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Interaction of Antioxidants with Ozone and Herbicide Stress

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Antioxidants are compounds used to prevent the reactions of organic materials with molecular oxygen. Antioxidants are used to protect foods and other products from discoloration and spoilage, thus sodium benzoate is added to bread to prolong its shelf life. Other antioxidants such as piperonyl butoxide (α-(2-(2-butoxyethoxy) ethoxy)-4,5-methylenedioxy-2-propyltoluene) and sesamex (a component of sesame oil) are used as insecticide synergists (O'BRIEN 1967a and 1967b). Both of these compounds are potent microsomal mixed function oxidase (m.f.o.) inhibitors (O'BRIEN 1967a and 1967b, REINBOLD & METCALF 1976, TANAKA et al. 1976) and when applied in combination with certain insecticides increase the insecticide's potency by decreasing the rate at which insects can detoxify the insecticides (O'BRIEN 1967a and 1967b, TANAKA et al. 1976). In plants, EDU (N-(2-(2-oxo-1-imidazolidinyl)ethyl)-N-phenylurea) has been reported to protect pinto beans (Phaseolus vulgaris L.) CARNAHAN et al. 1978) and piperonyl butoxide to protect tobacco (Nicotiana tabacum L.) (KOIWAI & KISAKI 1973) from ozone injury. The purpose of this study was to a) evaluate the antioxidants EDU, piperonyl butoxide, and n-propyl gallate, as protectants agains ozone injury in navy bean, and b) to determine if these antioxidants could function as herbicide synergists just as piperonyl butoxide functions as an insecticide synergist.

MATERIALS AND METHODS

Plant Culture. All plants were grown in a greenhouse soil mix (1:1:1 soil, sand, peat). Navy bean (Phaseolus vulgaris L. cultivars '0686' and '0670') and corn (Zea mays L. cultivar 'Pioneer 3780') were grown in 946-ml waxed cups and soybeans (Glycine max L.) Merr. cultivar 'Swift') were grown in 473-ml waxed cups. The waxed cups were filled 80% with soil, five seeds were placed upon the soil and then covered with 5 cm of soil. The navy beans were thinned to three plants per cup before ozone fumigation. Plants were grown in greenhouses with supplemental lighting (16 h day), a minimum

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temperature of 25 C and max of 33 C. The greenhouse used to grow the navy beans for ozone fumigation was fitted with charcoal filters to remove ambient ozone from the air entering the greenhouse to maintain the greenhouse ozone free.

The ozone fumigation chamber was located a substantial distance from the greenhouse.

Antioxidant-Ozone Interaction. The three antioxidants were evaluated for their ability to protect both navy bean cultivars from ozone injury in separate experiments. Navy beans were treated with EDU (50% wettable powder - Dupont) by spraying an aqueous solution onto the soil in the cups (2.24 kg/ha) immediately before placing and covering the seed, or when 10 days old by spraying the foliage (2.24 kg/ha) or by dipping 10-day-old plants (inverting the cups and dipping the plants into a 1-liter beaker filled with a 2000 ppm aqueous solution of the material for 15 sec). Piperonyl butoxide (Pfaltz-Bauer) was applied by spraying the soil before planting (4.48 kg/ha) or by spraying the foliage of 11-day-old plants (3.36 kg/ha) or by brushing (applying a 2000 ppm solution with a pencil brush to one-half of each primary leaf, both adaxial and abaxial sides, the non-treated half serving as a control). Propyl gallate (Sigma) was applied by spraying the soil before planting (4.48 kg/ha), or when the plants were 10 days old, by spraying the foliage (4.48 kg/ha), or by dipping (3000 ppm solution 15 sec). The antioxidants were sprayed in aqueous solution by a link-belt sprayer at 2.1 kg/cm^2 pressure in 935 L/ha spray volume. For all treatment methods the piperonyl butoxide was solubilized by 0.5% ethanol (v/v) and 0.1% cittowett surfactant (v/v). The plants were then fumigated with ozone at 0.25 ppm for 6 h two days after the antioxidant treatment of the foliage. Methods of fumigation and ozone monitoring are as previously described by Olson (1979). Visual injury ratings estimating the percent of leaf surface covered with ozone lesions were taken over the next 5 to 6 days. All treatments were replicated three times and percent data was converted to their arc sines prior to statistical analysis.

Antioxidant-Herbicide Interaction. The foliage of 16-day-old corn plants was sprayed with an atomizer until drip with 1500 ppm aqueous solutions (plus 0.5% ethanol, v/v, and 0.1% X-77, v/v) of the following antioxidants: piperonyl butoxide, EDU, sesame oil (Japan Food Corp.), and sodium benzoate (Sigma). Controls were treated with water plus ethanol and surfactant. Soybean foliage was treated with piperonyl butoxide, EDU, and propyl gallate (plus 0.1% X-77) when 14 days old. Immediately after antioxidant application, treatments of linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) (4.48 kg/ha) and oxadiazon (2-tert-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-Δ^2-1,3,4-oxadiazolin-5-one) (1.12 kg/ha) were applied to corn and oxadiazon (1.12 kg/ha) to soybeans by soil drench in 50 ml of solution. Twenty-four hours after antioxidant application treatments of atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) (11.2 kg/ha), bentazon (3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) (11.2 kg/ha), and endothall (7-oxabicyclo(2,2,1) heptane-2,3-dicarboxylic acid)

(4.5 kg/ha) were sprayed on the corn foliage, and atrazine (2.24 kg/ha), bentazon (5.6 kg/ha), and endothall (2.24 kg/ha) were sprayed on the soybean foliage by the linkbelt sprayer in aqueous solution plus 0.1% X-77 (v/v). Controls were sprayed with water and X-77 alone. Plants were harvested 10-12 days after herbicide application and fresh weights, dry weights, and visual injury determined. Because all parameters gave similar effects, only the fresh weights are reported and data expressed as percent of control. The antioxidant-herbicide interaction was also evaluated by dipping 10-day-old 'Swift' soybean plants into 1500 ppm solutions of piperonyl butoxide, EDU, and sodium benzoate for 15 sec. Twenty-four hours later atrazine (2.24 kg/ha), bentazon (2.24 kg/ha), and endothall (1.68 kg/ha) were sprayed on the foliage. Both antioxidants and herbicides were applied with 0.1% cittowett (v/v). Only dry weight was determined in this experiment and is expressed as percent of control. Values are the means of two experiments with five replications per treatment for the corn study and four replications per treatment with the two soybean studied.

RESULTS AND DISCUSSION

All three antioxidants decreased ozone injury to navy beans in at least one method of application to at least one cultivar. Cultivar '0670' was more susceptible to ozone injury than '0686' (Tables 1-3). The EDU completely prevented ozone injury to both navy bean cultivars by all methods of antioxidant application (Table 1). EDU was not phytotoxic by any application method (Table 1). Piperonyl butoxide prevented ozone injury to both cultivars when applied as a foliar spray but not as a soil treatment (Table 2). There was no significant difference between the ozone injury ratings to each half of the leaves brushed with piperonyl butoxide (Table 2). The piperonyl butoxide was slightly phytotoxic when applied as a spray, but the characteristic injury symptoms were different from those caused by ozone. Propyl gallate had no effect on ozone injury to cultivar '0686' but it significantly decreased injury to cultivar '0670' when applied by the dip method (Table 3). Propyl gallate was not phytotoxic.

TABLE 1

The effect of EDU on navy bean response to ozone fumigation.

EDU application method	Rate	Navy bean cultivar '0686' Hours after fumigation 72 (%)a	150 (%)	'0670' Hours after fumigation 72 (%)	150 (%)
None (ozone only)		5	20	30	60
Soil	2.24 kg/ha	0	0	0	0
Spray	2.24 kg/ha	0	0	0	0
Dip	2000 ppm/15 sec	0	0	0	0
Non-fumigated control		0	0	0	0

[a]Percent of primary leaf area covered by lesions.

TABLE 2

The effect of piperonyl butoxide on navy bean response to ozone fumigation.

Piperonyl butoxide application method	Rate	Navy bean cultivar '0686'		'0670'	
		Hours after fumigation			
		24 (%)a	144 (%)	24 (%)	144 (%)
None (ozone only)		17ab	33bc	40bcd	60d
Soil	4.48 kg/ha	34bc	47cd	40cd	47cd
Spray	3.36 kg/ha[b]	0a	0a	0a	0a
Non-fumigated control		0a	0a	0a	0a
Brushed (2000 ppm solution)		0a	0a	0a	0a
Brushed (control)		0a	17ab	20ab	27abc

[a]Percent of primary leaf area covered by lesions. Means followed by same letter or letters are not significantly different as judged by Duncan's multiple range test (5% level).

[b]This treatment slightly phytotoxic to navy bean.

TABLE 3

The effect of propyl gallate on navy bean response to ozone fumigation.

Propyl gallate application method	Rate	Navy bean cultivar '0686'		'0670'	
		Hours after fumigation			
		24 (%)[a]	96 (%)	24 (%)	96 (%)
None (ozone only)		17ab	43abcd	73def	73def
Soil	4.48 kg/ha	33abc	53bcd	87ef	93f
Spray	4.48 kg/ha	33abc	53bcd	60cde	60cde
Dip	3000 ppm/15 sec	33abc	33abc	13a	18ab

[a]Percent of primary leaf area covered by lesions. Means followed by the same letter or letters are not significantly different as judged by Duncan's multiple range test (5% level).

Most of the antioxidants tested acted as synergists to at least one of the herbicides in either corn or soybeans (Tables 4-6). It may be feasible to pursue the development of "herbicide synergists" similar to "insecticide synergists" now in use. None of the antioxidants affected the fresh weight of corn when applied alone, without herbicide. Piperonyl butoxide caused some discoloration of corn leaves in one of two experiments. Piperonyl butoxide significantly increased corn injury from oxadiazon, atrazine, and bentazon (Table 4). EDU increased corn injury from oxadiazon, endothall, and

bentazon (Table 4). Sesame oil did not affect the response of corn to any of the herbicides. Benzoate had an opposite effect and protected corn from endothall injury (Table 4). Endothall was the only herbicide toxic to corn at the rates used. When sprayed on soybean, propyl gallate alone significantly increased their fresh weight (Table 5), but piperonyl butoxide and EDU had no effect. When applied as a spray, both piperonyl butoxide and EDU increased soybean injury from endothall (Table 5). No other herbicide-antioxidant combination affected herbicide toxicity when the antioxidants were applied as foliar sprays (Table 5). Both atrazine and linuron were toxic to soybean in this set of experiments. Dipping soybeans into solutions of piperonyl butoxide caused a significant reduction in dry weight (Table 6). All three herbicides were toxic in this experiment. Applied by the dip method, piperonyl butoxide significantly increased injury caused by atrazine, EDU had no effect, and benzoate increased injury caused by atrazine (Table 6).

TABLE 4

The effect of foliar antioxidant applications on corn tolerance to herbicides.

Antioxidant	Herbicide[a]					
	None (%)	Oxadiazon (%)	Linuron (%)	Atrazine (%)	Endothall[b] (%)	Bentazon (%)
Control	100a[c]	100b	100a	100b	100bc	100b
Piperonyl but.	96a	75a	101a	76a	80b	74a
EDU	109a	74a	95a	92b	53a	85a
Sesame oil	106a	89ab	96a	91b	109c	96b
Benzoate	104a	92ab	86a	90b	136d	100b

[a]Data expressed as % of control of the fresh weights. Statistical analysis evaluated on the raw data; data converted to percent of control.

[b]Moderately phytotoxic to corn at the rate used.

[c]Means within columns followed by the same letter or letters are not significantly different as judged by Duncan's multiple range (5% level).

TABLE 5

The effect of foliar antioxidant applications on soybean tolerance to herbicides.

Antioxidant	Herbicide[a]					
	None (%)	Oxadiazon (%)	Linuron[b] (%)	Atrazine[b] (%)	Endothall (%)	Bentazon (%)
Control	100a[c]	100a	100a	100a	100b	100a
Piperonyl but.	120a	90a	87a	98a	88a	92a
EDU	113a	108a	98a	109a	84a	105a
Propyl gal.	153b	101a	118a	127a	108b	121a

[a]Data expressed as percent of control of the dry weights. Statistical analysis evaluated on raw data; data converted to percent of control.

[b]Slightly phytotoxic to soybeans at the rate used.

[c]Means within columns followed by the same letter or letters are not significantly different as judged by Duncan's multiple range test (5% level).

TABLE 6

The effect of antioxidants applied by dipping on soybean tolerance to herbicides.

Antioxidant	Herbicide[a]			
	None (%)	Atrazine[b] (%)	Endothall[b] (%)	Bentazon[b] (%)
Control	100b[c]	100c	100a	100b
Piperonyl butoxide	65a	35a	72a	62a
EDU	101b	78bc	96a	102b
Benzoate	90b	52ab	92a	77ab

[a]Data expressed as percent of control of the dry weights. Statistical analysis evaluated on raw data; data converted to percent of control.

[b]Slightly phytotoxic at the rate used.

[c]Means within columns followed by the same letter or letters are not significantly different as judged by Duncan's multiple range test (5% level).

As previously mentioned, many of these antioxidants have been shown to be m.f.o. inhibitors in insects (O'BRIEN 1967a and 1967b, TANAKA et al. 1976) as well as green sunfish (REINBOLD & METCALF 1976) and vertebrate liver (O'BRIEN 1967b). FREAR et al. (1972) have provided direct evidence for the presence of P420 and P450-type cytochromes in plants. They have also provided indirect

evidence that the microsomal m.f.o. system is similar in both plants and animals (FREAR et al. 1969). Furthermore, they have isolated a microsomal m.f.o. from cotton that demethylates N-methylphenyl urea herbicides (FREAR 1968, FREAR et al. 1972). The mechanism of antioxidant action in plants may also be to inhibit microxomal m.f.o. activity. This could in turn increase herbicide potency by decreasing the rate at which herbicides are metabolized. This hypothesis might also explain the protection offered by the compounds against ozone injury. The m.f.o. system requires molecular oxygen for activity. Since ozone is a "superactive" form of molecular oxygen, perhaps ozone fed m.f.o.'s become "superactive," abnormally oxygenating, peroxidating, or oxidizing many different cell constituents. Inhibition of the m.f.o. system by antioxidants could prevent this "superactivation" and thereby prevent ozone damage.

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