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Response of Beans to Simulated Ambient and Uniform Ozone Distributions with Equal Peak Concentration

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Abstract. **Response of *Phaseolus vulgaris* L. cv. California Dark Red Kidney to 2 different ozone concentration distributions was examined at 2 dose levels in controlled fumigations. When peak ozone concentrations were equal and total doses equivalent, there was no difference in injury, growth, or yield between a simulated ambient distribution with normal diurnal ozone fluctuations and a uniform distribution typical of laboratory fumigation at constant concentration. Plants fumigated with either ambient or uniform ozone distribution had oxidant stipple leaf necrosis and reduced growth and yield. There was significantly increased injury and reduced growth and yield at a high ozone dose for both types of distribution. The data indicate that with equal peak concentration and equivalent total dose, the constant square-wave, ozone concentration distributions in laboratory fumigations are adequate to describe mode of action and magnitude of response to ambient exposures.**

When plants are injured by exposure to an air pollutant, it is useful to characterize the pollutant exposure level in relation to the response. Pollutant exposure is commonly represented as a dose, the pollutant concentration integrated over the time interval of the exposure. Unfortunately, total dose ignores the importance of the components of the dose, including changing concentrations, peak concentrations, duration of exposure, and recovery time between exposures. Plants respond to the dynamics of these pollutant components. Increasing evidence shows that plant response is more dependent on the concentration component of the dose than on the time component (2, 3, 5, 6, 9, 10, 12).

Plant cells respond to an air pollutant stress by changing metabolic processes to adapt to the stress and to begin repair of injured tissue (11). At a constant pollutant concentration, the metabolic adjustment mechanisms should reach a steady state of response to the continuing stress. However, continuous changing of the pollutant concentration or removing the pollutant will result in continuous metabolic adjustments. The plant response to a pollutant varies over time as physiological status of the plant changes. Physiological status is dependent upon age or plant maturity, and is highly influenced by environmental conditions.

Most greenhouse or growth chamber experiments examining air pollutant effects on plants use fumigations with a constant or square wave ozone concentration distribution, where the fumigation is begun at a specific time, held constant for a period of several hours, than abruptly terminated. In contrast, typical ambient exposure of plants to ozone follows a near normal distribution, with ozone concentration rising during the morning, reaching a peak concentration in the afternoon, and dropping to near zero toward evening. Changing ozone concentration during controlled fumigations is more representative of ambient conditions than a constant concentration. Research has demonstrated the importance of fluctuating pollutant levels in controlled fumigations (1, 2, 4, 5, 7). Laboratory treatments simulating ambient fluctuations should result in a more realistic assessment of plant response to the pollutant.

The distribution of the pollutant concentration during exposure has been shown to influence plant response (8). Beans exposed to a normal distribution of ozone simulating ambient diurnal changes in concentration had significantly more injury, less growth, and lower yield than those exposed to a uniform (constant) distribution of equal dose. Length of fumigation was 6 hours. Total dose was the same for both exposure treatments, but peak concentration was higher for the normal than for the fixed distribution. Therefore, it could not be determined if the distribution differences alone were responsible for the treatment differences, or if the differences in peak concentration affected plant response. The experiment reported herein complements

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the previous one (8) by comparing ambient and uniform distributions at equal dose with equal peak concentrations.

Materials and Methods

The experiment compared simulated ambient and uniform ozone concentration distributions of 2 equivalent dose levels on injury, growth, and yield of 'California Dark Red Kidney' beans. Ozone concentrations chosen for the simulated ambient distribution (Table 1) followed the pattern of the ambient Riverside ozone frequency distribution described previously (8). Uniform distribution was selected at a constant concentration to match the total dose and peak concentration of the ambient distribution (Table 1). This matching required the uniform distribution to be exposed to the peak concentration during the entire fumigation period while the ambient distribution was exposed to the same peak concentration for only a half hour (Table 1). In order to produce equal total doses for the normal and uniform distribution treatments, total exposure time was 6 hr for the ambient distribution, and 2 hr, 18 min for the uniform distribution.

Plants were grown in 3.8-liter plastic pots filled with a standard greenhouse soil mix and were fertilized weekly. Pots were thinned to one uniform plant per pot at 2 weeks after germination. Plants were exposed to one ambient or uniform ozone treatment during week 6, during weeks 6 and 7, or during weeks 6, 7, and 8. One-third of the plants received one fumigation, one-third received 2 fumigations, and one-third received 3 fumigations. Plants were harvested 6 days after their last fumigation (weeks 7, 8, and 9 for the 3 groups, respectively).

Data included number of injured leaves, seeds, and pods; area of the injured leaves, total leaf area for the plants; and dry weights of leaves, stems, pods, and seeds. Plants were still in the vegetative growth stage at week 6, but had just begun to flower. Plants were well into the reproductive stage of development at week 7. No pods had formed for harvest by 6 days after the week 6 fumigation and no seeds were available for harvest after week 7 fumigation. Only pod and seed yield data were collected for the plants after week 8 fumigation due to excessive senescence and leaf drop after the 3rd fumigation. Harvest index was determined at the final harvest by dividing seed weight by total plant dry weight.

Plants were fumigated using 8 negative pressure chambers located within the greenhouse where the plants were grown (8). Ozone was adjusted manually to the treatment levels and monitored with a Dasibi Model 1003-AH Ozone Monitor (Dasibi Environment Corp). Both ozone distributions and both doses were applied to different plants on 2 consecutive days, with 2 replicate chambers for each treatment each day. The 8 chambers were randomized by treatment each day. Ten plants were grown as subsamples and averaged within each replication for each week of fumigation. Harvest data were analyzed using an analysis of variance (ANOVA) with treatments partitioned into single degree of freedom orthogonal contrasts for evaluating the effects of concentration distributions and doses, and with replication within days partitioned into contrasts of days and replicate chambers (Table 2). There was no treatment by day interaction, so this component of the ANOVA was included with the error term.

Table 1. Fumigation schedule for uniform and simulated ambient ozone concentration distribution at 2 dose levels. One-third of the plants were fumigated as described at week 6, one-third at weeks 6 and 7, and one-third at weeks 6, 7, and 8.

Time of day[z]	Time interval fumigated (hr)	Ozone concentration Low dose (pphm)	Ozone concentration High dose (pphm)	Total ozone dose Low dose (pphm/hrs)	Total ozone dose High dose (pphm/hrs)
Uniform distribution				69.0	92.0
10:51–1:09	2.3	30	40		
Ambient distribution				69.7	92.8
9:00–10:30	1.5	5.8	7.7		
10:30–11:15	0.75	12.6	16.8		
11:15–11:45	0.5	18.4	24.5		
11:45–12:15	0.5	30.0	40.0		
12:15–12:45	0.5	18.4	24.5		
12:45–1:30	0.75	12.6	16.8		
1:30–3:00	1.5	5.8	7.7		

[z]Pacific standard time.

Results

There were no significant differences between ozone distributions for any measured variable (Table 3). The orthogonal contrasts indicated no significant differences in injury, growth, or yield between a uniform, constant ozone distribution compared to an ambient ozone concentration distribution. Age of plants at fumigation, including both vegetative and reproductive stages of growth, did not result in differences in response.

For most variables, the high dose increased injury and reduced growth and yield (Table 4). Total leaf area and leaf dry weights responded less to dose after fumigation at weeks 6 and 7 than at week 6, probably a result of the change from vegetative to reproductive stage of plant growth during this period. Response after week 7 may have been a residual effect from the fumigation at week 6, since little leaf growth occurred after week 6. The number of injured leaves was not affected by treatment at week 6 (Table 3); but the high dose increased leaf injury significantly at week 7, indicating a change in leaf sensitivity with age or a cumulative effect of the 2 fumigations. Conversely, dry weight of roots was reduced more by dose during the vegetative growth stage at week 6 than at the reproductive stage of week 7. Concentration distribution by dose interactions occurred at week 7 for 4 of the growth variables, but they were not highly significant. The data suggest that growth may have been slightly decreased by the uniform concentration distribu-

Table 2. Analysis of variance with orthogonal treatment contrasts used to compare exposure of 'California Dark Red Kidney' beans to ozone concentration distribution (ambient vs. uniform) and ozone dose (high vs. low).

Sources of variation	df	Orthogonal comparisons[z] C_1D_1	C_2D_1	C_1D_2	C_2D_2
Chambers within days	3				
Chambers (C)	1	+1	−1	+1	−1
Days (D)	1	+1	+1	−1	−1
C × D (residual)	1	+1	−1	−1	+1
Ozone treatments (T)	3				
Ambient vs. uniform conc. (C)	1	+1	−1	+1	−1
Low vs. high dose (D)	1	+1	+1	−1	−1
C × D (residual)	1	+1	−1	−1	+1
Error	9				
Total	15				

[z]Comparisons codes: Chambers within days: C_1 = Chamber Set 1; C_2 = Chamber Set 2; D_1 = Day 1; and D_2 = Day 2. Treatments: C_1 = Ambient Concentration Distribution; C_2 = Uniform Concentration Distribution; and D_1 = Low Dose, D_2 = High Dose.

Table 3. Significance of injury, growth, and yield response of 'California Dark Red Kidney' beans to ambient or uniform ozone concentration distribution at 2 dose levels at 6 days after fumigation at age 6, 7, and 8 weeks. Only yield components were measured after the week 8 fumigation.

Variables	Ozone treatment	Concn distribution	Dose	C×D	Chambers within days	Chamber	Day	C×D
			Fumigated week 6					
Number/injured leaves	NS	NS	NS	NS	NS	NS	NS	NS
Area/injured leaves	NS	NS	*	NS	NS	*	NS	NS
Total leaf area	**	NS	***	NS	**	NS	***	NS
Total leaf dry wt	**	NS	**	NS	**	NS	***	NS
Dry wt roots	**	NS	**	NS	**	NS	***	NS
Dry wt stems	NS	NS	*	NS	*	NS	**	NS
			Fumigated weeks 6 & 7					
Number/injured leaves	**	NS	***	NS	NS	NS	NS	NS
Area/injured leaves	*	NS	**	NS	NS	NS	NS	NS
Total leaf area	NS	NS	*	*	NS	NS	NS	NS
Total leaf dry wt	NS	NS	*	NS	NS	NS	NS	NS
Dry wt roots	NS	NS	*	NS	NS	NS	NS	NS
Dry wt stems	*	NS	*	*	NS	NS	NS	NS
Number pods	*	NS	*	*	*	NS	**	NS
Dry wt pods	**	NS	**	*	***	NS	***	NS
			Fumigated weeks 6, 7 & 8					
Harvest index	NS	NS	NS	NS	*	NS	*	NS
Wt/seed	NS	NS	*	NS	*	NS	**	NS
Wt seeds/pod	NS	NS	NS	NS	*	NS	**	NS
Total dry wt seeds	NS	NS	*	NS	NS	NS	NS	NS
Total dry wt pods	*	NS	**	NS	NS	NS	NS	NS
Number/seeds	NS	NS	NS	NS	NS	NS	NS	NS
Number/pods	NS	NS	NS	NS	NS	NS	NS	NS

[z]wt = weight.
*,**,***,[NS]Significance at the 5% (*), 1% (**), or 0.1% (***) level, or nonsignificant.

Table 4. Treatment means for growth and yield of 'California Dark Red Kidney' beans exposed to ambient or uniform concentration at 2 dose levels. Ambient and uniform concentration distribution separated within each dose by *t* test.

Variable[z]	Low dose			High dose		
	Ambient	Uniform	Significance	Ambient	Uniform	Significance
			Fumigated week 6			
Total leaf area (cm^2)	1017	938	NS	734	761	NS
Total leaf dry wt. (g)	2.3	2.0	NS	1.5	1.6	NS
Dry wt roots (g)	0.76	0.79	NS	0.59	0.62	NS
Dry wt stems (g)	1.43	1.56	NS	1.13	1.17	NS
			Fumigated weeks 6 & 7			
Total leaf area (cm^2)	1327	1194	NS	1016	1202	NS
Total leaf dry wt (g)	3.4	3.3	NS	2.7	3.0	NS
Dry wt roots (g)	1.4	1.3	NS	1.2	1.3	NS
Dry wt stems (g)	3.8	3.6	NS	3.3	3.6	*
Number/pods	14.7	13.5	NS	12.0	13.5	NS
Dry wt pods (g)	4.2	3.8	NS	3.1	3.5	NS
			Fumigated weeks 6, 7, & 8			
Wt/seed (g)	0.3	0.3	NS	0.2	0.2	NS
Wt seeds/pod (g)	1.4	1.5	NS	1.4	1.4	NS
Total dry wt seeds (g)	6.7	7.3	NS	5.4	5.8	NS
Total dry wt pods (g)	5.0	4.7	NS	3.9	4.1	NS
Number/seeds	26.0	27.8	NS	24.4	25.8	NS
Number/pods	12.1	11.6	NS	10.9	11.4	NS

[z]wt = weight
*,[NS]Significant at the 5% level, nonsignficiant.

tion at low dose, but slightly increased by the uniform distribution at the high dose (Table 4).

There were significant replication-within-days effects for plant growth after one fumigation, a result of the large differences in response between the 2 days of fumigation at week 6. Normal daily temperature differences in the greenhouse where the plants were fumigated may account for the differences. Growth variables did not vary by day or replicate chamber after the first

fumigation. However, there was a significant day-of-fumigation effect on seed weight. A difference between replicate chambers was evident only for area of injured leaves at week 6, and this difference was not highly significant.

Discussion

This experiment clearly demonstrated that when peak concentrations were the same and total dose did not change, there was no difference in response of red kidney beans exposed to 2 different ozone concentration distributions. The ambient distribution had a longer duration of fumigation, shorter duration of time at the peak concentration, and had rising and falling concentration during the exposure, compared to the uniform ozone concentration distribution treatment. The results indicated that these combined differences were not sufficient to cause a significant difference in injury, growth, or yield of bean. The significance of the individual components of the distributions was not examined in this experiment.

There was a significant difference in growth and yield response to dose, with a high dose increasing injury and reducing growth and yield. The high dose was obtained by increasing the concentration levels for this treatment. Although this experiment examined plant response to total dose, the changing peak concentration may have been an important factor in that response.

In a previous, similar experiment (8), a significant difference was found in response to ambient and uniform ozone concentration distributions of equal dose. The ambient distribution increased injury and reduced growth and yield. However, in the previous experiment, total duration of fumigation remained the same for both distribution treatments. Peak concentration was higher for the ambient distribution. Several characteristics were changing between the 2 types of distributions studied in both the previous experiment and that reported here, but the evidence suggests that the peak ozone concentration may be an important factor in plant response to ozone.

The data presented confirm the finding of the previous experiment (8) that laboratory fumigations using uniform, square-wave fumigations are adequate to examine the mode of action of plant response to ozone. Beans responded to both ambient and uniform ozone distributions by having leaf injury, reduced growth, and reduced yield. Harvest index was the same for the 2 types of distribution. Data from this experiment indicated that when total dose and peak concentration were the same, the shape of the ozone distribution (normal vs. square wave) had no effect on the magnitude of plant response. Response to ambient and uniform ozone concentration distributions did not change with stage of growth of plants and was the same whether plants were in the vegetative or reproductive stage of growth.

Ambient, air-quality data for ozone are generally reported as hourly averages of concentration. The dynamics of changes in ozone concentration during the hour are not considered in summaries of air-quality data, although these have been shown to be important in plant response (8). Normal, diurnal ozone variation will result in peak concentrations of less than an hour duration, which will be missed in hourly summaries of ambient concentration. Other components of the exposure of ozone to the plant, such as total dose, and rate and direction of change in concentration within the hour, also may influence plant response.

The results of this experiment demonstrated that when peak concentration and total dose were equivalent, beans responded similarly to an ambient and a uniform ozone concentration distribution. Further research is needed to examine whether peak concentration is the most important component of the concentration distribution causing plant response.

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