7-wk-old plants, △, 9-wk-old plants/and ●, 11-wk-old plants.

TABLE 1. The effect of tobacco plant age on the susceptibility to blue mold of attached leaves and leaf disks inoculated by spray or drops containing sporangia of *Peronospora tabacina*

| | Disease severity[a] | | | |
|---|---|---|---|---|
| | Spray | | Drops | |
| Plant age (wk) | Attached half leaf | Leaf disks | Attached half leaf | Leaf disks |
| 9 | 3.8 A[b] | 3.3 A | 2.2 A | 0.6 A |
| 11 | 2.4 B | 1.8 B | 0.7 B | 0.4 A |
| 13 | 2.6 B | 2.2 B | 0.3 B | 0.0 B |
| 15 | 1.2 C | 1.6 C | 0.0 C | 0.0 B |

[a] Disease rating based on a 0–4 scale as described in Materials and Methods.
[b] Numbers within a column followed by different letters are significantly different ($P = 0.05$) according to Duncan's new multiple range test.

In some experiments, sporulation was induced on the attached leaves and/or on leaf disks. To induce sporulation, plants and leaf disks were kept under moist conditions at 20 C in the dark for 24 hr. The sporangia were washed from 10 leaf disks into a known volume of a fixative solution (ethanol, formaldehyde, and acetic acid [90:5:5, v/v]) and counted with the aid of a hemocytometer (four counts per 10 leaf disks).

**Effect of inoculum concentration and plant age on the susceptibility of tobacco leaf disks.** Fifty disks were taken from the fourth leaf from the top from each of four plants of each of three plant ages. The disks from each leaf (plant) were divided randomly into groups of 10, and each group was placed in a petri dish. Sporangial suspensions were diluted to obtain inocula containing 2, 8, 17, 83, and 166 sporangia per square centimeter. Each concentration was applied to 10 disks from each of the four plants of each plant age. Disks were kept for 7 days on filter paper.

**The effect of plant age on the susceptibility of attached leaves and leaf disks.** *Experiment I.* Two half leaves (leaves 3 and 4 from the top) of 9-, 11-, 13-, and 15-wk-old plants were detached from each of four plants of each age, and 10 disks were taken from each. The attached half and the leaf disks were inoculated either with drops or spray of a suspension containing $5 \times 10^4$ sporangia per milliliter.

*Experiment II.* Leaves 4, 7, and 9 (from the top) (fully expanded and without symptoms of senescence) were detached from 9-, 12-, and 15-wk-old plants, respectively. The position of each leaf on the plant was recorded. Leaf disks were taken from each leaf and inoculated as previously described. Blue mold severity and sporangial production were determined on the 7th day after inoculation.

*Experiment III.* Halves of three fully-expanded, nonsenescent leaves were removed from the top, middle, and the lower part of 9-, 12-, and 15-wk-old plants. The attached half leaves with midribs, as well as disks taken from detached half leaves, were inoculated with sporangial suspensions of *P. tabacina* as previously described.

**Susceptibility of field-grown tobacco plants to blue mold as determined by leaf disk assay.** Seedlings of burley tobacco (cultivar Ky-14), about 5 wk old, were transplanted to the field at the Spindletop Research Farm of the University of Kentucky. Leaves of the same developmental stage (leaf 4 from the top) were collected at various intervals after transplanting and brought to the laboratory in moistened plastic bags. Leaf disks were taken from each leaf and inoculated as previously described with 13 ±2 sporangia per square centimeter of leaf area.

TABLE 2. The effect of leaf position and tobacco plant age on the susceptibility of leaf disks to blue mold[v]

| Leaf position (from the top) | Disease severity[w] on plants at week: | | | Sporangial production[x] (sporangia/cm² of leaf disk) on plants at week: | | |
|---|---|---|---|---|---|---|
| | 9 | 12 | 15 | 9 | 12 | 15 |
| 1[y] | 2.15 A[z] | 2.70 A | 1.72 B | 2,043 A | 714 A | 298 A |
| 2 | 2.38 A | 3.03 A | 1.96 AB | 3,351 A | 633 A | 258 A |
| 3 | 3.03 A | 2.31 A | 2.28 AB | 4,260 A | 2,023 A | 179 A |
| 4 | 2.60 A | 2.90 A | 2.13 AB | 3,746 A | 752 A | 258 A |
| 5 | | 2.35 A | 2.55 AB | | 595 A | 694 A |
| 6 | | 2.90 A | 2.55 AB | | 1,448 A | 1,228 A |
| 7 | | 2.61 A | 2.33 AB | | 1,090 A | 1,269 A |
| 8 | | | 2.40 AB | | | 793 A |
| 9 | | | 2.96 A | | | 357 A |

[v] Airbrush sprayer was used for inoculation with approximately 15 sporangia per square centimeter of leaf disk area.
[w] Based on a 0–4 scale means are based on 20 leaf disks from each leaf with three plants of each age. Severity was determined on leaf disks placed on filter paper for 7 days and on sponge rubber pads following 4 days on filter paper as described in Materials and Methods.
[x] Numbers based on 10 leaf disks placed on sponge rubber pads following 4 days on filter paper, from each leaf with three plants of each age.
[y] Most recent fully expanded leaf.
[z] Numbers within a column (leaf position) followed by the same letter do not differ ($P = 0.05$) according to the Waller-Duncan $K$-ratio $t$-test ($K$-ratio = 100).

## RESULTS

**Effect of inoculum concentration and plant age on the susceptibility of tobacco leaf disks.** Disease was more severe on disks obtained from younger plants than on those obtained from older plants (Fig. 1). Blue mold severity on leaf disks of plants at all ages was positively related to inoculum density.

**The effect of plant age on the susceptibility of attached leaves and leaf disks.** *Experiment I.* All attached leaves and leaf disks of 9-wk-old plants were more susceptible than those from 11- and 13-wk-old plants and those from plants of the two intermediate ages were more susceptible than those from 15-wk-old plants (Table 1). Low disease severity was recorded when attached leaves and leaf disks of the oldest plants were inoculated by spraying, but no disease was recorded for those inoculated with drops. The overall means of blue mold severity from attached leaves and leaf disks were 1.6 and 1.4, respectively. This indicates no significant difference in their reaction to the pathogen.

*Experiment II.* Leaves at different positions on plants of the same age were equally susceptible to blue mold, except for a significant difference in disease severity between leaves 1 and 9 on 15-wk-old plants (Table 2). Separate analyses of the four leaves from the top or the bottom of plants supported these findings (Table 3). However, the age of the plants influenced their susceptibility to blue mold (Table 4). Sporangial production on infected leaf disks from 9-wk-old plants was significantly greater than on disks from 12- or 15-wk-old plants.

*Experiment III.* Results of overall factorial analyses of variance for disease severity indicated significant effects of age, age × detachment, and age × detachment × leaf position. There was no significant effect of detachment on leaf susceptibility. For both methods, detached and attached leaves of younger plants were more susceptible to blue mold than those of the older plants. A less significant effect of the leaf position was indicated (Tables 5 and 6). The significance of differences due to plant age was far greater ($P = 0.001$) than that of the differences due to leaf position ($P = 0.05$).

**Susceptibility of field-grown tobacco plants to blue mold as determined by leaf disk assay.** The susceptibility of tobacco plants to blue mold decreased with the age of the plants (Fig. 2). Disease severity recorded 42 and 49 days after transplanting was reduced by 50 and 90%, respectively, compared to that of plants 27 days after transplanting.

## DISCUSSION

Leaf disks obtained from field- and greenhouse-grown plants and attached half-leaves were used in this study. This enabled us to monitor and compare the susceptibility of plants to *P. tabacina* in the laboratory, greenhouse, and field, and to compare the susceptibility of leaf disks with that of attached leaves. The disk assay (disease rating and sporulation) was quantitative and reflected susceptibility of attached leaves.

Susceptibility of tobacco plants to blue mold changes with the age of plants. Overall, the younger tobacco plants were more susceptible to blue mold than older plants in terms of both severity of disease and increased sporangial production.

Similar changes in susceptibility have been reported for several other hosts and pathogens. Clayton (3) reported that resistance of several species of *Nicotiana* to blue mold increased with age. Hill (8) reported that immature tobacco plants produced more lesions of blue mold than mature plants. Dickinson and Crute (4) found that the susceptibility of lettuce seedlings to *Bremia lactucae* decreased with age. Mence and Pegg (10) found that the resistance of pea leaves to *Peronospora viciae* increased with age but declined again in senescence. The increasing resistance of the field-grown tobacco plants to *P. tabacina*, as determined by leaf disk assays, was probably due to an aging effect, and that supports our data from greenhouse-grown plants. However, environment may have had some effect on the susceptibility of plants in the field. Hill (6) observed that the first lesions of blue mold on field-grown tobacco plants appeared mostly on the large, succulent, and soft leaves. However, he concluded that, "in practice, the attack may occur at

TABLE 3. The effect of tobacco plant leaf position on the susceptibility of leaf disks to blue mold

| Disease severity[v] (0–4 scale) | | Sporangial production[w] (sporangia/cm² of leaf disk) | |
|---|---|---|---|
| Top[x] | Bottom[y] | Top | Bottom |
| 2.54 A[z] | 2.55 A | 1,018 A | 1,279 A |
| 2.54 A | 2.36 A | 1,415 A | 1,738 A |
| 2.46 A | 2.77 A | 2,155 A | 2,168 A |
| 2.18 A | 2,78 A | 1,587 A | 2,056 A |

[v] Means are based on 20 leaf disks from each leaf with three plants of each age. Severity was determined on leaf disks placed on filter paper for 7 days and on sponge rubber pads following 4 days on filter paper.
[w] Numbers based on 10 leaf disks placed on sponge rubber pads following 4 days on filter paper, from each leaf with three plants of each age.
[x] Means of all plants of the three ages from the top. Data are derived from Table 2. Top = leaves 1–4 (Table 2) of each age.
[y] Means of all plants of three ages from the bottom. Data are derived from Table 2. Bottom = Leaves 1–4 (9-wk-old plants), leaves 4–7 (12-wk-old plants), and leaves 6–9 (15-wk-old plants).
[z] Numbers within a column followed by the same letter do not differ ($P = 0.05$) according to the Waller-Duncan $K$-ratio $t$-test ($K$-ratio = 100).

TABLE 4. The effect of plant age on the susceptibility of tobacco leaf disks to blue mold

| Plant age (wk) | Disease severity[v] (0–4 scale) | | Sporangial production[w] (sporangia/cm² of leaf disk) | |
|---|---|---|---|---|
| | Top[x] | Bottom[y] | Top | Bottom |
| 9 | 2.54 AB[z] | 2.54 A | 3,351 A | 3,351 A |
| 12 | 2.74 A | 2.69 A | 1,031 B | 971 B |
| 15 | 2.03 B | 2.56 A | 248 B | 912 B |

[v] Means based on 20 leaf disks taken from each of four leaves of each plant of three plants of each age. Severity was determined on leaf disks placed on filter paper for 7 days and on sponge rubber pad following 4 days on filter paper.
[w] Numbers based on the ten leaf disks placed on sponge rubber pads following four days on filter paper. Disks were taken from the same leaves and planted as described above in footnote "v".
[x] Means of a total of four leaves per plant from the top. Data are derived from Table 2. Top = Leaves 1–4 (Table 2) of each age.
[y] Means of total four leaves per plant from the bottom. Data are derived from Table 2. Bottom = leaves 1–4 (9-wk-old plants), leaves 4–7 (12-wk-old plants), leaves 6–9 (15-wk-old plants).
[z] Numbers within a colum followed by different letters are significantly different ($P = 0.05$) according to the Waller-Duncan $K$-ratio $t$-test ($K$-ratio = 100).

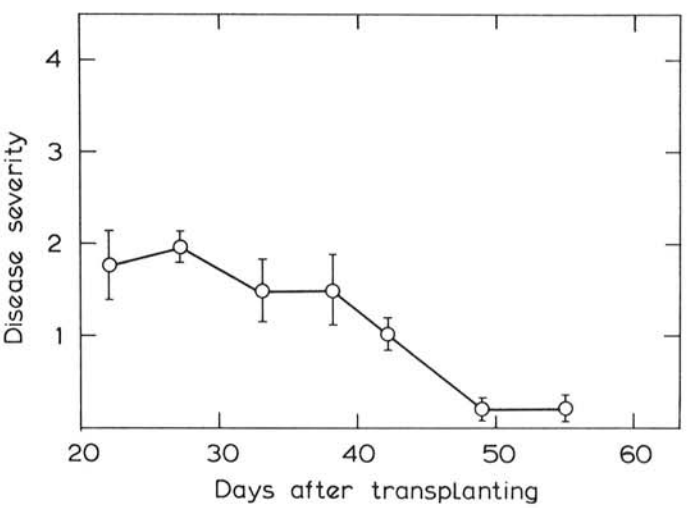


**Fig. 2.** Susceptibility to blue mold of leaf disks from tobacco plants grown in the field. Means ±S.D. (vertical bars) for six leaves (one leaf from each of six plants).

TABLE 5. The effect of leaf position and plant age on the susceptibility of tobacco leaf disks to blue mold[x]

| Leaf position on the plant | Disease severity (0–4 scale) on plants at week: 9 | 12 | 15 | Mean | Sporangial production (sporangia/cm² of leaf disk) on plants at week: 9 | 12 | 15 | Mean |
|---|---|---|---|---|---|---|---|---|
| Upper | 1.76 | 0.93 | 0.43 | 1.04 a[z] | 6,902 | 3,610 | 1,230 | 3,914 ab |
| Middle | 2.06 | 1.93 | 0.26 | 1.42 a | 9,084 | 6,664 | 516 | 5,421 a |
| Lower | 1.06 | 1.06 | 0.66 | 0.93 a | 1,190 | 873 | 516 | 859 b |
| Mean | 1.63 A[y] | 1.31 A | 0.46 B | | 5,725 A | 3,716 AB | 754 B | |

[x] Means are based on disks from three leaves from each of three plants of each age. Disks were cut from detached uninoculated leaves, placed on petri dishes and inoculated with about 13 sporangia of *P. tabacina* per square centimeter of leaf area. Four days after inoculation, disks were transferred to sponge rubber pads and on the 7th day they were rated for severity of disease and sporangial production.

[y] Numbers in rows (plant age) followed by different upper case letters are significantly different ($P = 0.001$) according to the same test.

[z] Numbers within a column (leaf position) followed by the same lower case letter do not differ ($P = 0.05$) according to the Waller-Duncan $K$-ratio $t$-test ($K$-ratio = 100).

TABLE 6. The effect of leaf position and plant age on the susceptibility of attached tobacco leaves to blue mold[x]

| Leaf position on the plant | Disease severity (0–4 scale) on plants at week: 9 | 12 | 15 | Mean | Sporangial production (sporangia/cm² of leaf disk) on plants at week: 9 | 12 | 15 | Mean |
|---|---|---|---|---|---|---|---|---|
| Upper | 2.53 | 1.33 | 0.16 | 1.34 a[z] | 135,422 | 62,356 | 3,967 | 67,248 a |
| Middle | 3.23 | 1.00 | 0.0 | 1.41 a | 37,644 | 27,608 | 635 | 21,962 a |
| Lower | 3.90 | 1.40 | 0.26 | 1.25 a | 79,373 | 38,735 | 198 | 26,240 a |
| Mean | 3.03 A[y] | 1.23 B | 0.14 C | | 85,510 A | 43,420 AB | 1,600 B | 26,240 a |

[x] Means are based on three leaves from each of three plants of each age. Half leaves were inoculated, while attached to the plants, with a suspension of $2 \times 10^4$ sporangia per milliliter. Seven days after inoculation, disks with sporulating lesions were cut from the same location on the leaf and in the same manner as those that were used to obtain the data reported in Table 5. Disks were also rated in the same manner as those in Table 5.

[y] Means in rows (plant age) followed by different upper case letters are significantly different ($P = 0.001$) according to the same test.

[z] Means within a column (leaf position) followed by the same lower case letter do not differ ($P = 0.05$) according to the Waller-Duncan $K$-ratio $t$-test ($k$-ratio = 100).

any stage of growth and leaves may be at any level on the plant." Our findings for leaf position are in general agreement with this conclusion. However, in a later study conducted by Hill (8) on greenhouse-grown tobacco plants, he reported that symptoms of disease caused by *P. tabacina* decreased from the lowest leaf to the youngest leaf of tobacco plants. The gradient was steeper on "immature" than "mature" plants. A similar gradient was observed on young plants at the three-leafstage (7).

Our data indicate that application of drops of inoculum to 15-wk-old plants did not result in lesion development (Table 1). In an additional experiment (*unpublished*) in which 15 or 150 sporangia per drop were applied to 10- and 17-wk-old plants, lesions developed only on the 10-wk-old plants. Although more lesions were produced on leaves at an intermediate position, the disease gradient as reported by Hill (8) was not observed. The use of 0.35 or 1.75 sporangia per drop as calculated from Hill's (8) data seems to us to be an unrealistically low inoculum concentration to produce lesions on all plants; especially on mature plants inoculated several weeks after flower heads were removed.

This report differentiates the effect of leaf position from plant age on the susceptibility of tobacco to blue mold. Our results indicate that plant age has a greater influence on the resistance to blue mold than leaf position. This suggests that older plants have a defense mechanism that inhibits disease development. The presence or production of anti-fungal substances could be responsible for the limited development of *P. tabacina* in older plants. The data presented in this paper emphasize the importance of considering the age of tobacco plants when challenged in screening for resistance as well as in determining the most effective timing for use of chemical controls.

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