Sulfur dioxide-enhanced Phytotoxicity of Ozone to Watermelon

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Additional index words. Citrullus lanatus, melon, air pollution

Abstract. Three watermelon [Citrullus lanatus (Thunb.) Matsum & Nakai] cultivars with different ozone (O₃) sensitivities were grown in a charcoal-filtered greenhouse and exposed in continuous-stirred tank reactor chambers to five levels (0, 100, 200, 300, or 400 nL·L⁻¹) of sulfur dioxide (SO₂) in the presence (80 nL·L⁻¹) or absence (0 nL·L⁻¹) of ozone (O₃) for 4 hours/day, 5 days/week for 22 days. In the presence of O₃, SO₂ increased foliar injury in all three cultivars, but the impact was greatest for the most O₃-sensitive cultivar, ‘Sugar Baby,’ moderate for ‘Crimson Sweet,’ and least for the least O₃-sensitive cultivar, ‘Charleston Gray.’ For all cultivars, SO₂ intensified O₃ suppression of leaf area for the first seven mainstem leaves and of dry weights for aboveground and total plant tissues. Root dry weight was independently suppressed by both pollutants, and the root : top ratio was linearly suppressed by SO₂ alone. Sulfur dioxide combined with O₃ can be detrimental to crop species such as watermelon. Thus, the potential for SO₂ phytotoxicity should not be summarily dismissed, especially in the vicinity of SO₂ point sources where O₃ co-occurs.

Recent air-quality research in the United States has often focused on ozone (O₃), because it occurs over large areas and causes economically significant crop yield suppression (Heck et al., 1990). Sulfur dioxide (SO₂), a by-product from the combustion of fossil fuels, is neither as prevalent nor as phytotoxic as O₃ (Reinert, 1984). Under some conditions, SO₂ can stimulate plant growth (Weigel et al., 1990), but deleterious effects of SO₂ include foliar injury (Beckeerson and Hofstra, 1979) and growth and yield suppression (Weigel et al., 1990). Sulfur dioxide can be more damaging combined with O₃ (Reinert, 1984; Reinert et al., 1975), but some effects of the combination appear to be species specific (Beckeerson and Hofstra, 1979; Reinert, 1984).

The impact of O₃ on watermelon and muskmelon has been examined recently because of air-quality problems in the melon-producing regions of southwestern Indiana (Simon et al., 1986) and the Ebro Delta of Spain (Reinert et al., 1992). Ambient levels of O₃ at both locations induced foliar injury in watermelon (Decoteau et al., 1986; Reinert et al., 1992; Snyder et al., 1991) and muskmelon (Simini et al., 1989; Snyder et al., 1988), but a wide range of cultivar sensitivities was identified for both crops (Decoteau et al., 1986; Simini et al., 1989). Open-top field chamber research was conducted to compare relative sensitivities to O₃. Three watermelon (Citrullus lanatus) cultivars were selected to represent different relative sensitivities to O₃: ‘Charleston Gray’ (O₃-insensitive), ‘Crimson Sweet’ (intermediately O₃-sensitive), and ‘Sugar Baby’ (extremely O₃-sensitive) (Decoteau et al., 1986).

Materials and Methods

Three watermelon (Citrullus lanatus) cultivars were selected to represent different relative sensitivities to O₃: ‘Charleston Gray’ (O₃-insensitive), ‘Crimson Sweet’ (intermediately O₃-sensitive), and ‘Sugar Baby’ (extremely O₃-sensitive) (Decoteau et al., 1986). For each cultivar, three seeds were planted on 3 Feb. 1987 and 5 Feb. 1988 in each of eighty 15-cm plastic pots filled with an artificial peat–perlite–vermiculite medium (Metro Mix 220; Grace-Sierra Horticultural Products Co., Milpitas, Calif.). Pots were placed in a greenhouse supplied with charcoal-filtered (CF) air. Two weeks after seeding, plants were thinned to one per pot and allowed to acclimate for 5 days before assignment to blocks and treatments. Since ‘Charleston Gray’ seeds did not germinate well in 1987, that cultivar had fewer plants per experimental unit in 1987 than the other two cultivars.

Plants were watered daily with tap water. Daylength was extended to 12 h with supplemental lighting from metal halogen lamps (PAR = 250 µmol·m⁻²·s⁻¹ at plant height). The first week after transplanting, plants were fertilized with a dilute fertilizer solution (2.5 g Peter’s 20N–20P–20K/L water; Grace-Sierra). Thereafter, plants were fertilized weekly with a more concentrated fertilizer solution (5 g Peter’s 20N–20P–20K/L water). Vines were attached to bamboo stakes to facilitate transport to and from exposure chambers and to maximize exposure of foliage to pollutants. When necessary, (2 methyl[1,1´-biphenyl]-3-yl) methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropane carboxylate (bifenthrin) was used at the manufacturer’s recommended rate to control white flies and spider mites.

Exposures to O₃ and SO₂ were conducted in 10 continuous-stirred tank reactor (CSTR) chambers (Heck et al., 1978) in a greenhouse bay supplied with CF air and adjoining the CF air growing bay. To expose two blocks of plants each day, the first block of plants was exposed in the morning and the second block...
of plants was exposed in the afternoon. Plants were returned to the CF air growing bay immediately after each exposure. Each block of plants was exposed for 4 h/day, 5 days/week for 22 days beginning 24 Feb. 1987 or 29 Feb. 1988. Ozone was generated by electrostatic discharge in compressed air (model GTC-1A; Griffin Technics Corp., Lodi, N.J.), and SO2 was supplied from a tank containing 1% SO2 in dry nitrogen. Each gas was dispensed into CSTR chambers through rotometers (Heck et al., 1978). The concentrations of O3 and SO2 in each chamber were monitored by ultraviolet photometry (model 49; Thermo Electron Instruments, Hopkinton, Mass.) and flame photometry (model 8450; Monitor Labs, San Diego), respectively. Gas concentrations in CSTRs were sampled sequentially for 3-min intervals every 30 min by an automated time-shared monitoring system.

One day before the first exposure, the width of the first leaf (LFWIDTH) on each plant was measured at the widest point. Individual measurements ranged from 0.4 to 7.8 cm over the 2 years. We believed this measurement indicated vigor and/or size of the future plant and, thus, chose it as a potential covariate for post-experimental data analyses. Young leaves that have received only short periods of exposure to chronic doses of O3 and SO2 do not express visible foliar injury. Thus, only the first five mainstem leaves were sampled for visible injury on 15 Mar. 1987 or 22 Mar. 1988. The percentage of leaf area visibly injured was estimated for each of the five leaves, and the averages of these leaf ratings for each plant were used in data analyses. One day after the last exposure, plants were destructively harvested. Leaf area for the first seven leaves on the main stem was measured to include leaf tissue with and without visible injury. Roots from each plant were washed and bagged separately from the aboveground tissue (top). Tissues were dried at 60 °C for 48 h and weighed. The total plant dry weight and the root : top dry weight ratio were calculated.

The treatment design for each experiment was a 2 × 5 × 3 factorial with two O3 concentrations (0 or 100 nL·L–1 O3), five SO2 concentrations (0, 100, 200, 300, or 400 nL·L–1 SO2), and three watermelon cultivars. The experimental design was a split plot with the 10 pollutant combinations serving as main-plot treatments and arranged in a randomized complete-block design with two blocks. The watermelon cultivars were the subplot treatments. Blocking was against plant size and exposure time: block one contained the larger plants that were exposed to pollutants in the mornings. The integrity of the blocks was maintained regardless of the greenhouse bay in which plants were located. Four containers of each cultivar (except for three containers of ‘Charleston Gray’ in 1987) were randomly placed in each chamber for a total of 220 and 240 plants in 1987 and 1988, respectively. The means for each cultivar within each CSTR were used for all statistical analyses.

To preserve the orthogonality of the split-plot design, the covariate, LFWIDTH, was split into two orthogonal parts, the sum of which equalled the centered original covariate (Pantula et al., 1989). The first term, designated as cov1, and used to adjust the whole-plot error term (error1) in each analysis by year, was calculated by subtracting the grand mean for the appropriate year from the whole-plot mean for LFWIDTH. The second term, designated cov2, and used to adjust the subplot error term (error2) in each analysis by year, was calculated by subtracting the whole-plot mean for LFWIDTH from the subplot mean for LFWIDTH. Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were conducted for each variable and experiment to determine the need for the covariate terms in residual plots. If the subplot error term was reduced in the ANCOVA compared to the ANOVA, then ANCOVA was used for residual plots.

Residual plots for each variable were examined for outliers, heterogeneous variances, and nonnormal data. Injury data were transformed by arcsin (Y0.5), but transformation was not necessary for any of the other variables. A separate analysis was conducted for each variable and each experiment, and Bartlett’s chi-square test was used to determine whether or not variances were heterogeneous between years. Leaf area, transformed injury readings, and root, top, and total dry weights did not have homogeneous variances between the 2 years, so all combined-experiment analyses for these variables were weighted by the inverse of the appropriate mean square errors (1/SE2) from the analyses by year.

After determining the need for weighting of variances, data for the 2 years were combined, with year considered to be a fixed factor. If neither covariate term was significant in the combined analysis of a variable, the covariates were deleted from all subsequent analyses. Consequently, ANCOVA was replaced with ANOVA for injury, leaf area, and root : top ratio data. The dose response of SO2 and all SO2 interactions were partitioned by orthogonal polynomial coefficients with contrast statements. Varietal trials indicated that the O3 response of ‘Crimson Sweet’ was more similar to ‘Charleston Gray’ than to ‘Sugar Baby’ (Decoteau et al., 1986). Therefore, hypotheses were developed to compare the response of ‘Sugar Baby’ to the average of the responses of ‘Charleston Gray’ and ‘Crimson Sweet’ and to compare the response of ‘Charleston Gray’ with that of ‘Crimson Sweet.’ In accordance with these hypotheses, cultivar effects (and cultivar × pollutant treatment interactions) were partitioned using orthogonal contrasts.

Results

Foliar injury was first visible 4 days after gas exposures began in 1987 on ‘Sugar Baby’ plants placed in chambers with the two highest SO2 concentrations combined with O3. In 1988, foliar injury was not obvious until 10 days after exposures began, and injury was then visible on ‘Sugar Baby’ plants exposed to 200, 300, or 400 nL·L–1 SO2 combined with O3 and on ‘Crimson Sweet’ plants exposed to 300 nL·L–1 SO2 combined with O3. All visible injury resembled O3 injury as described by Decoteau et al. (1986), and no visible injury occurred in either year on plants that were not exposed to O3. Although foliar injury occurred earlier and tended to be greater (leaf area affected and symptom severity) in 1987 than in 1988, the difference between years was not significant, and treatment effects on injury did not depend on year (Table 1). The intensity of foliar injury was significantly affected by O3 and SO2 concentrations and by cultivar, but the main effects of these factors were all interdependent. As expected, ‘Sugar Baby’ exhibited more O3 injury than the less O3-sensitive cultivars, and ‘Charleston Gray’ had less O3 injury than ‘Crimson Sweet.’ The foliar injury caused by O3 was increased in the presence of SO2 for all three cultivars, but the SO2 enhancement of O3 injury was greatest for ‘Sugar Baby’ (Fig. 1).

Total area of the first seven mainstem leaves was not affected by year or by a year × gas interaction (Table 1). Leaf area was suppressed by O3 and SO2, and the interaction of the pollutants was significant. In the absence of O3, the linear suppression of leaf area by SO2 was minimal, but the suppression of leaf area by O3 increased linearly with SO2 concentration (Fig. 2). As expected, cultivars had different mean leaf areas (693.7, 781.2, and 796.5 cm2 for ‘Sugar Baby,’ ‘Crimson Sweet,’ and ‘Charleston Gray,’ respectively), but the cultivars did not significantly differ in the degree of pollutant suppression of leaf area.

All dry weight variables were affected significantly by year and cultivar (Table 1). Final biomass was generally greater in 1988 than in 1987, but this was especially true for ‘Sugar Baby’ plants.
Table 1. Mean squares for foliar injury ratings, leaf area, and dry weights of three watermelon cultivars exposed to SO$_2$ and O$_3$ in 1987 or 1988.

| Source$^a$ | df | Injury$^{x,y}$ | Leaf area$^{x,w}$ | Top$^x$ | Root$^v$ | Total$^v$ | Root : top$^v$ |
|------------|----|---------------|-------------------|--------|---------|---------|-------------|
| Year (Y)   | 1  | 19.2          | 652.9             | 15.5   | 299.4*  | 30.0*   | 122.09**    |
| Blk (Y)    | 2  | 12.5          | 61.8              | 0.5    | 3.4     | 0.7     | 0.31        |
| SO$_2$     | 4  | 24.1**        | 7.8*              | 0.6    | 2.4     | 0.5     | 1.63*       |
| SL         | 1  | 90.9**        | 30.3**            | 7.8*   | 3.66**  |         |             |
| O$_3$      | 1  | 292.2**       | 23.2**            | 9.4*   | 8.0*    | 10.3*   | 1.00        |
| SO$_2$ × O$_3$ | 4 | 24.1** | 3.5 |
| SL × O$_3$ | 1  | 90.9**        | 12.7              | 15.5** | 15.5**  |         |             |
| SO$_2$ × Y | 4  | 0.9           | 0.3               | 1.7    | 1.7     | 1.7     | 0.53        |
| O$_3$ × Y  | 1  | 19.2          | 1.4               | 2.4    | 0.1     | 2.3     | 0.04        |
| SO$_2$ × O$_3$ × Y | 4 | 0.9 | 1.6 |
| Cov        | 1  | 7.6           | 6.9*              | 8.5*   |         |         |             |
| Error      | 17,18u | 5.3 | 2.3 | 1.9 | 1.4 | 1.9 | 0.43 |
| Total      | 119 |                |                  |        |         |        |             |

$^a$SL = SO$_2$ linear; CG, CS, and SB = ‘Charleston Gray’, ‘Crimson Sweet’ and ‘Sugar Baby’, respectively.

$^b$Data were recorded for the first five leaves as percentage of leaf area injured, and the averages of these individual leaf ratings were transformed by the arcsin of the square-root function.

$^w$Leaf area (cm$^2$) was measured for the first seven mainstem leaves.

$^v$Mean squares have been multiplied by 10$^4$.

$^u$Error terms from ANOVAs have 1 df more than error terms from ANCOVAs.

$^x$Significant at 0.01 < $P$ ≤ 0.05 or $P$ ≤ 0.01, respectively. Only significant partitions of treatment effects are given.

The difference in magnitude of cultivar responses between years and/or the difference in ‘Sugar Baby’ plant size for the 2 years resulted in year × cultivar interactions. Root dry weight, the only dry weight variable for which no cultivar × year interaction occurred, was greatest for ‘Charleston Gray’ and smallest for ‘Sugar Baby.’ The year × cultivar interaction for top dry weight was significant because ‘Sugar Baby’ had the greatest top weight in 1988 and the lowest weight in 1987. In turn, the total dry weight for ‘Sugar Baby’ was much greater in 1988 than in 1987 but was similar between the 2 years for the other two cultivars. Although the relative rankings of the cultivars were the same in both years for the root : top ratio, the ratios for ‘Charleston Gray’ and ‘Crimson Sweet’ were more similar in 1988 than in 1987. Year and cultivar effects per se were of less interest in these experiments than their potential to influence pollutant effects, and no significant interactions with pollutant treatments were found for either factor.

Root dry weight was suppressed linearly by SO$_2$ (Fig. 3a) and slightly suppressed by O$_3$ (1.11 and 1.06 g, respectively, for 0 and 100 nL·L$^{-1}$). Ozone suppressed aboveground biomass, and suppression increased linearly with SO$_2$ dose (Fig. 3b). The influence of O$_3$ on top weight response to SO$_2$ was most apparent with SO$_2$ concentrations >200 nL·L$^{-1}$. The enhanced top growth in response to SO$_2$ in the absence of O$_3$ was equivalent to the suppression that occurred in response to SO$_2$ in the presence of O$_3$; thus, the main effect of SO$_2$ on top growth was not significant. Aboveground tissue accounted for a much greater percentage of the total biomass than the belowground tissue (93% vs. 7%, respectively) (Table 2). Thus, pollutant effects on total dry weight (Fig. 3c) resembled those found for top dry weight. Whereas root and top dry weights were linearly suppressed by SO$_2$ in the presence of O$_3$, suppression of root biomass was greater than that of the top (13% and 6%, respectively, at 400 nL·L$^{-1}$SO$_2$), and SO$_2$ did not increase root weight in the absence of O$_3$. Thus, the root : top ratio was suppressed linearly by SO$_2$ (Fig. 3d) with no significant O$_3$ effect nor any significant O$_3$ × SO$_2$ interaction in the course of these experiments.

Discussion

Ideally, pollutant concentrations in controlled studies approximate those found in the ambient environments in which the crop is
grown. Ambient hourly O₃ concentrations have been reported for the melon-growing seasons in Vincennes, Ind., in 1984 and 1985 (Decoteau et al., 1986; Simini et al., 1989; Simon et al., 1986) and for Decker, Ind., in 1986 and 1987 (Snyder et al., 1988). These hourly averages include data from overcast or rain days when O₃ concentrations are naturally lower. In all 4 years, the hourly averages over the growing seasons peaked between 55 and 70 nL·L⁻¹, with concentrations fluctuating around the peak for 5 to 8 h. In our experiments, square wave exposures were restricted to 4 h/day and only 5 days/week. Therefore, we chose to exceed the ambient hourly O₃ average in Indiana without exceeding the U.S. national ambient air quality standard of 120 nL·L⁻¹ maximum hourly average O₃ concentration.

Ambient SO₂ levels near point sources are generally more variable throughout the day than ambient O₃ levels, which follow a diurnal pattern. In addition, SO₂ levels are occasionally elevated during darkness. Simon et al. (1986) monitored SO₂ levels at a Decker site, which was selected to receive maximum SO₂ exposures from local point sources. They found higher SO₂ levels during the hours when O₃ concentrations are generally high. For example, in 1984, elevated SO₂ levels were recorded as often as 39,
Ozone reduces plant growth, and stems and leaves on O₃-stressed plants usually retain assimilates resulting in stunted root systems (Cooley and Manning, 1987). The root : top dry weight ratio is a general indicator of assimilate partitioning, and the suppression of the root : top ratio by SO₂ observed in our experiments is consistent with reports that SO₂ and O₃ can have similar effects on partitioning (Winner, 1994).

Only foliar injury varied according to cultivar × pollutant treatment interactions. Differences in O₃ sensitivity ratings among the three cultivars in these controlled greenhouse studies were consistent with those observed in the field (Decoteau et al., 1986). Sulfur dioxide linearly increased the O₃-induced injury for all three cultivars, but the increase was significantly greater for ‘Sugar Baby’ than for the less O₃-sensitive watermelon cultivars. ‘Crimson Sweet’ had an O₃ injury response intermediate to that of O₃-insensitive ‘Charleston Gray’ and O₃-sensitive ‘Sugar Baby,’ but it did not differ significantly from ‘Charleston Gray’ in its response to SO₂ alone or combined with O₃. Although foliar injury is an unreliable indicator of pollutant impacts on biomass, ‘Sugar Baby’ plants had suppressed marketable yields and increased foliar injury in response to ambient ozone in open-top field chambers (Snyder et al., 1991).

Information provided by this research contributes to an understanding of the complex environmental stresses on melon crops, but important interactions remain undescribed. Mixtures of O₃ and SO₂ are more phytotoxic than either gas alone during vegetative growth, but watermelon response to the combination of SO₂ and O₃ during fruit set is still unknown. Furthermore, early-season watermelon cultivars may be more O₃-sensitive than late-season cultivars (Simini et al., 1989), and the effects of O₃ on watermelon in these experiments. Ozone induced foliar injury and reduced leaf area and root, top, and total dry weights, but combined with SO₂, the toxic effects of O₃ were intensified. In the absence of O₃, no visible injury occurred, suppression of leaf area was minimal, and top and total dry weights increased with increased SO₂ concentrations. Root biomass and the root : top ratio were linearly suppressed by SO₂, and for these variables the phytotoxicity of SO₂ was independent of O₃.

### Table 2. Adjusted dry weight means with standard errors by year and watermelon cultivar.

| Year | Cultivar     | Top (g) | Root (g) | Total (g) | Root:topa |
|------|--------------|---------|----------|-----------|-----------|
| 1987 | Charleston Gray | 14.6 (0.1) | 1.1 (0.1) | 15.7 (0.1) | 7.8 (0.02) |
|      | Crimson Sweet | 13.0 (0.1) | 0.9 (0.1) | 13.9 (0.1) | 7.0 (0.02) |
|      | Sugar Baby    | 11.9 (0.1) | 0.7 (0.1) | 12.6 (0.1) | 6.2 (0.02) |
| 1988 | Charleston Gray | 14.2 (0.1) | 1.4 (0.1) | 15.6 (0.1) | 10.0 (0.02) |
|      | Crimson Sweet | 12.9 (0.1) | 1.2 (0.1) | 14.1 (0.1) | 9.5 (0.02) |
|      | Sugar Baby    | 14.4 (0.1) | 1.1 (0.1) | 15.5 (0.1) | 7.5 (0.02) |

aMeans have been adjusted for the covariate (where applicable). The 1987 experiment included 60 ‘Charleston Gray’ plants. All other year and cultivar combinations included 80 plants.

bRatio values and standard errors should be multiplied by 10⁻² and 10⁻⁴, respectively.
assimilate sink caused by fruit set may contribute to O₃ sensitivity differences due to earliness. If fruit set results in greater vulnerability to pollutant toxicity, then earlier cultivars would be impacted earlier in the growing season than later cultivars, and higher ambient O₃ levels are more probable during the earlier part of the watermelon season in most regions of the United States. Field chamber experiments with multiple cultivars and mixtures of SO₂ and O₃ should address the relationships of fruit set and earliness to pollutant sensitivity. The importance of cation-exchange capacity, soil pH, and nitrogen source have been examined for muskmelon and watermelon cultivation (Elamin and Wilcox, 1986; Simon and Wilcox, 1984; Gerald Wilcox, personal communication) and SO₂ exposures (Kaiser et al., 1993; Kropff, 1991), but almost no information is available on the relationship of these factors to SO₂ and O₃ effects on watermelon. Such research might lead to recommendations that could be implemented by growers to ameliorate the impacts of pollutants, even though ambient concentrations of pollutants cannot be directly controlled.

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