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Significance of Pollutant Concentration Distribution in the Response of 'Red Kidney' Beans to Ozone[1]

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Abstract. **Bean plants (*Phaseolus vulgaris* L. cv. Red Kidney) exposed to ozone with a simulated ambient concentration distribution showed significantly more injury, less growth, and lower yield than those exposed to an equivalent dose of ozone with a uniform concentration distribution. The concentration distribution did not alter the type of biological response of 'Red Kidney' beans to ozone, an indication that uniform concentration distribution fumigations are appropriate for investigations of mode of action of pollutants on plants. However, this study suggests that research using a uniform concentration distribution of pollutants may underestimate the magnitude of growth and yield responses to ambient pollutants.**

Pollutant dose, a quantitative description of pollutant exposure, has been defined as the product of pollutant concentration and exposure duration (17, 22). The components of pollutant dose to which plants are exposed are now recognized to be much more complex (16, 21). Exposure concentration should consider concentration distribution, peaks, and means, while exposure duration includes length of time exposed to zero concentration to indicate time intervals between exposures as well as duration of individual exposures. Sequence and patterns of intermittent pollutant exposures are also involved in describing dose.

The flux of pollutant into the plant (16), absorbed dose (3, 22), or effective dose (24) is more meaningful in studies relating plant physiological responses to pollutants. However, for practical purposes most air pollution research deals with ambient (24) or exposure dose (8, 11), since this can be directly measured with air quality monitors and related to ambient air quality standards.

Dose response relationships between plants and pollutants have been considered to be one of the most important air pollution research needs (13). Dose must be understood to quantify growth and yield responses to pollutants. The use of regression techniques produces response models useful for predictive purposes. Heck et al. (10) examined the response of pinto bean and tobacco to the concentration and time components of ozone dose. By varying both concentration and exposure time for specific doses and utilizing a uniform concentration distribution (square wave fumigation), they determined that ozone produced a sigmoidal leaf injury response for pinto beans and tobacco. Heck et al. (10) suggest that either concentration or exposure time may be more important in the dose response, depending upon the location on the dose response surface. Maas et al. (18) also examined the response of pinto beans to ozone dose and concluded that the sigmoidal response was dependent on ozone concentration.

Guderian (5) found that for a specific dose of sulfur dioxide, growth of sunflowers, corn, field peas, and vetch was reduced more by increased concentration than by increased exposure time. High concentration peaks of SO_2, especially when occurring at frequent intervals, were more critical in inducing leaf injury and reducing growth, even though uptake of sulfur may be more related to exposure time than to concentration (6). Van Haut (25) also demonstrated that SO_2 concentration was an important component of dose in leaf injury to radish. Although these studies with SO_2 used several pollutant concentrations and exposure times to characterize dose, specific fumigations used a uniform distribution of concentrations.

Experiments of air pollution effects on plants using greenhouse or controlled-environment exposure chambers generally utilize fumigation with a uniform distribution of pollutant concentrations. The fumigation is initiated at a specified concentration, held constant for a period of time, and then abruptly stopped. These fumigations, although easier to define and control for experimental purposes, do not represent typical ambient exposures.

Heck et al. (13) recognized that laboratory exposures to pollutants having fluctuating instead of uniform concentrations during fumigation would more closely simulate natural exposure of plants. They suggested using fluctuating ambient exposures in laboratory studies examining effects of air pollutants on crop growth and yield. McLaughlin et al. (21) also recognized that changing the distribution of pollutant concentrations may effect plant response. Their experiments examined the effects of 3 different SO_2 exposure patterns on 'Red Kidney' beans. Both concentration distribution and total SO_2 dose varied among treatments. They concluded that concentration distribution affected plant response and suggested that additional research should examine plant response to fluctuating exposures (20, 21). Bell (1) and Garsed et al. (4) reported that fluctuating SO_2 exposures caused a greater reduction in growth of plants than uniform concentration exposures at equivalent mean concentration, although the dose to which the plants were exposed was not reported for their experiments.

The experiment described here tested the importance of concentration distribution and dose on plant response. the commonly used uniform concentration distribution and a simulation of ambient Riverside concentration distribution were compared at 2 equivalent dose levels.

Materials and Methods

The experiment was designed to compare the effects of 2 different distributions of ozone concentrations and 2 levels of ozone dose on injury, growth, and yield of 'Red Kidney' beans. Beans seeds were germinated in 400-ml Styrofoam cups using a standard greenhouse soil mix (University of California II-sand, peat, redwood shavings at 2:1:1 plus nutrients), thinned to one plant per pot at the first leaf stage, and transplanted to 4-liter

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pots at 4 weeks of age. Styrofoam cups were cut away from the soil-root medium with little disruption at transplanting. Plants were grown in a carbon-filtered glasshouse equipped with evaporative air coolers. Minimum glasshouse temperatures were maintained at 21°C, maximum temperatures ranged from 30 to 35°. Relative humidity fluctuated with weather and efficiency of the evaporative coolers.

Plants were exposed to a 6-hr ozone fumigation at 6, 7, and 8 weeks of age. Six days after each of the 3 fumigations, one-third of the plants were measured for leaflet oxidant stipple injury and destructively analyzed for leaf area and dry weights of plant parts. Thus, one-third of the plants received one fumigation at 6 weeks of age, one-third received 2 fumigations at 6 and 7 weeks of age, and the remaining one-third received 3 fumigations at 6, 7, and 8 weeks of age.

Fumigations were made in 8 continuous-stirred tank reactors similar to those previously described (12), which were situated within the same glasshouse. Maximum temperatures were 20°, 23°, and 35° for the 3 fumigation dates. All ozone fumigations had identical exposure times from 0915 to 1515 HR PST according to the schedule in Table 1. The 4 treatments tested were: 1) ambient distribution of ozone concentrations, low dose (AL); 2) uniform ozone concentration, low dose (UL); 3) ambient distribution of concentrations, high dose (AH); and 4) uniform concentration, high dose (UH). Low ozone dose was chosen as 120 pphm-hr, high dose was 168 pphm-hr. Ozone was generated by passing O_2 through a tube containing UV lamps to which voltage was adjusted to vary O_3 output. Ozone concentrations were adjusted manually to each chamber after monitoring with a Dasibi Ambient Ozone Monitor calibrated periodically by the California State Air Resources Board.

Diurnal ambient ozone concentrations in Riverside, Calif. generally follow a typical distribution pattern—ozone increases in the morning, peaks in the afternoon, and then decreases in the evening (15). A typical frequency distribution of diurnal ambient ozone levels from 0600 to 2000 HR during 1977 at Riverside, Calif., is shown in Fig. 1A, where the number of hours decrease nonlinearly as a function of increasing concentration.

Ozone concentration distributions were chosen for our ambient treatments using the Riverside ambient functional relationship (Fig. 1A) to generate simulated ambient distributions (Fig. 1B). Our exposures required adjustment of time to a typical 6-hr laboratory fumigation schedule, and adjustment of concentrations to higher levels used in single laboratory exposures. However, the functional relationships between time of exposure and concentration of ozone remained similar between the Riverside ambient situation (Fig. 1A) and our simulated ambient exposures (Fig. 1B). The proportionality of time to concentration for the Riverside ambient function between 100 and 600 hr of occurrence was chosen to determine a similar proportionality between half-hour time intervals and ozone concentration during our 6-hr simulated ambient fumigations. Each treatment was replicated twice, with 7 plants measured and averaged per replicate after each fumigation. The study used a one-way analysis of variance design with treatments partitioned into single degree of freedom contrasts (Table 2).

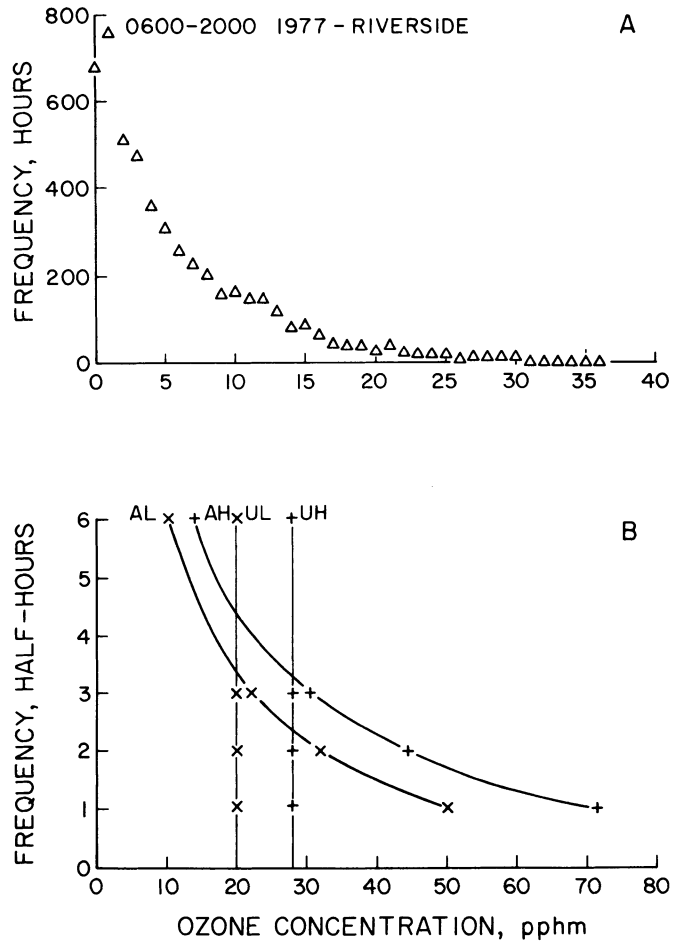

Fig. 1. (**A**) Frequency distribution of hourly average ozone concentrations occurring in ambient air between 0600 and 2000 HR from Jan. 1–Dec. 31, 1977, at Riverside, Calif. (**B**) Frequency distribution of ozone concentrations used in a 6-hr laboratory fumigation to simulate the Riverside ambient distribution of Fig. 1(**A**), where AL is ambient concentration disbribution, low dose, and AH is ambient concentration distribution, high dose. Also shown are the frequency distributions for the uniform laboratory fumigations, where UL is uniform concentration, low dose, and UH is uniform concentration, high dose. Actual fumigation concentrations and durations from Table 1 are indicated by "x" or "+" for low or high doses, respectively.

Table 1. Fumigation schedule of uniform and simulated ambient ozone concentration distributions at 2 equivalent dose levels.

Time of day[z]	Time fumigated (hr)	Low dose Ambient (pphm)	Low dose Uniform (pphm	High dose Ambient (pphm)	High dose Uniform (pphm)
0915–1045	1.5	10	20	14.0	28
1045–1130	0.75	22	20	30.5	28
1130–1200	0.5	32	20	44.5	28
1200–1230	0.5	50	20	71.5	28
1230–1300	0.5	32	20	44.5	28
1300–1345	0.75	22	20	30.5	28
1345–1515	1.5	10	20	14.0	28
	6 Dose[y]:	120	120	168	168

[z]Pacific Standard Time.
[y]Sum of [(hours fumigated) × (concn)], in pphm-hr.

Results

The treatment component contrast comparing uniform vs. typical ambient concentration distributions (Table 2) indicated that simulated ambient distribution of ozone produced significantly greater leaf injury and reduced growth and lower yield response than the uniform distribution (Table 3). The treatment compo-

Table 2. Experimental design describing a one-way analysis of variance with orthogonal treatment component contrasts used to compare the effect of distribution of ozone concentrations (ambient vs. uniform) and ozone dose (high vs. low) on 'Red Kidney' bean.

Analysis of variance table		Orthogonal comparisons			
Source of variance	d.f.				
Replication (R)	1				
Treatments (T)	3				
		Ambient		Uniform	
		Low dose	High dose	Low dose	High dose
Concn distribution (C) (ambient vs. uniform)	1	1	1	−1	−1
Dose (D) (low vs. high)	1	1	−1	1	−1
C vs. D (residual)	1	1	−1	−1	1
Error	3				
Total	7				

nent contrast comparing the 120 pphm-hr and 168 pphm-hr dose levels produced significant differences in only 3 of the dependent variables (number of uninjured leaflets, dry weight of uninjured leaflets, and dry weight of seeds) and these were restricted to small differences only at 6 and 7 weeks of age. There were no treatment differences between the 120 and 168 pphm-hr dose levels at the final harvest. The treatment component contrast for the interaction between concentration distribution and dose level was insignificant for all dependent variables at all 3 harvests.

The simulated Riverside ambient ozone concentration distribution treatments significantly reduced the total number of leaflets, and consequently their area and dry weight at both the 6- and 7-week fumigations. Both pod and seed weights were reduced by the ambient distribution exposures. However, the percentage of reduction in dry weight of seed due to the ambient distribution resulted from the first fumigation at 5 weeks of age, and did not change with subsequent fumigations.

Much of our data shows a pattern of plant response to ozone similar to previously published research for beans (7, 9, 14, 18, 19, 23, 26). Ozone fumigations caused oxidant stipple necrosis and chlorosis on leaflets and subsequent reductions in their number, area, and dry weight. Although we noted some evidence of stimulation of axial meristems due to fumigations as previously reported (2), our data indicate that there was little growth of these initiated laterals after the first fumigations. This difference is likely due to our selection of a determinate dry bean variety which was well into the reproductive stage when stressed. Our exposures were not initiated until 6 weeks of age, after anthesis was complete and beans had switched from the vegetative to the reproductive growth phase.

At 8 weeks, the plants had begun to senesce. Senescence, in conjunction with high temperature during the 3rd fumigation at 8 weeks, resulted in stressed plants which showed massive leaf injury and subsequent abscission due to fumigation. Dry weight data indicate that there was little leaf tissue left for assay at 9 weeks, particularly on plants fumigated with simulated ambient ozone concentration distributions. Immature pods also abscised after the 3rd fumigation. The harvest data reflected enormous loss of leaflets and an increase in pod and seed dry weights. Although ozone-induced loss of leaflets likely reduced yield below its potential, the increase in pod and seed weights over time suggested by the data was likely due to normal translocation to the seeds as they matured.

Despite the obvious difference in response to ambient and uniform distribution treatments, harvest indices (dry weights of seeds per total dry weight of plants) were not affected by treat-

Table 3. Growth and yield response of 'Red Kidney' beans to uniform or ambient distribution of ozone concentrations at 6, 7, and 8 weeks of age.

Dependent variables	Means, 6th week fumigation			Means, 7th week fumigation			Mean, 8th week fumigation		
	Ambient[z]	Uniform[z]	Sig.	Ambient	Uniform	Sig.	Ambient	Uniform	Sig.
Number of									
Injured leaflets	12.0	5.7	*	13.7	7.9	*	1.3	3.2	NS
Uninjured leaflets	14.0	25.5	**	7.1	22.6	***	1.0	5.8	NS
Total leaflets	26.0	31.2	*	20.8	30.4	*	2.3	9.0	NS
Pods	9.5	9.5	NS	8.4	7.6	NS	5.1	5.8	**
Seeds	5.5	7.5	*	8.8	13.5	**	11.0	14.1	**
Leaf area (cm^2)									
Injured leaflets	39.2	17.2	NS	37.8	26.2	NS	1.3	10.5	*
Uninjured leaflets	52.1	101.2	*	24.5	88.0	***	3.6	28.2	NS
Total leaflets	91.3	118.4	*	62.3	114.2	*	4.9	38.6	NS
Dry weight (g)									
Stems and petioles	2.8	2.9	NS	2.2	2.7	*	1.7	1.9	*
Injured leaflets	0.89	.33	*	0.95	0.65	NS	0.04	0.24	*
Uninjured leaflets	1.17	2.32	***	0.72	2.02	***	0.10	0.70	*
Total leaflets	2.06	2.66	*	1.67	2.68	*	0.14	0.94	*
Pods	2.32	2.74	*	3.64	5.64	**	5.10	8.19	*
Seeds	0.12	0.20	*	1.39	2.37	***	3.58	5.83	**
Roots	0.78	0.80	NS	0.86	1.00	NS	0.63	0.70	NS
Harvest index[y]	0.015	0.022	NS	0.14	0.16	NS	0.32	0.33	NS

[z]Distribution of ozone concentration.
[y]Harvest index = seeds dry wt/total dry wt.
[NS,*,**,***]Nonsignificant (NS) or significant at 5% (*), 1% (**), or 0.1% (***) level.

ment (Table 3). This would indicate that yields were proportionately reduced with overall plant growth. The data show a clear pattern of injury to susceptible leaves, with subsequent reductions in leaf biomass due to ozone stress. This pattern was consistent with both the uniform and ambient distribution treatments, but clearly was more severe with the ambient.

Discussion

The standard uniform concentration distribution adopted by most researchers for greenhouse or controlled-environment experiments clearly does not produce plant response characteristic of the ambient distributions. Fumigations at a fixed concentration underestimate plant response relative to the Riverside ambient distribution at equivalent dose levels. Furthermore, when one considers the additional environmental differences between greenhouse or chamber exposures and ambient field conditions, it is clear that these controlled experiments should be used with caution when modeling ambient effects. This brief study with its limited objectives is not comprehensive enough to indicate whether a method can be developed to extrapolate from uniform distribution effects to ambient distribution effects.

Previous research, with few exceptions, has focused on the relative contributions of concentration and time components of dose to plant response (5, 7, 10, 18) but has not considered concentration distributions. This study has compared the effect of different concentration distributions of equivalent dose, and has demonstrated that plant response to ozone is markedly affected by the concentration distribution.

Pollutant dose, the product of pollutant concentration and exposure duration, is determined by integrating the concentration function over a specific duration of exposure time. We conclude that simply integrating the concentration function over time without regard to the distribution may be inadequate to describe the exposure of plants to ozone. Our experiment has shown that 2 different patterns of concentration distribution at a specific dose resulted in a difference in magnitude of plant response. The concentration distribution must be adequately described as well as integrated for the specific exposure duration. Plants respond to a pollutant exposure comprised of the complex interaction of the exposure duration and concentration of the pollutant. Distribution pattern of the pollutant concentration is an important component of pollutant exposure. It is important to define the role of concentration in the dose response of plants, since present U.S. air quality standards do not consider concentration distribution. The results of our study suggests that ambient air quality standards based on data generated from experiments utilizing uniform pollutant concentration distribution may not be useful to indicate the magnitude of plant response to ambient concentrations.

The usefulness of uniform concentration distribution exposures lies in the simplicity of controlling fumigations. The results of this study indicate that the pattern of biological responses of 'Red Kidney' beans in the reproductive stage of development was not dependent upon the concentration distribution used. The mechanisms involved with the ozone stress appear to be the same regardless of the concentration distribution. Only the magnitude of the response seemed to be altered by the concentration distribution. Therefore, uniform distribution exposures appear to be suitable for experimentation investigating the mode of action of pollutants on plants. It is only in the area of quantifying the magnitude of growth and yield loss that uniform concentration distributions appear to be unsuitable.

This study compared plant response of one cultivar of bean to uniform distribution of ozone and a simulated ambient distribution concentration representing Riverside, Calif. Since our simulated ambient distribution is specific to one geographic area, ambient ozone distribution patterns should be determined for several areas and related to plant response in controlled fumigations. The response of many additional species at different developmental stages also needs to be investigated to put our findings into perspective.

Although our ambient distribution fumigations more closely simulated the Riverside ambient situation than did our uniform distribution fumigations, it was not feasible in our limited study to completely duplicate actual ambient distribution. Our simulated ambient distribution function (Fig. 1B) did not account for the portions of the actual frequency distribution above 600 or below 200 hr of occurrence for the year, nor did our ambient distribution include hours before 0600 HR or after 2000 HR during the day (Fig. 1A). Our fumigation also allowed weekly recovery periods not normally encountered in normal ambient air. Even though ambient hourly concentrations of ozone as high as 66 pphm have occurred in Southern California (15), concentrations used in our fumigations are not common. Fumigations with a different distribution using lower concentrations may have caused a different response. However, our results underscore the necessity to consider distribution of pollutant concentrations when designing studies of plant response to pollutants and suggest that fumigation distributions resembling ambient may allow more realistic estimates of plant response to pollutants.

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Inheritance of a Leaf Distortion Tendency in Bush Lines of Beans, *Phaseolus vulgaris* L., of Blue Lake Background[1]

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Abstract. **'Gallatin 50', a bush cultivar not of 'Blue Lake' background, and 'Oregon 1604', a bush cultivar of 'Blue Lake' type, were crossed with distortion susceptible lines of 'Blue Lake' type. Environment affected distortion expression; the best expression was obtained in the greenhouse while expression was much reduced in the field. Ratios obtained suggested distortion is primarily controlled by a single dominant gene, designated *Ld*. 'Oregon 1604' carries the recessive allele for normal. 'Gallatin 50' carries a major dominant gene, designated *Ds*, that supresses the expression of the dominant gene *Ld* for leaf distortion tendency. Results also suggested that modifying factors are involved and are responsible for levels of susceptibility among parents and progenies. Additional crosses between susceptible lines showing severe to mild distortion indicated that these lines carried the same major genes for distortion, but differed in modifying factors.**

An abnormality similar to injury from phenoxy herbicides was observed in Oregon State Univ. bean breeding lines in 1958 (3). The lines were characterized by a bush growth habit and had been derived from a series of backcrosses to the pole bean 'Blue Lake'. The distortion was initally thought to be cytoplasmically inherited because it did not appear in progenies when the normal pole 'Blue Lake' was used as female in crosses with distorted lines. The distortion has persisted as a problem in the breeding program, requiring elimination of many bush breeding lines. It has also been observed in breeding lines or cultivars of 'Blue Lake' bush type developed by seed companies.

The distortion reported here appears to be similar to pseudomosaic, observed by Burkholder and Muller (1) in a line derived from a cross of 'White Marrow' x 'Flat Morrow' and controlled by 2 complimentary recessive genes. Zaumeyer (5) reported that a mosaic-like abnormality was controlled by 2 recessive genes

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