# AN INTEGRATED STATISTICAL APPROACH TO ESTIMATING PLANT RESPONSES TO SEQUENTIAL AND CONCURRENT GASEOUS POLLUTANTS

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# AN INTEGRATED STATISTICAL APPROACH TO ESTIMATING PLANT RESPONSES TO SEQUENTIAL AND CONCURRENT GASEOUS POLLUTANTS

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HALE B., RYAN D., ORMROD D. P. and ALLEN O. B. *An integrated statistical approach to estimating plant responses to sequential and concurrent gaseous pollutants.* BIOTRONICS 22, 35–46, 1993. Controlled environment studies of plant response to multiple environmental stresses frequently have physical (such as chamber space) and analytical (such as experimental design and data summary) limitations. This study demonstrates the use of an efficient, integrated statistical approach in evaluating rutabaga (*Brassica napus* L. ssp. *rapifera* (Metzg.) Sinsk cv. Laurentian) and cabbage (*Brassica oleracea* L. var. *capitata* cv. Market Prize) shoot growth responses to sulphur dioxide ($SO_2$) followed by ozone ($O_3$) at acute doses. The approach combines analysis of covariance, an incomplete factorial experimental design, polynomial dose response functions and a reduced-rank regression procedure to the comparison of functions. Young plants were exposed to $SO_2$ on one day followed by $O_3$ on the next day. Growth responses to sequential exposure were compared with previously reported growth responses to concurrent exposure to the same doses. Rutabaga growth was sensitive to both gases in both exposure regimes, whereas cabbage growth was sensitive to only $SO_2$ in the sequential exposure. Rutabaga leaf area and shoot fresh weight responses to sequential exposures followed the same pattern as concurrent responses, and their magnitudes following sequential exposures were approximately 60% of the concurrent responses. Rutabaga shoot dry weight and cabbage shoot fresh and dry weight, and leaf area responses to the sequential exposures were different in both magnitude and pattern from responses to the concurrent exposures. The importance of this work lies in the method for quantification and comparison of plant response to pollutant mixtures, in different exposure patterns. Using a suite of off-the-shelf statistical techniques, plant response to sequential versus concurrent exposure has been mathematically generalized over a broad range of pollutant mixture concentrations. Particularly for the purpose of environmental risk assessment, this integrated statistical technique has broad application to controlled environment studies of plant response to multiple stresses.

**Key words:** stress, models, covariate, cabbage, rutabaga, ozone, sulphur

dioxide, exposure dynamics.

## INTRODUCTION

Environmental stresses in combination may have plant effects of greater magnitude than predicted from single stress studies; a good example of this phenomenon is the phytotoxicity of gaseous pollutant mixtures (*12, 18, 19, 15, 6, 3*). While concurrent exposure to $SO_2$ and $O_3$ is possible in the ambient environment, particularly in less well-developed countries lacking state-of-the-art control technology, it is more likely that plants would be exposed to these gases sequentially (*10, 11*). Plant response to the first pollutant gas may modify plant uptake or detoxification of the second pollutant gas, thus altering response to the second gas. Several mechanisms for sequential effects of pollutant gases have been proposed, whether the second exposure is to the same or to a different gas. Pre-exposure to $O_3$ increased the leaf diffusive resistance, thereby reducing gas flux to the leaf interior during a subsequent $O_3$ episode (*21*). Hypothetically, pre-exposure may activate detoxification or repair mechanisms (for example, elevated oxidase enzymes), which then minimize the effect of the second episode; however, evidence of this has been difficult to establish (*5*).

The assessment of plant response to mixtures of pollutant gases is usually carried out with a full factorial experimental design (*4*). Given that each treatment combination needs a separate exposure chamber, most studies are quite restricted in either the number of discrete exposure concentrations of each pollutant, or the number of replications. Either of these limitations can reduce the strength with which the plant response is estimated: the former by limiting the range of inference, and the latter by decreasing the precision of the estimate. It is possible to counter these limitations, using experimental designs and data analyses which increase the range of inference (without increasing the number of treatments) and also increase the experimental precision. Four distinct statistical methods have been separately demonstrated to be very effective at enhancing the collection, analysis or interpretation of data describing plant responses to pollutant doses:

1) Incomplete factorial designs are well suited for efficient determination of pollutant dose-plant response relationships: the number of discrete exposure chambers is greatly reduced, often by more than 50%, with little loss of information (*17, 1*);

2) Analysis of covariance can increase precision in randomized experiments by reducing the contribution of pre-treatment variation to experimental error. Depending on the degree of correlation between the covariate and response variable, analysis of covariance will reduce the experimental error, thus increasing precision (*16*);

3) Multiple regression can generalize the individual treatment responses into dose-response relationships. In this form, the data may be used to predict plant response to treatment combinations not included in (but within the boundaries of) the incomplete factorial design (*13*);

4) Reduced-rank multivariate analysis is suitable for comparing the shapes of several dose-response relationships (*22*).

These four procedures can be integrated into an efficient, simple statistical protocol for the determination of plant response to multiple environmental stresses, with particular application to gaseous pollutants. This protocol is well suited for assessing the responses of plants to global climate change, particularly in controlled environments: elevated carbon dioxide, ultraviolet-B radiation and air temperature are all difficult to apply on an experimental unit basis, and a study of their interaction needs an experimental approach which maximizes resource use. The objective of this study is to describe growth responses of young cabbage and rutabaga plants to sequential exposure to $SO_2$ and $O_3$, and to determine the similarity of the responses to concurrent exposure to the same gases. These determinations were made using the previously described statistical procedures, and their effectiveness at investigating plant responses to multiple stresses is demonstrated.

## MATERIALS AND METHODS

Rutabaga (*Brassica napus* L. ssp. *rapifera* (Metzg.) Sinsk cv. Laurentian) and cabbage (*B. oleracea* var. *capitata* cv. Market Prize) seeds were sown in Promix BX medium (60% peatmoss, 20% perlite and 20% vermiculite, v/v) in 10 cm diameter green plastic pots which were placed in a controlled environment chamber. The photoperiod was 16 h (0400–2000h) at 325±20 $\mu$E. $m^{-2}$. $s^{-1}$, measured with a Li-Cor quantum sensor and meter at canopy height. Input wattage was 77% from fluorescent and 23% from incandescent lamps. The thermoperiod was 12 h (0600–1800) with a day temperature of 25±1℃ and a night temperature of 20±1℃. Relative humidity was maintained at 75±5% during both day and night. The pots were irrigated with half-strength Hoagland's complete nutrient solution (*9*). Seven days after sowing, the seedlings were thinned to one per pot. The two species developed at similar rates; the plants had one pair of large expanding true leaves by the day of pollutant exposure (14 d after sowing).

*Pollutant exposure*

On day 14 after sowing, planar leaf area (*PLA*) was determined by placing a transparent acetate sheet with a 1 $cm^2$ grid over each plant, and recording the number of grid intersections directly over plant tissue. Planar leaf area differs from true leaf area (*LA*) in that it does not include leaf area hidden under upper leaves. This number was used, untransformed, as the covariate for all response variables except visible injury (*PLI*). Immediately following covariate measurement, three plants of each cultivar were placed in a Continuous Stirred Tank Reactor chamber (CSTR, for details see *8*) for pollutant exposure. Canopy light level was 290±20 $\mu$E. $m^{-2}$. $s^{-1}$, supplied by one 400 W metal halide and one 400 W high pressure sodium lamp over each CSTR chamber; the lamps were angled so that the beams intersected in the area of the plant canopy. Relative humidity

was 70±5%, and temperature was 25±2℃. The pollutant exposure period was 6 h (1000–1600) for each gas on two consecutive days, after which the plants were returned to the growth chamber for two days. Ozone was produced by a Grace high-voltage generator (Model LG-2-LI) and monitored with a Dasibi analyzer (Model 1003 AH). Sulphur dioxide was supplied by a pressurized cylinder containing 0.5% $SO_2$ in nitrogen and monitored with a Thermo Electron Series 43 pulsed fluorescent $SO_2$ analyzer. Sulphur dioxide exposure occurred first, followed by $O_3$ on the second exposure day; the treatment concentrations were ($O_3/SO_2$) 0/0.5, 0/1.3, 0.2/0, 0.2/0.9, 0.2/1.7, 0.4/0.5, 0.4/1.3 $\mu l \cdot l^{-1}$.

*Harvesting procedures*

On the second day after exposure (17 d after sowing) the plants were harvested. The shoot was designated as all parts of the plant above the junction of the cotyledons with the stem, including the cotyledons. Leaf area was determined photometrically and shoot fresh and dry (after 48 h at 60℃) weights were determined gravimetrically. Percent foliar injury was determined as that proportion of total leaf area showing chlorotic or necrotic lesions, or abaxial pitting.

*Experimental design and data analysis*

The experimental design was an incomplete factorial design: six pairs of gas mixture treatments were chosen such that they formed a hexagon with the seventh gas mixture in the centre of the hexagon (*1*). Since four CSTR's constituted a block, each replicate was divided into two blocks in such a way that the fitted response surface was orthogonal to blocks (*2*). Each block was repeated four times, giving eight replicates of the central treatment (0.2/0.9) and four replicates of the peripheral treatments (all others). The extra replication of the central treatment allows for estimation of lack of fit. There were three subsamples (pots) per experimental unit. The data were analyzed using the General Linear Models (GLM) and Regression (REG) procedures of SAS (Statistical Analysis System, SAS Institute Inc, Cary, NC.). The data were first analyzed using proc GLM to determine treatment, block and covariate effects, as well as the partitioning of sums of squares amongst the various error terms, represented by the simplified covariance model:

$$Y_{ijk}=\mu+R_i+T_j+RT_{ij}+B(R)_{k(i)}+a(PLA)+E_{ijk}$$

where $R$=rep (1,…4), $T$=treatment (1,…7) and $B$=block (1, 2). Covariate adjusted treatment means were calculated for the reduced data sets and inspected for trends. For each variable, the simplified covariance model was compared (using an F test) to the full polynomial model, where treatment effects were expanded and replication effects were pooled with residual error $E_{ij}$:

$$Y_{ij}=\beta_0+\beta_1(SO_2)+\beta_2(O)_3+\beta_{12}(SO_2xO_3)+\beta_{11}(SO_2)^2+\beta_{22}(O_3)^2+\alpha(\ln PLA)+E_{ij}.$$

In no case was there a significant lack of fit ($P \leq 0.1$) and most P values were greater than 0.35, suggesting that the polynomial model was an adequate

estimator of the treatment effects described by the simplified covariance model.

This full polynomial model was reduced by the method of backward selection (*6*). Lack of significant increase in error sum of squares (ESS) in comparison to the full model ESS, indicated the simplest model of good fit to describe the response of each growth variable of each cultivar. The first reduced model to be tested was that which did not include the interaction term ($SO_2 \times O_3$). If the error associated with this model was not greater than the full model, progressively simpler models were tested until the simplest good fitting model was arrived at. The ESS were compared by:

$$F = (EMS_r - EMS_f)/EMS_f$$

with r–f, f degrees of freedom. The selection of an $\alpha$ level for the inclusion/exclusion of model term is not highly critical (*6*); a value less than 0.20 is usually used.

The final reduced model was then tested for lack of fit by comparison with the full polynomial model ($P \leq 0.15$). The exclusion of important terms from the model leads to a biased estimate of the response surface, as suggested by significant lack of fit, whereas inclusion of non-significant terms increases the variability of the surface. For most of the equations, there was no significant lack of fit suggesting that the final reduced equations were appropriate descriptions of plant response to $SO_2$ and $O_3$. If significant lack of fit was detected, a higher order model which did not have significant lack of fit was used to describe these growth responses.

The partial $R^2$ for each dose response relationship was calculated to evaluate the contribution of the pollutant dose factors to the fit of the data to the regression relationship. The partial $R^2$ was defined as RSS/(RSS+ESS), where RSS was the additional reduction in variation from fitting the response surface after consideration of the covariates and block/replicate effects, and ESS was the error sum of squares (*4*). The F test for the significance of the partial $R^2$ was calculated as:

$$\frac{(\text{partial } R^2)(\text{error df})}{(1-\text{partial } R^2)(\text{regression df})}$$

and was used to evaluate the predictive strength of the relationship (*23*).

One of the limitations of describing plant response to pollutants in terms of dose–response relationships, is that a general comparison of responses (for example, under different exposure scenarios) is often desirable. In this study, plant responses to sequential and concurrent exposures were compared in order to test whether predisposition to or protection from $O_3$ (or neither) was conferred by pre–exposure to $SO_2$, or whether the plant responded similarly to concurrent and sequential exposures. The shapes of these functions describing plant growth response to sequential $SO_2/O_3$ exposure were then compared to functions describing plant growth response to the same concentrations of $SO_2/O_3$ except that the pollutants were administered concurrently (*13*). The comparison

was accomplished using reduced-rank regression (*22*). This procedure compares the shapes of two or more response surfaces by testing whether the coefficients of one response surface are similar in proportion to the coefficients of another. The coefficients of each response surface are tested as a group against the coefficients of another response surface, rather than individually. The surfaces must be described by the same regression terms; this condition can be easily met by fitting the full model to the data, and not removing any terms, no matter how small the coefficient is. The data must also have been collected using the same experimental design. The elevations of the surfaces (i.e., the intercepts) are not compared by this method, so the response variables need not be measured using the same units.

In mathematical terms, this reduced-rank regression procedure arranges the coefficients of the equations to be compared into a matrix of two rows and five columns and determines whether one row can be expressed as a multiple of the other, i.e.; whether the rank (p) of the matrix is equal to 2 or 1. The general form of the matrix was as follows:

$$\begin{bmatrix} C_1 & C_2 & C_{11} & C_{22} & C_{12} \\ S_1 & S_2 & S_{11} & S_{22} & S_{12} \end{bmatrix}$$

where $C_1$ and $S_1$ are the linear $SO_2$ coefficients for the plant growth response functions 1 (concurrent) and 2 (sequential), $C_2$ and $S_2$ are the linear $O_3$ coefficients, $C_{11}$ and $S_{11}$ are the quadratic $SO_2$ coefficients, $C_{22}$ and $S_{22}$ are the quadratic $O_3$ coefficients and $C_{12}$ and $S_{12}$ are the interaction coefficients. Whether one row could be expressed as a multiple of the other (i.e., calculating the rank of the matrix) was tested using a likelihood ratio statistic. This is equivalent to testing whether the concurrent response was a rescaling of the sequential response,

$$H_0: S_{(i..ij)} = K(C_{(i..ij)}), \text{ where } K = (1, \cdots k).$$

If the null hypothesis was accepted, K was calculated by forming a matrix from the equations to be compared, and determinig whether the rank of that matrix was one (indicating that one response was the same as the other) or different from one, indicating that one response was a rescaling of the other (*22*).

## RESULTS AND DISCUSSION

An examination of the treatment means for cabbage shoot size after exposure to $SO_2$ followed on the next day by exposure to $O_3$ indicated that fresh and dry weights were strongly modified by the gases, whereas leaf area was slightly modified (Table 1). Cabbage specific leaf area and specific water

Table 1. Treatment means of cabbage and rutabaga shoot size following sequential exposure to $SO_2$ followed by $O_3$

| $O_3$ ($\mu l.l^{-1}$) | 0 | | .2 | | | .4 | | | |
|---|---|---|---|---|---|---|---|---|---|
| $SO_2$ ($\mu l.l^{-1}$) | .5 | 1.3 | 0 | .9 | 1.7 | .5 | 1.3 | s.e.† | P‡ |
| CABBAGE | | | | | | | | | |
| Leaf area ($cm^2$) | 135§ | 112 | 120 | 112 | 80.1 | 104 | 85.9 | .137 | .131 |
| Dry Weight (g) | .543 | .405 | .410 | .389 | .303 | .381 | .315 | .116 | .036 |
| Fresh weight (g) | 9.77 | 4.78 | 5.35 | 4.96 | 3.34 | 4.47 | 3.79 | .240 | .099 |
| Specific leaf area ($cm^2 \cdot g^{-1}$) | 248 | 277 | 293 | 287 | 265 | 273 | 273 | .114 | .969 |
| Specific water content ($g \cdot g^{-1}$) | 16.9 | 10.8 | 12.0 | 11.7 | 9.98 | 10.7 | 11.0 | .150 | .283 |
| Leaf injury (%) | .017 | .782 | 1.18 | 5.98 | 13.0 | 52.5 | 47.4 | 6.01 | .0001 |
| RUTABAGA | | | | | | | | | |
| Leaf area ($cm^2$) | 162 | 138 | 133 | 137 | 92.8 | 111 | 80.3 | .111 | .003 |
| Dry weight (g) | .427 | .450 | .414 | .438 | .264 | .331 | .290 | .106 | .006 |
| Fresh weight (g) | 5.74 | 5.39 | 5.00 | 5.18 | 3.49 | 3.67 | 2.90 | .116 | .003 |
| Specific leaf area ($cm^2 \cdot g^{-1}$) | 379 | 307 | 320 | 313 | 359 | 335 | 277 | .089 | .299 |
| Specific water content ($g \cdot g^{-1}$) | 12.4 | 10.9 | 11.0 | 10.8 | 12.2 | 10.1 | 8.97 | .104 | .350 |
| Leaf injury (%) | .068 | 8.57 | 9.54 | 7.47 | 12.6 | 26.5 | 45.0 | 5.87 | .004 |

† Standard error of the transformed means; applicable to mean values after conversion to arcsine (leaf injury) or $\log_e$ (all others).

‡ P values for F=treatment MS/error MS, where n=4.

§ Values are the means of four replicates and three subsamples per replicate, and are adjusted to mean covariate and replicate effects.

Table 2. Polynomial dose response relationships describing cabbage and rutabaga shoot size (transformed to natural log) and leaf injury (transformed to arcsine) following sequential exposure to $SO_2$ and $O_3$.

| CABBAGE | $R^{2\dagger}$ | P‡ |
|---|---|---|
| ln Leaf area=4.87−.242 ($SO_2$) | .503 | ** |
| ln Dry weight=−0.613−.212 ($SO_2$) −.756 ($O_3$) | .664 | ** |
| ln Fresh weight=2.17−.347 ($SO_2$) −1.29 ($O_3$) | .579 | ** |
| ln Specific leaf area (n. s.)§ | | |
| ln Specific water content (n. s.) | | |
| arcsine Leaf injury=−2.96+6.96 ($SO_2$) −.294 ($O_3$)+259 ($O_3$)$^2$ | .837 | ** |
| RUTABAGA | | |
| ln Leaf area=5.22−.241 ($SO_2$) −1.15 ($O_3$) | .768 | ** |
| ln Dry weight=−.713+.266 ($SO_2$) −.869 ($O_3$) −.269 ($SO_2$)$^2$ | .447 | ** |
| ln Fresh weight=1.92−.208 ($SO_2$) −1.33 ($O_3$) | .771 | ** |
| ln specific leaf area (n. s.) | | |
| ln Specific water content (n. s.) | | |
| arcsine Leaf injury=7.24+68.2 ($O_3$) | .701 | |

† partial $R^2$

‡ P of F for $H_0$: partial $R^2$ is equal to zero

§ n. s. indicates that no regression relationship could be fitted

content were apparently not changed by any of the treatments, whereas leaf injury was related to the two gases. The dose response equations for cabbage shoot fresh and dry weights were described by a negative linear relationship to both $O_3$ and $SO_2$, whereas leaf area was negatively related to $SO_2$ only (Table 2). Leaf injury was positively related to $SO_2$ (linear) and related to $O_3$ in a mixed quadratic function. The level of $O_3$ at which the minimum response occurred ($.00057\,\mu l \cdot l^{-1}$) was not different from zero, so this response was essentially a positive relationship to both $SO_2$ and $O_3$ (Table 2).

The treatment means for rutabaga demonstrate a similar pattern of response. Leaf area, and shoot fresh and dry weights were altered by the pollutant gases, and the appearance of visible injury was related to $SO_2$ and $O_3$. Specific leaf area and specific water content were not influenced by either gas (Table 1). The dose response equations for rutabaga were also generally consistent with the treatment means (Table 1). Leaf area and shoot fresh weight were negatively related to $SO_2$ and $O_3$ (linear). Shoot dry weight was negatively related to $O_3$ and to $SO_2$ with a mixed quadratic relationship (Table 2). The level of $SO_2$ which produced the maximum shoot dry weight was $0.49\,\mu l \cdot l^{-1}$, which indicated that low levels of $SO_2$ likely had a positive effect on shoot dry weight. As in cabbage, specific leaf area and specific water content were not related to $SO_2$ nor $O_3$ concentration, consistent with the treatment means (Table 1). Leaf injury was positively related to $O_3$ only (linear) (Table 2). The F test of whether the partial $R^2$ was equal to zero was significant for all of the equations at $P \leq 0.05$ (Table 2).

The dose response relationship coefficients for $O_3$ were much larger than for $SO_2$: a comparison of the linear coefficients indicates that $O_3$ was 3–4 fold as phytotoxic as $SO_2$. The variable responses were similar for both species. The shoot size of both was generally depressed by the gases, and both $SO_2$ and $O_3$ were involved in most of the responses. There was no evidence of interaction between the gases.

The shoot growth dose response functions were compared to those for concurrent exposure (Table 3, from *13*). On a qualitative basis, the responses were different. Cabbage was sensitive to $SO_2$ in sequential exposure only, and rutabaga response to $SO_2$ was different for concurrent and sequential exposures. The $O_3$ coefficients for cabbage growth responses were very similar for concurrent and sequential exposures, indicating some consistency between responses to the two regimes. When the full models were tested using the reduced-rank regression approach, the shoot response of cabbage to sequential exposure was a different shape compared to concurrent exposure response (Table 4). Leaf area and shoot fresh and dry weight responses were different because of the inclusion of a negative linear $SO_2$ term in sequential responses. This difference suggests that during concurrent exposure, elastic strain in response to $SO_2$ (for example, changes in leaf diffusive resistance) was not integrated into plant growth strain. However, if the gases were delivered sequentially, the plant appeared to integrate stress from both gases into growth strain. Rutabaga leaf area and shoot fresh weight responses did not differ in

Table 3. Final polynomial dose response relationships describing cabbage and rutabaga shoot size (transformed to natural log) and leaf injury (transformed to arcsine) following concurrent exposure to $SO_2$ and $O_3$ (*13*).

| CABBAGE | $R^{2\dagger}$ | $P^{\ddagger}$ |
|---|---|---|
| ln Leaf area = 4.46 − 1.25 ($O_3$) | .653 | ** |
| ln Dry weight = −1.20 − .816 ($O_3$) | .649 | ** |
| ln Fresh weight = 1.33 − 1.18 ($O_3$) | .661 | ** |
| ln Specific leaf area = 5.66 − .433 ($O_3$) | .473 | ** |
| ln Specific water content = 2.45 − .393 ($O_3$) | .441 | ** |
| arcsine Leaf injury = .813 − 47.9 ($O_3$) + 355 ($O_3$)$^2$ | .845 | ** |
| RUTABAGA | | |
| ln Leaf area = 5.01 − .422 ($SO_2$) − 1.02 ($O_3$) + .191 ($SO_2$)$^2$ | .704 | ** |
| ln Dry weight = − .663 − .606 ($SO_2$) − .800 ($O_3$) + .282 ($SO_2$)$^2$ | .667 | ** |
| ln Fresh weight = 1.79 − .501 ($SO_2$) − 1.13 ($O_3$) + .227 ($SO_2$)$^2$ | .702 | ** |
| ln Specific leaf area (n. s.)[§] | | |
| ln Specific water content = 2.45 − .366 ($O_3$) | .561 | ** |
| arcsine Leaf injury = −2.74 + 4.18 ($SO_2$) − 22.8 ($O_3$) + 262 ($O_3$)$^2$ | .721 | ** |

† partial $R^2$

‡ P of F for $H_0$: $R^2$ is equal to zero

§ n. s. indicates that no regression relationship could be fitted

Table 4. Comparison of sequential and concurrent dose response relationships (Tables 3 and 4) describing cabbage and rutabaga shoot size (transformed to natural log) and leaf injury (transformed to arcsine) following sequential and concurrent exposure to $SO_2$ and $O_3$.

| Shoot size variable | $X^{2\dagger}$ | df‡ | P§ | K¶ |
|---|---|---|---|---|
| CABBAGE | | | | |
| ln Leaf area | 5.30 | 1 | * | — |
| ln Dry weight | 3.56 | 1 | * | — |
| ln Fresh weight | 4.97 | 1 | * | — |
| RUTABAGA | | | | |
| ln Leaf area | 5.29 | 2 | ns | .59 |
| ln Dry weight | 8.16 | 2 | * | — |
| ln Fresh weight | 3.36 | 2 | ns | .65 |

† Chi-squared test for $H_0$: Dose response A = K (Dose response B) where K is a constant to be estimated.

‡ Degress of freedom for $X^2 = m\ (q_2 - p + 1)$ where m is the hypothesized number of restrictions (in this case m = 1), $q_2$ is the number of pollutant terms in the model and p is the number of response surfaces.

§ P value for $H_0$ tested by $X^2$.

¶ Estimated value for K, where $P > 0.05$.

shape for concurrent versus sequential exposure, so one could be expressed as a rescaling of the other; rutabaga shoot dry weight responses were different for the two types of exposure. The similarity between the leaf area responses is clear: both sequential and concurrent functions have negative linear $O_3$ coefficients of approximately the same magnitude. However, $SO_2$ is a mixed quadratic for concurrent versus negative linear for sequential; the linear $SO_2$ coefficients for sequential functions were approximately one half of those for the concurrent functions.

The values for K (0.59 (leaf area) and 0.65 (shoot fresh weight)) indicate that the sequential responses were scaled down versions of the equations describing the concurrent responses. These results suggest that for rutabaga, although there was no statistical interaction between $SO_2$ and $O_3$, the additivity of the responses to each of the two gases was somewhat dependent on the exposure timetable. In contrast, in the presence of $O_3$, $SO_2$ clearly had a stimulatory effect on leaf area and dry weight, at concentrations above 1.0 $\mu l \cdot l^{-1}$; in the absence of $O_3$, $SO_2$ had a negative effect at all concentrations. The difference between the response relationships for dry weight was clear: the response to $SO_2$ was opposite for the two exposures. Although there was no statistical interaction between the two gases, the additivity was very dependent on the exposure timetable. The extrema (maxima and minima) for $SO_2$ were 0.49 $\mu l \cdot l^{-1}$ and 1.1 $\mu l \cdot l^{-1}$ for sequential and concurrent, respectively.

The response of plants to pre-exposure and exposure to the same gas may be different from plant response to different gases presented sequentially. Bean, exposed to 0.02 $\mu l \cdot l^{-1}$ $O_3$ prior to being exposed to $O_3$ concentrations up to 0.4 $\mu l \cdot l^{-1}$ $O_3$ was less injured as a result of acute exposure than as a result of pre-exposure to filtered air (*20, 21*). The pretreated plants had a lower stomatal conductance prior to the initiation of the acute exposure and had less than one half as much leaf injury as plants which were not pretreated with a chronic level of $O_3$. Since the same gas was used for both treatments, it is possible that the pre-exposure not only closed stomates, but also initiated detoxification mechanisms, which were then well in place for the subsequent exposure. It is unlikely that a similar sequence of events would occur for two different gases, for example $SO_2$ and $O_3$, as the proposed detoxification mechanisms are different for each gas (*14*).

## SUMMARY

These data for cabbage and rutabaga clearly indicate the potential for interaction between gases in their effects on plant growth response to gaseous pollutant mixtures, depending on the exposure regime. Although interaction was not detectable by examining for a statistically significant interaction term ($SO_2 \times O_3$), growth response to the gases depended upon whether the gases were presented sequentially or concurrently. The nature of the joint action was species dependent: cabbage seemed to be more sensitive to sequential exposure, whereas rutabaga was more sensitive to concurrent. The integration of the four

statistical techniques (analysis of covariance, incomplete factorial experimental design, polynomial dose response functions and comparison of dose response functions using reduced-rank regression) constituted an efficient, simple approach to determination of plant response to pollutant mixtures using commercial statistical software. Analysis of covariance, the incomplete factorial design and development of dose-response functions have been proven in earlier studies to increase the precision of detecting treatment effects and improve the efficiency of data collection. Polynomial dose-response functions enable estimation of plant response to treatment concentrations not utilized in the experiment, and through differentiation, reveal treatment levels at which plant response changes. Both of these goals are very difficult to achieve using other data comparison techniques, such as multiple range tests. A reduced-rank regression approach to comparison of polynomial dose-response functions is a good method for quantitative determination of similarity between two dose responses. This latter technique can be applied to comparison of exposure regimes, and would also be suitable for comparing species responses or responses of different growth parameters.

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