Responses of Closely Related Bush Blue Lake Snap Bean Cultivars to Increasing Concentrations of Ozone

G. Eason¹

North Carolina State University, Raleigh, NC 27695

R.A. Reinert²

U.S. Department of Agriculture, Agricultural Research Service, Raleigh, NC 27695

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Abstract. Eight Bush Blue Lake type snap bean (*Phaseolus vulgaris* L.) lines and cultivars with similar genetic backgrounds were container-grown to green-pod maturity in open-top field chambers while being exposed to chronic doses of O₃ for 7 hours·day⁻¹ for 42 consecutive days. Treatments included charcoal-filtered air, nonfiltered air, and 0.02, 0.04, or 0.08 ppm O₃ added to nonfiltered air. Visible injury was estimated during the 2nd week of exposure and compared to the green pod yield data. The presence of four yield response groups, as determined via regression analysis, indicated the presence of variation for O₃ sensitivity in the germplasm pool, but all eight lines were O₃-sensitive with yield losses at O₃ levels exceeding a 7-hour daily mean of 0.085 ppm. Foliar injury may be a good indicator of general yield loss; however, estimates of visible injury lack the precision necessary to distinguish subtle differences among a collection of O₃-sensitive snap bean lines.

Bean has been established as an O₃-sensitive horticultural crop (Davis and Kress, 1974; Haas, 1970; Middleton, 1956). Foliar injury has been characterized (Butler and Tibbitts, 1979; Haas, 1970; Middleton, 1956), and O₃ has been shown to reduce yields (Heck et al., 1988; Heggstad et al., 1977, 1980; MacLean and Schneider, 1976; Reinert and Eason, 1986). Although immunity to O₃ does not exist (Reinert et al., 1982), genetic variation exists within the germplasm pool (Butler and Tibbitts, 1979; Davis and Kress, 1974; Hucl and Beversdorf, 1982a, 1982b; Meiners and Heggstad, 1979; Reinert et al., 1984). Insensitivity to O₃ has been identified among commercial cultivars (Butler and Tibbitts, 1979; Davis and Kress, 1974; Hucl and Beversdorf, 1982a; Meiners and Heggstad, 1979) and is especially prevalent among snap bean cultivars (Meiners and Heggstad, 1979). Most germplasm evaluations for O₃ insensitivity have been based on ratings of foliar injury, but, where the selection pressure has been intense, snap bean cultivars classed as insensitive on the basis of their foliar response to O₃ have also been classed as insensitive based on their yields under O₃ stress in field experiments (Heck et al., 1988; R. A. R., unpublished).

Ambient levels of O₃ have increased during the last few years, and, indeed, the oxidant problem in general is considered ubiquitous (Heck et al., 1983). Cultivars produced under polluted conditions may be the products of unintentional selection for insensitivity to O₃ (Reinert et al., 1982). Bush Blue Lake snap

beans for processing, however, have been developed in one of the historically clean air basins of the United States, the Willamette Valley of Oregon, and the 'Blue Lake Pole' germplasm, frequently used in the Oregon State Univ. breeding program, has generally been classed as O₃-sensitive (Meiners and Heggstad, 1979). Therefore, information on existing variability in this closely related gene pool would be useful in case of a decline of air quality in the Willamette Valley and in cases where bean breeders might desire to use the germplasm in O₃-stressed areas. The objective of this research was to examine a collection of eight cultivars and/or breeding lines from the Oregon State Univ. snap bean breeding program for variability in yield response to O₃ stress. A second objective was to determine whether visible injury response was correlated with yield response.

Materials and Methods

Seven snap bean lines developed by the Oregon State Univ. breeding program for commercial processing in the Willamette Valley ['Oregon (ORE) 1604', 'ORE 83', 'ORE 91', 'ORE 17', 'ORE 55', 'ORE 58', and 'OSU 4907'] and 'Bush Blue Lake (BBL) 290' (Asgrow Seed Co., Kalamazoo, Mich.) were evaluated for O₃ sensitivity. Seed of each snap bean line were planted in a greenhouse supplied with charcoal-filtered (CF) air on 18 May in 236-cm³ styrofoam cups filled with Metro Mix 220 (W.R. Grace Co., Cambridge, Mass.), an artificial peat-perlite-vermiculite medium. Nine days after seeding, 30 uniform seedlings of each line were selected, transported to a field site 5 km south of Raleigh, N. C., and transplanted into 7.6-liter plastic containers filled with Metro Mix 220. Containers were placed in open-top chambers (Heagle et al., 1973), and seedlings were allowed to acclimate to field conditions for 3 days before O₃ exposures. When exposures began, plants had one fully expanded trifoliolate leaf.

When temperatures exceeded 32°C, plants were watered twice daily; otherwise, plants were watered once daily. The 1st week after transplanting, 500 ml of a fertilizer solution (3.2 g Peter's 20N-20P-20K/liter H₂O) was added to each container. Thereafter, 500 ml of a more concentrated solution (9.7 g Peter's 20N-20P-20K/liter H₂O) was added weekly to each container.

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¹Dept. of Plant Pathology, Box 7616, North Carolina State Univ., Raleigh, NC 27695.

²U.S. Dept. of Agriculture/Agricultural Research Service, Dept. of Plant Pathology, North Carolina State Univ.

When necessary, 1-naphthyl- *N*-methylcarbamate (carbaryl, 1.4 cm³·liter⁻¹), tricyclohexylhydroxystannane (cyhexatin, 1.0 cm³·liter⁻¹), or O, S-dimethyl acetylphosphoramidothioate (acephate, 1.4 g·liter⁻¹), was used to control insects and mites.

Plants were exposed to O₃ for 7 h each day (1000 to 1700 EDT) for 42 consecutive days beginning on 1 June. An O₃ generator (OREC, Model 03B2-O; Ozone Research and Equip. Corp., Phoenix, Ariz.) using an electrostatic discharge converted dry O₂ into O₃ that then was dispensed into the open-top chambers via a control system (Heagle et al., 1979). The concentration of O₃ at plant canopy level in each chamber and in the ambient air was monitored using a chemiluminescence O₃ analyzer (Monitor Labs 8410; Monitor Labs, San Diego, Calif.) and shared-time system (Heagle et al., 1979). A seasonal (duration of the experiment) 7-h mean concentration was calculated for each O₃ treatment and the ambient air.

The treatment design was a factorial with eight snap bean lines and five O₃ levels: CF air, nonfiltered ambient air (NF), and 0.02, 0.04, or 0.08 ppm O₃ added to NF air. The experimental design was a split-plot design with two blocks of the five O₃ treatments. Thus, 10 open-top chambers were used in the experiment. Snap bean lines served as the subplot factor. One container of each line was randomly assigned to each third of each chamber. The total number of plants used in the experiment was 240.

On 13 June, each primary leaf and the first two trifoliolate leaves were rated for percent (0% to 100%, in 5% increments) of the total leaf surface exhibiting O₃ injury. These individual leaf readings were averaged for each plant, and the average was used for analyses. Marketable bean pods from all lines were harvested and weighed on 5, 14, and 15 July.

Yield data were examined for normality and homogeneity of variances among the individual snap bean lines. The Box-Cox Test (Rawlings, 1988) did not indicate that the data needed to be transformed. Total marketable bean fresh weight for each snap bean line was analyzed separately using analysis of variance (ANOVA), and variances among bean lines were not found to be different according to Bartlett's χ^2 test of homogeneity (Steel and Torrie, 1980). Consequently, an overall ANOVA was run to test for a significant interaction between O₃ treatments and bean lines. Regression analyses were then used to determine which lines differed in yield response to O₃ dose. Initially, each bean line's response to O₃ was regressed individually, and these response curves were then compared to each other. Since grouping of responses is somewhat arbitrary from a statistical viewpoint, common models were fit for lines based on similar genetic backgrounds and homogeneous response curves. Homogeneity was determined by testing the response errors in a "full" and "reduced" models test (Rawlings, 1988). Since the purpose of these models was to compute the residuals needed to test homogeneity and not to predict yield response to O₃ dose, individual intercepts were allowed for each of the snap bean lines in any combined model, and models were expanded to the highest order necessary to result in insignificant lack of fit when compared to pure error. When two models of different polynomial order were combined, the higher order model was the common model first tested and then, if possible, reduced to a lower order common model by testing lack of fit. The success of a common response model to fit the data was determined with an F test (Rawlings, 1988). When a common model adequately fit the data from two or more lines, it replaced the original snap bean line models in subsequent tests. These procedures allowed de-

termination of similarity between the bean lines and the parents present in their pedigrees.

Injury data were also examined for normality and heterogeneity of variances. The arcsin of the square root function was used to transform the injury data. Data from each O₃ treatment were separately analyzed, and Bartlett's χ^2 test (Steel and Torrie, 1980) found a lack of homogeneity among variances. Consequently, all further analyses were weighted by 1/s from the individual O₃ analyses. Since injury data were only of interest in relation to yield response, bean lines with a common dose response model for yield were grouped for analysis. Variation due to snap bean lines was partitioned into variation among yield response groups and variation among snap bean lines within a group. For the purpose of comparison, a similar ANOVA was conducted on yield data.

Results

The 7 h-day⁻¹ mean ambient O₃ level for the duration of the exposure period was 0.073 ppm. Generally, activated charcoal filters can remove nearly all ambient O₃, but through the growing season there was an ingress of ambient air containing O₃ through the opening at the top of the chamber. Nonfiltered air chambers will have below ambient O₃ due to some loss of O₃ through the dust filter. Thus, the seasonal 7 h-day⁻¹ O₃ treatment means—0.035, 0.063, 0.085, 0.104, 0.132 ppm (for CF, NF, and 0.02, 0.04, and 0.08 ppm O₃ added to NF air, respectively)—were close to the expected values.

Snap bean lines yielded differently in response to O₃ ($P < 0.0001$). Yield was sharply reduced for all lines in response to O₃ levels exceeding 0.085 ppm (Table 1). All of the bean lines had significant linear regression components, and four of the bean lines also had significant nonlinear components.

After individual regression analyses had been run for each snap bean line, lines were tested for similarity of response to O₃ dose. Since pairing is arbitrary for statistical purposes, lines were compared beginning with those lines having more similar genetic backgrounds. Published pedigrees were used as aids in grouping (Baggett et al., 1981a, 1981b, 1981c, 1982, 1984; Frazier et al., 1972b), and portions of available pedigrees are shown in Fig. 1. 'ORE 58', one of the earliest bush beans with true Blue Lake qualities, has been used extensively in the Oregon State Univ. breeding program. Having evolved from a bulk planting of complex crosses, its exact pedigree is unknown, but contributing parents included 'Blue Lake Pole', 'Puregold',

Table 1. Effects of O₃ dose on mean fresh weight yields (g) of eight snap bean lines.^z

Bean line ^y	Mean concn of O ₃ (ppm)				
	0.035	0.063	0.085	0.104	0.132
ORE 1604	267	225	241	116	56
ORE 58	256	193	212	164	77
ORE 55	304	288	246	142	78
OSU 4907	286	238	194	112	54
ORE 83	282	252	241	142	80
ORE 17	260	222	191	108	51
ORE 91	257	190	140	81	34
BBL 290	297	215	160	95	85

^zEach value represents the mean of six plants.

^yAll bean lines had significant linear regression components; the following bean lines also had significant nonlinear components: 'ORE 1604', 'ORE 58', 'ORE 55', and 'ORE 83'.

Fig. 1. Pedigrees for snap bean breeding lines 'ORE 1604' (Frazier et al., 1972b), 'ORE 17' (Baggett et al., 1981a), 'ORE 91' (Baggett et al., 1981c), 'ORE 55' (Baggett et al., 1982), 'ORE 83' (Baggett et al., 1981 b), and 'OSU 4907' (Baggett et al., 1984, references a sister line released instead of 'OSU 4907').

'Tendercrop', 'Gallatin 50', and 'Calpac 77' (Frazier et al., 1968). Its successor in the processing industry, 'ORE 1604' (Frazier et al., 1972b), was derived from selections from a cross between 'ORE 58' and 'ORE 190', another bush bean of complex parentage and with some 'Blue Lake' qualities (Frazier et al., 1972a). Because 'ORE 58' and 'ORE 1604' were closely related and were involved in the parentage of most of the other snap bean lines in this experiment (Fig. 1), a combined model was attempted for these two lines. However, the responses of these two bean lines were not similar enough to be combined into a single model.

'BBL 290' is a commercial cultivar and consequently does not have a published pedigree. It had been involved with 'ORE 58' and 'ORE 1604' in the parentage of some of the other lines in this experiment (Fig. 1). A common model could not be fit between 'BBL 290' and either of the other two parental lines, 'ORE 58' and 'ORE 1604'.

The five remaining snap bean lines were grouped by similarities in their pedigrees. 'ORE 17' (Baggett et al., 1981a) and 'ORE 91' (Baggett et al., 1981c), essentially sister lines, were the first nonparental lines tested for similar responses. As expected, a single model adequately expressed their yield response to O_3 . The pedigree for both 'ORE 83' (Baggett et al., 1981b) and 'OSU 4907' (Baggett et al., 1984, references a sister line released instead of 'OSU 4907') included 'BBL 290', 'ORE 58', and 'ORE 1604'. The pedigree for 'ORE 55' (Baggett et al., 1982) included 'BBL 290', 'ORE 58', and a cross between the parents of 'ORE 1604', 'ORE 190' and 'ORE 58'. A common model was fit first for 'ORE 83' and 'OSU 4907' and then for 'ORE 55' with 'ORE 83' and 'OSU 4907'. Finally, a common model was successfully fit for all five of the nonparental snap bean lines.

Common yield response models were attempted for the group

of five progeny lines with each of the three parental lines, but no additional common models could be fit. Thus, the eight snap bean lines could be divided into three groups of one line each ('ORE 58', 'ORE 1604', 'BBL 290') and one group of five lines ('ORE 91', 'ORE 17', 'OSU 4907', 'ORE 83', and 'ORE 55') (Fig. 2).

Injury began as necrotic stippling, a response of bean to O_3 that is well-defined (Butler and Tibbitts, 1979; Haas, 1970; Middleton, 1956). As the injury progressed, the foliage developed a bronze appearance on all of the bean lines except 'BBL 290', which became increasingly chlorotic and could be identified by its yellow foliage in chambers with O_3 additions. Some bean plants had begun to defoliate in 0.132 ppm O_3 before fruit maturity. Overall correlation between fresh weight yield and injury rating was large in absolute value and highly significant ($R = -0.819$, $P = 0.0001$), indicating that foliar injury could be a good indicator of yield loss.

Based on the ANOVA for the injury ratings (Table 2), O_3 dose significantly affected foliar injury, but injury response to O_3 differed for the yield response groups, as indicated by the significant group $\times O_3$ interaction. As with the fresh weight yield data, lines within the progeny group responded similarly to increasing O_3 dose. Thus, 23 days before the first bean harvest, bean lines that would yield similarly in response to O_3 stress were also showing similar increases in foliar injury in response to O_3 dose. However, although 'BBL 290' had the least injury for O_3 concentrations >0.063 ppm (Table 3) of all of the groups, its yield was significantly and linearly reduced by O_3 (Fig. 2).

Discussion

As indicated by the four different responses identified (Fig. 2), variation in yield response to O_3 dose existed among the

Fig. 2. Predicted values for the 7 h-day⁻¹ mean O₃ levels from this experiment have been plotted for total marketable fresh weight yield (y) of the four groups of snap bean lines. Lines within the last group (D) have the same response curve but different yields. The model given for group D is for the first snap bean line ('ORE 91') listed in the group and is adjusted for the other four line(s) in that group via a dummy variable, I, which equals 1 for a particular line and otherwise equals 0: I₁ represents 'ORE 17'; I₂, 'OSU 4907'; I₃, 'ORE 83'; and I₄, 'ORE 55'. Standard errors are from the regression error estimate. (A) 'ORE 58': $y = 500 - 11,219 (*5426) O_3 + 142,174 (f 70,599) O_3^2 - 618,203 (~ 279,753) O_3^3$. (B) 'ORE 1604': $y = 2582 - 141,972 (\pm 33,578) O_3 + 2,976,870 (* 692,236) O_3^2 - 25,833,124 (* 5,895,275) O_3^3 + 78,275,131 (\pm 17,711,527) O_3^4$. (C) 'BBL 290': $y = 364 - 2310 (\pm 265) O_3$. (D) 'ORE 91', 'ORE 17', 'OSU 4907', 'ORE 83', and 'ORE 55': $y = 138.1 + 25.9 I_1 + 36.512 + 58.913 + 71.314 + 5745.6 (\pm 2554) O_3 - 94,783.2 (\pm 33,231) O_3^2 + 336,283.5 (\pm 131,680) O_3^3$.

eight snap bean lines in this experiment. Ozone sensitivity of the bean groups in this experiment was based on the rapidity of decline in yields in response to O₃ dose. 'Gallatin 50' and 'Tendercrop', two lines involved in the parentage of 'ORE 58', have been classed as insensitive and moderately insensitive to O₃, respectively (Davis and Kress, 1974; Heggstad et al., 1977, 1980; Meiners and Heggstad, 1979). Consequently, less sensitivity in 'ORE 58' and 'ORE 1604' to concentrations of O₃ < 0.104 ppm was not unexpected. 'BBL 290' is O₃ sensitive (Heck et al., 1988; Heggstad et al., 1977, 1980) and has even been used previously as a standard of reference for sensitivity (Reinert et al., 1984). Therefore, its extreme sensitivity in this experiment "was also expected. 'ORE 91' and 'ORE 17' initially appeared to be more sensitive than the other three lines ('ORE 83', 'ORE 55', and 'OSU 4907') in their yield response group, but the difference was not statistically significant, i.e., one response model could be used to express the fresh weight yield of all five bean lines.

As previously stated, the equations given are not for predicting yields but for comparing responses to O₃ among related snap

bean lines. Therefore, biological interpretation was not considered when fitting the polynomial orders. In fact, bean lines may have a greater variation in response to O₃ at very low doses than at high doses. Perhaps this variation could have been better addressed [particularly in the model for 'ORE 1604' (Fig. 2)] if additional O₃ treatments between 0.032 ppm and 0.085 ppm had been included in this experiment.

Data from the 13 June injury rating agreed with yield data in that different yield response groups also exhibited different foliar injury responses to O₃ dose, and bean lines yielding similarly in response to O₃ dose showed similar foliar injury responses to O₃. The rating method chosen for this experiment has been used effectively in determining extremes in bean sensitivity to O₃ (Heck et al., 1988; Reinert et al., 1984) and should be a relatively objective method of estimating injury when used before the increased plant stress induced by fruiting results in natural senescence and possibly increased O₃ injury (Simini et al., 1989). This early injury rating, however, did not indicate the extreme yield sensitivity of 'BBL 290'. Thus, as indicated by the negative correlation between yield and injury, bean plants

Table 2. Sum of squares and probability values for total bean fresh weight and foliar injury ratings of eight snap bean breeding lines exposed to O₃.^z

Source	df	Total bean fresh wt		Injury rating ^y 13 June	
		SS ^x	P > F ^w	S ^x	P > F ^w
Block (Blk)	1	14,184	0.0290	1	0.6963
O ₃	4	1,063,947	0.0001	545	0.0030
Blk × O ₃	4	5,099		18	
Group (G)	3	2,269	0.4705	2	0.4215
Line (G)	4	94,239	0.0001	2	0.5677
O ₃ × G	12	55,581	0.0001	44	0.0002
O ₃ × Line (G)	16	16,437	0.3376	13	0.4709
Blk × O ₃ × G + Blk × O ₃ × Line (G)	35	30,756		28	
Residual	160	137,565		136	

^zO₃ concentrations were 0.035, 0.063, 0.085, 0.104, and 0.132 ppm.

^yThe primary leaves and the first two trifoliolates were rated for percentage of leaf surface injured by O₃. Before analysis, the average of these ratings was transformed using the arcsin of the square root function and weighted by 1/s from analyses by O₃ dose.

^xSS = sum of squares.

^wPr > F = Probability > F.

Table 3. Effects of O₃ dose on foliar injury of eight snap bean lines^z after 12 days of exposure.

Bean line	Mean concn of O ₃ (ppm)				
	0.035	0.063	0.085	0.104	0.132
	<i>Average foliar injury (%)^y</i>				
ORE 1604	0.0	9.6	26.9	56.5	81.7
ORE 58	0.0	11.7	24.4	36.2	52.7
ORE 55	0.0	15.0	29.2	48.3	62.3
ORE 4907	0.0	6.0	12.5	36.7	55.4
ORE 83	0.0	9.0	20.2	41.2	45.4
ORE 17	0.0	11.5	27.5	49.6	60.4
ORE 91	0.0	23.8	39.0	51.7	59.8
BBL 290	0.0	12.1	15.6	26.7	38.3

^zEach value represents the mean of six plants.

^yData were recorded on the primaries and first two trifoliolates as the percentage of the foliar surface injured by O₃. Before analysis, data were transformed with the arcsin of the square root function and weighted by 1/s from analyses by O₃ dose. Pretransformation values are shown.

expressing greater foliar O₃ injury generally showed greater yield loss, but visible estimates of injury lacked the precision necessary for discerning the subtle variations in yield response among closely related, generally O₃-sensitive bean lines.

Although different yield response groups were identified among the eight snap bean lines, all of the lines responded negatively to levels of O₃ > 0.085 ppm, a level not very different from the mean ambient O₃ level during this experiment. Another concern is that only 'ORE 58', the oldest of the bean lines examined in this experiment, had <50% yield loss at 0.104 ppm O₃. Thus, all of the snap bean lines in this experiment were considered O₃ sensitive, and existing variability would probably be insufficient to prevent yield losses under O₃ stress. Furthermore, this experiment indicates that as new snap bean lines with improved horticultural qualities and resistances to biotic diseases are developed in the absence of O₃ stress, the genetic potential for O₃ insensitivity is being lost. Since crop loss due to O₃ sensitivity is one aspect of the global climate issue, the genetic basis for O₃ sensitivity needs to be thoroughly studied for bean and other O₃-sensitive crop species.

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