Ozone Response of Tobacco Cultivars Unrelated to Root System Characteristics

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ABSTRACT

Using the technique of reciprocal grafts we have provided decisive evidence that the mechanism of ozone resistance in Bel-B tobacco plants does not depend on the root system. Although Bel-B root systems are consistently smaller than those of Bel-W3, the foliage of each cultivar retains its characteristic response to ozone when grafted onto roots of the other. We therefore concluded that the resistance of Bel-B and sensitivity of Bel-W3 must be due to some structural and/or biochemical characteristic of the leaves themselves.

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Additional key words: air pollution, reciprocal graft technique.

Many investigators have sought an explanation regarding the basis for observed cultivar differences in ozone sensitivity. Only in the case of onion has a mechanism been proven, and that involves stomatal closure by resistant plants in the presence of ozone (1). Studies with sensitive and resistant tobacco cultivars have failed to demonstrate consistent stomatal responses which might explain the resistance of certain cultivars on the basis of exclusion of ozone from the leaf (5, 6, 8).

Ting and Dugger (7) suggested that observed differences in the size of the root systems of Bel-B, an ozone-resistant tobacco cultivar, and Bel-W3, an ozone-sensitive tobacco, were an important factor in determining response to ozone. They maintained that Bel-B is in a continual state of water stress due to its significantly smaller root system. This would be consistent with the general phenomenon that plants under water stress are more resistant to ozone damage than plants with optimal water relations. However McKee (4) showed that an ozone-sensitive Maryland tobacco cultivar, Catterton, had a smaller root system than Wilson, a resistant cultivar.

In order to test the hypothesis that root system characteristics are significant in determining the response of Bel-B and Bel-W3 tobacco to ozone we used reciprocal grafts. We subjected the grafted plants to levels of ozone sufficient to injure Bel-W3 both in the ambient outdoor atmosphere and in controlled fumigations.

MATERIALS AND METHODS.—Bel-W3 and Bel-B tobacco plants were grown from seed in a charcoal-filtered greenhouse. After the plants had three-to-four fully expanded leaves, the stems were cut approximately 3 cm above the soil level and cleft grafts were made with Bel-B scions on Bel-W3 root stock and vice versa. Self grafts of Bel-B scions on Bel-B roots and Bel-W3 scions on Bel-W3 roots were also made. The grafted plants were maintained in a high humidity chamber within the filtered-air greenhouse for 2 weeks until the graft unions were formed, after which the plants were grown under normal greenhouse conditions of light and humidity. After several new leaves had expanded the grafted plants were repotted in 15 × 15 cm plastic pots and maintained in filtered air for 4-5 weeks prior to treatment. The plants were fertilized regularly.

On 18 September 1974, a group of grafted plants at the 15-leaf stage was placed in an open-top outdoor module as described by Mandl et al. (3) through which ambient air was continuously circulated. Ambient oxidant levels were monitored using a Mast Ozone Meter (Mast Development Co., Inc., Davenport, Iowa) calibrated by the buffered neutral KI method (2). The first group consisted of ten grafts of Bel-W3 scions on Bel-B roots and an equal number of reciprocal grafts plus four grafts of Bel-B scions on Bel-B roots and four Bel-W3 self grafts. By 26 September enough injury had occurred as a result of ambient oxidant levels to rate the plants as to ozone sensitivity.

On 28 September 1974, a second group of grafted plants was placed in the open top module. This group consisted of representatives of each graft type as in the first group. On 8 October the second group of plants was removed from the chamber for rating of visible leaf injury due to ambient oxidant concentrations.

Roots of representative plants from each group were washed under running water, damp dried, and weighed.

Concurrently with the outdoor exposure two additional groups of grafted plants were exposed to ozone in a 6 m^3 glass-enclosed fumigation chamber located in the greenhouse. Air flow in the chamber was sufficient to cause a complete air change every 45 seconds, temperature was maintained at 24-27 C and relative humidity at 75-80%. Use of a turntable within the chamber assured that all plants received equal exposure. Ozone was generated by passing pure oxygen through a commercial ozone generator and fed into the charcoal-filtered air stream. The control chamber was the same in all respects except for the addition of ozone to the air stream. Fumigations were at 287-382 μg ozone/m^3 air (0.15 - 0.20 ppm) for 2 hours and 573 μg ozone/m^3 air (0.30 ppm) for 2 hours. Ozone levels in the chamber were monitored with a Mast Ozone Meter.

Measurements of leaf resistance were made with a Diffusive Resistance Meter (Lambda Instrument Co., Lincoln, Nebraska Model L1-60 equipped with an L1-15S sensor) just prior to the beginning of the fumigation period and again immediately after. Duplicate readings were taken on the sixth and seventh leaves from the apex which leaves we had observed to be the most sensitive to ambient oxidant damage.

RESULTS.—*Ambient exposures.*—On 19 and 20 September and 6 October we recorded ambient oxidant levels in excess of 95 μg O_3/m^3 air (0.05 ppm). This was sufficient to injure the sensitive tobacco cultivar. In both sets of plants exposed to ambient oxidant levels, Bel-W3 shoots developed typical symptoms of oxidant fleck regardless of the root system on which they had been grown. The injury on Bel-W3 was moderate to severe in all cases (40-60% of the leaf surface affected). The Bel-B shoots, whether grown as reciprocal or self grafts, were free of injury.

Controlled fumigations.—Fumigation at 287-382 μg O_3/m^3 air (0.15 - 0.20 ppm) for 2 hours resulted in moderate to severe injury to all Bel-W3 shoots (40-60% of the leaf surface necrotic) but produced no visible effect on Bel-B. Fumigation at 573 μg ozone/m^3 (0.30 ppm) caused very severe injury on all Bel-W3 shoots (60-80% of the leaf area necrotic) and moderate injury on Bel-B (25-40% of the leaf area affected). As with the ambient exposures, the identity of the root system did not alter the visible response of the foliage to ozone.

Root size.—Bel-B tobacco roots were significantly smaller than those of Bel-W3, whether they were grown as reciprocal or as self grafts (Table 1). The shoots of the Bel-W3 and Bel-B grafted plants were at all times indistinguishable in overall size, vigor, and general appearance.

TABLE 1. Mean fresh weight of roots of grafted tobacco plants exposed to ambient oxidant levels

Root systems	Mean fresh weight (g)	
	Self graft	Reciprocal graft
Bel-W3	27.3 ± 1.2[z]	28.0 ± 3.7
Bel-B	7.3 ± 0.9	11.5 ± 0.8

[z] ± Standard error.

TABLE 2. Mean leaf resistance in sec cm^{-1} of the sixth and seventh leaves of ozonated and control tobacco grafts, before and immediately after ozonation

Graft	Ozonated		Control	
	Before	After	Before	After
W3/B[y]	5.0 ± 0.5[z]	8.3 ± 0.9	2.8 ± 0.3	3.1 ± 0.1
W3/W3	4.6 ± 0.5	7.3 ± 0.9	3.3 ± 0.1	3.5 ± 0.1
B/W3	4.9 ± 0.3	9.0 ± 1.6	4.1 ± 0.1	4.1 ± 0.5
B/B	5.8 ± 1.0	6.8 ± 0.7	3.6 ± 0.5	4.0 ± 0.5

[y]W3/B = Bel-W3 scion grafted onto Bel-B root.
[z] ± Standard error.

Leaf resistance.—Although we observed a tendency for leaf resistance to increase immediately after ozonation, this was equally true for Bel-W3 and Bel-B scions (Table 2). There was no difference between or within the two cultivars regardless of the root stock on which they had been grown.

DISCUSSION.—Using reciprocal grafts we have provided unequivocal evidence that root system characteristics do not determine ozone response in Bel-W3 and Bel-B tobacco, contrary to the suggestion of Ting and Dugger (7). We are in agreement with Ting and Dugger on the question of relative root size; however, the fact that Bel-B root systems are consistently smaller does not appear to have any relationship to the foliar response to ozone. Bel-W3 shoots retained their extreme sensitivity to ozone when grown for 5-6 weeks on Bel-B roots and Bel-B remained resistant to ozone injury when grafted onto the larger Bel-W3 roots. Even after 12 weeks the grafted scions of Bel-W3 were still much more sensitive to ozone than the Bel-B scions. Ting and Dugger reported consistently lower water potentials in the foliage of intact Bel-B plants but found that when excised leaves were kept with petioles in water, leaf resistance of Bel-B and Bel-W3 was similar. They implied that ozone response of the excised leaves should be similar, but did not provide any evidence on this point. We did not observe any indication of water stress in Bel-B under the conditions of our experiment; leaf resistance was similar to that of Bel-W3 and at no time did we observe wilting. Furthermore when we fumigated excised leaves of Bel-W3 and Bel-B both cultivars retained their differential response to ozone.

Our porometer measurements failed to show a correlation between leaf injury and leaf permeability. Therefore we must agree with previous authors (5, 6, 8) who have been unable to relate resistance to stomatal closure. We can only conclude that the basis for ozone resistance in tobacco lies in structural and/or biochemical characteristics of the leaf tissue.

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