Lipid Peroxidation, a Result of Injury in Bean Leaves Exposed to Ozone

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ABSTRACT

Bean leaves exposed to ozone or treated with a solution of α-iodoacetamide showed no evidence of lipid peroxidation until after symptoms of injury developed. Evidence of lipid peroxidation appeared immediately in bean leaves injured by crushing or by a spray of chloroform. Unsaturated fatty acids were not selectively destroyed in bean leaves exposed to ozone. Phytopathology 60:1531-1532.

Additional key words: Phaseolus vulgaris L., malonyl dialdehyde, thiobarbituric acid.

Scott et al. (5) proposed that ozone attacks cell membranes by oxidizing unsaturated lipids. We reported the loss of unsaturated fatty acids in tobacco leaves exposed to ozone at 100 parts/hundred million (pphm) (6). By using thiobarbituric acid reagent (TBA) Goldstein & Balchum (1) looked for the formation of malonyl dialdehyde (MD) as evidence that ozone reacts with unsaturated lipids to produce organic peroxides. They were able to detect peroxidation in red blood cells exposed to high concn of ozone, and suggested that the organic peroxides themselves are toxic.

We have examined the possibility that lipid peroxides are formed in green leaves exposed to ozone. With the equipment and procedures previously reported (7), we exposed 10- to 14-day-old pinto beans (*Phaseolus vulgaris* L.) to 25 pphm ozone for 3 hr. Control plants were treated exactly the same, except that there was no ozone in the air stream entering the chamber. We chose 25 pphm ozone because this concn is representative of that usually found outdoors in the USA when the air is polluted (10 to 40 pphm).

In addition to ozonated and control leaves, we also analyzed leaves injured by crushing or spraying with chloroform, and excised leaves allowed to take up a 2 mM solution of α-iodoacetamide (IA) for 2 hr. The leaves treated with IA, a sulfhydryl-binding reagent, were included in this study because such leaves show symptoms and metabolic changes similar to those caused by ozone (8). Because the symptoms caused by ozone and IA are distinctive, leaves so injured were considered to be selectively damaged. Leaves injured by crushing or chloroform were considered as nonselectively damaged. Each analysis represents a min of three experiments, each containing three replications.

The following method was used to measure the MD produced in the leaves of the ozonated and control plants. A test tube containing a mixture of 1 ml 0.8% TBA, 0.5 ml 7% perchloric acid, and 1.5 ml distilled water was heated in a boiling water bath. To this heated solution was added a 3-cm diam disc of leaf tissue, and the test tube was heated in the bath for 10 min more, then removed and cooled. The material in the test tube was extracted with 3 ml normal butanol, and 2.5 ml of the butanol extract was further clarified with 0.5 ml absolute ethanol. The OD of the clarified butanol extract was measured at 532 mμ in a Beckman Model B spectrophotometer, and expressed as n moles of MD per disc (2). The MD concn reported are the average of a min of nine measurements for each experiment.

The results (Table 1) show that the MD content of ozonated leaves does not increase significantly until injury develops. This is also true of the leaves treated with IA. The MD content of the IA-treated leaves was compared to that of excised leaves allowed to take up water for the same period. Samples from both treatments contained 0.8 n moles/disc at the time (2 hr after treatment began) that the IA-treated leaves began to show injury. Three hr after injury appeared, the MD content of the IA-treated leaves was 1.2 n moles/disc, and by the next day had increased further to 1.8. During this period, the MD content of the water-treated controls remained at 0.8 n moles/disc.

In contrast, the MD content of the leaves damaged by crushing or by chloroform increased to 3 to 10 n moles/disc within 10 min after being injured. This was also true of leaves ozonated or treated with IA before being crushed or damaged by chloroform. This last experiment demonstrates that neither ozone nor IA inhibits peroxidation induced by nonselective injury.

That lipid peroxidation occurs only after ozone injury appears is also supported by an analysis of the fatty acid content. Methyl esters were prepared and analyzed by methods reported previously (8). In agreement with others (4), we found that palmitic, linoleic, and linolenic acids make up about 90% of the total fatty acids in bean leaves. Our data (Table 2) show that ozonation of bean leaves produces only slight changes in these fatty acids, and the changes occur only after injury develops.

There was no evidence of lipid peroxidation occurring before injury appears on bean leaves treated with ozone or IA. This agrees with Robinson (3), who reported that the membrane function of microsomes can be altered by sulfhydryl-binding reagents without the appearance of lipid peroxidation. Lipid peroxidation in the bean leaves could be detected only after they were

TABLE 1. Malonyl dialdehyde (n mole/disc) in bean leaves following exposure to air or to 25 pphm ozone for 3 hr

Hr after exposure	Air	Ozone	
		Not injured	Injured
1	0.90 ± 0.1	0.86 ± 0.15	
3	0.80 ± 0.1	0.82 ± 0.1	1.04 ± 0.15
18	0.70 ± 0.1		1.60 ± 0.4

TABLE 2. Fatty acid composition of bean leaves after exposure to 25 pphm ozone for 3 hr. Expressed as per cent of sum of the three fatty acids

Fatty acid	Control[a]	Hr after ozonation	
		3	18
Palmitic	14.6	14.7	14.8
Linoleic	14.0	14.5	13.1
Linolenic	71.4	70.8	72.1

[a] Not exposed to ozone.

visibly injured, whether by ozone, IA, chloroform, or crushing. Lipid peroxidation in bean leaves results from damage to the tissue, and does not appear to be a part of the process by which ozone injures leaves.

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