

Utilization of a Response-Surface Technique in the Study of Plant Responses to Ozone and Sulfur Dioxide Mixtures¹

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DOUGLAS P. ORMROD*, DAVID T. TINGEY, MARCIA L. GUMPERTZ, AND DAVID M. OLSZYK
Department of Horticultural Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1 (D. P. O); United States Environmental Protection Agency and Northrop Services, Inc., Corvallis, Oregon, 97333 (D. T. T., M. L. G); and Statewide Air Pollution Research Center, University of California, Riverside, California, 92521 (D. M. O.)

ABSTRACT

A second order rotatable design was used to obtain polynomial equations describing the effects of combinations of sulfur dioxide (SO₂) and ozone (O₃) on foliar injury and plant growth. The response surfaces derived from these equations were displayed as contour or isometric (3-dimensional) plots. The contour plots aided in the interpretation of the pollutant interactions and were judged easier to use than the isometric plots. Plants of 'Grand Rapids' lettuce (*Lactuca sativa* L.), 'Cherry Belle' radish (*Raphanus sativus* L.), and 'Alsweet' pea (*Pisum sativum* L.) were grown in a controlled environment chamber and exposed to seven combinations of SO₂ and O₃. Injury was evaluated based on visible chlorosis and necrosis and growth was evaluated as leaf area and dry weight. Covariate measurements were used to increase precision. Radish and pea had greater injury, in general, than lettuce; all three species were sensitive to O₃, and pea was most sensitive and radish least sensitive to SO₂. Leaf injury responses were relatively more affected by the pollutants than were plant growth responses in radish and pea but not in lettuce. In radish, hypocotyl growth was more sensitive to the pollutants than was leaf growth.

Plant responses to one factor frequently depend upon the levels of other factors. This dependence demands that treatment combinations be considered in studies of environmental, nutritional, or growth regulator effects on plants. A full set of factorial combinations with several levels of each factor may require large, costly experiments. The use of a response-surface technique, the rotatable experimental design, minimizes the number of treatments required to cover the desired range of levels of each factor in the experiment (5). In spite of its efficiency, only a few studies have used the technique (1, 8). With a rotatable design, one obtains a response surface using fewer experimental treatments than with a full factorial design. Additional measurements, called covariates, taken on each plant prior to pollutant exposures can be incorporated into this design to increase the precision of statistical tests. These covariate measurements can be used to eliminate some of the variation among plants from the error of parameter estimates (21). The characterization of response surfaces may facilitate an understanding of the nature of the inter-

action in relation to changing levels of each factor and help to interpret the physiological mechanisms of the interaction.

The objective of this research was to demonstrate the utility of the rotatable design in providing response surfaces for the interpretation of the effects of O₃ and SO₂ on visible foliar injury and growth of lettuce, radish, and pea. While there have been many studies on the effects of O₃ and SO₂ mixtures on plants (16), the use of a rotatable experimental design for pollutant combination studies has not hitherto been evaluated nor have covariate measurements been incorporated to increase precision in such designs.

MATERIALS AND METHODS

Plant Culture. Plants of three species (leaf lettuce, *Lactuca sativa* L. cv Grand Rapids; garden pea, *Pisum sativum* L. cv Alsweet; and radish, *Raphanus sativus* L. cv Cherry Belle) were grown from seed in 10-cm diameter pots containing Pro-Mix BX (1 sphagnum peat moss:1 vermiculite:1 perlite by volume) in Conviron Model E15 growth chambers. Several seeds were sown per pot and seedlings were thinned to one per pot at 7 d. Environmental conditions were similar to those used for base line growth studies of lettuce (9): 25/30 ± 1°C day/night temperature, 72 ± 5% RH, 325 ± 10 μmol m⁻² s⁻¹ PAR at the top of the plant canopy for 16 h each d from 75% input wattage cool-white fluorescent and 25% input wattage incandescent lamps. The plants were watered daily with North Carolina State University Phytotron nutrient solution (6).

Experimental Design. For the rotatable design, seven O₃ and SO₂ exposure combinations were selected based on a hexagon in which the treatments included six points at the vertices of the hexagon plus two points at the center (Fig. 1). The seven treatments were divided into two orthogonal blocks (4) so that the estimated coefficients for SO₂, O₃, and SO₂ × O₃ would be independent of differences between the blocks (values in ppm):

Block 1	SO ₂	0.45	0.80	0.10	0.45
	O ₃	0.40	0.10	0.10	0.20
Block 2	SO ₂	0.80	0.45	0.10	0.45
	O ₃	0.30	0.00	0.30	0.20

The two blocks were run on two consecutive days because only four exposure chambers were available. Seeding dates were adjusted accordingly. Four plants were exposed to each treatment at one time. The entire experiment was replicated five times for lettuce and radish and four times for pea.

Pollutant Exposures. Plants were exposed to each pollutant combination for 6 h in modified exposure chambers (10). Two exposure chambers were housed in each of two Sherer Model

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CEL37-14² growth chambers and maintained at environmental conditions similar to those of the plant culture chambers. Modifications to the exposure chambers included increased air flow, placement of a perforated upper barrier for better mixing and uniformity in the chamber, increased pressure drop in the recirculating system, and a slightly larger size than originally used. O₃ was generated by the intermittent operation of a UV lamp in the base of each chamber and monitored with Dasibi 1003AH analyzers. SO₂ was metered from a tank containing 1% SO₂ in N₂ and monitored with a fluorescent SO₂ analyzer. The O₃ analyzers were calibrated with a Dasibi Model 10008PC UV standard source, and the SO₂ analyzers calibrated with a Bendix Model 8861 DATM Field Calibration system using tank SO₂ traceable to the National Bureau of Standards.

Harvest Procedures. At 14 d each pot was randomly numbered and covariate measurements PLA,³ HD14, and PH14 were taken. For lettuce and radish, PLA was determined using a transparent sheet with a 1-cm grid placed gently over the plant (17). Height of pea plants was measured from the second stipular node to the shoot apex. After these nondestructive measurements were made, the plants were transferred to exposure chambers. Foliar injury, leaf area, and weight were measured on each plant 3 d after exposure. Dry weight was determined after 48 h in a forced draft oven. Leaf area was measured photometrically. Radish plants were separated into leaf and hypocotyl components. For peas, plant heights, and shoot weights (leaf + stem) were determined. Foliar injury was visually estimated as the per cent of leaf area showing chlorotic or necrotic injury and expressed as both PLI and mean PLAI for each plant.

Data Analysis. All growth variables (*i.e.* leaf area, dry weight, and plant height) were transformed to natural logarithms before further analysis in order to normalize the responses and stabilize their variances. Foliar injury did not require transformation.

A second order multiple regression response surface was fitted for each response variable. The form of the linear model for the response surface used was:

$$Y_{ijk} = R_i + B_j + RB_{ij} + \alpha_1(\text{covariate}) + \alpha_2(\text{O}_3) + \alpha_3(\text{SO}_2) + \alpha_4(\text{O}_3)^2 + \alpha_5(\text{SO}_2)^2 + \alpha_6(\text{O}_3 \times \text{SO}_2) + \epsilon_{ijk}$$

where R = replicates, B = blocks.

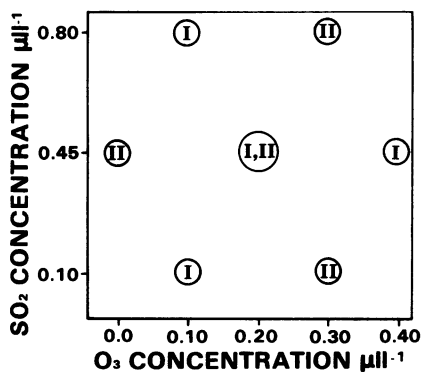


FIG. 1. Diagram of treatment combinations. Roman numerals indicate block assignment.

² Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

³ Abbreviations: PLA, planar leaf area; HD14 HD17, hypocotyl diameter at 14 and 17 days from seeding, respectively; HDW, hypocotyl dry weight; LA, leaf area, one surface; LDW, leaf dry weight; PH14 PH17, plant height at 14 and 17 days from seeding, respectively; PLAI, per cent of leaf area injured; PLI, per cent of leaves injured; SDW, shoot dry weight; SGE, standard geometric error.

The covariate PLA was used for lettuce and radish leaf dry weight and leaf area; the covariate HD14 was used for radish hypocotyl dry weight and diameter; and the covariate PH14 was used for pea growth variables. No covariate was used for leaf injury data. After the surface was fitted, the interaction coefficient was tested using an F test with a significant level of 0.05. If the SO₂ × O₃ interaction was not significant, the effects of O₃ and SO₂ alone were then tested by dropping terms out of the model and comparing these reduced models to the full model above. Contour plots and isometric (3-dimensional) graphs were made of the final models.

The aptness of the model was checked by comparing the fitted response surface with the model:

$$Y_{ijk} = R_i + B_j + RB_{ij} + \alpha_1(\text{covariate}) + T_k + \epsilon_{ijk}$$

where R = replicates, B = blocks, T = treatment.

For lettuce there was no significant lack of fit. For radish, all but HD, HDW, and PLAI were adequately estimated by the model. For pea, all but PLI fit the model. For the response variables that showed significant lack of fit, caution is required in interpretation of the response surfaces. The calculation of coefficients of determination (R^2) provided additional interpretation of the variation. If there was no significant lack of fit but the R^2 value was low, then there was either (a) a large replicate × treatment interaction, (b) wide scatter of response in each replicate, or (c) the magnitude of the SO₂ and O₃ effects was not large.

RESULTS AND DISCUSSION

Response surfaces developed from experiments using two factors can be illustrated graphically using either isometric (3-dimensional) or contour graphs. Contour graphs are two-dimensional representations of three-dimensional surfaces. The individual factors of the experiment form the abscissa and the ordinate and the response is shown as a series of isoeffect (7) or contour lines. Each isoeffect line represents a certain effect or degree of response. The shapes of the isoeffect lines in the X-Y plane illustrate cross-sections of the surface, whereas the spacing of the isoeffect lines shows the rate of change or curvature of the surface in the third dimension. The interpretation of isoeffect lines is similar to that of contour lines on a map; the closer the lines, the more rapid the rate of change. If the lines are uniformly spaced, then the rate of change is constant over the surface.

Plant response data from studies of the effect of O₃ and SO₂ were used to illustrate the usefulness of response surfaces to

Table I. Arithmetic Means for Foliar Injury

No adjustment for initial size has been made. The model used to calculate standard errors was $Y_{ijk} = \mu + R_i + T_j + \epsilon_{ijk}$, where $i = 1, \dots, 5$ replicates, $j = 1, \dots, 7$ treatments, and $k = 1, 2, 3, 4$ plants per replicate.

Response Variable	0.10 ^a		0.45		0.80		SE	
	0.10	0.30	0	0.20	0.40	0.10		0.30
	%							
'Grand Rapids' Lettuce ($n = 20$)								
PLAI	0.50	4.1	0.54	1.9	3.4	2.6	3.3	0.5142
PLI	10	32	8	24	32	26	34	3.478
'Cherry Belle' Radish ($n = 20$)								
PLAI	5.0	55	0	31	63	9.6	49	1.864
PLI	32	69	0	68	88	47	65	5.218
'Alsweet' Pea ($n = 16$)								
PLAI	14	61	0	62	70	40	66	1.646
PLI	61	86	0	82	90	74	84	1.612

^a SO₂/O₃.

Table II. Geometric Means for Growth Variables

Each response has been adjusted to a common initial size (analysis of covariance). The model used for the analysis of covariance was $\ln(Y_{ijk}) = \mu + R_i + T_j + \alpha \text{ covariate} + \epsilon_{ijk}$, where $i = 1, \dots, 5$ replicates, $j = 1, \dots, 7$ treatments, and $k = 1, 2, 3, 4$ plants per replicate.

Response Variable	0.10 ^a		0.45		0.80		Geometric SE	
	0.10	0.30	0	0.20	0.40	0.10		0.30
%								
'Grand Rapids' Lettuce ($n = 20$)								
LA (cm ² plant ⁻¹)	141	129	144	137	136	134	129	1.023
LDW (g plant ⁻¹)	0.242	0.237	0.267	0.228	0.221	0.221	0.223	1.031
'Cherry Belle' Radish ($n = 20$)								
LA (cm ² plant ⁻¹)	108	87.4	114	98.5	67.5	99.5	86.5	1.049
LDW (g plant ⁻¹)	0.364	0.317	0.364	0.340	0.287	0.330	0.333	1.035
HD (mm)	16.1	12.9	17.5	14.9	11.4	15.2	12.1	1.033
HDW (g plant ⁻¹)	0.304	0.159	0.307	0.202	0.106	0.247	0.131	1.096
'Alsweet' Pea ($n = 16$)								
LA (cm ² plant ⁻¹)	118	99.5	124	97.5	85.6	106	88.2	1.038
SDW (g plant ⁻¹)	0.453	0.370	0.492	0.368	0.330	0.400	0.340	1.039
PH (cm)	35.5	31.5	35.2	31.8	30.6	33.4	30.3	1.011

^a SO₂/O₃.

describe various responses. The means of various plant responses to combinations of O₃ and SO₂ are shown in Tables I and II. Table III contains the equations of all the response surfaces developed; only statistically significant terms were retained in the regression equations. To illustrate the range of various response surfaces, the effects of combinations of O₃ and SO₂ on foliar injury (PLA) and plant growth (LA), and LDW of lettuce, radish, and pea are graphed in Figure 2 with points along each line representing combinations of concentrations that produce the same effect.

Two categories of plant response are possible when the effects of two factors are evaluated. When one factor has no effect on the plant response, it is termed no joint action. The term joint action implies that both factors have some effect on the plant response. Joint action is frequently divided into subcategories (7):

additive response: $\text{effect}_{12} = \text{effect}_1 + \text{effect}_2$

interaction: $\text{effect}_{12} \neq \text{effect}_1 + \text{effect}_2$

There are two possible types of interaction:

synergism: $\text{effect}_{12} > \text{effect}_1 + \text{effect}_2$

antagonism: $\text{effect}_{12} < \text{effect}_1 + \text{effect}_2$

The simplest case of no joint action is a linear response to one factor, *i.e.* a linear effect of O₃ within the concentration range represented. The generalized equation for this pattern is $Y = b_0 + b_1(O_3)$. PLAI (Fig. 2D) and LA of radish (Fig. 2E) illustrate this pattern. In these figures, the isoeffect lines are parallel to the SO₂ axis and equally spaced on the O₃ axis, indicating that at the SO₂ concentrations used, SO₂ had no effect on the plant response. A more complex type of response to O₃ with no SO₂ effect is illustrated by LA and LDW of lettuce (Fig. 2, B and C). As shown by the nonuniform spacing of the isoeffect lines, the relative response to O₃ decreased with increasing O₃ concentration; a linear and quadratic effect of O₃ ($Y = b_0 - b_1[O_3] + b_2[O_3]^2$). The spacing of the contours widened with increasing O₃ because the quadratic component was significant.

The joint action of two factors can take numerous forms, but the simplest form is a linear additive response, as seen in LA of pea (Fig. 2H). The equation for this type of response is $Y = b_0 - b_1(O_3) - b_2(SO_2)$. The isoeffect lines are equally spaced diagonal lines and the concentration of O₃ required to produce a constant response decreases as the SO₂ concentration increases. The relative importance of the two factors can be estimated from the regression equation by using the linear regression coefficients. They provide an estimate of the ratio (volume or moles) of O₃

and SO₂ required to produce the same level of effect. In this example (Fig. 2H), it required about 6 mol of SO₂ to produce the same response as 1 mol of O₃; therefore, if the O₃ concentration was decreased by 0.01 $\mu\text{l l}^{-1}$, then the SO₂ concentration would have to be increased by 0.06 $\mu\text{l l}^{-1}$ to maintain the same response.

If any quadratic terms are introduced into the model, the contour lines become curved and unequally spaced. An example of this is the pea SDW (Fig. 2I), which has the form $Y = b_0 - b_1(S_2) - b_2(O_3) + b_3(O_3)^2$. At low concentrations of either factor, the response was essentially additive. Only when the O₃ concentration was relatively high (above 0.25 ppm) did the quadratic term have a major influence on the shape of the surface. The concentration of O₃ required to elicit a specific level of response decreased nonlinearly with increasing SO₂ concentration.

Interaction is a more complex form of the joint action of two pollutants and may be either synergistic or antagonistic when the dose-response function includes an interaction term (7). If the dose-response function is linear with an interaction such as $Y = b_0 + b_1(O_3) + b_2(SO_2) - b_3(O_3 \times SO_2)$, then the isoeffect lines will be hyperbolic (20), as illustrated by PLAI of lettuce and LDW of radish (Fig. 2, A and F). If the coefficient of the interaction term has the same sign as the coefficients for O₃ and SO₂, the magnitudes of the response to the mixture will be larger than the sum of the responses to SO₂ and O₃ alone, hence the interaction is synergistic. If the interaction coefficient has the opposite sign from the O₃ and SO₂ coefficients, then the interaction is antagonistic. In these examples (Fig. 2, A and F), the curvature of the isoeffect lines indicates that the response is less than would be expected if the effects of O₃ and SO₂ were additive, providing a visible illustration of the antagonistic interaction. To more clearly illustrate the antagonistic response shown in Figure 2F, draw a straight line connecting the points where the 336-g isoeffect line intersects the O₃ and SO₂ axes. This hypothetical line illustrates the additive effect of SO₂ and O₃. A comparison of the 336-g isoeffect line with the hypothetical additive response line clearly shows that more O₃ and SO₂ are required to cause a radish leaf dry weight of 336 g than would be expected if the effects of O₃ and SO₂ were additive.

Quadratic dose-response functions of the form $Y = b_0 + b_1(O_3) + b_2(SO_2) - b_3(O_3)^2 - b_4(SO_2)^2$ give concentric elliptical isoeffect lines. If an interaction term is introduced into the model, the ellipses are rotated (3, 20) as seen in the pea PLAI response (Fig. 2G).

Table III. Equations for Fitted Response Surfaces (Final Models) and R^2 Values, Including Those for Response Variables Not Presented Graphically

	R^2
'Grand Rapids' Lettuce	
PLAI = $-1.692 + 5.156(\text{SO}_2) + 17.88(\text{O}_3) - 21.31(\text{SO}_2 \times \text{O}_3)$ (Fig. 2A)	0.429
PLI = $-3.827 + 33.20(\text{SO}_2) + 109.4(\text{O}_3) - 101.8(\text{SO}_2 \times \text{O}_3)$	0.427
$\ln(\text{LA}) = 1.747 + 0.8985(\ln \text{PLA}) - 0.6041(\text{O}_3) + 0.9803(\text{O}_3)^2$ (Fig. 2B)	0.911
$\ln(\text{LDW}) = -5.511 + 1.157(\ln \text{PLA}) - 0.9894(\text{O}_3) + 1.679(\text{O}_3)^2$ (Fig. 2C)	0.896
'Cherry Belle' Radish	
PLAI = $-5.385 + 179.1(\text{O}_3)$ (Fig. 2D)	0.770
PLI = $-25.26 + 100.8(\text{SO}_2) + 493.0(\text{O}_3) - 71.28(\text{SO}_2)^2 - 592.9(\text{O}_3)^2 - 141.4(\text{SO}_2 \times \text{O}_3)$	0.760
$\ln(\text{LA}) = 1.341 + 0.8495(\ln \text{PLA}) - 1.195(\text{O}_3)$ (Fig. 2E)	0.682
$\ln(\text{LDW}) = -4.479 + 0.8874(\ln \text{PLA}) - 0.2512(\text{SO}_2) - 0.9890(\text{O}_3) + 1.101(\text{SO}_2 \times \text{O}_3)$ (Fig. 2F)	0.760
$\ln(\text{HDW}) = -3.985 + 1.318(\ln \text{HD14}) - 2.813(\text{O}_3)$	0.719
$\ln(\text{HD17}) = 1.418 + 0.6766(\ln \text{HD14}) - 0.09077(\text{SO}_2) - 1.083(\text{O}_3)$	0.799
'Alsweet' Pea	
PLAI = $-41.84 + 128.8(\text{SO}_2) + 520.8(\text{O}_3) - 84.26(\text{SO}_2)^2 - 680.4(\text{O}_3)^2 - 155.0(\text{SO}_2 \times \text{O}_3)$ (Fig. 2G)	0.944
PLI = $1.144 + 6.612(\text{SO}_2) + 601.9(\text{O}_3) + 24.64(\text{SO}_2)^2 - 932.8(\text{O}_3)^2 - 107.5(\text{SO}_2 \times \text{O}_3)$	0.934
$\ln(\text{LA}) = 1.490 + 1.086(\ln \text{PH14}) - 0.1554(\text{SO}_2) - 0.9162(\text{O}_3)$ (Fig. 2H)	0.687
$\ln(\text{SDW}) = -4.850 + 1.354(\ln \text{PH14}) - 0.1553(\text{SO}_2) - 1.788(\text{O}_3) + 2.040(\text{O}_3)^2$ (Fig. 2I)	0.770
$\ln(\text{PH}) = 1.080 + 0.8226(\ln \text{PH14}) - 0.2243(\text{SO}_2) - 0.7597(\text{O}_3) + 0.1696(\text{SO}_2)^2 + 0.8470(\text{O}_3)^2$	0.921

Isometric (3-dimensional) graphs (Fig. 3) were prepared for some response variables to compare their usefulness to the contour plots. In our opinion, presentation of the response surfaces as isometric plots rather than contour plots frequently produced surfaces that were difficult to interpret. For example, the contour plot of lettuce LA (Fig. 2B) was much easier to interpret than the corresponding isometric surface (Fig. 3A). Radish LDW, which had significant SO_2 , O_3 , and $\text{SO}_2 \times \text{O}_3$ terms in the equation, yielded an isometric surface that was difficult to interpret because the surface was almost hidden from view (Fig. 3B). Similarly, the isometric surface for pea PLAI (Fig. 3C) has complex features, increasing the interpretative problems in contrast to the contour plot (Fig. 2G).

As seen in the example (Fig. 2, A to I), response patterns were dependent on species and measured response variable, as noted in earlier studies of foliar injury responses in which several combinations of SO_2 and O_3 were used (13, 24). Lettuce was quite insensitive to the pollutants; the contour lines for PLAI (Fig. 2A) represent only about 1% increments in leaf injury, those for LA and LDW (Fig. 2, B and C) represent less than 2% and about 2% decrements in area and dry weight, respectively. Identification of significance of such small differences was greatly enhanced by using covariate measurements (17, 21). Lettuce

showed significant interactive effects for foliar injury (PLAI and PLI) (Fig. 2A, Table III). In contrast, only O_3 had significant effects on LA and LDW (Fig. 2, A to C; Table III). There have been very few studies of SO_2 and O_3 sensitivity of lettuce. Grand Rapids lettuce is sensitive to O_3 compared with other cultivars (12). Substantial growth retardation of lettuce by outdoor air pollution in California has been reported (11).

For radish, the nature of the foliar injury response depended on the response measured; PLAI was affected only by O_3 (Fig. 2D; Table III), while PLI showed an interaction (Table III). Radish growth responses also showed this complex response pattern; leaf area (Fig. 2E) was affected only by O_3 , displaying a linear decrease with increasing O_3 concentration. LDW was decreased by exposure to the gas mixtures and showed an interaction (Fig. 2F; Table III). The interaction was antagonistic, as can be seen in the signs of the regression coefficients; more O_3 and SO_2 were required to produce an effect than would be expected based on the effects of the single gases. HDW displayed a linear decrease with O_3 concentration, while HD17 showed an additive decrease in weight in response to the joint action of SO_2 and O_3 . Based on the linear regression coefficients, about 12 mol of SO_2 are required to cause the same level of decrease as 1 mol of O_3 .

The sensitivity of radish to SO_2 and O_3 has been studied extensively (16) and Cherry Belle, a sensitive cultivar (19), has been used in several research projects. Tingey and Reinert (22) found that single exposures to SO_2 and/or O_3 resulted in growth reductions in the mixture that were no different from the additive effects of the single gases. In two studies (12, 22), the mixtures caused greater foliar injury than that calculated as the sum of the individual components. However, in this study, PLAI of radish was significantly affected only by O_3 (Fig. 2D). In two studies, hypocotyl growth was more sensitive to O_3 than leaf growth (18, 22), as in the present experiment (Table II). In a long-term study with continuous exposure to low concentrations of SO_2 and/or O_3 , most response variables were affected by either gas alone as well as by the mixture (23).

Pea foliar injury displayed a complex interaction pattern influenced by linear and quadratic responses and a significant interaction of the two gases (Fig. 2G; Table III). Foliar injury was enhanced by the addition of SO_2 up to about 0.25 ppm O_3 , but SO_2 was much less effective than O_3 in increasing PLAI. At higher O_3 concentrations, the O_3 effect was decreased by either low or high concentrations of SO_2 . Pea PLAI was very sensitive to these gas mixtures; the contour lines each represented large increases in PLAI. The response of PLI paralleled PLAI in peas. Additive joint linear action of O_3 and SO_2 occurred throughout the concentration range in decreasing LA of peas, while SDW was affected by a quadratic response to O_3 . The decrements represented by contour lines are about 7% for LA and LDW (Fig. 2 H and I), indicating considerable sensitivity but less than for PLAI. Plant height (PH) showed a significant additive decrease (linear and quadratic effects), from SO_2 and O_3 (Table III). Pea cultivars differ widely in sensitivity to O_3 (15). Injury responses to SO_2 and/or O_3 in Alsweet pea have been characterized indicating that necrosis, Chl concentration, dry weight, and surface area were useful response variables (14).

The physiological explanation for the features of the contour graphs will require supplementary studies. The use of a similar methodology, perhaps modified to include more treatment levels, will facilitate the evaluation of the effects of combinations of treatments in comparative studies. For example, studies of stomatal action on pollutant flux rates may explain the interaction of SO_2 and O_3 in causing lettuce leaf injury on a leaf area (PLAI) or leaf number (PLI) basis, or in causing radish LDW decreases. However, Beckerson and Hofstra (2) were unable to establish a relationship between leaf diffusive resistance and low concentra-

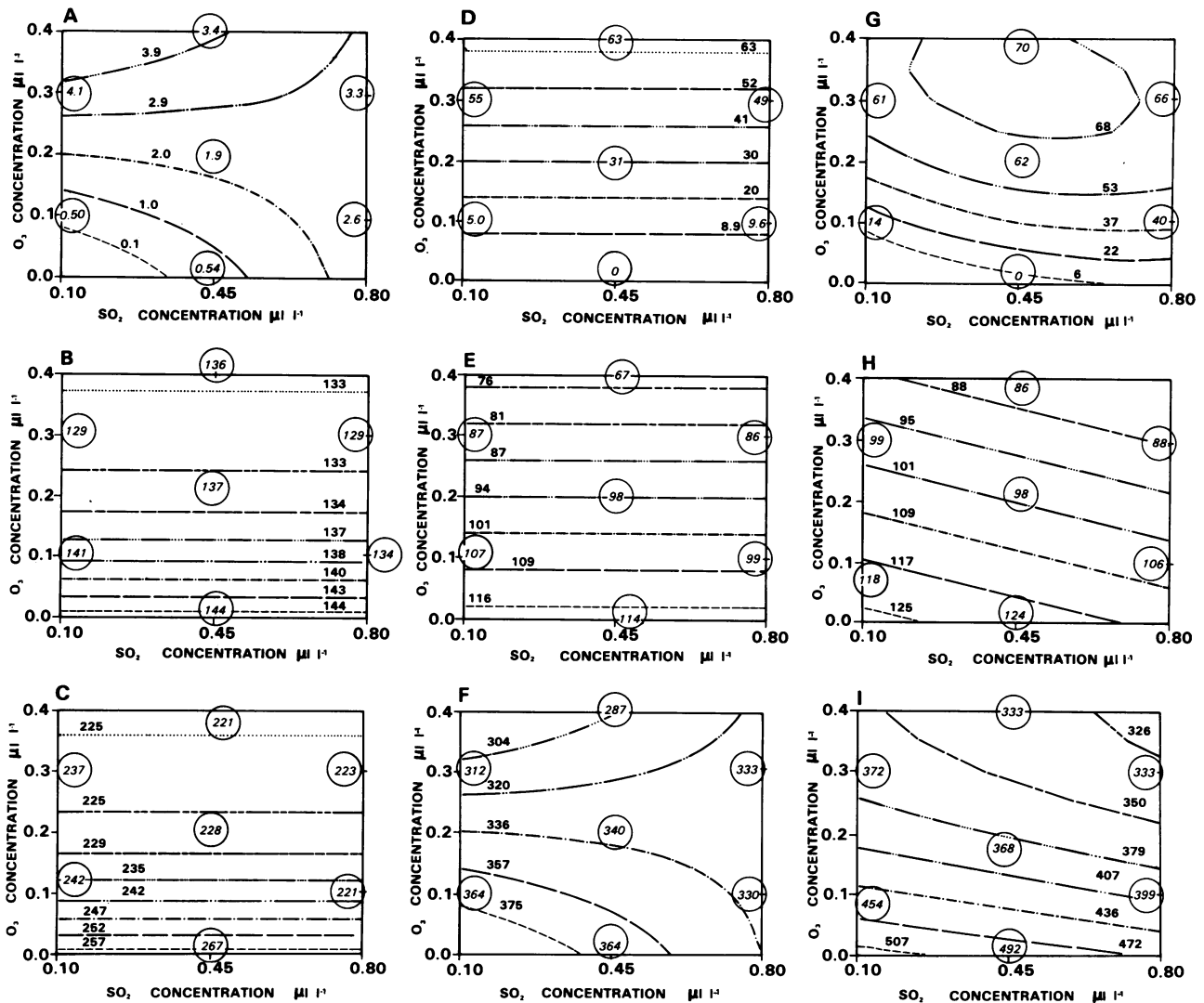


FIG. 2. Examples of contour plots illustrating the responses of lettuce, radish, and pea to combinations of O_3 and SO_2 ; similar response variables were used for each species. The circled numbers in each figure represent the actual mean responses. The numbers on the isoefficient lines indicated the fitted value for the line. For 'Grand Rapids' lettuce, PLAI, LA, and LDW are shown in A, B, and C, respectively. For 'Cherry Belle' radish, PLAI, LA, and LDW are shown in D, E, and F, respectively. For 'Alsweet' pea, PLAI, LA, and LDW are shown in G, H, and I, respectively.

tion SO_2/O_3 mixture injury but their studies did not include flux rate determinations.

In those cases for which both SO_2 and O_3 had significant positive effects on leaf injury or negative effects on growth, SO_2 was always much less effective than O_3 on an equal concentration v/v or molar basis. Usually about five to ten times as much SO_2 was required than O_3 to elicit the same leaf injury or growth retardation response as indicated by many of the contour plots (Fig. 2) and coefficients for linear and quadratic effects (Table III).

The relationship of leaf area injured to growth retardation varied among species. For lettuce, there was less leaf area injured (about 4% maximum) than growth retardation (about 17% for LDW), perhaps indicating that the visual estimates of injury did not adequately represent all injured tissue. In contrast, the radish and pea leaf injury was greater than growth retardation on a percentage basis. While adjustments should be made for differences in plant weight at the time of exposure, it is possible that uninjured tissue in these two species may have been induced to greater levels of assimilatory activity to compensate for loss of injured tissue, or the plants had excess leaf area when compared

to that required for growth.

Altered distribution of dry matter during and subsequent to pollutant exposure was implied by the differential response patterns of leaves and hypocotyls in radish. Hypocotyl weights were reduced much more than leaf weights by high O_3 concentrations. Also, SO_2 affected LDW but did not have a significant effect on hypocotyl weights.

The use of a second order rotatable design illustrates the utility of response surface designs and contour plots to study and explain the interaction of pollutants. Many of the contradictions among earlier reports (16) can be explained by the use of particular combinations that represented only part of a response surface. For example, with either radish or pea PLAI or PLI or radish LDW, the use of any one combination to represent the overall response surface could lead to incomplete assessment of the nature of joint action and interaction. Only a response surface based on a range of joint concentrations can fully illustrate the nature of the interaction. Furthermore, the use of quadratic terms in the response surface equation serves to provide clues as to the mode of action of the pollutants in combinations. The experimental design used in this study could be further improved

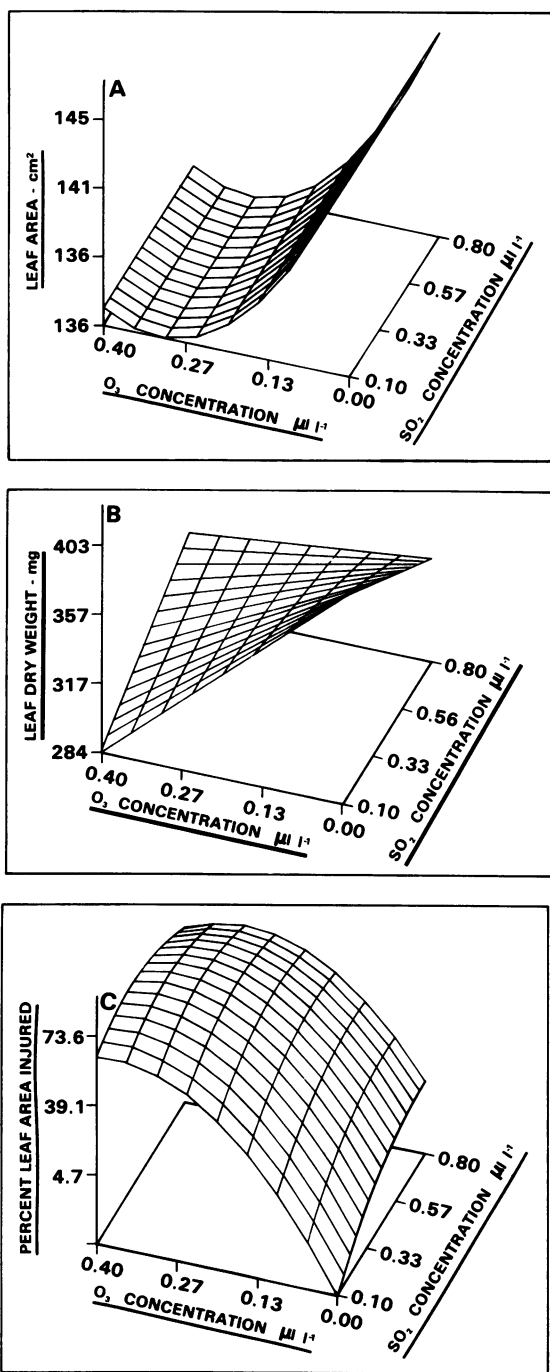


FIG. 3. Isometric (3-dimensional) response surfaces for comparison with selected contour plots in Figure 2; the same data were used to develop both types of response surfaces. A, LA of 'Grand Rapids' lettuce, corresponds to Figure 2B. B, LDW of 'Cherry Belle' radish, corresponds to Figure 2F. C, PLA of 'Alsweet' pea, corresponds to Figure 2G.

by including more SO_2 and O_3 concentrations.

The use of efficient experimental designs combined with covariance measurements provides a useful avenue for elucidation of the nature of plant responses to combinations of environmental factors. This technique will apply equally well to nutritional and growth regulation studies and deserves consideration as an important complementary technique in plant physiology research.

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