# Comparative Effects of Alternaria alternata Infection and Other Leaf Injuries on Growth of Tobacco

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## ABSTRACT

Postinoculation measurements of leaf growth, stem elongation, and time until flowering were made on 'Coker 187-Hicks' tobacco plants that were inoculated with *Alternaria alternata* or *Colletotrichum destructivum* or mechanically injured. When young leaves were heavily inoculated or wounded, subsequent expansion was inhibited. *A. alternata, C. destructivum*, severe wounding, and removal of most or two to four of the youngest leaves caused an increase in the rate of subsequent growth and resulted in significant increases in the numbers of leaves per plant at 14 and 28 days after treatment. However, only plants affected by *A. alternata*, severe wounding, or removal of most leaves had significantly more leaves than controls by the flowering date. These three treatments delayed flowering, but the delay was less with *A. alternata* than with the other two treatments. All treatments except removal of two to four of the youngest leaves inhibited stem elongation in the area treated. All treatments except severe wounding or removal of most leaves inhibited stem growth above the treated area. The results suggest that A. alternata infection caused both nonspecific and specific effects on growth of the tobacco plant.

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We reported that expansion of young tobacco leaves that were less than 75% of their potential size at the time of inoculation was markedly inhibited following heavy infection by *Alternaria alternata* (Fr.) Keissl., incitant of brown spot (10). A cicatrix of densely packed, angular cells was formed around each of the small lesions that developed on these leaves, but not around the larger lesions that developed on older leaves. The cicatrix response to A. alternata infection was similar to the response of young leaves to mechanical wounding. The production of new leaves seemed to be more rapid on infected plants than on noninoculated control plants.

The purpose of the present study was to determine whether infection by *A. alternata* affects growth as measured by changes in leaf and stem dimensions and time until flowering. The effects of A. alternata infection were compared with the effects of infection by *Colletotrichum destructivum* O'Gara, another tobacco leaf-spotting pathogen (4), and those of various types of mechanical leaf damage. Some of our results have been reported previously (8).

MATERIALS AND METHODS.—Seedlings of flue-cured tobacco cultivar 'Coker 187-Hicks', highly susceptible to brown spot, were transplanted to 6-inch clay pots about 4-6 weeks prior to treatment. When about 11 leaves had emerged, plants were arranged into consecutively numbered groups of 10 and a different treatment was applied to each group. Numbered plants in each group were matched for size.

Each test included nontreated plants and A. alternata-inoculated plants as controls. The highly pathogenic isolate A3 was utilized for inoculum. The inoculation methods were the same as those described previously; inoculum concentration was 120,000 conidia/ml (10). The controls were compared in various tests with plants that were wounded moderately on the four youngest leaves or severely on all leaves; plants that had two, three, or four of the youngest leaves excised; plants that had all but the two oldest leaves removed; and plants inoculated with 400,000 or 600,000 C. destructivum conidia/ml of water containing wetting agent (6). An eighth treatment involved inoculation with a lower concentration (60,000/ml) of A. alternata conidia. Inoculum was applied to all aboveground surfaces of all inoculated plants. Wounds were inflicted with straight pins. Leaves were removed by cutting them off at the stem. At the time of treatment the youngest leaf of sufficient size on each plant was tagged for use as a reference point for future measurements.

After inoculation or injury, all plants were incubated at 20 C, half in each of two Sherer-Gillette CEL 37-14 plant growth chambers, as described previously (9). Seven days later the plants were transferred from the growth chambers to a greenhouse kept at a minimum temperature of 24 C. They were fertilized biweekly with 1 level teaspoonful of 7-7-7 fertilizer spread over the soil surface.

On the day of treatment and at 14-day intervals thereafter until the first flower opened, we measured the width and length of all but the four basal leaves, the number of leaves, and plant height. The number of days from treatment until the first flower opened was recorded; and on the flowering date, records were made of leaf dimensions, the numbers of leaves per plant, plant height, and the distance from the highest

TABLE 1. Depression in growth of youngest leaves of tobacco plants 14 days after heavy inoculation with *Alternaria alternata* or *Colletotrichum destructivum*, or after mechanical wounding

| Treatment | Leaf position and depression in width and length[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Width (mm) | | | | Length (mm) | | | |
| | −2 | −1 | 0 | +1 | −2 | −1 | 0 | +1 |
| *A. alternata* | 93.8 c | 82.2 c | 40.8 b | + 3.6 b | 159.7 c | 153.0 c | 111.6 b | 39.9 b |
| *C. destructivum* | 45.8 b | 61.8 bc | 19.1 ab | + 8.9 b | 31.1 ab | 51.1 b | 35.0 a | 1.0 a |
| Wounded | 45.7 b | 49.9 b | 9.9 a | +57.2 a | 64.5 b | 42.7 b | 31.8 a | +13.8 a |
| Nontreated | 0 a | 0 a | 0 a | 0 b | 0 a | 0 a | 0 a | 0 a |

[a] All values preceded by a + sign represent increases over the nontreated controls. Each figure is an average of 20 measurements. The 0 leaf measured 8±2 (width) × 25±5 (length) mm on the day of treatment; (−) leaf positions are lower and (+) leaf positions are higher on the stem. Any two figures in the same column followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

point of treatment to the flower.

RESULTS.—Measurements of leaves of severely affected plants at 14 days after treatment showed that growth of the youngest treated leaves was inhibited by *C. destructivum*, wounding, and *A. alternata* (Table 1). *A. alternata* caused a greater inhibition of leaf expansion than did the other two treatments, which differed little in their effects. Inhibition was more severe at the first and second position below the zero leaf (measured 8 ± 2 × 25 ± 5 mm on the day of treatment) than at the zero and the next higher leaf positions. Increases in leaf widths tended to be greater at the next higher leaf position, but the increase was significant only on wounded leaves. Table 1 gives the results from heavy inoculation, whereas the averages in Fig. 1 include the results from less severe infections, which resulted in less inhibition.

Apparent stimulation of leaf length occurred in the upper, nontreated leaves of treated plants (Fig. 1). Curves were similar for leaf widths. This stimulation was measurable from 14 days after treatment until flowering. However, if these leaves were numbered down the stalk from the apex instead

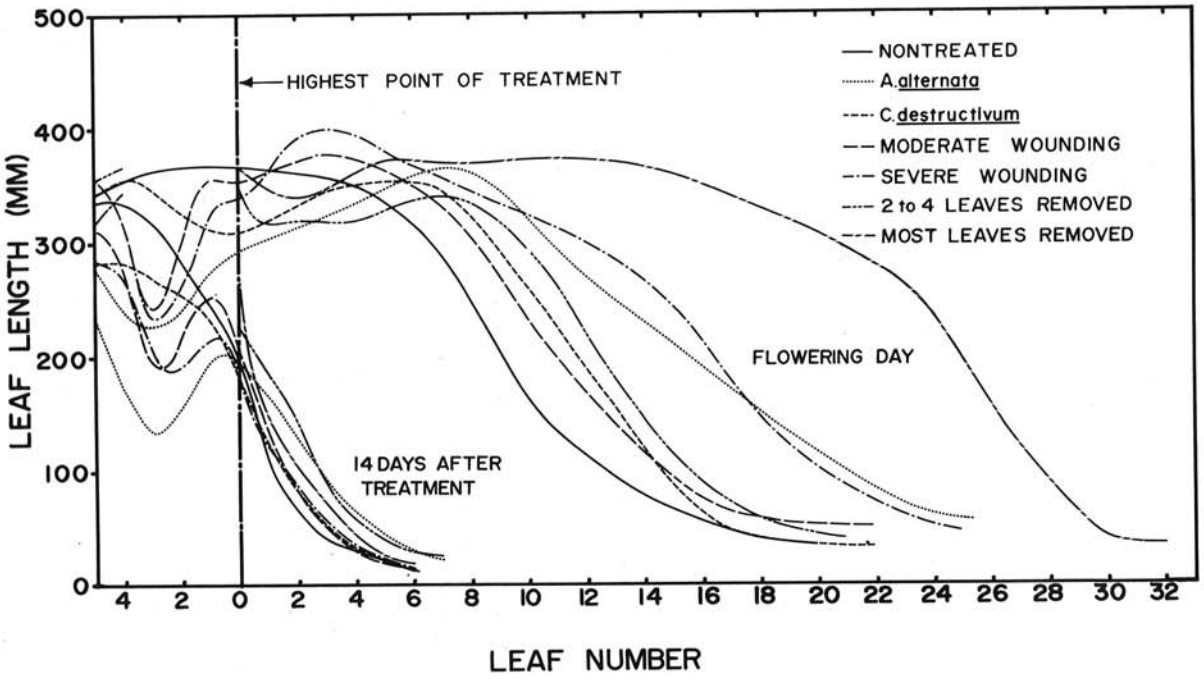


Fig. 1. Average leaf lengths on the 14th day after treatment and the flowering date of 'Coker 187-Hicks' tobacco plants that received six different treatments. The leaf numbered zero measured 8±2 × 25±5 mm on the day of treatment and was the approximate location on the plant of the highest extent of a treatment. Leaves lower or higher on the stalk are shown to the left and right, respectively, of the zero leaf. Curves were plotted from averages for four replicates, each with 10 plants.

of up from the zero leaf position as was done for Fig. 1, leaf sizes at equivalent nodes on plants having the various treatments would differ little from those on the nontreated plants at approximately the top 14 nodes. Thus, an increase in leaf number rather than an increase in leaf size should be considered the principal effect of the treatments.

Leaf counts showed that all treatments caused an increase in the average number of leaves per plant by 14 days after treatment (Table 2). With most treatments, most of this growth stimulation resulting in additional leaves occurred within the first 28 days after treatment. Only removal of most of the leaves or severe wounding, both of which caused significant delays in flowering, resulted in a major increment in leaf number that occurred almost entirely at a later time. These two treatments caused a relatively small increment in leaf number through the first 42 days. A. alternata-infected plants also showed a sizeable increase in leaf number between the 42nd day and the flowering date, although this increase was less than the earlier one on these plants. This was also accompanied by a small delay in flowering. Removal of two to four of the youngest leaves tended to cause earlier flowering, but no significant effect on the number of leaves present on the flowering date. However, these plants averaged 3.1 additional leaves in the earlier, 42nd day, count. *C. destructivum* and moderate wounding caused a slight increase in leaf number only during the first 28 days.

Above the treated area (Fig. 2-A) stem growth that occurred after inoculation and included the region where additional leaves were produced, was somewhat inhibited by all treatments except severe wounding and removal of most leaves. These were the same two treatments that delayed flowering so markedly.

Infection with either pathogen or severe wounding inhibited elongation of that part of the stem that was present at the time of treatment (Fig. 2-B). Both pathogens caused stem lesions, but wounding did not include the apical region of stems. Moderate wounding and leaf removal had little effect on elongation of the treated stem area, although all treatments except removal of two to four of the youngest leaves tended to inhibit elongation of this part of the stem.

Most A. alternata-infected leaves died within 3 weeks after inoculation and by 46 days all infected leaves had died.

DISCUSSION.–Inhibition of expansion appeared to be a general, direct effect when young tobacco leaves were injured by leaf pathogens or mechanical factors. Increased subsequent leaf growth, resulting in more leaves, was a common response to injuries that directly inhibited expansion. However, each of the treatments had certain unique effects. The differences in degrees of reduction in expansion of young leaves by *A. alternata*, *C. destructivum*, and mechanical wounding were probably partially due to the relative severities of the various treatments. Inoculation with *A. alternata* was the most severe treatment. Inoculum of both pathogens and resultant infection were present on the +1 and next younger leaves, although coverage was not as thorough as on leaves that were lower and more completely expanded. However, the +1 leaves could not be as intensively wounded as the zero and older leaves, and the youngest leaves were not wounded. Thus, growth stimulation exceeded inhibition more in the +1 and next younger leaves when they were wounded than when they were inoculated.

The increase in leaf numbers in the first 28 days after treatment may be distinct from the increase observed after the 42nd day on plants affected by *A. alternata*, severe wounding, and removal of most

TABLE 2. Effects of infection by two fungi, *Alternaria alternata* and *Colletotrichum destructivum*, and other treatments on the average number of leaves and days to flower of 'Coker 187-Hicks' tobacco plants

| Treatment | Average no. of additional leaves at four time intervals after treatment[a] | | | | Days to flower[c] |
|---|---|---|---|---|---|
| | 14 days | 28 days | 42 days | FD[b] | |
| Inoculation | | | | | |
| *A. alternata* | 2.5 a | 3.9 a | 3.6 a | 5.2 b | + 5.9 c |
| *C. destructivum* | 1.0 c | 1.6 c | 2.0 abc | 1.9 bc | – 0.8 de |
| Wounding | | | | | |
| Moderate | 1.3 bc | 1.5 c | 1.6 bc | 1.8 bc | + 2.6 cd |
| Severe | 0.9 c | 1.3 c | 1.5 bc | 4.8 b | +12.9 b |
| Leaves removed[d] | | | | | |
| 2-4 Youngest | 2.0 a | 2.8 b | 3.1 ab | 1.0 c | – 5.7 e |
| Most | 1.9 ab | 2.0 bc | 2.2 ab | 11.1 a | +25.9 a |
| Nontreated | 0 d | 0 d | 0 c | 0 c | 0 de |

[a] Each value is an average of four replicates with 10 plants per replicate. Any two values in a single column followed by the same letter are not significantly different at the 5% level (Duncan's multiple range test).

[b] FD = flowering date, which was the day the first anther dehisced.

[c] Number of days from treatment to anther dehiscence. Nontreated plants averaged 64 days to flower. Numbers given are the differences from the average of the nontreated plants.

[d] Leaves were removed from the youngest apical leaf progressively down the stem. All but the two basal leaves were removed on plants having most leaves removed.

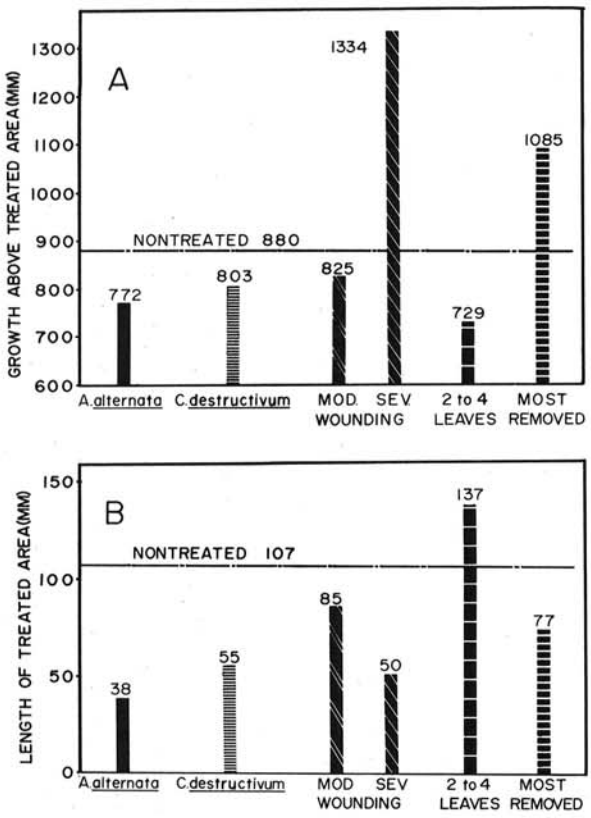


**Fig. 2, A-B.** Average stem lengths on the flowering date of 'Coker 187-Hicks' tobacco plants that had been inoculated at the 11-leaf stage with *Alternaria alternata* or *Colletotrichum destructivum* or mechanically injured. Mechanical injuries included moderate wounding of the four youngest leaves, severe wounding of all leaves, or removal of two to four of the youngest or all but the two oldest leaves. Each value is an average of four replicates, each consisting of 10 plants. **A)** Length from the highest treated leaf to lowest flower. **B)** Length from the base of the plant to the highest treated leaf.

leaves. Only these latter three treatments resulted in significant increases in leaf number through the flowering date. On the other hand, in any or all three of these treatments both increments may be due to a similar mechanism, the critical factor for a sustained effect being the relative extent and severity of the treatment. The increase in leaf number was smaller and was confined to the earlier, 28-day period on plants infected less severely by *A. alternata*.

When the effects on days to flower and number of leaves are considered together, the response to *A. alternata* appears unique. If a mechanism similar to that in plants with the more severe mechanical injuries was involved, then the flowering of A. alternata-infected plants should have been delayed more. In addition, removal of two to four of the youngest leaves and C. destructivum infection caused a different response than A. alternata infection or severe mechanical injury.

Since the pathogens caused stem lesions, their effect on elongation of the inoculated part of the stem could be direct. However, the similarity of the effect of severe leaf wounding on elongation of this part of the stem suggests that inhibited leaf expansion or leaf injury per se could play a role in inhibition of stem elongation.

Stem growth above the treated leaves showed two opposite patterns of response to the various treatments. Severe wounding and the more extensive leaf removal, the two mechanical treatments that involved most of the leaves, stimulated growth in this area. The pathogens had inhibitory effects similar to moderate wounding and removal of two to four of the youngest leaves. Three of the treatments causing this inhibition, *C. destructivum*, moderate wounding, and removal of two to four leaves, caused little or no injury to those older leaves present at the time of treatment. Thus, *A. alternata* was unique in being the only treatment causing injury to all the treated leaves and causing a reduction in subsequent stem elongation. *A. alternata* inhibited elongation more than any other treatment.

We might conclude that many of the effects of *A. alternata* on the growth of the tobacco plant are characteristic, nonspecific, injury responses. The severity of infection by this pathogen is probably responsible for some of the observed differences from other treatments. On the other hand, the precise effects of *A. alternata* on flowering and postinoculation elongation of new stem tissue could be unique.

An increased rate of leaf production, reduction in plant height, delay in flowering, and reduction in internode length were reported by Stein (13) for severe etch virus-infected tobacco. Other instances in the literature of effects such as we have reported here are rare (1). At least one report (3) indicates a quite different effect by disease on postinfection growth than we observed.

That an aggressive and virulent pathogen such as *A. alternata* would affect the regulation of growth and differentiation in the host is not surprising. This pathogen or any other form of injury might be expected to affect the precise balance of growth-regulating substances in the host. Such pathogenic effects could result from direct production of growth-regulating substances or from production of or effects upon inhibitory systems or other compensatory mechanisms controlling response of plant cells to such biologically active substances. When tobacco plants were sprayed with gibberellic acid, the effect was more like that which we obtained by removing two to four of the youngest leaves than that from any of our other treatments (13).

Steadman & Sequeira (11) reported that the wilt bacterium, *Pseudomonas solanacearum*, caused growth inhibition in tobacco plants that was correlated with the activity of a seemingly host-produced factor later identified as abscisic acid (12). When applied to noninfected tobacco plants, abscisic acid caused maximum inhibition of stem elongation in 6 days. This inhibition may have been due to a reduction in the gibberellin content of the

affected tissues caused by abscisic acid. Most interesting, in light of our results, was their finding that growth resumed at a rate faster than normal between the 6th and 12th day after treatment (12).

Another unidentified growth inhibitor, which was neither abscisic acid nor an aromatic compound, was recently reported from young expanding tobacco leaves by Cutler (2).

A distinctive halo of yellow tissue characteristically surrounds the central brown necrotic lesion incited by *A. alternata* on sensitive tobacco cultivars. Halo tissue and the proximal green prehalo tissue differed chemically from each other and from necrotic and noninfected tissue (5). Most of the halo area is not invaded by the fungus (10). Two metabolites produced by *A. alternata* in culture, alternariol monomethyl ether (7) and tenuazonic acid (6), have been implicated as halo-inducing factors. The latter compound also occurred in the halo tissue in infected leaves, had an effect on cultivars correlated with that of the pathogen, and was produced by various isolates of the pathogen in direct relationship to pathogenicity. Tenuazonic acid also inhibited root growth of lettuce seedlings. However, it appeared to be localized in occurrence and disappeared from affected tissues as the disease progressed. Furthermore, treatment of noninoculated tobacco plants with this or a similar substance (J. R. Stavely, *unpublished data*) failed to cause effects on growth such as we have described here.

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