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 (O3), were exposed to chronic doses of O3 for 7 hr/day in early and late-season studies. Plants were pot-cultured in open-top field chambers. Greater than ambient O3 doses were applied by supplementing the O3 present in nonfiltered air with additional O3 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 O3 concentrations showed that 'BBL-254' and 'BBL-290' were more sensitive to O3 than were 'BBL-274' and 'Dwarf Horticural'. 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 O3 at current levels of O3, but that resistance is lost with increasing O3 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 (O3) 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 soybeans, peanuts, tobacco, and cotton at current ambient concentrations of O3 (7). Although peak concentrations of O3 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 O3 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 O3 (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 O3 effects on vegetation (5). More recently, studies have evaluated the relative effects of O3 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 O3 concentrations used (1, 8–10, 12). However, mechanisms of resistance may exist at current ambient concentrations of O3.

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 O3 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 O3 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 cultivars could be used as indicators of ambient concentrations of O3 and to determine the effects of ambient O3 on bean growth and development.

Materials and Methods

'Bush Blue Lake 290' ('BBL-290') and 'Bush Blue Lake 254' ('BBL-254') were O3-sensitive and 'Bush Blue Lake 274' ('BBL-274') and 'Dwarf Horticural' were O3-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 250-cm³ 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.
located 8 km south of Raleigh, N.C. In the second study, plants were transplanted (6 Aug.) in the large pots 12 days after seed
and moved to the field site. Plants at the field site were watered once daily if the temperature was <3°C, and twice daily
if the temperature was >32°C. Plants were fertilized with 600 cm3 of Peters 20-20-20 (20 g liter−1) weekly follow-

Plants were kept at the field site for 10 days (early) study and then were moved into open-top field chambers (2, 3) for expo-

sures to O3. 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-naph-
thalenyl methylcarbamate (carbaryl) (Sevin, 7.4 cm3 liter−1),
triclocarbanophenol cyanide (cyperazine) (Piggin, 1.0 cm3 li-
ter−1), and O3-dimethyl acetylenedicarboximide (ace-
phate) (Oreathox, 1.4 g liter−1).

Ozone was generated by passing O3 through an O3 generator
(OREC, Model O3B2G-0) and dispensing the O3−O2 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 chemilumines-
cent O3 monitor. A shared-time system was used (3) so that
several chambers could be monitored with a single instrument.
Ozone was dispensed for 7 h/day (1600 to 1700 std 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. (36 days from planting) in the late
study; exposures continued through final fresh bean harvest in
both studies. The experiment involved four O3 treatments: a) carbon-fil-
tered air (CF); b) a filtered air (NF, all chambers used a par-
tial pressure of O3 equivalent to c) 0.04 ppm of O3 (NF−4); or d) 0.08 ppm of O3 (NF−8) for 7 h/day. The O3 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 among 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), beans and dry weight of bean
pods, and dry weights of plant parts (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 Heart” 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 (ANO-
VAs). Except for yield, where a regression analysis was used,
responses across seasons were not compared because of the
differences shown in seasonal O3 concentration. Plant responses showed a significant O3 × cultivar interaction for both the early and late studies. Thus, tables for injury, top and root dry weight, byin 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 numbers for the CF treat-
ment, all other O3 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
nonparametric 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
establish efficacy of O3 in terms of a decrease in economic loss
and sensitive germination, to determine if bean contain germination res-

istat to ambient concentrations of O3, and to determine yield
losses at ambient concentrations. The response of pod fresh
weight to O3 for each of the four cultivars was evaluated using a regression approach. This approach tested the homo-
geneity of the relative yield response 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 O3 effect was significant for each of the four cultivars in
both seasons. These plots mean then were regressed against the
respective 80 O3 mean concentrations (seasonal 7 h/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. Yoda using the Weibull
function then were conducted to compare the differences in
the intercepts and slopes of the eight response curves. First, for
each cultivar, the nature of the response for the two seasons
was tested. The shape of the intercept of the response curve
was similar over the two seasons for three cultivars. Therefore,
a Weibull model was fit for each of those cultivars using the
seasons as the replicate factor (10). The following equation
was fit for each cultivar, where y is the yield value, O3 is
the O3 concentration when yield is 0.737a; and c, a dimen-
sionless shape parameter. The s1 and s2 are used as “dummy”
variables (11), where s1 is the least season and s2 is for
the later season crop. For the fourth cultivar (“BBL-254”, sensitive), the shape of the curve for each season was different (hetero-
geneous); thus, the Weibull model for this cultivar for each
season was 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-h/day season mean O3 concentration in ambient air was
0.055 ppm for the early study and 0.055 ppm for the late
study. The higher ambient concentration in the early study was expected because O3 concentrations in September are usually
lower than in June and July. The 7 h/day seasonal O3 treatment
means for the CF, NF, NF + 4, and NF + 8 in the early study
were 0.055, 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 to 56 days of
exposure in each of the two studies. The NF seasonal values
are lower than the ambient concentration because some O3 is
47
### Table 1. Effects of ozone on injury severity in four bean cultivars in two studies.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Relative sensitivity to O&lt;sub&gt;3&lt;/sub&gt;</th>
<th>Percent leaf injury*</th>
<th>Percent leaf injury*</th>
<th>O&lt;sub&gt;3&lt;/sub&gt; Conc (ppm)</th>
<th>O&lt;sub&gt;3&lt;/sub&gt; Conc (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.035</td>
<td>0.050</td>
<td>0.087</td>
<td>0.119</td>
<td>0.028</td>
</tr>
<tr>
<td>BBL-254</td>
<td>Sensitive</td>
<td>17</td>
<td>56</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>BBL-290</td>
<td>Sensitive</td>
<td>9</td>
<td>54</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>BBL-274</td>
<td>Resistant</td>
<td>12</td>
<td>58</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Dw Hurt</td>
<td>Resistant</td>
<td>26</td>
<td>48</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

*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 h/day seasonal means for each treatment.

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

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Early study</th>
<th>Late study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry wt (g/plant)</td>
<td>Dry wt (g/plant)</td>
</tr>
<tr>
<td></td>
<td>% change from control</td>
<td>% change from control</td>
</tr>
<tr>
<td>BBL-254</td>
<td>37.3</td>
<td>69</td>
</tr>
<tr>
<td>BBL-290</td>
<td>37.3</td>
<td>69</td>
</tr>
<tr>
<td>BBL-274</td>
<td>47.6</td>
<td>69</td>
</tr>
<tr>
<td>Dw Hurt</td>
<td>26.4</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>6.3</td>
<td>69</td>
</tr>
</tbody>
</table>

* *Root dry wt*

| BBL-254   | 6.9         | 69         | 55                   | 78                     | 7.4                    | 20                    | 58                     |
| BBL-290   | 6.6         | 69         | 61                   | 78                     | 7.2                    | 22                    | 87                     |
| BBL-274   | 10.4        | 69         | +11                  | 12                     | 9.2                    | 1                     | 24                     |
| Dw Hurt   | 7.7         | 69         | +33                  | 8                      | 9.1                    | 7                     | 30                     |

*Percent change (increase (+) or reduction) from the respective CF treatment.*

*The raw value for percentages was calculated by dividing the lab by the highest CF number or weight.

Comparisons are estimates for use across O<sub>3</sub> concentrations within cultivars.

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

Bean cultivars designated as sensitive or resistant based on prior acute screen exhibited similar relative sensitivities to foliar injury at all three elevated O<sub>3</sub> 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<sup>2</sup> values: top dry weight = 0.77, root dry weight = 0.87, pod number = 0.44, pod dry weight = 0.55, and pod fresh weight = 0.49). The percent injury is generally less (especially at the highest O<sub>3</sub> 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<sub>3</sub> concentrations greater than the NF treatment; the trend is clear even in the NF treatment. In the early study, the effects of O<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> more in the early than in the late study, the differences were less than for pod number.

The Weibull model showed a heterogeneous 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.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Early study</th>
<th>Late study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O$_3$ conc. (ppm)</td>
<td>O$_3$ conc. (ppm)</td>
</tr>
<tr>
<td></td>
<td>0.035</td>
<td>0.050</td>
</tr>
<tr>
<td>Pod/plant (no.)</td>
<td>(% change from control)</td>
<td>(% change from control)</td>
</tr>
<tr>
<td>BBL-254</td>
<td>44.1</td>
<td>5</td>
</tr>
<tr>
<td>BBL-290</td>
<td>44.0</td>
<td>16</td>
</tr>
<tr>
<td>DW Hort</td>
<td>40.8</td>
<td>20</td>
</tr>
<tr>
<td>lsd (0.05)*</td>
<td>8.3</td>
<td>18.8</td>
</tr>
</tbody>
</table>

*Pod dry wt (g/plant) (% change from control)* = (g/plant - control) / control * 100.

BBL-254 and BBL-290 are sensitive cultivars, BBL-274 and DW Hort are resistant cultivars.

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.

Percent change (increase (+) or reduction) from the respective CF treatment.

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 Horticulturale") across seasons. For the two O$_3$-resistant cultivars, one model adequately represented the yield response over both seasons (Table 4); the relative responses of the two homogenous cultivar models across seasons were homogenous. 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 homogenous model for "BBL-290" across seasons) were compared, a single homogenous model was found to fit the two cultivars across the two seasons (Table 4). Trans 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 on) for the resistant cultivars are shown in Fig. 1 and the models (different on) 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 26.5%.

Discussion

The suppression of pod dry weight with increasing O$_3$ was related to cultivar sensitivity and the season in which the study involved.
Table 5. Relative yield suppression of sensitive and resistant bean cultivars to increasing O₃ concentrations assuming a 2% daily seasonal mean of 0.02 ppm as a background O₃ concentration.

<table>
<thead>
<tr>
<th>Ozone conc (ppm)</th>
<th>Yield suppression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cultivars</td>
</tr>
<tr>
<td>0.03</td>
<td>0.1</td>
</tr>
<tr>
<td>0.04</td>
<td>0.8</td>
</tr>
<tr>
<td>0.05</td>
<td>1.9</td>
</tr>
<tr>
<td>0.06</td>
<td>2.6</td>
</tr>
<tr>
<td>0.07</td>
<td>3.7</td>
</tr>
<tr>
<td>0.08</td>
<td>12.1</td>
</tr>
<tr>
<td>0.09</td>
<td>19.6</td>
</tr>
<tr>
<td>0.10</td>
<td>26.3</td>
</tr>
<tr>
<td>0.11</td>
<td>41.1</td>
</tr>
<tr>
<td>0.12</td>
<td>54.1</td>
</tr>
<tr>
<td>0.13</td>
<td>67.0</td>
</tr>
</tbody>
</table>

*Seven hours/day seasonal mean.*

was conducted. Yield suppression in the sensitive cultivars was related primarily to a suppression of pod/seed yield in the early study, whereas pod/seed yield was highest in the second study. Yield suppression in the resistant cultivars appears to be related to pod and seed weight in both studies. Flowers or small fruits may have benefited from the sensitive cultivars during the early study due to the timing of the O₃ exposure. Sensitivity to O₃ 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 O₃ on yield of field-grown plants. Results from Hud and Beverdifer (9) support the validity of such extrapolations, but field exposures were only to ambient levels of O₃ 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 O₃ exposure in field-grown plants, since two sensitive and two O₃-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 O₃ 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 screens (12). Yield losses in the resistant cultivars may reflect a low tolerance in common bean germplasm for O₃. It is apparent that, in common bean, resistance is related to the concentration of O₃ the plants receive. This relationship is seen in the marked separation of the sensitive and resistant cultivars at O₃ concentrations around and just above ambient. However, at the highest seasonal O₃ concentrations, the yield suppression in the resistant cultivars increased markedly, suggesting that O₃ tolerance in bean has a fairly narrow range.

Analysts suggested that the yield responses of the two resistant bean cultivars to O₃ are homogeneous with respect to chronic levels of O₃ 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 homogeneous, 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 O₃ between the sensitive and resistant cultivars tends to become blunted at high O₃ 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 species, years, and cultivars, even though the actual yield yields may vary greatly.

**Literature Cited**


9. Hud, P. and W.D. Beverdoff. 1982. The response of selected \textit{Phaeolus vulgaris} L. cultivars to ozone under controlled fumiga-

