The Impact of Ozone on Peanut Exposed in the Laboratory and Field

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ABSTRACT

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Laboratory studies and observations for 2 yr in the field demonstrated that ambient levels of ozone (O_3) have the potential to injure peanut (*Arachis hypogaea*) crops. Assessment of O_3 injury following exposure in the laboratory showed that the Spanish-type USDA PI 268661 peanut was more sensitive than the Valencia-type McRan peanut. The antioxidant, EDU (ethylene diurea), reduced foliar injury on USDA PI 268661 in both the laboratory and field. EDU protected McRan from injury in laboratory exposures. Little or no injury was observed on McRan peanut grown in the field, and EDU treatment had no effect on yield. High levels of foliar injury on USDA PI 268661 were associated with significant reductions in yield, reduced N fixation, and increased leaf drop.

Ozone (O_3) injury on field-grown crops in Ontario has been observed for at least 20 yr. Injury symptoms on tobacco, bean, onion, and grape have been summarized by Ormrod et al (10), and those on red clover by Ensing and Hofstra (5).

Peanut (*Arachis hypogaea* L.), a crop recently introduced to Ontario, has been reported to be sensitive to O_3 in controlled experiments where high levels of O_3 caused chlorosis (1). Heagle et al (6), using supplemental O_3 in open-top chambers, noted the occurrence of white and beige necrotic flecks and chlorosis on peanut leaves followed by decreases in overall growth and yields. However, the relative sensitivity of peanut cultivars has not been documented, nor has the impact of ambient O_3 on field-grown plants been described.

In this study, laboratory fumigations were conducted to determine the sensitivity of two peanut cultivars (USDA PI 268661 and McRan) to O_3 and to determine the effectiveness of the antioxidant, ethylene diurea (EDU), in protecting peanut against O_3. EDU decreases oxidant injury on bean (3), resulting in increased yields (7,13). On O_3-tolerant cultivars of navy bean, EDU application generally did not affect foliage or yields (12). It was also shown that in seasons when few or no O_3 episodes occur, EDU has no effect on yield components of either tolerant or sensitive cultivars of navy bean (12,13). Therefore, field work was also conducted to establish the effect of O_3 on growth and yield of peanut by using EDU to reduce O_3-injury symptoms.

MATERIALS AND METHODS

Laboratory study. Peanut seed (Valencia-type cultivar McRan and Spanish-line USDA PI 268661) were planted, one seed per 12.5-cm-diameter pot, in a mixture of sandy loam and perlite (60:40, v/v). The plants were watered with deionized water, fertilized once per week with 1 g of 20-20-20 (N-P-K) per liter, and grown in a greenhouse supplemented with high-pressure sodium lamps (minimum 250 $\mu mol/m^2/sec$ photosynthetic photon flux density [PPFD] on heavily overcast days) at a 16-hr photoperiod. Four weeks after planting, treated plants were sprayed to leaf wetness with 2.0 g of 50% WP EDU (*N*-2-(2-oxo-1-imidazolidinyl)ethyl-*N*-phenylurea) per liter obtained from E. I. du Pont de Nemours & Co., Wilmington, DE.

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Two days after EDU application, both treated and untreated plants were moved to controlled-environment exposure chambers with a 16-hr photoperiod, $34 \pm 3\%$ relative humidity, a PPFD of 350 ± 25 $\mu mol/m^2/sec$, and an average day temperature of 30 ± 1 C. Charcoal-filtered air was supplied to each chamber at a flow rate of approximately 6,000 cm^3/sec to give a complete air change every 3 min. Both EDU-treated and untreated plants of both peanut types were subjected to either 0 or 430 ± 60 $\mu g/m^3$ O_3 for 7 hr per day for 4 consecutive days. Ozone was produced by an electric-arc generator and monitored in the exposure chambers with a Dasibi model 1003 AH analyzer (Dasibi Environmental Corp., Glendale, CA). Two days after O_3 treatment, the plants were rated for foliar injury on a per-plant basis by using a modified Horsfall-Barratt rating system (15). The experiment was replicated four times with each experimental unit consisting of five pots as subsamples. The data were examined with analysis of variance.

Field studies. Field experiments were established at the Agriculture Tobacco Research Station, Delhi, Ontario, in 1982 and 1983. On 13 May 1982, McRan and USDA PI 268661 peanut were each planted in two plots. In each plot, 2-m sections of eight rows were sprayed with EDU and eight rows were not sprayed. Plants were sprayed to leaf wetness with a backpack sprayer at the same concentration of EDU used in the laboratory. Navy bean plants not sprayed with EDU were not sprayed with water because earlier work has shown this to have no effect on plant growth, even when sprayed with a urea solution to mimic the nitrogen added from EDU (12). Rows within each plot were assigned treatments on a random basis. A 1.5-m center portion of each 2-m row was harvested to determine yields on 25 September 1982.

Both McRan and USDA PI 268661 were planted on 17 May 1983 in a split-plot design of eight replicates with cultivars as main plots and EDU treatments as subplots. Seven-meter rows were used. On 19 August 1983, N fixation was estimated by the acetylene-reduction assay as described earlier (5). Two plants were sampled from each experimental unit. Nodules were removed, counted, and dry weights were determined. The percent nitrogen (Kjeldahl) of the seeds, analyzed after the final harvest (by the Department of Land Resource Science, University of Guelph) was converted to percent protein by multiplying by 5.46. A 6-m section of row from each experimental unit was harvested for yield analysis on 30 September 1983.

In both years, the rows of experimental plots were planted mechanically with 13 seeds per meter. Inoculum of *Rhizobium* (The Nitragin Company, Milwaukee, WI) was applied to the soil at a rate of 6 g per meter. Weeds were controlled with vernolate, metachlor, and dinoseb at recommended rates. In both years, EDU was applied at 14-day intervals after flowering had initiated.

However, if meteorological conditions were such that high O_3 ($\geqslant 170\ \mu g/m^3$) was present 7 days after spraying, plants were sprayed on 7-day intervals. Plants were sprayed six times in 1982 and eight times in 1983.

Percent leaf area affected, estimated by using a modified Horsfall-Barratt rating system (15), was used to rate O_3 injury to the foliage on a per-plot basis. In 1983, leaf drop was also rated on a per-plot basis, after 25 August, by using the same rating system. O_3 levels in the atmosphere were monitored by the Ontario Ministry of the Environment at the Simcoe Horticultural Research Station, located 21 km east of the Delhi Station. O_3 episodes were assumed to have occurred when hourly averages exceeded Ontario Ministry of the Environment criteria of 170 $\mu g/m^3$. Previous work showed that O_3 isolines parallel the shoreline of Lake Erie, resulting in O_3 concentrations at Delhi which were usually the same at Delhi and Simcoe (4). Work by Gillespie and Hofstra in 1979 (*unpublished*) affirmed this pattern.

RESULTS

Laboratory studies. Treatment with EDU significantly reduced O_3-induced injury to the foliage of both cultivars tested (Table 1). USDA PI 268661 was more sensitive than McRan. Injury symptoms, consisting of white flecks, were observed similar to those reported by Heagle et al (6) along with bronzing similar to that reported on white bean (14). Injury was largely confined to mature leaves near the base of the plant. Chlorosis was observed mainly on injured leaves of USDA PI 268661 and appeared subsequent to other symptoms.

TABLE 1. Foliar injury to two peanut cultivars treated or untreated with ethylene diurea (EDU) and ozone (O_3) under laboratory conditions

Cultivar	EDU[x]	O_3[y]	Foliar injury[z]
PI 268661	−	−	0 a
	−	+	33.4 c
	+	−	0 a
	+	+	4.1 a
McRan	−	−	0 a
	−	+	11.2 b
	+	−	0 a
	+	+	2.8 a

[x]Treated plants (+) sprayed with 1 g/L EDU 2 days before O_3 exposure.
[y]Plants treated with 0 (−) or 430 $\mu g/m^3$ (+) O_3 in controlled-environment exposure chambers.
[z]Injury rated as percent bronzing on a per-pot basis, using a modified Horsfall-Barratt rating system. Numbers followed by the same letter are not significantly different (protected LSD; $P = 0.05$).

Field studies. The field trials demonstrated that EDU also reduces oxidant injury under ambient conditions. O_3 injury, consisting of foliar bronzing followed by extensive chlorosis, was observed on USDA PI 268661 (Table 2) with injury ratings as high as 38% in 1982 and 76% in 1983 on plants not sprayed with EDU. Injury to plants sprayed with EDU was less than 5% in 1982 and 10% in 1983. No injury was observed on McRan peanut in 1982 and a minor amount (5%) was observed in 1983 on unsprayed plants. Although there was some chlorosis of the oldest leaves on McRan, there was no prior bronzing. In the split-plot design of 1983, cultivar × treatment interactions were significant at all dates following 9 July. EDU provided significant protection to USDA PI 268661.

The appearance of injury on the foliage of USDA PI 268661 corresponded well with the occurrence of O_3 episodes. O_3 exceeded 170 $\mu g/m^3$ throughout June, on 5, 6, 10, 15, 16, 17, 25, and 30 July, and on 14, 15, and 16 August in 1982. In 1983, episodes occurred throughout June, on 1, 3, 11, 12, 14, and 28 July and on 7, 8, 16, 17, 19, and 26 August. O_3 concentrations were generally lower in 1982 than in 1983 at Simcoe, totaling 20.5 $mg/m^3/hr$ more in 1983 than in 1982. O_3 episodes that occurred in June, before flowering, did not induce foliar injury in either year.

On unsprayed USDA PI 268661, injury appeared most frequently on the lower, mature leaves. Adaxial surfaces of the leaves developed small, white flecks similar to those reported by Heagle et al (6). As the injury progressed, the symptomatology became more similar to that reported on white bean (15), with widespread bronzing, chlorosis, premature senescence, and leaf drop. Table 3 shows the effect of EDU on leaf drop of the two peanut types in 1983. As with bronzing, the cultivar × treatment interaction was significant. USDA PI 268661 plants not sprayed with EDU began to drop leaves at a significant rate 18 days before

TABLE 2. The effect of ethylene diurea (EU) on ozone (O_3)-induced bronzing of foliage of two peanut cultivars grown in Ontario, Canada, in 1982 and 1983

Year	Plot	Cultivar	EDU[x]	Foliar injury on[y]				
1982					19 July	3 Aug	16 Aug	31 Aug
	1	PI 268661	−		5.6	7.9*	26.6*	32.6*
			+		4.0	0.1	2.3	2.8
	2	PI 278661	−		6.2*	15.8*	30.2*	38.9*
			+		4.2	1.9	4.9	5.2
	3	McRan	−		0	0	0	0
			+		0	0	0	0
	4	McRan	−		0	0	0	0
			+		0	0	0	0
1983[z]					17 June	1 July	9 July	16 July
		PI 268661	−		0	0	9.9	16.2
			+		0	0	1.7	5.3
		McRan	−		0	0	0	0.3
			+		0	0	0	0
		LSD			−	−	2.6	4.7
				26 July	9 Aug	17 Aug	29 Aug	12 Sept
		PI 268661	−	13.8	43.8	76.4	70.4	69.4
			+	3.0	3.1	9.0	6.4	4.9
		McRan	−	0.8	1.6	5.0	0.5	0
			+	0.5	1.5	0.2	0	0
		LSD		2.3	6.8	4.4	6.4	5.2

[x]Plants were sprayed with EDU at 1 g/L at 7-day intervals during O_3 episodes (at 14-day intervals if O_3 levels are low), from flower initiation until frost (+, treated; −, untreated).
[y]Injury was rated as percent flecking, bronzing, and chlorosis on a per-plot basis, by using a modified Horsfall-Barratt rating system. Asterisk indicates EDU-treated significantly different from untreated (ANOVA, $P = 0.05$).
[z]LSD given is for between EDU treatments of each peanut cultivar ($P = 0.05$).

EDU-treated plants in 1983. By mid-September 1983, defoliation of EDU-treated plants was only half that observed on untreated plants.

The effect of EDU on yield of USDA PI 268661 was variable among plots in 1982 (Table 4). In the first plot, spraying with EDU significantly increased kernel and sound-mature-kernel (SMK) yields; in the second plot, however, EDU had no significant effect on these yield components. EDU had no significant effect on yield parameters of McRan in either plot (Table 4). In 1983, the higher amount of injury to the foliage is reflected in significant yield reductions. Although the cultivars did not differ significantly in yields, treatment × cultivar interactions were significant for both total and SMK yields. Total seed yields were 24% more in USDA PI 268661 plants treated with EDU compared with untreated plants (Table 4). SMK yields of EDU-treated rows were 23% greater than in rows not treated (Table 4). No significant effect of EDU on yield of McRan was observed.

EDU application, in 1983, doubled acetylene-reduction rates, nodule number, and nodule dry weight of USDA PI 268661 when compared with unsprayed plants (Table 5), as indicated by a significant cultivar × treatment interaction. No significant differences were observed in the N fixation statistics of McRan peanut. The percent protein of the mature kernels at final harvest did not differ significantly for either cultivar (Table 5).

DISCUSSION

This study has demonstrated that ambient levels of O_3 will induce injury symptoms on peanut which are similar to those associated with injury and yield losses in other crops. The antioxidant, EDU, protects plants against the visible symptoms of O_3 injury. In years when O_3 injury to the foliage is substantial, as in 1983, significant increases in yield may be obtained by protecting the foliage with an antioxidant. When less injury is observed, yield increases following application of an antioxidant are also expected to be less.

Previous work has shown that the effects of EDU on yields are variable. Although EDU reduced O_3 injury to the foliage of white bean (13), potato (8), and now peanut, yield increases were not consistently obtained. The reasons for this apparent lack of effect on yield are not well understood. Oshima et al (11) and Mosley et al (9) demonstrated that foliar injury is not always correlated with yield losses even though O_3 reduced the foliar growth of sensitive species. Compared with other years, peanut yields in Ontario were generally depressed by more than 50% in 1982. Rainfall during July was 85% of normal with only 7.4 mm falling between 4 and 27 July. August was a cool month with temperatures averaging 2.0 C below normal. In addition, an early ground frost on 29 August resulted in the wilt and necrosis of approximately one third of the foliage. These factors appear to have resulted in overall low yields in 1982 and they most likely masked treatment differences between O_3-protected and control plants, even though foliar injury was observed. In 1983, the experiment was irrigated when drought appeared likely. Monthly temperatures were near normal for July and averaged 1.4 C above normal for August with no killing frost until the third week of September (meteorological data courtesy of Atmospheric Environment Service, Environment Canada). Therefore, environmental conditions in 1983 were more favorable for growing peanut than in 1982 and yields of both cultivars were greatly increased in 1983.

In addition to reduced yield of USDA PI 268661, the appearance of foliar injury was also associated with reduced N fixation, when assayed near the end of several days of episodic levels of O_3. These results are similar to the effects of O_3 on the acetylene reduction potential of red clover (5), a species also known to be O_3-sensitive (2). However, although N fixation was decreased on plants injured by O_3, the percent protein of the kernels was not affected, suggesting that protein content and N fixation are closely related to yield and growth potential of peanut. Oxidant stress would appear to affect the amount of kernel produced, and not the quality in terms of protein.

Our results also indicated that O_3 shortens the length of time to final senescence of USDA PI 268661. Even though USDA PI 268661 is often viewed as a short-season peanut, the growing season of this line in the absence of O_3 may actually be similar to that of Ontario-grown McRan. EDU, as a research tool, is very effective as

TABLE 3. The effect of ethylene diurea (EDU) on leaf drop of two peanut cultivars grown in Ontario, Canada, in 1983

Cultivar	EDU[x]	Leaf drop on[y] 25 Aug	5 Sept	12 Sept	19 Sept
PI 268661	−	67.9	78.7	68.3	72.9
	+	2.8	9.3	34.7	41.3
McRan	−	4.5	14.8	26.7	27.8
	+	4.2	12.7	27.4	28.4
LSD[z]		5.0	4.3	7.5	8.5

[x] EDU applied as 1 g/L of spray at 7-day intervals during O_3 episodes (at 14-day intervals if O_3 levels are low), from flower initiation until frost (+, treated; −, untreated).
[y] Percent leaf drop was rated on a per-plot basis using a modified Horsfall-Barratt rating system.
[z] LSD given is for between EDU treatments of each peanut cultivar (P = 0.05).

TABLE 4. The effect of ethylene diurea (EDU) on kernel and sound-mature-kernel (SMK) yields of two peanut cultivars grown in Ontario, Canada

Year	Plot	Cultivar	EDU[y]	Kernel yield[z] (g/m)	SMK[z] (g/m)
1982	1	PI 268661	−	67.3	37.4
			+	79.2*	43.3*
	2	PI 268661	−	74.8	42.9
			+	83.4	47.8
	3	McRan	−	54.2	27.1
			+	54.9	22.7
	4	McRan	−	58.1	29.4
			+	56.8	31.7
1983	1	268661	−	154	135
			+	203	179
	1	McRan	−	175	154
			+	163	140
		LSD		20.6	17.7

[y] EDU applied as a 1 g/L spray at 7-day intervals during O_3 episodes (at 14-day intervals if O_3 levels are low), from flower initiation until frost (+, treated; −, untreated).
[z] Yields calculated at 10% moisture. In 1982, yields of EDU treatments followed by an asterisk are significantly different from untreated (ANOVA, P = 0.05). In 1983, treatments within each peanut type significant if difference is greater than LSD (P = 0.05).

TABLE 5. The effect of ethylene diurea (EDU) on peanut-*Rhizobium* symbiosis of two peanut cultivars grown in Ontario during 1983

Cultivar	EDU[w]	Nodules per plant	Nodule dry wt per plant (g)	N_2-fixation activity[x]	Kernel protein[y] (%)
PI 268661	−	50	0.052	6.0	24.9
	+	92	0.106	14.8	24.8
McRan	−	108	0.266	18.9	24.4
	+	88	0.203	14.8	23.6
LSD[z]		34.8	0.074	6.2	1.3

[w] EDU applied as a 1 g/L foliar spray at 7-day intervals during O_3 episodes (at 14-day intervals if O_3 levels are low), from flower initiation until frost (+, treated; −, untreated).
[x] μM C_2H_4 evolved per plant per hour (acetylene reduction assay).
[y] Percent Kjeldahl N of kernels multiplied by conversion factor of 5.46.
[z] LSD given is for between EDU treatments of each peanut cultivar (P = 0.05).

an antioxidant chemical. It has the distinct advantage of allowing the assessment of O_3 impact on plants growing under actual field conditions. As such, the chemical deserves further research attention to determine if its effects on yield can be attributed in whole to its antioxidant properties. This is especially desirable since this would allow for the assessment of O_3 effects on crops without the uncertainty involved in conclusions based on research conducted in the laboratory, greenhouse and open-top chamber designs where confounding environmental factors exist.

Our results indicate that even though significant levels of O_3 may result in injury, other environmental factors appear to affect O_3 dose effects, as in 1982, when rainfall, frost, and temperature overshadowed O_3 effects. When used in conjunction with other designs, antioxidants could be useful in describing the nature of the relationship between dose and other equally important ambient factors.

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