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Differential Responses of Four Bean Cultivars to Chronic Doses of Ozone

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Abstract. **Four cultivars of bean (*Phaseolus vulgaris* L.), with different sensitivities to ozone (O_3), were exposed to chronic doses of O_3 for 7 hr/day in early and late-season studies. Plants were pot-cultured in open-top field chambers. Greater than ambient O_3 doses were applied by supplementing the O_3 present in nonfiltered air with additional O_3 at a constant rate for 7 hr/day. Cultivar sensitivity, as determined using an acute exposure screening protocol, was maintained in both studies. Regression of yield against O_3 concentrations showed that 'BBL-254' and 'BBL-290' were more sensitive to O_3 than were 'BBL-274' and 'Dwarf Horticultural'. Results suggest that the acute screen used can predict the relative yield response of cultivars grown under field conditions when very sensitive and very resistant cultivars are compared. The results support the contention that bean germplasm has traits for resistance to O_3 at current levels of O_3, but that resistance is lost with increasing O_3 concentration. Predicted relative yield suppression at a 7 hr/day seasonal mean of 0.04 to 0.06 ppm (the common ambient range in eastern United States) was 2% to 4% for the two resistant cultivars and 10% to 26% for the two sensitive cultivars.**

Ambient concentrations of ozone (O_3) suppress yields of many agronomic crops throughout the United States (6, 7). Reports from the National Crop Loss Assessment Network (NCLAN) have shown major suppression of yield of crops such as soybean, peanut, tobacco, and cotton at current ambient concentrations of O_3 (7). Although peak concentrations of O_3 in the southeastern United States are generally less than in the east and northeast, seasonal 7 hr/day mean values tend to be as high or higher due to meteorological conditions that favor O_3 production throughout the growing season. The extended growing season also may result in more severe effects on crops growing in the southeast.

Common bean is sensitive to O_3 (4, 5, 12). A commercially grown pinto bean was used as an indicator of oxidant pollution in California (4) and was used as a test plant in many early studies of O_3 effects on vegetation (5). More recently, studies have evaluated the relative effects of O_3 on a number of bean cultivars (1, 8–10, 12) and selections (12). These studies have been carried out to determine whether resistant germplasm is present in the species. Although considerable variation in sensitivity has been found, all cultivars and selections have shown injury at the high O_3 concentrations used (1, 8–10, 12). However, mechanisms of resistance may exist at current ambient concentrations of O_3.

A major concern in the evaluation of the relative sensitivities of cultivars within species is whether the results reported in short-term acute screening designs (12) are a useful indicator of relative yield suppression in field-grown cultivars. This concern was tested at our field site in North Carolina, where the length of the growing season permits the growth of two successive bean crops. The first crop (mid-May planting) can be harvested by late July; the second crop (late July to early August planting) can be harvested in late September or early October. Thus, plant responses (including yield) of spring and fall crops can be compared within the same growing season. This comparison permits some information on changing response under different environmental conditions and O_3 stress levels.

Two such studies were conducted to determine if responses across the two growing periods were comparable. Two sensitive and two resistant cultivars—as determined by an acute cultivar screen (12)—were included in each study to determine if relative sensitivity to O_3 was the same in the field studies (measured as final yield) and in the acute screen (measured as foliar injury). Finally, the two studies were undertaken to determine if common bean carried some resistant germplasm to current ambient concentrations of O_3 and to determine the effects of ambient O_3 on bean growth and development.

Materials and Methods

'Bush Blue Lake 290' ('BBL-290') and 'Bush Blue Lake 254' ('BBL-254') were O_3-sensitive and 'Bush Blue Lake 274' ('BBL-274') and 'Dwarf Horticultural' were O_3-resistant common bean cultivars. The sensitivity determination was based on an acute screen. The four cultivars were grown in early and late season field experiments (studies) with similar cultural procedures used in both experiments. Seeds of the four cultivars were planted in Metro-mix (W.R. Grace) in 236-cm^3 styrofoam cups and watered as needed. After emergence, plants were watered twice a day. In the early study, plants were transplanted (29 May) 14 days after seeding to 7.6-liter plastic containers, using the same potting mix, and transferred from the greenhouse to the field site

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located 8 km south of Raleigh, N.C. In the second study, plants were transplanted (6 Aug.) to the large pots 12 days after seeding and moved to the field site. Plants at the field site were watered once daily if the temperature was <32.2°C, and twice daily if the temperature was >32.2°. Plants were fertilized with 600 cm^3 of Peters 20N–20P–20K (20 g·liter^{-1}) weekly following transplant.

Plants were kept at the field site for 10 days (early study) and then were moved into open-top field chambers (2, 3) for exposures to O_3. Plants in the second study were placed in the open-top chambers at time of transplant. Weeds were controlled in the chambers. Insects were controlled as needed with 1-naphthalenyl methylcarbamate (carbaryl) (Sevin, 1.4 cm^3·liter^{-1}), tricyclohexylhydroxystannane (cyhexatin) (Plictran, 1.0 cm^3· liter^{-1}), and *O*,*S*-dimethyl acetylphosphoramidethioate (acephate) (Orethene, 1.4 g·liter^{-1}).

Ozone was generated by passing O_2 through an O_3 generator (OREC, Model 03B2-0) and dispensing the O_3–O_2 mixture through a dispensing control system to the open-top chambers (3). Ozone concentrations in the chambers were monitored at plant canopy height using a Monitor Labs 8410 chemiluminescent O_3 monitor. A shared-time system was used (3) so that several chambers could be monitored with a single instrument. Ozone was dispensed for 7 hr/day (1000 to 1700 HR EDT) from 8 June (24 days from planting) through 27 July (50 days from planting) in the early study, and from 6 Aug. (12 days from planting) through 30 Sept. (56 days from planting) in the late study; exposures continued through final fresh bean harvest in both studies.

The experiment involved four O_3 treatments: a) carbon-filtered air (CF); b) nonfiltered air (NF, all chambers used a particulate filter) and nonfiltered air supplemented with either; c) 0.04 ppm of O_3 (NF+4); or d) 0.08 ppm of O_3 (NF+8) for 7 hr/day. The O_3 treatments were replicated four times; thus, 16 open-top chambers were used in the study. Four pots of each cultivar were placed in each chamber and arranged in a Latin square design; thus, pot placement across the four replications included all 16 positions for each cultivar.

Plants were grown to maturity and pods were harvested twice as they matured (>5 cm) before final harvest. Shoots and roots were harvested at the last green bean harvest, at which time leaf abscission was prevalent. Data were taken for the number of large bean pods (>5 cm long), fresh and dry weight of bean pods, and dry weights of plant tops (stems and leaves) and roots. Plant leaf area injured (a subjective evaluation of the entire plant) was determined at final harvest on a 1 to 5 scale (1 = 0%, 2 = 1% to 25%, 3 = 26% to 50%, 4 = 51% to 75%, and 5 = 76% to 100% injury). The scale values (averaged to the nearest 0.1) then were changed to percentage values (e.g., a scale value of 3 is 37.5%). Most plants had some injury in the CF in both the early and late studies ('BBL-254' had 11% and 5%; 'BBL-290' had 7% and 5%; 'BBL-274' had 0% and 0%; 'DW Hort' had 2% and 2%, respectively) at harvest. Thus, the CF values were set to 0 and subtracted from original injury values in other treatments to determine values used in the table. All responses were subjected to analyses of variance (ANOVAs). Except for yield, where a regression analysis was used, responses across seasons were not compared because of the differences shown in seasonal O_3 concentration.

Plant responses showed a significant O_3 × cultivar interaction for both the early and late studies. Thus, tables for injury, top and root dry weight, bean number, and bean dry weight were developed to show this interaction. Mean separations were determined using least significant differences (LSD) at the 5% probability level. Top and root dry weights, pod number, and pod dry weight are given in grams or number for the CF treatment: all other O_3 treatments are reported as percent change [reduction or increase (+)] from the respective CF treatments, which simplified comparison of treatments within cultivars and across responses. To aid in determining significance levels, a nonstatistical LSD (in percent) was calculated using the highest CF weight or number for dividing the LSD.

Economic yield (pod fresh weight) was the parameter used to test the efficiency of the acute screen to separate resistant and sensitive germplasm, determine if bean contains germplasm resistant to ambient concentrations of O_3, and to determine yield losses at ambient concentrations. Thus, the response of pod fresh weight to O_3 for each of the four cultivars was evaluated using a regression approach. This approach tested the homogeneity of the relative yield responses of the four cultivars across the two seasons. Initially, ANOVAs were conducted using pod fresh weight plot means for each cultivar and season (eight tests). The O_3 effect was significant for each of the four cultivars in both seasons. These plot means then were regressed against the respective plot O_3 mean concentrations (seasonal 7 hr/day) for each cultivar and season. From preliminary plots of the data obtained for each cultivar, nonlinear behavior among cultivar responses was apparent. The nonlinear Weibull function (11) was chosen as the regression model because it can be used to test the homogeneity of cultivar responses and has the flexibility to cover a wide range of responses. Tests using the Weibull function then were conducted to compare the differences in the intercepts and shapes of the eight response curves. First, for each cultivar, the nature of the response for the two seasons was tested. The shape but not the intercept of the response curve was similar over the two seasons for three cultivars. Therefore, a Weibull model was fit for each of these cultivars using the general form: $y = (\alpha s_1 + \alpha s_2) \exp[-(x/\sigma)^c]$; where y is yield and x is the O_3 concentration. The three parameters to be estimated are α, the hypothetical maximum yield at zero O_3; σ, the O_3 concentration when yield is 0.37α; and c, a dimensionless shape parameter. The s_1 and s_2 are used as "dummy" variables (11), where s_1 is for the early season and s_2 is for the late season crop. For the fourth cultivar ('BBL-254', sensitive), the shape of the curve for each season was different (heterogeneous); thus, the Weibull models for this cultivar for each season were used. The next step compared the two resistant cultivars (two Weibull models) and the two sensitive cultivars (three Weibull models) for homogeneity. The final step in the analysis investigated whether the resistant and sensitive cultivars could be combined into 1 dose-response model.

Results

The 7 hr/day mean seasonal O_3 concentration in ambient air was 0.058 ppm for the early study and 0.055 ppm for the late study. The higher ambient concentration in the early study was expected because O_3 concentrations in September are usually lower than in June and July. The 7 hr/day seasonal O_3 treatment means for the CF, NF, NF+4, and NF+8 in the early study were 0.035, 0.050, 0.087, and 0.119 ppm, respectively. The same treatment values for the late study were 0.026, 0.045, 0.087, and 0.126 ppm, respectively. These mean concentrations represent 15% of the monitoring data for the 50 or 56 days of exposure in each of the two studies. The NF seasonal values are lower than the ambient concentration because some O_3 is

Table 1. Effects of ozone on injury severity in four bean cultivars in two studies.

Cultivars	Relative sensitivity to O_3	Percent leaf injury[z], early study O_3 concn (ppm) 0.035	0.050	0.087	0.119	Percent leaf injury[z], late study O_3 concn (ppm) 0.026	0.045	0.087	0.126
BBL-254	Sensitive	0	3	22	47	0	17	56	56
BBL-290	Sensitive	0	7	26	51	0	9	54	58
BBL-274	Resistant	0	0	0	12	0	2	12	38
Dw Hort	Resistant	0	0	2	26	0	0	20	48
LSD (0.05)			14%				11%		

[z]Injury values, on a whole-plant basis, were determined at harvest on a 1 to 5 scale and translated to percent injury in the table (see Materials and Methods). Ozone concentrations are the 7 hr/day seasonal means for each treatment.

Table 2. Effect of ozone on growth of four bean cultivars over two studies.

Cultivars[z]	Early study, O_3 concn (ppm)[y] 0.035: Dry wt (g/plant)	0.050: Dry wt (% change from control)[x]	0.087	0.119	Late study, O_3 concn (ppm)[y] 0.026: Dry wt (g/plant)	0.045: Dry wt (% change from control)[x]	0.087	0.126
			Top dry wt					
BBL-254	37.3	6	49	78	36.7	13	42	73
BBL-290	37.3	7	55	71	34.8	6	44	72
BBL-274	47.6	2	29	51	41.7	0	23	63
Dw Hort	26.4	+25	8	66	32.4	+5	12	55
LSD (0.05)[w]	6.3		13.2[w]		4.4		10.5	
			Root dry wt					
BBL-254	6.9	8	56	86	7.4	20	58	86
BBL-290	6.6	3	61	78	7.2	20	62	87
BBL-274	10.4	+11	12	59	9.2	1	24	68
Dw Hort	7.7	+33	8	70	9.1	7	30	70
LSD (0.05)[w]	1.6		15.3		1.1		12.1	

[z]'BBL-254' and 'BBL-290' are sensitive cultivars, 'BBL-274' and 'Dw Hort' are resistant cultivars.
[y]Control is the CF treatment (0.035 and 0.026 ppm O_3 for the early and late studies, respectively) and ozone concentrations are the 7 hr/day seasonal means for each treatment.
[x]Percent change [increase (+) or reduction] from the respective CF treatment.
[w]The LSD value for percentages was calculated by dividing the LSD by the highest CF number or weight. Comparisons are estimates for use across O_3 concentrations within cultivars.

destroyed when air passes through the chamber air-handling system.

Bean cultivars designated as sensitive or resistant based on prior acute screens exhibited similar relative sensitivities to foliar injury at all three elevated O_3 concentrations for both the early and late study (Table 1). Foliar injury was significantly correlated with the yield and biomass parameters shown in Tables 2 and 3 (R^2 values: top dry weight = 0.77, root dry weight = 0.87, pod number = 0.44, pod dry weight = 0.53, and pod fresh weight = 0.49). The percent injury is generally less (especially at the highest O_3 concentration) than the percent reductions in plant biomass and pod number, lending credence to the hypothesis that yield and biomass can be reduced without evidence of visible injury.

Biomass data (Table 2) show a clear separation between the sensitive and resistant cultivars at all O_3 concentrations greater than the NF treatment; the trend is clear even in the NF treatment. In the early study, the effects of O_3 on tops and roots were similar for each cultivar, whereas in the late study, the root growth was apparently more sensitive than top growth in three of the four cultivars; a definite trend for greater sensitivity of roots was shown in all data. The biomass of individual cultivars (CF treatments) during the two studies was similar, but dry weight pod yield was much greater in the late study than in the early study (Table 3). Sensitive cultivars showed similar biomass responses (CF treatments), whereas the resistant cultivars were different (e.g., 'BBL-274' was the largest and 'Dwarf Horticultural' was the smallest of the four cultivars tested).

Pod number and dry weight yields of sensitive cultivars in response to O_3 were clearly different from those of resistant cultivars (Table 3). The number of pods (CF treatments) in the two sensitive cultivars was greater in the late study than in the early study; this difference was not found for the resistant cultivars. Pod number was affected by O_3 more in the early study than in the late study for both sensitive and resistant cultivars. Pod weight had almost doubled in the late study (CF treatments) for the sensitive cultivars and increased about 50% for the resistant cultivars. Thus, environmental conditions favored increased yields in the late study, even though vegetative growth was similar (Table 2). Although pod weight was affected by O_3 more in the early than in the late study, the differences were less than for pod number.

The Weibull model showed a homogeneous response of relative yields (fresh weight of pod per plant) for three of the four

Table 3. Effect of ozone on yield of four bean cultivars over two studies.

Cultivars	Early study				Late study			
		O_3 concn (ppm)[y]				O_3 concn (ppm)[y]		
	0.035	0.050	0.087	0.119	0.026	0.045	0.087	0.126
			Pods/plant					
	(no.)	(% change from control)[x]			(no.)	(% change from control)[x]		
BBL-254	44.1	0	48	84	54.6	4	42	64
BBL-290	44.0	16	54	83	52.5	7	35	62
BBL-274	40.8	+20	15	46	41.9	+3	0	40
Dw Hort	23.8	22	25	54	19.5	+6	4	40
LSD (0.05)[w]	8.3		18.8		5.0		9.2	
			Pod dry wt					
	(g/plant)	(% change from control)[x]			(g/plant)	(% change from control)[x]		
BBL-254	18.4	0	49	89	43.8	9	55	80
BBL-290	21.3	33	66	89	34.4	4	47	75
BBL-274	22.4	+18	12	62	35.8	3	12	54
Dw Hort	20.2	28	38	61	30.1	+6	8	53
LSD (0.05)[w]	4.2		18.9		5.6		12.9	

[z]'BBL-254' and 'BBL-290' are sensitive cultivars, 'BBL-274' and 'Dw Hort' are resistant cultivars.
[y]Control is the CF treatment (0.035 and 0.026 ppm O_3 for the early and late studies, respectively) and ozone concentrations are the 7 hr/day seasonal means for each treatment.
[x]Percent change [increase (+) or reduction] from the respective CF treatment.
[w]The LSD value for percentages was calculated by dividing the LSD by the highest CF weight. Comparisons are estimates for use across O_3 concentrations within cultivars.

cultivars ('BBL-290', 'BBL-274', and 'Dwarf Horticulture') across seasons. For the two O_3-resistant cultivars, one model adequately represented the relative yield response over both seasons (Table 4); the relative responses of the two homogeneous cultivar models across seasons were homogeneous. For the two sensitive cultivars, although 'BBL-254' did not show a homogeneous response across seasons, when all three models (two for 'BBL- 254' and the one homogeneous model for 'BBL-290' across seasons) were compared, a single homogeneous model was found to fit the two cultivars across the two seasons (Table 4). Tests of homogeneity of the two models, the resistant (two cultivars across two seasons) and the sensitive (two cultivars across two seasons) cultivars, indicated a heterogeneous response, showing that the relative yield responses for the sensitive and resistant cultivars were different. Thus, the models (different αs) for the resistant cultivars are shown in Fig. 1 and the models (different αs) for the sensitive cultivars are shown in Fig. 2 for each cultivar and season. The relative responses of the two resistant or the two sensitive cultivars are similar in each figure. The relative yield suppression for the sensitive and resistant cultivars is summarized in Table 5 for all seasonal O_3 values from 0.03 to 0.13 ppm.

Results indicated that, in the ambient air of Raleigh, N.C. (0.055 to 0.060 ppm O_3), the resistant bean cultivars show yield reductions of up to 3.5% and the sensitive cultivars up to 26.3%.

Discussion

The suppression of pod dry weight with increasing O_3 was related to cultivar sensitivity and the season in which the study

Table 4. Weibull models of fresh pod weight for snapbean plants exposed to chronic doses of O_3 in open-top field chambers.

Resistant cultivars (BBL-274; Dwarf Horticultural)[z]
$y = (208x_1 + 124x_2 + 374x_3 + 205x_4) \exp[-(x/0.127)^{4.42}]^y$
(12, 12, 13, 12, 0.003, 0.89)[x]

Sensitive cultivars (BBL-290; BBL-254)[z]
$y = (172x_1 + 202x_2 + 406x_3 + 362x_4) \exp[-(x/0.096)^{2.36}]^w$
(16, 17, 21, 20, 0.004, 0.37)[v]

[z]y = fresh pod weight (g), x = O_3 dose (seasonal 7 hr/day mean) in ppm.
[y]Dummy variables ($x_1 - x_4$) for αs of each cultivar by season study: x_1 = 'BBL-274', early; x_2 = 'Dw Hort', early; x_3 = 'BBL-274', late; x_4 = 'Dw Hort', late; to compute yields for a particular cultivar, the dummy variable is equal to 1, otherwise it is 0.
[x]Standard errors for Weibull parameters in order of occurrence: $\alpha_1, \alpha_2, \alpha_3, \alpha_4, \sigma$, c.
[w]Dummy variables ($x_1 - x_3$) for αs of each cultivar by season study: x_1 = 'BBL-290', early; x_2 = 'BBL-254', early; x_3 = 'BBL-254', late, x_4 = 'BBL-290', late; to compute yields for a particular cultivar, the dummy variable is equal to 1, otherwise it is 0.
[v]Standard errors for Weibull parameters in order of occurrence: $\alpha_1, \alpha_2, \alpha_3, \alpha_4, \sigma$, c.

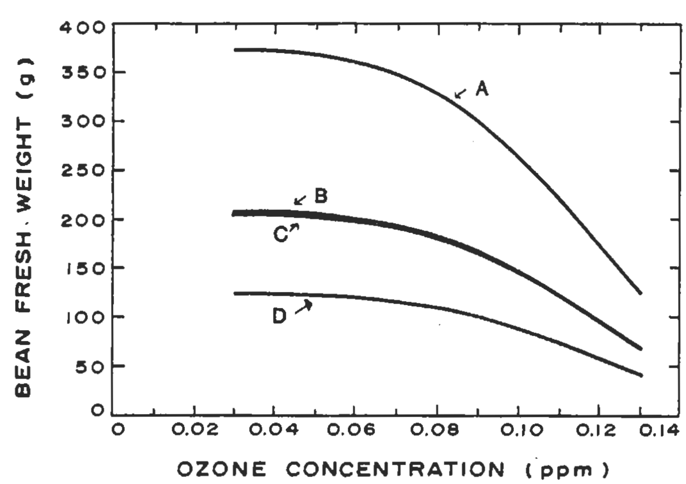

Fig. 1. Weibull model for fresh weight of pod from two resistant cultivars ('BBL-274' and 'Dwarf Horticultural') for early and late season crops. Curves have the same relative response with different maximum yields (α): A = 'BBL-274' (late); B = 'BBL-274' (early); C = 'Dw Hort.' (late); D = 'Dwarf Horticultural' (early).

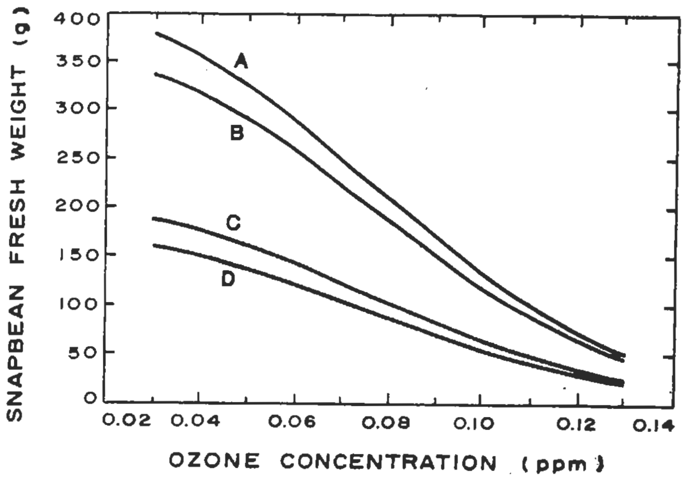

Fig. 2. Weibull model for fresh weight of pod from two sensitive cultivars ('BBL-290' and 'BBL-254') for early and late-season crops. Curves have the same relative response with different maximum yields (α): A = 'BBL-254' (late); B = 'BBL-290' (late); C = 'BBL-254' (early); D = 'BBL-290' (early).

Table 5. Relative yield suppression of sensitive and resistant bean cultivars to increasing O_3 concentrations assuming a 7 hr/day seasonal mean of 0.02 ppm as a background O_3 concentration.

Ozone concn[z] (ppm)	Yield suppression (%) Cultivars Resistant	Sensitive
0.03	0.1	3.9
0.04	0.6	9.7
0.05	1.6	17.3
0.06	3.5	26.3
0.07	6.9	36.3
0.08	12.1	46.5
0.09	19.6	56.6
0.10	29.3	65.9
0.11	41.1	74.2
0.12	54.1	81.1
0.13	67.0	86.7

[z]Seven hours/day seasonal mean.

was conducted. Yield suppression in the sensitive cultivars was related primarily to a suppression of pods/plant in the early study, whereas pods/plant and pod weight both were affected in the second study. Yield suppression in the resistant cultivars apparently was related to pods/plant and pod weight in both studies. Flowers or small fruits may have abscised from the sensitive cultivars during the early study due to the timing of the O_3 exposures. Sensitivity to O_3 may increase in mature plants if they have not had some opportunity to acclimate to the stress during early development.

Controversy (5) has continued on the validity of extrapolating foliar injury data obtained from acute screens to expected effects of chronic doses of O_3 on yield of field-grown plants. Results from Hucl and Beversdorf (9) support the validity of such extrapolations, but field exposures were only to ambient levels of O_3 and the controls were protected by chemical spray. The results presented in this paper are the first strong support for the concept that foliar injury in an acute screen can be used to predict yield reduction from chronic O_3 exposure in field-grown plants, since two sensitive and two O_3-resistant cultivars (as determined by foliar injury in acute screens) maintained relative sensitivities (as determined by pod yield and biomass) after season-long exposures to chronic O_3 concentrations in field chambers in both early and late-season plantings. It should be understood that the sensitive and resistant selections chosen represented the extremes of sensitivity in the cultivars actually handled in the acute screen (12). Yield losses in the resistant cultivars may reflect a low tolerance in common bean germplasm for O_3.

It is apparent that, in common bean, resistance is related to the concentration of O_3 the plants receive. This relationship is seen in the marked separation of the sensitive and resistant cultivars at O_3 concentrations around and just above ambient. However, at the highest seasonal O_3 concentrations, the yield suppression in the resistant cultivars increased markedly, suggesting that O_3 tolerance in bean has a fairly narrow range.

Analyses suggested that the yield responses of the two resistant bean cultivars to O_3 are homogenous with respect to chronic levels of O_3 as are the yield responses of the two sensitive cultivars. Thus, the relative yield response of all the cultivars designated as sensitive in the acute screen may be homogenous, but different from those classified as resistant in the acute screen. This concept requires further validation before acceptance. Cultivars of intermediate sensitivity presumably exhibit yield responses intermediate to those of the sensitive and resistant selections. However, the results presented here suggest that cultivars of intermediate sensitivity may not separate well from the sensitive or resistant selections because the reaction to O_3 between the sensitive and resistant cultivars tends to become blurred at high O_3 concentrations. A study of selections from all three levels of sensitivity is warranted.

It should be noted that characterization of nonlinear response curves is frequently difficult with only four data points. Although prior knowledge of the nature of the response curve would allow a choice of treatment levels that would optimize the precision of the response curve, where this knowledge is lacking, the use of five or six treatment levels would improve characterization of the response.

Results reported here support the concept developed in the NCLAN studies (7) that comparing relative yield losses may permit comparisons of results across seasons, years, and cultivars, even though the actual yields may vary greatly.

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