Soybean Cultivar Hodgson Response to Ozone

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ABSTRACT

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Acute response (visible injury) of soybean unifoliolate and first trifoliolate leaves to ozone conformed well to a log-probability model with pollutant concentration and exposure time as the independent variables. Reduction in leaf chlorophyll concentration was correlated with visible injury. Reduction in chlorophyll concentration was also observed in leaves fumigated with low concentrations of ozone (0.08–0.10 μL[μl/L = ppm]) for 2 hr/day for 5 consecutive days.

Several models based on pollutant concentration and exposure time have been proposed for predicting ozone injury in plants (4,7,8,12). Heck and Brandt (8) discussed the variables involved in mathematical modeling of pollutant injury and suggested that separate models may be necessary to describe the acute and chronic responses of vegetation. The log-probability model proposed by Larsen and Heck (12) effectively related short-term acute injury to pollutant concentration and exposure time. They found that percent leaf injury as a function of pollutant concentration and exposure time tends to fit a log normal frequency distribution.

Evaluation of ozone-induced injury can be difficult; a visual estimate of injury is often the only method used for measuring response. Other methods of evaluating pollutant injury have utilized quantitative measures of a physiological response (10,14).

In this paper we report the results of a log-probability analysis of ozone-induced acute visible injury to leaves of soybean cultivar Hodgson. In addition, measurements of leaf chlorophyll concentration were used to further quantify the acute response and to relate visible injury to reductions in leaf chlorophyll. Leaf chlorophyll concentrations were also used to evaluate plant response to fumigations on consecutive days with low concentrations of ozone, to approximately simulate short-term ambient conditions in Minnesota (11).

MATERIALS AND METHODS

Soybean (*Glycine max* (L.) Merr. 'Hodgson') seeds were planted in 10-cm-square plastic pots in a layer of sand overlying steamed soil. Five seeds were placed in each pot. After 2 wk the plants were thinned (leaving two plants of approximately equal size in each pot), fertilized with 20-20-20 (N-P-K) fertilizer, and grown under greenhouse conditions supplemented with 14 hr/day of fluorescent light at 26 ± 3 C. Plants 15, 20, 25, and 30 days old were used in the fumigations.

Two days before fumigation, all plants were transferred from the greenhouse to a growth chamber in which the incoming air was filtered through activated charcoal (control chamber). The chamber was maintained at 26 ± 3 C, 80 ± 10% relative humidity and approximately 26 klux illumination with a 14-hr photoperiod. The plants were watered uniformly and exposed to light for 2 hr before the fumigations. Plants to be fumigated were transferred to a second growth chamber (exposure chamber) with modifications permitting pollutant introduction and monitoring. A group of plants was maintained in the control chamber during the fumigations. Immediately after each fumigation the exposed plants were returned to the control chamber. Two days after fumigation all plants were returned to the greenhouse, and on the third day injury ratings were recorded. All fumigations were begun at either 0800 or 1000 hours.

Ozone was generated with an OREC ozone generator (Model 03V5-0, Ozone Research and Equipment Corp., Phoenix, AZ 85019) and the chamber pollutant concentration was monitored with a Columbia Scientific Industries Model 1100 chemiluminescent ozone monitor (CSI, Austin, TX 78766). Ozone concentrations in the chamber were maintained to ±0.01 μl/L (μl/L = ppm) of the desired concentration with an automated feedback mechanism for pollutant dispensation. The ozone monitor was calibrated routinely with a CSI 1000 ozone generator precalibrated against 1% neutral-buffered potassium iodide and verified by gas-phase titration (1).

The two unifoliolate and the first two trifoliolate leaves on each plant were evaluated for injury by rating the percentage area showing symptoms on each leaf. A rating of zero indicated no injury and a rating of 100 represented a completely necrotic leaf. Generally, ratings were given to the nearest 5%, but below 20% the ratings were given to the nearest 1% to distinguish more clearly the low levels of injury. A severity index (mean percent

TABLE 1. Mean injury ratings for unifoliolate (UNI) and first trifoliolate (TRI) leaves of soybean cultivar Hodgson exposed to ozone

		Ozone leaf injury rating after			
		25 days		30 days	
Ozone (μl/L)	Exposure (hr)	UNI	TRI	UNI	TRI
0.10	0.5	0.42	0.25	0.33	0.65
	1.0	0.25	0.04	1.39	1.38
	2.0	0.71	0.01	1.00	1.63
	4.0	3.17	0.42	6.79	4.00
0.15	0.5	1.87	0.75	1.21	2.54
	1.0	6.04	2.83	6.09	9.59
	2.0	6.23	5.13	14.0	13.4
	4.0	6.00	4.63	7.62	12.5
0.20	0.5	1.13	0.11	0.42	1.25
	1.0	2.44	0.78	1.64	9.54
	2.0	3.73	2.25	21.0	26.6
	4.0	4.52	4.63	11.2	23.0
0.25	0.5	2.96	1.79	3.13	4.71
	1.0	8.83	3.96	20.6	21.0
	2.0	7.83	7.25	31.4	30.8
	4.0	11.3	6.88	23.6	27.3

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visible injury) for a given treatment was obtained by summing the ratings for all leaves on all plants that had received that treatment and dividing by the number of leaves rated. Unifoliolate and trifoliolate leaves were compared separately. Ozone concentrations and exposure times were 0.10, 0.15, 0.20, and 0.25 μl/L each for 0.5, 1, 2, and 4 hr. Twelve plants of a given age were exposed to each of the 16 concentration/exposure time combinations. Plants of four ages were used. Each of the 64 age/pollutant concentration/exposure time combinations was replicated twice.

The chlorophyll extraction method of Knudson et al (10) was used to provide another measure of ozone-induced injury. Six, eight, or 12 leaves were selected at random from the plants that had received a given treatment and the chlorophyll analyses were performed.

Additionally, the effect of 5 days of successive fumigation with 0.08 and 0.10 μl/L ozone for 2 hr/day was investigated. On the first day all except six control plants were fumigated. On the second day all plants except the controls and a subgroup of six experimental plants were refumigated. Each successive day another subgroup of six experimental plants was left untreated so that on the fifth day only the residual subgroup of six plants was fumigated. After the fifth day of fumigation, all plants were kept in the control chamber for 2 more days of postfumigation conditioning and then returned to the greenhouse. The postfumigation conditioning time thus differed among treatments. However, postconditioning beyond 2 days did not change the injury ratings in our study. On the third day after the final fumigation, plants were rated for visible injury and samples were taken for chlorophyll concentration measurements.

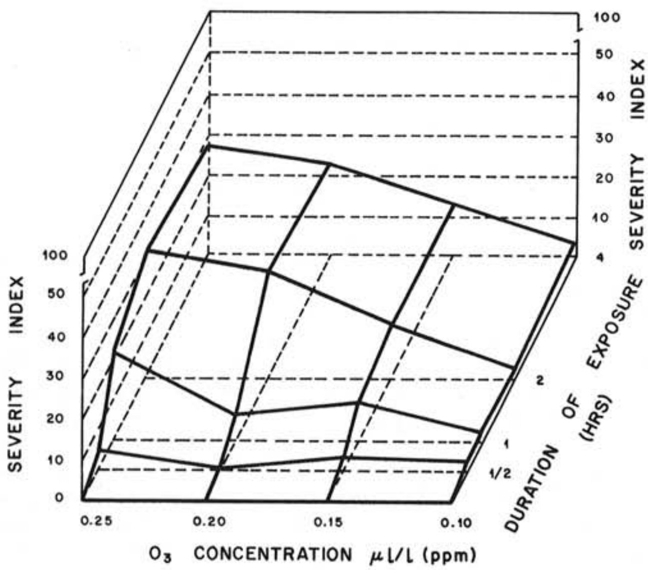

Fig. 1. Response surface of first trifoliolate leaves of 30-day-old soybean cultivar Hodgson plants exposed to ozone as a function of ozone concentration (ppm as μl/L) and duration exposure.

RESULTS

Symptoms on soybean leaves after exposure to ozone consisted of generalized chlorosis, stippling, flecking, and bleaching. Table 1 gives the mean injury ratings for unifoliolate and first trifoliolate leaves of 25- and 30-day-old plants exposed to the 16 combinations of concentration and exposure time. The means for first trifoliolate leaves of the 30-day-old plants are graphed as an exposure surface in Fig. 1. This data set is representative of the results obtained with plants of three other ages. However, ozone sensitivity increased with age; both unifoliolate and trifoliolate leaves of 30-day-old plants were most sensitive of the four age groups evaluated.

The reduction in chlorophyll concentration of the exposed leaves correlated well with the estimate of visible injury (Fig. 2). Chlorophyll extraction data showed similar trends in dose and age response relationships to that determined by estimates of visible injury.

Plants exposed to 0.08 or 0.10 μl/L ozone for up to 5 consecutive days (2 hr/day beginning when plants were 20 days old) showed few or no visible symptoms. The severity index for leaves exposed for 5 successive days at these concentrations was less than 1.0%. The chlorophyll concentration of the first trifoliolate leaves decreased significantly with successive days of ozone fumigation at 0.10 μl/L (Fig. 3). The regression equation predicts a loss of 0.36 mg of chlorophyll per gram of dry tissue for each day of exposure to 2 hr of 0.10 μl/L ozone (approximately 2% loss after 5 days). Unifoliolate leaves of plants of the same age (20–25 days) were more susceptible to visible injury than first trifoliolate leaves, and they also exhibited a greater reduction in chlorophyll concentration

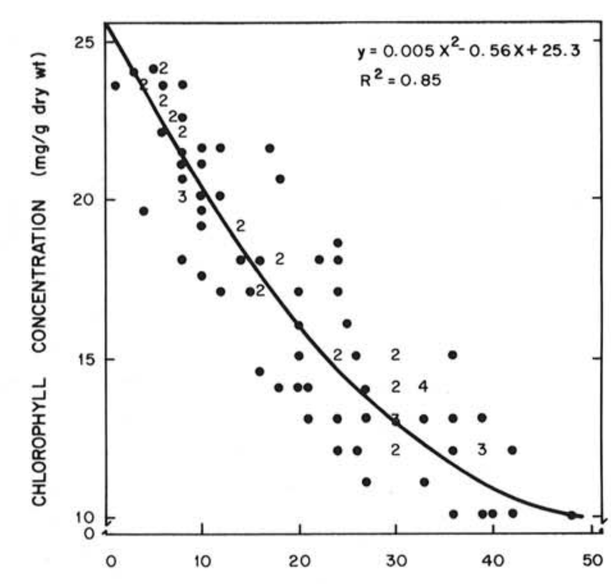

Fig. 2. Relationship between leaf chlorophyll concentration and percent of soybean leaf area with ozone-induced injury symptoms. Unifoliolate leaves of 25-day-old plants were exposed to 0.25 μl/L for 0.5–4 hr.

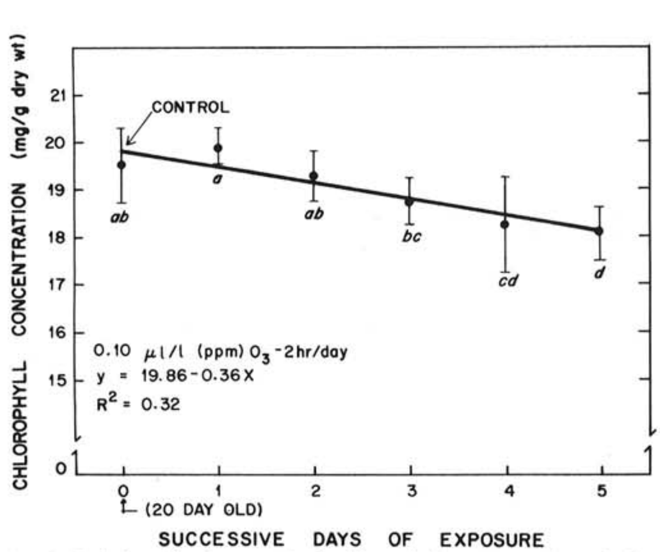

Fig. 3. Relationship between leaf chlorophyll concentration of first trifoliolate leaves and successive days of fumigation with 0.10 ppm ozone for 2 hr/day (different lowercase letters indicate significant difference between treatment means at $P<0.05$ using Newman-Keul's procedure; each point is the mean of 12 observations).

(Fig. 4). In these more susceptible leaves >1.0 mg of chlorophyll per gram of dry leaf tissue was lost during each day of fumigation. Plants exposed to 0.08 μl/L ozone for 2 hr/day for 5 successive days also showed a reduction in chlorophyll concentration in the unifoliolate and first two trifoliolate leaves; however, the reduction was less than in those that had been fumigated with higher concentrations.

DISCUSSION

The response of a plant to ozone should be related to variations in the concentration of pollutant, the exposure time, the age of the plant, the environmental conditions before, during, and after exposure, and any genetic differences in the sensitivity of the plant population. These factors were discussed by others (6,9). In our study, environmental and genetic variations were minimized by use of environmentally controlled growth and exposure conditions and a single test cultivar.

The log-probability model of Larsen and Heck (12) was applied to the visible injury data from Table 1. The modeled parameters are presented in Table 2 and Fig. 5. The assumption of a normal distribution of the log of the dose (pollutant concentration and exposure time combination) necessary to produce a given response leads to log and probit transformations that achieve linearization of the regression (3). The mean response to a given pollutant dose was used rather than the median since the mean is a better point estimate of the response when the magnitude of injury is small. In this model, the pollutant concentration expected to injure a certain percentage of leaf surface is expressed by the equation:

$$\text{Probit}(Y) = \ln(\text{MGHR})/\ln(\text{SG}) - (P)\ln(D)/\ln(\text{SG}) + \ln(C)/\ln(\text{SG})$$

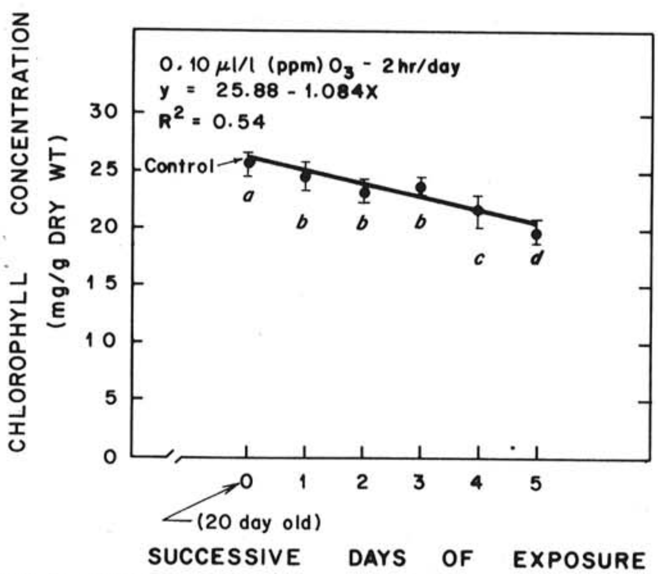

Fig. 4. Chlorophyll concentration of unifoliolate soybean leaves on successive days of fumigation with 0.10 μl/L (ppm) ozone for 2 hr/day (different lowercase letters indicate significant difference between treatment means at $P < 0.05$ using Newman-Keul's procedure; each point is the mean of 12 observations).

in which: Y = the percent of leaf surface injured, MGHR = the geometric mean concentration in microliters per liter for a 1-hr exposure, SG = the geometric standard deviation, P = the slope on log probability graph paper, D = the exposure duration in hours, and C = the pollutant concentration in microliters per liter. With this model, visible injury to soybean cultivar Hodgson is predictable based on the pollutant concentration and exposure duration (Fig. 5 and Table 2). In comparison with the modeled responses of other crops (12), soybean cultivar Hodgson is relatively susceptible to ozone-induced visible injury. Using ambient ozone concentration data from a given location, Larsen and Heck (12) have proposed that it may be possible to predict the level of ozone-induced injury that will occur under appropriate environmental conditions on crops grown in that area.

Defoliation of soybean leaves during the growing season was related to yield (13), and the yield can be reduced significantly when

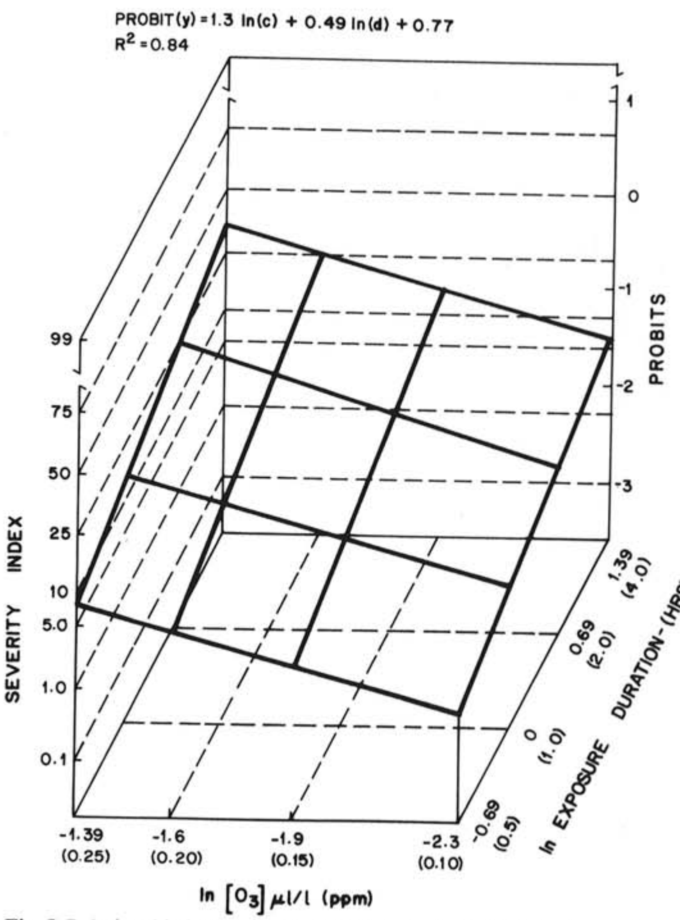

Fig. 5. Relationship between ozone concentration and visible injury at four exposure durations for first trifoliolate leaves of 30-day-old soybean plants (cultivar Hodgson). The vertical axis is in probit units and the concentration and duration axes are in natural log units. Values in parentheses indicate the concentrations in parts per million (as μl/L) and the exposure times in hours.

TABLE 2. Calculated visible injury parameters from log-probability model for soybean plants exposed to ozone

Plant age (days)	Leaf type	MGHR[a]	SG[b]	Slope	R^2	Coef. on ln[b]	Coef. on ln[c]	1-hr threshold[c]	3-hr threshold	8-hr threshold
30	UNI[d]	0.814	2.42	−0.502	0.72	1.13	0.57	0.104	0.060	0.036
	TRI[e]	0.586	2.15	−0.375	0.84	1.31	0.49	0.094	0.62	0.043
25	UNI	1.46	2.94	−0.341	0.80	0.928	0.316	0.119	0.082	0.059
	TRI	1.56	2.63	−0.367	0.65	1.033	0.379	0.164	0.110	0.077

[a] Geometric mean concentration in μl/L (ppm) for a 1-hr exposure.
[b] Geometric standard deviation.
[c] Threshold injury levels were arbitrarily set at 1% visible injury. Concentrations are in μl/L.
[d] Unifoliolate leaves.
[e] Trifoliolate leaves.

the reduction in photosynthetic surface occurs during or immediately before the pod-filling stage (5). If pollutant concentrations are sufficient to produce visible symptoms and thereby reduce photosynthetic surface area, a decrease in yield is possible (2). Most soybeans have a determinate reproductive cycle, but cultivar Hodgson and some others have indeterminate reproductive cycles and flower and fruit over a longer period. Thus, under field conditions where episodic high concentrations of ozone occur, a longer period for potential yield effects exists with these indeterminate cultivars.

A decrease in leaf chlorophyll concentration occurred in soybean plants fumigated with low concentrations of ozone over several days. In these plants, visible symptoms were few or absent or consisted of a generalized chlorosis that was distinguishable only by careful comparison with controls or by measurement of leaf chlorophyll concentration. This loss in photosynthetic potential may also affect the efficiency of plant biomass production without being directly observable. Ambient atmospheric conditions in soybean-growing regions of Minnesota often include low concentrations of ozone ($<0.10\ \mu l/L$) for weeks or months with intermittent hourly peaks of higher concentrations (11). The results reported here suggest that such a pattern of exposure to ozone could affect soybean growth.

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