Response of Plants to Air Pollutants. III. A Relation Between Ascorbic Acid Levels and Ozone Susceptibility of Light-Preconditioned Tobacco Plants

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The effects of fumigation light regimes on plants indicate that light modifies the response of plants to ozone. Taylor et al. (11) reported that ozone injury to Pinto bean was reduced when plants were grown at the higher level of 2 light intensities. Menser et al. (7) found that ozone damage to tobacco plants was diminished by an extended 22-hour photoperiod immediately before fumigation.

Ascorbic acid is known to protect plants from ozone and other oxidizing agents. Freebairn (3) increased the tolerance of Pinto bean plants to ozone either by supplying ascorbic acid to the roots or by foliar applications. Freebairn and Taylor (4) made tissue analysis of smog-sensitive plants sprayed with ascorbic acid. They found that concentrations of ascorbic acid in the leaves increased and resulted in partial to complete protection from air-pollution injury.

This paper presents results of a study to determine the effects of light preconditioning on ascorbic acid levels in tobacco plants; and the relation of ascorbic acid levels to ozone injury of 2 tobacco varieties, one resistant and the other sensitive to ozone. Ascorbic acid concentrations reported represent the excess formed in synthesis over that utilized in metabolism. The results show that light preconditioning caused notable differences in ascorbic acid levels. These differences were associated with changes in the degree of ozone injury.

Materials and Methods

Ascorbic acid concentrations were determined by using greenhouse-grown plants of the cigar tobacco (Nicotiana tabacum L.) varieties Bel-B (ozone resistant) and Bel-W3 (ozone sensitive). These varieties are designated B and W3, respectively, in this report. Vigorous, uniform 8-to-9-week-old plants with 5 or 6 leaves were selected for experiments and 2 lighting cycles were imposed on 4 plants of each variety during the 27-hour period before ascorbate extraction. The essential difference between the cycles was a 12-hour exposure to 900 ft-c of incandescent light imposed on one plant group from 4 pm to 4 am. Control plants were in the dark during this period. Lighting conditions (shaded greenhouse) were the same for both plant groups before and at the conclusion of the 4 pm to 4 am period. Details of cultural procedures and manipulations of lighting cycles were reported previously (7).

The ascorbic acid concentration of tobacco leaves was determined by using a colorimetric procedure developed by Freebairn (2) and adapted from Roe (9). The method is based on the reduction of 2,6-dichlorobenzenoneindophenol by ascorbic acid at pH 3.5. Transmission of samples was measured at 520 nm. Extraction consisted of grinding leaves in a mortar using a 10-ml solution of 3.0% trichloroacetic acid containing 0.005 M disodium-EDTA. Half-leaves from uniform, paired plants were quickly detached, weighed, dried, and ground with quartz sand in the extracting solution for 1 to 2 minutes. The homogenate was filtered through Whatman No. 42 paper, washed with an additional 10 ml of extracting solution, and diluted to 25 ml. Extractions were conducted at the completion of the respective preconditioning periods. At the same time, a second pair of plants from each treatment was placed in an ozone fumigation chamber and fumigated at 0.28 ppm while ascorbic acid extractions progressed. The fumigation procedure and the arbitrary evaluation of ozone injury were reported previously (7, 8).

Potassium ascorbate uptake by detached tobacco leaves and the effect of time of day on ascorbic acid concentrations were studied. These corollary investigations were intended to support the study of light preconditioning and ascorbic acid levels.

An attempt was made to relate ascorbate levels to ozone injury of detached, fumigated leaves. Leaves were detached and the petioles were inserted immediately in beakers containing 40 ml of 10−2 M potassium ascorbate solution. Detachment was done in the fumigation chamber in darkness and was followed 2.5 to 3 hours later by turning on the lights (500 ft-c) to induce stomatal opening. After 45 minutes of light, duplicate pairs of leaves were removed from the chamber and extracted for ascorbic acid or were fumigated for 1.5 hours at 0.45 to 0.55 ppm ozone. Leaves of representative plants were observed at 80 × magnification to determine the initial condition of stomata.

The study of the effects of day period on ascorbic acid levels was designed to measure the progress of ascorbic acid metabolism as affected by exposure to normal daylight conditions. The greenhouse-grown plants (Bel-W3) used for this work were selected from the same supply as others used in ascorbic acid
investigations and assayed for ascorbic acid at 9 AM, 12 noon, and 3 PM.

Results

Light preconditioning resulted in a considerable increase of ascorbic acid concentrations in the leaves of the 2 tobacco varieties (fig 1, lower portion). The mean increase expressed as a ratio to the control was 2.4 (second leaf), 2.6 (third leaf), and 3.3 (fourth leaf). These 3 leaves represent stages of leaf maturity in which the second leaf was recently-expanded, the third leaf was newly-expanded, and the fourth leaf was continuing to expand. At each leaf position, actual differences in ascorbic acid levels between the 2 varieties were small. In contrast, when plants were kept in the dark, ascorbic acid levels of Bel-W3 slightly exceeded Bel-B.

Ozone injury to each variety (fig 1, upper portion) was reduced by light preconditioning. Comparisons of the upper and lower portions of figure 1 show that the injury reduction tends to correspond with the increase in ascorbic acid concentrations. The average amount of ozone injury to light preconditioned plants was approximately 50% of the amount that occurred to control plants. The data further indicate that light-induced ozone protection as related to changes in ascorbic acid levels is more effective in older than younger leaves. For example, the general decline in ozone damage was about 50% in all leaves, but the ascorbic acid increase in the oldest leaves was only two-thirds as much as occurred in the youngest leaves.

Results of the detached-leaf experiments (table I) show that potassium ascorbate supplied to detached leaves caused large increases in ascorbic acid levels. The average increase in ascorbate compared with the control, was 2.5 times as much in the oldest leaves and 3.0 times as much in the youngest leaves. The concentration gradient corresponded with that of intact plants; i.e., concentration increased from old to young leaves. The rate of ascorbate uptake was rapid; concentrations more than doubled 3 hours after leaves were placed in ascorbate solutions. Ascorbate levels were about equal (both varieties) in the water check, but ascorbate treatment caused higher ascorbate levels in variety Bel-B than Bel-W3.

Table I. Ascorbic Acid Levels of Detached Tobacco Leaves Treated with 10\(^{-4}\) M Potassium Ascorbate

<table>
<thead>
<tr>
<th>Stage of leaf development</th>
<th>Ascorbate treated</th>
<th>Water control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bel-W3</td>
<td>Bel-B</td>
</tr>
<tr>
<td>(ascorbic acid, (\mu g/g) fr wt)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Youngest</td>
<td>549</td>
<td>646</td>
</tr>
<tr>
<td>Mature</td>
<td>530</td>
<td>587</td>
</tr>
<tr>
<td>Oldest</td>
<td>424</td>
<td>440</td>
</tr>
</tbody>
</table>
* Mean of 3 determinations.

Table II. Ozone Injury to Detached Tobacco Leaves Treated with 10\(^{-4}\) M Potassium Ascorbate

<table>
<thead>
<tr>
<th>Variety</th>
<th>Ascorbate treated</th>
<th>Water control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bel-W3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bel-B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(leaf area ozone damaged, % of total)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Youngest</td>
<td>24.29</td>
<td>2.00</td>
</tr>
<tr>
<td>Mature</td>
<td>40.95</td>
<td>4.76</td>
</tr>
<tr>
<td>Oldest</td>
<td>28.81</td>
<td>10.00</td>
</tr>
</tbody>
</table>

* Mean of 5, 1.5-hour fumigations at 0.45 to 0.55 ppm ozone.

Ascorbate treatment of detached leaves resulted in partial protection from ozone damage (table II). The results generally correspond with the evidence obtained from the light-preconditioning experiments. Untreated leaves of Bel-W3 and Bel-B were injured the most severely, regardless of the stage of leaf development. Ozone injury to ascorbate-treated Bel-W3 was one-third to one-half as much as the control, while injury to Bel-B was one-fourth to one-ninth of the control. Although ascorbate concentrations of the 2 varieties usually were about equal, much more injury was inflicted on Bel-W3.

Ascorbic acid concentrations in greenhouse-grown plants (Bel-W3) increased steadily from mid-morning (9 AM) to mid-afternoon (3 PM). Amounts of ascorbate were lowest in the oldest leaves and highest in the young leaves. At 3 PM, the ascorbate concentrations of the oldest and youngest leaves were 175 and 440 \(\mu g/g\) fresh weight, respectively. The rate of ascorbate increase was most rapid in young leaves and least rapid in the oldest leaves.

Differences in stomatal behavior following light preconditioning were studied as a possible cause of the decreased ozone susceptibility of tobacco plants. Transpiration rates of the 2 varieties were used as indicators of the stomatal condition. The rates did not differ appreciably although the average rates of moisture loss from light-preconditioned plants were about 15% less than the control. These observations indicate that differences in stomatal condition were not a probable cause of the decline in ozone susceptibility.

Discussion

This investigation confirms results of earlier studies (7) that light preconditioning causes a partial protection of plants from ozone damage. The results of ascorbic acid analysis provide a biochemical basis for interpreting frequently observed differences in the degree of ozone injury. A 22-hour photoperiod caused more than 2 to 3 times as much ascorbic acid in the leaves of both ozone-sensitive and resistant plants. These increases corresponded to a decrease in ozone susceptibility. The partial protection of tobacco leaves from ozone possibly was caused by
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preferential oxidation of the ascorbic acid produced by the 22-hour photoperiod.

Dugger et al. (1) observed that high light intensity and a 15-hour photoperiod caused an increase in sugar levels above an ozone susceptibility threshold. The cause of ozone protection was uncertain, but a close association with sugar metabolism was established. In the present report, the evidence suggests that ascorbic acid is a possible cause of the light-induced partial protection of tobacco plants from ozone damage.

The gradient of ascorbic acid concentrations in the leaves of tobacco plants (youngest > mature > oldest) is inversely related to the ozone susceptibility of these stages of leaf development. Mapson (5) reported that young, vegetative tissues contained the highest concentrations of ascorbic acid. The specificity of the oxidant peroxynitrate for young tissues (10) presents an interesting contrast to the pattern of ozone damage to tobacco leaves.

Photo-oxidation of ascorbic acid after illumination of leaves was reported recently by Mapson (6). Ascorbate was oxidized to dehydroascorbic acid and a steady-state concentration of the latter resulted without appreciably changing the proportions of the 2 compounds. The photooxidation effect possibly influenced ascorbic acid synthesis in the present study. The ascorbate produced by extended light preconditioning, however, more than exceeded any losses caused by a change in the position of the ascorbic acid to dehydroascorbic acid equilibrium.

The results of this report indicate that ascorbic acid is not involved as a fundamental cause of inherent differences in the ozone susceptibility of ozone-resistant Bel-B and ozone-sensitive Bel-W3. No significant differences were found in ascorbate concentrations at times of widely contrasting ozone sensitivity of the two varieties.

Summary

Light preconditioning caused increased ascorbic acid concentrations in the leaves of two tobacco (Nicotiana tabacum L.) varieties. The increases occurred in leaves representing developmental stages of rapid leaf expansion, maturity, and recent maturity. Ozone injury to these leaves declined as ascorbate levels increased. Ascorbate concentrations in light-preconditioned leaves were 2.5 to 3.3 times as much as controls while ozone damage declined to about 50 per cent of controls. Preferential oxidation of ascorbic acid was regarded as the probable cause of the partial ozone protection induced by light preconditioning. Results of this work, however, do not show an obvious or direct relation of ascorbic acid to fundamental differences in varietal resistance or susceptibility to ozone.

Acknowledgments

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Literature Cited


Fig. 1. Effects of light and control preconditioning on ascorbic acid levels and ozone susceptibility of two tobacco varieties. Light preconditioning consisted of a 22-hour photoperiod immediately before fumigation, while control preconditioning consisted of 10 hours of light and 12 hours of darkness during the same (6 AM to 4 AM) 22-hour pre-fumigation period. Plants were fumigated 5 hours later at 9 AM. Leaf positions 2, 3, and 4 represent 3 stages of leaf maturity in which the second leaf is recently-mature, the third leaf is newly-mature and the fourth leaf is expanding. Data are averages of 5 ascorbate extractions and ozone fumigations (2 hr at 0.28 ppm) conducted simultaneously.