Stomatal Conductance, Fleck Injury, and Growth of Tobacco Cultivars Varying in Ozone Tolerance

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ABSTRACT

Four tobacco (*Nicotiana tabacum*) cultivars that vary in ozone sensitivity were grown in the field and in two greenhouses, one with charcoal-filtered air and one with unfiltered air. The plants were compared for stomatal conductance, stomatal frequency, height, oven-dry weight, leaf area, and amount of fleck injury.

The total leaf conductance, measured with a ventilated diffusion porometer, of the ozone-sensitive cultivars was greater than that of the ozone-tolerant cultivars; both adaxial and abaxial conductances were greater. However, the differences in stomatal conductance were not sufficient to account completely for the differences in fleck damage.

In ozone-free air, the two ozone-sensitive cultivars grew more than did the two ozone-tolerant cultivars. The converse was true when the plants were grown in unfiltered air. The reduction of dry weight in the unfiltered air accompanied a reduction in the green leaf area caused by flecking and early senescence of damaged leaves.

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Weather fleck, a serious problem in the production of tobacco, is caused by the presence of the photochemical oxidant, ozone, in the atmosphere (5); sulfur dioxide in the absence or presence of ozone can also cause leaf flecking. The ozone enters the leaf through the stomata and attacks the palisade parenchyma; for damage to occur, the stomata must be open. Recently, Rich et al. (12) showed that the uptake of ozone is, in fact, regulated by the same factors that control the exchange of water vapor between leaf and atmosphere.

Tobacco cultivars vary in their sensitivity to ozone (7, 8), and the sensitivity of a given plant varies with leaf maturity. Recently matured leaves were more sensitive than old matured leaves or young, rapidly expanding leaves (7). Although genetic variation in ozone tolerance has been recognized, the only known mechanism of resistance in ozone injury is stomatal closure. The guard cells of tolerant onions are so sensitive that they close and protect the plant, whereas in sensitive onions they remain open (3). In tobacco, stomatal closure in tolerant cultivars has also been reported (2, 11), and has been implicated in the apparent tolerance of young and old leaves (7).

Photosynthesis is reduced in the presence of ozone whether the stomata close or not (1, 6). Both the characteristic flecking and the reduction in photosynthesis would be expected to reduce growth. Indeed, Feder (4) observed reduced growth and floral productivity in petunias and carnations from long exposures to ozone, and Menser et al. (7) observed a reduction in the leaf expansion rates in an ozone-sensitive tobacco cultivar after a short exposure to the pollutant.

This paper reports differences in stomatal conductances in two pairs of genetically similar cultivars known to differ in sensitivity to ozone. Stomatal conductances were compared in a greenhouse with charcoal-filtered air, and in the field on a day with moderate ozone levels in order to determine whether the previously reported differences in stomatal conductance in the field (11) arose from the presence of ozone. Furthermore, the growth and leaf areas of the four cultivars were compared after a long exposure to ozone and in ozone-free conditions in order to determine whether the reported differences in stomatal conductance are reflected in yield differences, and to evaluate the magnitude of yield reduction from ozone.

MATERIALS AND METHODS.–The following tobacco (*Nicotiana tabacum* L.) cultivars were used: 6524, Connecticut 49, 6590, and Bel W-3. Cultivar 6524 is an ozone-tolerant selection genetically similar to Conn. 49, which is moderately ozone-sensitive. Cultivar 6590 is a moderately ozone-sensitive selection from Bel W-3, which is highly sensitive to ozone.

Four plots, each containing one row of the four cultivars, were planted in a well-fertilized soil at the Lockwood Farm, Mt. Carmel, Conn. Each row of 10 plants was 1.3 m from the adjacent rows, and plants were spaced at 1-m intervals along the row. The seedlings were transplanted into the plots on 14 June 1967 and 12 June 1968. In addition, six seedlings of each cultivar, grown in a greenhouse with an activated carbon air-filtering system, were transferred to pots on 5 June or 6 December 1968, and either left in the greenhouse with charcoal-filtered air or transferred to an adjacent greenhouse that had no air filtration. They were placed on a sand bench with automatic watering, and fertilized weekly with a complete nutrient solution.

All the field plants were rated for fleck damage on 8 September 1967 and 13 September 1968. The plants grown in the greenhouse were similarly rated 42 days after transplanting. These ratings were made by estimating the average per cent of flecked area of

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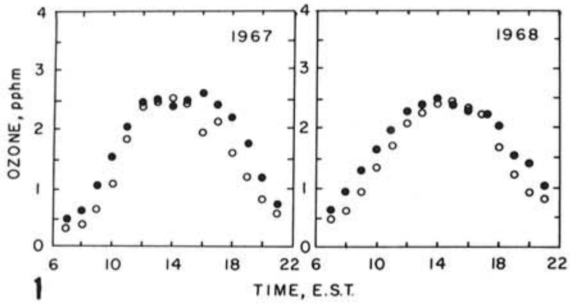

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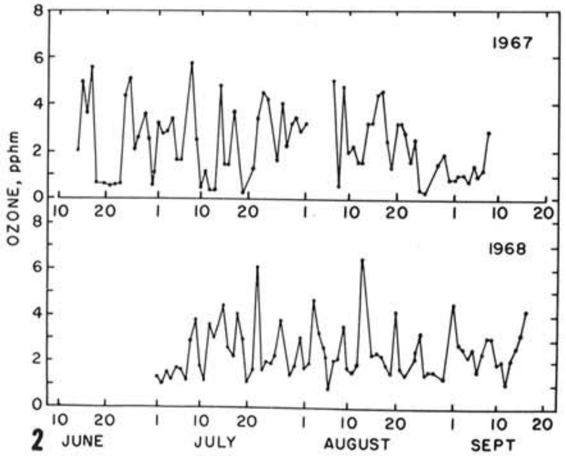

Fig. 1-2. 1) The mean change in ozone concentration in parts per hundred million (pphm = μliters/100 liters) between 0700 and 2100 hr, Eastern Standard Time (E.S.T.), during the months of July (●) and August (○) in 1967 and 1968. Each point represents the mean concentration of ozone for 30 min prior to the hour of observation. **2)** The daily peak concentration of ozone in pphm between 14 June and 8 September 1967 and 1 July and 16 September 1968. Each point is the mean of four observations of ozone concentration measured for 30 min prior to 1300, 1400, 1500, and 1600 hr E.S.T.

the newly matured leaves for each plant. The plot mean and standard error were then calculated.

The stomatal conductance of the upper and lower surfaces of four randomly selected plants, one from each cultivar, was measured with a ventilated diffusion porometer (14). This porometer has been calibrated in conductance units of centimeters per second. For each plant, four measurements were made on each surface of a well-illuminated portion of the fifth leaf below the terminal; in the ozone-sensitive plants, this was the most fully expanded leaf showing no signs of injury. The conductances of the field plants were measured 63 days after planting in the field, and those of the greenhouse plants were measured 39 days after transplanting. The measurements were obtained between 0900 and 1530 hr, Eastern Standard Time.

In each instance, the illumination incident upon the leaves was measured with a sunshine illumination meter (Model 756, Weston Instrument Co., 614 Frelinghuysen Ave., Newark, N. J.) immediately after measuring stomatal conductance. At the same time, a silicon rubber impression (15) was made of each epidermis of one sampled leaf/cultivar. These were used later to obtain stomatal frequencies from 10 microscope fields of each replica.

On 16 September 1968, five randomly selected plants from each row were harvested, and the total oven-dry weight of the aboveground portions of each plant was obtained. Growth measurements of the greenhouse plants, taken 42 days after transplanting, included height, leaf area, and oven-dry weight.

Ozone concentrations in the air were measured with a Mast ozone meter (Model 724-2, Mast Development Co., 2212 East 12th St., Davenport, Iowa) equipped with an SO_2 trap.

RESULTS.—The mean concentrations of ozone for the months of July and August showed a steady increase of ozone in the morning, with peak readings generally observed at 1400 hr, Eastern Standard Time (Fig. 1). In both 1967 and 1968, the ozone concentrations in August were lower than those in July at 0700 hr, rose slowly to the same peak at 1400 hr, then declined earlier in the late afternoon. The mean concentration of ozone over the 4 peak hr/day, i.e., from 1200 hr to 1600 hr, fluctuated from day to day throughout the entire growing season (Fig. 2). The number of days on which the mean level over this 4-hr period was equal to, or greater than, 5 pphm (5 μliters/100 liters), the level generally considered to cause fleck injury in sensitive tobacco cultivars was 5 days in 1967 and 2 days in 1968. However, the number of days on which the ozone levels were equal to or above 5 pphm for at least 30 min was similar in the 2 years, with 16 days in 1967 and 15 days in 1968. Therefore, we conclude that the ozone concentrations were essentially similar in the 2 years, but the high concentrations of ozone persisted for a longer period during 1967 than 1968. The ozone concentration reached 7 pphm in the period that stomatal conductances were measured in the field.

Cultivar 6524 had consistently less fleck, and Bel W-3 had consistently more fleck than the other cultivars (Table 1). Conn. 49 and Cultivar 6590 had intermediate injury ratings and, except for the 1967 field plots, gave similar fleck responses; possibly the greater amount of fleck in 1967 arose from the slightly higher concentrations of ozone observed in 1967. No fleck was observed on the plants grown in the greenhouse with charcoal-filtered air.

The total stomatal conductance (Table 2) of the Conn. 49 and Bel W-3 was greater than that of their more ozone-tolerant relatives: both adaxial and abaxial conductances were greater. Moreover, this was true in the filtered air of the greenhouse and in the field. In Cultivars 6524 and 6590, the abaxial stomata had a slower conductance in the field than in the greenhouse with charcoal-filtered air, whereas Conn. 49 and Bel W-3, the more ozone-sensitive cultivars of each genetically related pair, showed no differences in stomatal conductance between field and filtered greenhouse. Except for the major difference between

TABLE 1. Fleck ratings (± standard error) for four tobacco cultivars that differ in sensitivity to ozone; the ratings are the average per cent of flecked area of the newly matured leaves for each plant

Location and Year	Cultivars			
	6524[a]	Conn. 49[b]	6590[b]	Bel W-3[c]
Field 1967[d]	7	40	13	54
Field 1968	7 ± 1	9 ± 2	11 ± 1	65 ± 2
Unfiltered greenhouse 1968	10 ± 1	22 ± 1	20 ± 2	71 ± 3

[a] Highly ozone-tolerant.
[b] Moderately ozone-tolerant.
[c] Highly ozone-sensitive.
[d] Standard errors not available.

TABLE 2. Stomatal frequencies and stomatal conductances (± standard error) of four tobacco cultivars differing in sensitivity to ozone

Location and year	Cultivars			
	6524[a]	Conn. 49[b]	6590[b]	Bel W-3[c]
Stomatal frequency (per mm^2)				
Adaxial	44 ± 2	65 ± 3	68 ± 5	58 ± 3
Abaxial	131 ± 9	134 ± 5	126 ± 5	128 ± 5
Stomatal conductance (cm/sec)				
Filtered greenhouse				
Adaxial	0.07 ± .01	0.11 ± .01	0.09 ± .01	0.11 ± .01
Abaxial	0.29 ± .02	0.35 ± .03	0.32 ± .02	0.39 ± .02
Total	0.36 ± .02	0.46 ± .05	0.40 ± .02	0.51 ± .03
Field				
Adaxial	0.12 ± .02	0.13 ± .01	0.08 ± .01	0.12 ± .01
Abaxial	0.18 ± .03	0.33 ± .03	0.15 ± .02	0.31 ± .02
Total	0.30 ± .04	0.45 ± .04	0.23 ± .02	0.43 ± .03

[a] Highly ozone-tolerant.
[b] Moderately ozone-tolerant.
[c] Highly ozone-sensitive.

the adaxial and abaxial epidermis in all four cultivars, the differences in conductance were not correlated with the stomatal frequencies.

The mean illumination incident upon the measured leaves was 4,400 ft-c in the field and 3,100 ft-c in the greenhouse. In both cases this is more than the illumination required to open stomata in tobacco (13). That there was sufficient light for maintaining open stomata is further supported by the similar conductance of the sensitive cultivars both in the field and in the greenhouse with charcoal-filtered air.

In filtered air, the ozone-sensitive Conn. 49 and Bel W-3 were taller, heavier, and had larger leaf areas than their more tolerant counterparts, Cultivars 6524 and 6590 (Table 3). In the greenhouse with unfiltered air, the reverse was true for the most part. The highly ozone-sensitive Bel W-3 was significantly smaller in all three measurements than its ozone-tolerant partner, Cultivar 6590. In the other genetic pair, Conn. 49 yielded significantly less dry weight and tended to be shorter and have less leaf area than its ozone-tolerant partner, Cultivar 6524. In the field, the highly sensitive Bel W-3 yielded significantly less dry weight than the other three cultivars, which did not differ significantly in dry weight.

Since growing conditions in the greenhouse with filtered air were not identical to those in the unfiltered greenhouse, the growth of the cultivars in the two greenhouses is best compared on a relative basis. The magnitude of the effect of the polluted air can be seen in Fig. 3, Above, where the dry weight of the most ozone-tolerant cultivar, Cultivar 6524, is used as the standard. Compared to Cultivar 6524, the moderately sensitive Conn. 49 and 6590 produced 30% to 60% more dry weight, respectively, while the very sensitive cultivar, Bel W-3, produced 77% more dry weight in the filtered greenhouse. The reverse was true in the unfiltered greenhouse. There the ozone-tolerant Cultivar 6524 produced 35% more dry weight than the moderately sensitive Conn. 49 and Cultivar 6590, and 80% more dry weight than the very sensitive Bel W-3. Both leaf and stem dry weights were equally affected by the presence of ozone.

Again, using the ozone-tolerant Cultivar 6524 as the standard, the areas of green and flecked or yellow leaves are compared in Fig. 3, Below. In the filtered greenhouse, Cultivar 6590 had a similar total leaf area to that of Cultivar 6524. Conn. 49 and Bel W-3 also had similar areas, and both had 30 to 35% more area

TABLE 3. Height, oven-dry weight, and leaf area (± standard error) of four tobacco cultivars differing in sensitivity to ozone; plants grown in 1968 in a greenhouse either with charcoal-filtered air or with unfiltered air, or in the field

	Cultivars			
	6524[a]	Conn. 49[b]	6590[b]	Bel W-3[c]
Filtered greenhouse				
Height, cm	22 ± 1	39 ± 2	36 ± 4	64 ± 2
Dry weight, g	2.6 ± 0.2	4.1 ± 0.2	3.4 ± .5	4.8 ± 0.3
Leaf area, dm^2	10 ± 1	14 ± 2	11 ± 1	14 ± 1
Unfiltered greenhouse				
Height, cm	132 ± 10	120 ± 3	117 ± 4	72 ± 6
Dry weight, g	30 ± 3	20 ± 1	20 ± 2	6 ± 1
Leaf area, dm^2	47 ± 5	43 ± 4	55 ± 5	11 ± 3
Field				
Dry weight, g	286 ± 20	279 ± 25	319 ± 21	128 ± 9

[a] Highly ozone-tolerant.
[b] Moderately ozone-tolerant.
[c] Highly ozone-sensitive.

of leaf than did their more resistant genetic counterparts. In the unfiltered greenhouse, on the other hand, Conn. 49 had a total leaf area only slightly less (8%) than Cultivar 6524; Cultivar 6590 was 15% larger, whereas the very sensitive Bel W-3 had an area 75% smaller than that of Cultivar 6524.

In the greenhouse with charcoal-filtered air, the leaves were principally green; no flecked leaves were observed, but senescence had begun. In the unfiltered greenhouse, however, 79% of the leaf area of Bel W-3 and 58% of Conn. 49 had been injured by air pollution, whereas flecked and yellowing leaves only contributed 35 and 43% to the total leaf area in Cultivars 6524 and 6590, respectively.

DISCUSSION.–The four tobacco cultivars that differ in susceptibility to weather fleck (Table 1) also differed in their stomatal conductance (Table 2). The two ozone-tolerant cultivars not only had a lower conductance in the field on a day with moderate ozone levels (11), but also had lower conductances in the ozone-free greenhouse. Clearly, in the selection for ozone tolerance, an increase in the stomatal resistance at equal illumination has also been selected.

Although the palisade cells lying close to the adaxial epidermis are the primary site of damage by ozone (9), the aperture of the abaxial stomata appears to regulate ozone injury. Rich (10) reported that closure of the abaxial stomata prevented leaf fleck in the presence of ozone, whereas closure of the adaxial stomata provided no such immunity. In the present study, both the adaxial and abaxial conductance were higher in the ozone-sensitive cultivars. But, because of the low conductance of the

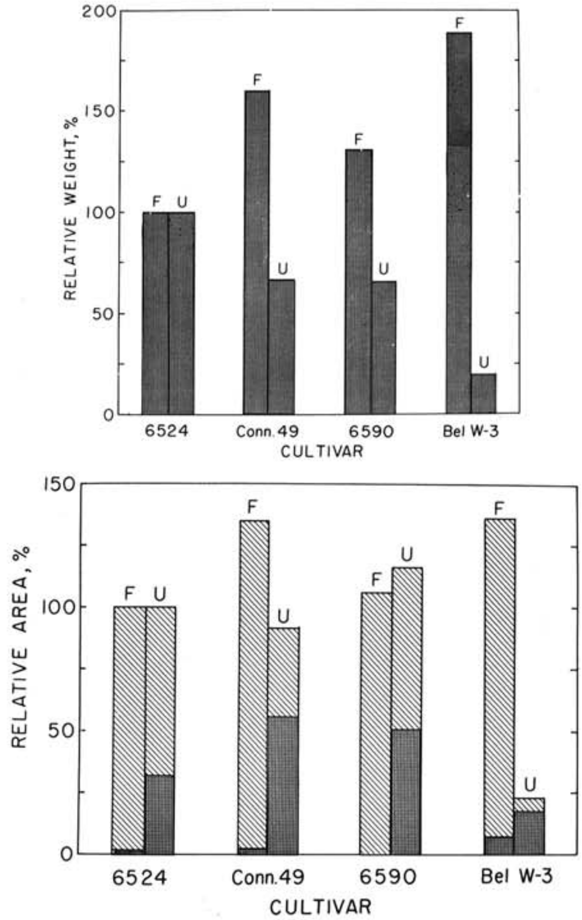

Fig. 3. (Above) The relative dry weights of four tobacco cultivars that differ in sensitivity to ozone. The dry weight of ozone-tolerant Cultivar 6524 is used as the standard. The plants were grown for 42 days in a charcoal-filtered greenhouse (F) and in a greenhouse with unfiltered air (U). (Below) The relative leaf areas of four tobacco cultivars that differ in sensitivity to ozone. The leaf area of ozone-tolerant Cultivar 6524 is used as the standard. Hatched portions depict the green leaf area and shaded portions depict the area of flecked and yellowing leaves; in the charcoal-filtered house the shading depicts yellowing only. The plants were grown for 42 days in either a filtered greenhouse (F) or a house with unfiltered air (U).

adaxial epidermis, closure of these stomata would not materially affect the differences in conductance between the cultivars. This suggests that the lack of correlation between fleck damage and the aperture of the adaxial stomata observed previously arises from the low conductance of this epidermis.

The conductance of the two ozone-tolerant selections was not as high under the moderate ozone conditions in the field as in the filtered air of the greenhouse. Since the difference in illumination between the field and greenhouse was insufficient to create differences in stomatal aperture (13), this indicates that the tolerant strains closed their stomata slightly in the presence of ozone. Rich & Turner (*unpublished data*) have observed a decrease in stomatal conductance of a tolerant tobacco cultivar in a dry atmosphere. Since Rich et al.(12) showed that ozone uptake was proportional to stomatal conductance, we expect greater damage in the plants with wider stomata.

The observed differences in stomatal conductance do not fully account, however, for the differences in ozone injury. Conn. 49 had a similar total conductance to Bel W-3 in both the field and filtered greenhouse, but the fleck damage was much less. Therefore, we conclude that although differences in stomatal conductance may provide the plant with a measure of tolerance to ozone injury, other mechanisms of tolerance are also involved and need to be sought.

Since the stomata are primary regulators of the uptake of carbon dioxide as well as the uptake of ozone, other things being equal, the plants with wider stomatal apertures should yield better than those with narrower stomata. This was indeed the situation in the greenhouse with charcoal-filtered air, but with ozone in the atmosphere, the situation was reversed (Fig. 3). Clearly, this arose from the damage inflicted by the ozone upon the photosynthetic apparatus. Not only was the green leaf area reduced by flecking (Table 1, Fig. 4), but the injured leaves senesced earlier, particularly in the highly sensitive selection, Bel W-3. Moreover, the observation that the cultivars that yield best where ozone is present, but yield relatively poorly in an environment free of ozone, indicates the sacrifice that had to be made in the past to avoid losses in areas of air pollution. Clearly, any method of increasing the tolerance to ozone that does not depend on closing of the stomata will be of great benefit, and may again allow production of previously high yielding strains of tobacco now adversely affected by leaf flecking.

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