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Short Communication

Ethylene Air Pollution

EFFECTS OF AMBIENT LEVELS OF ETHYLENE ON THE GLUCANASE CONTENT OF BEAN LEAVES

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We reported earlier that ethylene increased the levels of glucanase (endo-β-1,3-glucan-3-glucanohydrolase, EC 3.2.1.6) in bean (*Phaseolus vulgaris* L. cv. Red Kidney) leaves (1, 2) at Frederick, Maryland. While the function for glucanase has not been firmly established, a number of roles have been proposed and include digestion of storage glucans in seeds, control of cell elongation, regulation of pollen tube growth, yeast cell expansion, fertilization, removal of phloem callose, and an antibiotic role of attacking the cell walls of invading fungi and bacteria (1, 2). When we attempted to repeat the experiment in Beltsville, Maryland, we found the addition of ethylene failed to increase enzyme activity (see Table I). Though care was taken to use similar extraction and assay procedures, we observed considerable variation between the base levels of glucanase from one experiment to another. The reasons for these fluctuations are unknown at this time. We examined but rejected differences in cultural practices and seed stock as explanations for the inability of ethylene to increase glucanase activity and turned our attention to the possibility that the air at Beltsville contained sufficient levels of ethylene to cause the observed effects.

Ethylene levels at Frederick vary in amounts not measurable by gas chromatography (less than 1 nl/l, which is equal to 1.2 $\mu g/m^3$) to 5 nl/l. The Plant Industry Station at Beltsville is located on Route 1 and a few hundred feet north of the exit to the Washington, D.C., Beltway. Levels there vary from undetectable, on windy or rainy days, to 40 nl/l during the morning rush hour on calm days. Average values during the day are between 10 and 20 nl/l. Since glucanase induction is ethylene-sensitive, we examined the possibility that growing plants in chronic low levels of ethylene disrupted the ability of additional quantities of ethylene to induce glucanase activity.

Bean plants were grown in 0.6 m^3 plastic chambers located in a greenhouse equipped with carbon filters to remove ozone. The 2-liter per min air supply for the chambers was passed through a 6- × 30-cm column of $KMnO_4$ absorbed on alumina pellets (Purafil, Marbon Division, Borg Warner, Washington, W. Va. 26181)[1]. The data in Table II show that there was less glucanase in plants grown in $KMnO_4$-filtered air compared to those grown in unfiltered air. Since the plants were grown in enclosed chambers and the rate of air movement was low, the temperature inside the chambers rose as high as 45 C during midday on sunny days and returned to 18 C during the night. In addition to changes in glucanase levels, we noticed that plants grown in unfiltered air had smaller leaves and shorter internodes than those grown in filtered air.

The data in Table III show that a 3-day exposure of leaves to ethylene (10–28 nl/l) doubled glucanase activity. The $KMnO_4$ absorbant used in these experiments was prepared by mixing 120 ml of 0.1 M $KMnO_4$ with 100 g of 16-mesh silica gel and drying the slurry at 110 C for 16 hr.

Ambient levels of ethylene in unpolluted air vary between 1 and 5 nl/l (4, 14, 16), whereas in urban areas the levels increase to a maximum of approximately 130 nl/l (3, 7, 16). Average urban values are in the order of 10 to 40 nl/l (3, 16). Whereas most of the literature deals with ethylene levels in California, we have made essentially similar observations in Beltsville.

Automobiles account for 90% of the ethylene released in the air (Abeles *et al.*, submitted for publication), and gas chromatographic analysis of urban air suggests that the atmosphere is essentially dilute auto exhaust with small amounts of photooxidants and SO_2 added. Levels of ethylene vary with the amount of traffic, and a number of workers have shown that a peak in ethylene levels coincides with the morning rush hour (3, 7, 15). The evening rush hour levels are generally lower because of increased wind speeds later in the day. Highest ethylene levels are associated with calm days (5, 9, 10), whereas lower levels are reported during windy or rainy weather. Seasonal fluctuations also occur. Using "dry sepal" orchid damage as a criterion, highest levels of ethylene are found during the fall and winter (10) when photochemical degradation is reduced. Highest levels of ethylene are found at ground level. Little difference in ethylene levels were observed in the first 100 feet (6, 16), while smaller amounts were found at 3,000 feet (6). Other important point sources of ethylene are industry and fires. Cotton plants growing within a 2-mile radius of a polyethylene plant showed damage, and a 3 μl/l ethylene level was observed in the immediate vicinity of the plant (7). Ethylene levels in smoke vary from 0.2 to 4 μl/l (6, 12) and are diluted to ambient levels 2 or more miles down wind from the fire (6, 12).

Since the first report of dry sepal damage on orchids by Davidson in 1947 (5), a number of other investigators have reported similar phenomena. Estimates of loss to California

[1] Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be available.

Table I. *Effect of Ethylene on Levels of Glucanase in Bean Leaves from Plants Grown in Frederick and Beltsville, Maryland*

Leaves were treated 24 hr with 10 µl/l ethylene.

Location	Trial	Units of Glucanase[1]	
		Initial	24 Hr of ethylene
Frederick[2]		0.89	28.0
Beltsville	1	3.2	3.7
	2	0.96	0.72
	3	3.7	4.4
	4	12.0	25.0

[1] Milligrams of glucose released from laminarin per hour per ml enzyme solution at 50 C.

[2] Data from reference 1.

Table II. *Effect of Filtering Ambient Air Through Columns of $KMnO_4$ Absorbed on Alumina on the Levels of Bean Leaf Glucanase*

Treatment	Units of Glucanase
Unfiltered air	10.6
Filtered air	3.4

Table III. *Effect of Low Levels of Ethylene on Glucanase Levels in Bean Plants Grown in the Frederick, Maryland Area*

Treatment	Units of Glucanase
Initial control	30
3 Days outside fumigation chamber	32
3 Days inside fumigation chamber + $KMnO_4$ absorbant	36
3 Days inside fumigation chamber + 10 nl/l ethylene[1]	67

[1] Final level of ethylene after 3 days was 28 nl/l.

flower growers range from $60,000 to $700,000 a year in 1963 to 1964 (10, 11), and the amount of damage has been increasing in a steady fashion since the initial reports in 1952 (10).

However, floral damage is a striking effect of ethylene, and the economic loss focuses attention on this problem. Other effects of low ethylene levels are more subtle, and the changed appearance is difficult to visualize, especially when compared with the striking effect of other air pollutants such as oxidants, SO_2, and fluorides.

The data reported here suggest that sublethal levels of ethylene can change the biochemical nature of the plant while having no obvious effects on the plant's appearance. Whereas growth reduction is a result of ethylene, it is difficult to visualize in the absence of unfumigated plants. This is in contrast to damage by other gases which cause a variety of necrotic lesions and abnormal discolorations on plant tissue. What this means in terms of productivity, and in insect and disease resistance, remains to be shown. However, it is clear that plants grown in the urban environment may be biochemically different from those in a rural area, and the differences may cause problems in comparing research with plants grown in clean *versus* polluted air.

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