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INDUCTION OF ASCORBATE PEROXIDASE AND GLUTATHIONE REDUCTASE ACTIVITIES BY INTERACTIONS OF MIXTURES OF AIR POLLUTANTS

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The response of ascorbate peroxidase and glutathione reductase activities in peas (*Pisum sativum* var. Waverex) was investigated after three weeks of exposure to mixed fumigations with SO_2, NO_2 and O_3 (0.050 parts per million each) and increasing concentrations of O_3 (0–0.150 parts per million). The results show that plants respond similarly to a high concentration (0.150 parts per million) of a single air pollutant (ozone) and to mixtures of air pollutants (SO_2, NO_2 and O_3) when individual concentrations are low (0.050 parts per million each). In both cases, levels of ascorbate peroxidase and glutathione reductase activites were approximately twice those to be found in plants grown in charcoal-filtered air ($p < 0.01$).

KEY WORDS: sulphur dioxide, nitrogen dioxide, ozone, vitamin C, antioxidants, lipid peroxidation.

INTRODUCTION

In atmospheric chemistry it is well established, that air pollutants such as sulphur dioxide and ozone initiate the formation of free radicals:[1–3]

$$(1)\quad SO_2 + O_2 \longrightarrow SO_3^{\cdot}$$

$$(2)\quad O_3 \longrightarrow O(1D) + O_2$$

$$O(1D) + H_2O \longrightarrow 2\,OH^{\cdot}$$

$$(3)\quad NO_2 + O_3 \longrightarrow NO_3^{\cdot} + O_2$$

These free radicals are highly reactive and *in vitro* they have been shown to induce peroxidative breakdown of polyunsaturated fatty acids via formation of peroxy- and alkoxy-radicals.[4] In plants, this eventually leads to membrane damage, cell death and leaf necrosis.

However, the way in which free radical mechanisms affect plants in air pollution toxicity is unclear. Free radicals themselves may either directly cause the damage or chemical reactions within the cell, initiated by the air pollutants, may lead to the formation of free radical products which subsequently cause leaf injury. Peroxidases may well prevent this from happening in plants by reducing the levels of hydroperoxides. It is known, for example, that these enzymes increase in activity during exposure to oxidative stress.[5] Unfortunately, it is not clear in plants if the physiologi-

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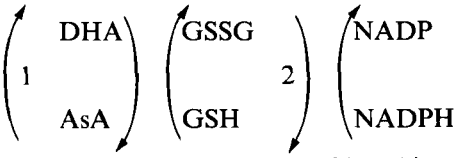
cal significance of this response is brought about by virtue of the removal of toxic hydroperoxides. There is evidence, however, that hydroperoxides are detoxified in plants via ascorbic acid.[6] In such a reaction, ascorbic acid would be oxidised and then recycled via glutathione and glutathione reductase.[7,8]



1 = Ascorbate peroxidase 2 = Glutathione reductase

Consequently, a simultaneous increase of both ascorbate peroxidase and glutathione reductase activities may be expected if ascorbate peroxidase detoxifies hydroperoxides during oxidative stress in plants.

The purpose of the research described in this paper was to answer two questions: "Are the biochemical changes that may precede leaf injury caused by interactions between the common air pollutants SO_2, NO_2 and O_3, and, if so, do ascorbate peroxidase and glutathione reductase activities increase simultaneously during this process?".

MATERIALS AND METHODS

Plant Material

Seeds of a commercial pea variety (*Pisum sativum* var. Waverex) were sown in Vermiculite and allowed to germinate under green house conditions. After seven days, the seedlings were pricked out into individual plastic 9 cm-pots containing John Innes No. 2 compost (7:3:2; sterilized loam, peat and sand, together with 6.44 kg m^{-3} of John Innes Base Fertilizer). Of these, seventy-two uniform plants (six per treatment) were selected and placed in controlled environment fumigation cabinets although the distribution of the light was improved from that previously described[9] by having two lamps (400 W, Kolorlux, Thorn-EMI) so as to give a more even distribution of light (250 W m^{-2}). In both experiments, the photoperiod lasted twelve hours with day/night temperatures of 17/9°C and a relative humidity of 68%.

Fumigations

Ozone was generated using an electric discharge tube ozone generator (Wallace and Tiernan Ltd., Tonbridge, UK) and introduced into the cabinet for seven hours each day, two hours after the lights were switched on. In the mixed fumigation experiments, levels of ozone, sulphur dioxide and nitrogen dioxide concentrations were checked periodically using a Dasibi 1008 ozone analyser (Analysis Automated, Oxford, UK), a Meloy SA 285 flame photometric SO_2 analyser, and a Meloy NA 530 chemiluminescence NO_x-analyser (Techmation Ltd., Middlesex, UK). In the experiment with ozone alone, peas were fumigated with charcoal-filtered air containing 0.050, 0.100 and 0.150 parts per million (ppm) of ozone for three weeks corresponding to 100, 200 and 300 $\mu g\, m^{-3}$, respectively; whilst, in the mixed fumigation experiments, plants were exposed to 0.050 ppm of sulphur dioxide, nitrogen dioxide and ozone (individually and in all combinations) in charcoal-filtered air.

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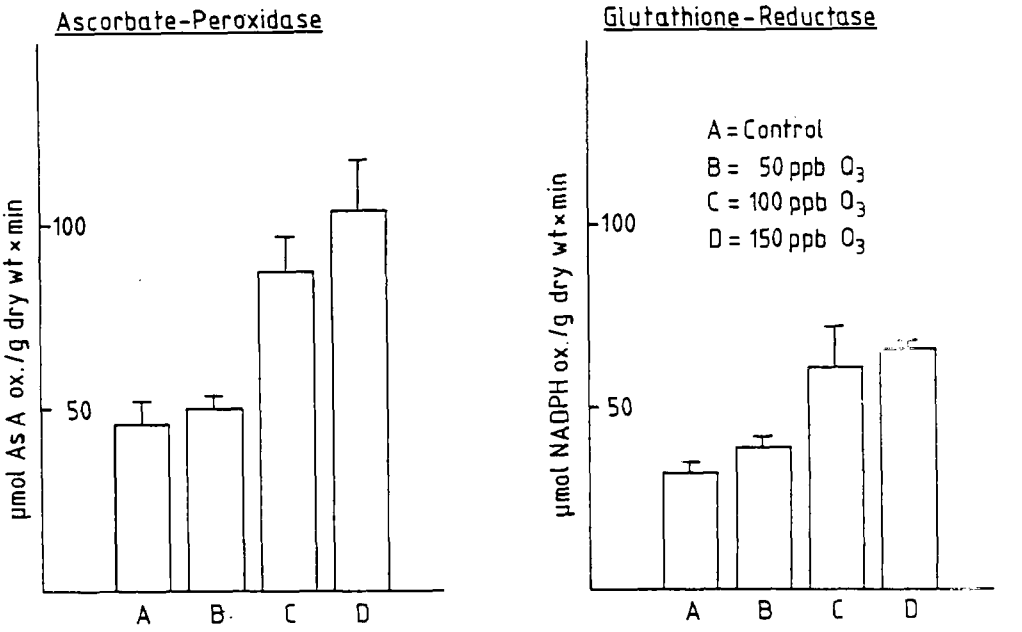

FIGURE 1 Ascorbate peroxidase and glutathione reductase activities in peas after three weeks of exposure to increasing concentrations of ozone (0–0.150 ppm).

Enzyme Analysis

Ascorbate peroxidase and glutathione reductase activities were estimated as described by Nakano and Asada[6] and Law *et al.*,[10] except that a 66 mM sodium phosphate buffer, pH 7.0, containing 2 mM EDTA was used.

RESULTS

Fumigations with Ozone

Conditions for the dose-response experiment were chosen such that the concentrations used resembled approximately one, two and three times ambient mean concentrations of ozone in moderately polluted areas of Central Europe. None of the ozone levels used caused any visible leaf injury; although, in the experiments with 0.100 ppm and 0.150 ppm, there was a slight inward curling of the leaves.

After three weeks of fumigation, both ascorbate peroxidase and glutathione reductase activities were found to have doubled ($p < 0.01$) in pea plants that had been exposed to 0.100 and 0.150 ppm of ozone. No effect was observed in plants that had experienced only 0.050 ppm of ozone (Fig. 1).

Mixed Fumigations

In mixed fumigations, concentrations of the pollutants were chosen so that, when all three pollutants were applied together, a similar total concentration was applied as in

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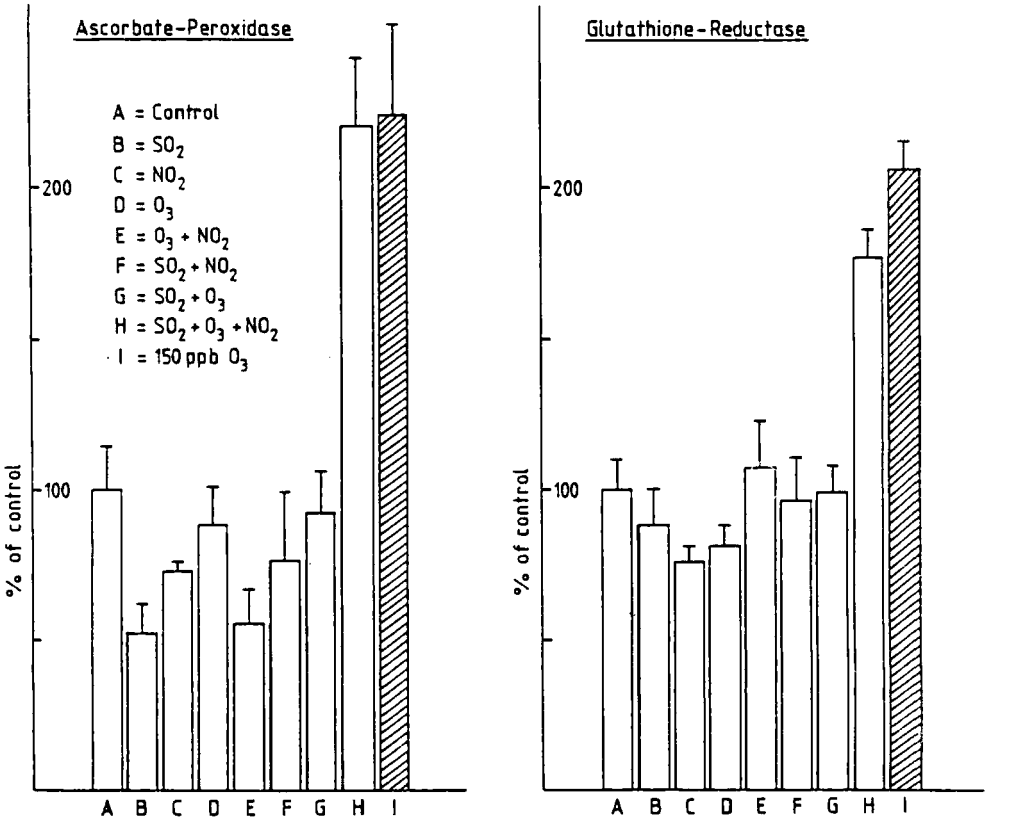

FIGURE 2 Ascorbate peroxidase and glutathione reductase activites in peas after three weeks of fumigation with SO_2, NO_2 and/or O_3 (0.050 ppm each; alone and in all combinations). Results are given as means ± standard error of six replicates. Activities of ascorbate peroxidase and glutathione reductase were 35.6 ± 5.3 and 27.8 ± 2.9, respectively (data are given as a mean ± standard error, $n = 6$).

the ozone experiment (i.e. 0.150 parts ppm in total or 0.050 ppm each). As in the ozone experiment, no visible leaf injury was observed although there was a slight inward curling of the leaves when two or three air pollutants were applied together giving concentrations corresponding to 0.100 and 0.150 ppm.

There was an interactive increase of both ascorbate peroxidase and glutathione reductase activities only when all three air pollutants were present together ($p < 0.01$). In this case (Treatment H in Fig. 2), the response was approximately the same as in peas that had been fumigated with 0.150 ppm of ozone alone (Treatment I). Sulphur dioxide, nitrogen dioxide and ozone alone (Treatments B, C and D respectively) had no significant effect at 0.050 ppm ($p < 0.05$). Combinations of two atmospheric pollutants (Treatments E, F and G), each at a total concentration of 0.100 ppm, had no significant effect on either of the two enzyme activities ($p > 0.05$). This contrasts with treatments with ozone alone at 0.100 ppm which caused a two-fold increase in the levels of both enzyme activities.

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DISCUSSION

More-than-additive effects of combinations of atmospheric pollutants have often been reported.[12,13] Unfortunately, in most of these experiments, the results from mixed treatments were not compared to those employing single pollutant dosages of equivalent quantities. This is necessary in order to establish that the response to increasing concentrations of a single pollutant is linear, and that the response to the mixture is truely additive or more-than-additive, and that no dosage- or concentration-dependent threshold has been passed in the mixed treatment.

In most rural parts of Europe and North America, concentrations of ozone are often higher than those of sulphur dioxide and nitrogen dioxide.[11] If free radical reactions are involved in air pollution toxicity, it may be that an interaction of ozone with other atmospheric pollutants, such as sulphur dioxide and nitrogen dioxide, is possible. Consequently, evidence for changes in the free radical scavenging systems in plants was sought and found.

Current levels of ambient ozone expressed as an average would appear to indicate that no changes to the free radical scavenging systems might be expected. However, this misses the point that atmospheric ozone rarely occurs in isolation and higher concentrations are frequently measured as peak concentrations in industrialised areas. In such circumstances, they would be capable of doubling levels of both ascorbate peroxidase and glutathione reductase activities. Moreover, these results indicate that ozone, sulphur dioxide and nitrogen dioxide act together via a similar mechanism to cause leaf injury. In addition, the simultaneous increase in levels of ascorbate peroxidase and glutathione reductase activities provides further evidence that hydroperoxides are involved in air pollution toxicity and that peroxidases appear to be responsible for the detoxification of these hydroperoxides in peas.

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