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Sulfur Dioxide and Nitrogen Dioxide Meet Growth, Gas Exchange, and Water Relations of Potato Plants

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Abstract. **The effects of SO_2 and NO_2, singly and in combination, on the growth and physiology of nontuberizing *Solarium tuberosum* L. 'Russet Burbank' plants were studied in controlled conditions. Plants were exposed to 0.11 µl SO_2 and/or 0.11 µl NO_2/liter for 24 hours a day up to 10 days. Statistically significant effects were observed mainly in the SO_2 + NO_2 treatments compared with the control plants. Leaf area was reduced from day 2 onward, and root fresh and dry weights were reduced from day 4 onward. Significant reductions in leaf and stem dry weights occurred on day 6. Net CO_2 exchange rates were reduced for SO_2 exposed compared with control plants beginning on day 3, while water loss rates were increased with SO_2 + NO_2 beginning on day 3. The increases in water loss rate were possibly due to the development of cuticular injury observed as abaxial glazing on the upper and middle canopy leaves. Leaf osmotic potential (π) of plants with SO_2 + NO_2 became more negative within the first 24 hours of the exposure. This reduction was accompanied by an increase in reducing sugar concentration. Xylem water potential was reduced in the mature and expanding leaflets by day 2 of the SO_2 + NO_2 exposure. The most sensitive aspect of the action of SO_2 + NO_2 appeared to be the increase in reducing sugars that affected osmotic potential in the leaves. Considering the retardation of root growth, these data suggest that the pollutant gases may have interfered with partitioning of dry matter from the leaves to the roots.**

Potato plants demonstrate various responses to SO_2 and NO_2. Sulfur dioxide exposure causes reductions in tuber yield and mean tuber size (Foster et al., 1983), while chronic NO_2 exposures throughout the growing season cause reductions in tuber number and fresh weight (Sinn and Pen, 1984). Sulfur dioxide + NO_2 alters potato plant water status and interferes with the partitioning of dry matter from the leaves to the roots (Petitte and Ormrod, 1988).

At low doses, air pollutants may alter biochemical or physiological cell processes (e.g., stomatal function in photosynthesis) resulting in lowered plant productivity without visible injury (Heath, 1980). These alterations can be the result of several factors. Exposure of soybean (Amundson and Weinstein, 1981) and garden bean (Ashenden, 1979b) to SO_2 and NO_2 caused parallel decreases in transpiration and photosynthesis rates due to increased stomatal resistance. However, Sinn and Pen (1984) demonstrated that 'Kennebec' and 'Atlantic' potato plants chronically exposed to levels of NO_2 ranging from 0.12 to 0.34 µl·liter^{-1} did not experience significant increases in leaf diffusive resistance. Reinert (1984) stated that the reductions in photosynthesis rates, as a result of plant exposure to some combinations of SO_2 and NO_2, suggest an increase in respiration. Carlson (1983) believed that where reductions in photosynthesis were not the result of increases in respiration (Black and Unsworth, 1979), the reduction was due to increased internal diffusive resistances. A reduction in photosynthesis also may be caused by the actions of pollutant by-products formed in the leaves. Malhotra and Khan (1984) state that SO_2 can affect photosynthesis by influencing the carboxylation reactions and by attacking photosynthetic electron transport and photophosphorylation reactions. Reductions in photosynthesis in the potato affect metabolism of assimilates needed to maximize tuber production and quality (Harris and Pittman, 1923). No work has been reported concerning the effects of SO_2 and/or NO_2 on photosynthesis or water loss in the potato.

Maintenance of water status in the potato plant is necessary for continued production of photosynthetically active leaf area (Gandar and Tanner, 1976). However, ψ_{leaf} can be reduced by SO_2 in combination with O_3, as was observed in 'White Cascade' *Petunia* (Elkiey and Ormrod, 1979). The reduction in ψ_{leaf} may be due to an alteration in π as influenced by increasing free sugars in the leaf tissue. Koziol and Jordan (1978) observed in garden bean an increase in free sugars at low SO_2 concentrations, which they suggested was important in the repair process when little or no injury was visible. We found no reports elucidating the effects of SO_2 and/or NO_2 on ψ_{leaf} and its components.

Air quality data on SO_2 and NO_2 in the United States (Tingey et al., 1971) and the United Kingdom (Ashenden, 1979a) have indicated that the occurrence of combinations of these gases is possible (Ormrod, 1982). Combinations of SO_2 and NO_2 can affect growth (Ashenden, 1979a; Tingey et al., 1971) in many plant species, even when the same concentration of either pollutant alone does not cause injury. The concentrations of SO_2 and NO_2 in our study are based upon the maximum 24-h level for a single gas as presented in the Recommended Air Quality Objectives of Environment Canada (Fisheries and Environment Canada, 1976). The purpose of this experiment was to determine the time course of effects of the single and mixed gases upon shoot and root growth, CO_2 exchange, water loss, ψ_{leaf}, and reducing sugar content of leaves of the potato plant.

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Abbreviations: CSTR, continuously stirred tank reactor; PLA, planar leaf area; π, osmotic potential; ψ_{leaf}, leaf water potential.

Materials and Methods

Propagation of experimental plants. Stem cuttings were taken from plants grown from 12-month-old 'Russest Burbank' tubers and rooted as described by Petitte and Ormrod (1988). Rooted stem cuttings were transplanted into a mixture of 1 Premix BX (Premier Brands, Stamford, Corm.) :1 Fox sandy loam (v/v). Ten days later, plants were taken at random for experimentation.

Experimental design. There were two studies. Plant growth, photosynthesis, water loss, stomatal conductance, and water potential were measured in the first. Reducing sugars were measured in the second. Both studies were repeated three times over succeeding months with extra replications conducted for water potential measurement in the first study. The design for each study was a randomized complete block with the repetitions of the study as blocks. Data for each response variable were analyzed separately for each time of measurement. The growth variables were transformed to their natural logarithms before analysis of covariance with PLA (Ormrod et al., 1983) before treatment as the covariate. Geometric least mean squares were obtained for the growth variables by computing the antilog of means of transformed values (Snedecor and Cochran, 1967). Data for all other response variables were subjected to analysis of variance (ANOVA). The onset of treatment effects with increasing duration of treatment was detected on the basis of significance of contrasts between treated and control plants measured at the same hourly time. When the contrast was significant, the onset of treatment effects was deemed to have taken place.

Exposure conditions. In each replicate of the first study, 10 plants were placed into each of four CSTR chambers (Le Sueur-Brymer, 1982). In each replicate of the second study, 11 plants were placed into each CSTR. The exposure conditions inside the CSTR for both studies were: day/night at 25/18C ± 1C; relative humidity, 79% ± 5%; photosynthetically active radiation (PAR), 325 ± 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a 16 $h \cdot day^{-1}$ photoperiod (0600-2200 HR) supplied by a 400-W high-pressure sodium lamp and a 400-W metal halide lamp. A LI-COR LI-185 (LI-COR, Lincoln, Neb.) meter was used to measure PAR at mid-canopy level. Air temperature and relative humidity inside each CSTR chamber were monitored using a Taylor hygrometer (Model 5522, Taylor Instruments/Sybrong Corp., Arden, N. C.) situated at midcanopy level. Air flow rate through each CSTR chamber was 130 $liters \cdot min^{-1}$, measured periodically with an Alnor thermoanemometer (Type 8500, Alnor Instrument Co., Skokie, Ill.). The plants were irrigated daily with full strength complete nutrient solution (Hoagland and Agnon, 1950). Following ≈ 18 h of acclimation to the CSTR conditions and beginning at ≈ 0900 HR on Day 0, the plants were exposed for 24 $h \cdot day^{-1}$ to one of the following treatments: 1) filtered air (no detectable SO_2 or NO_2); 2) 0.11 SO_2; 3) 0.11 NO_2; or 4) 0.11 SO_2 plus 0.11 µl NO_2/liter for a maximum of 10 days. Sulphur dioxide and NO_2 were supplied from gas cylinders; a Beckman Model 953 SO_2 analyzer, and a Beckman Model 952A NO_2 analyzer comprised the monitoring system (Beckman Instruments, Fullerton, Calif.). Analyzers were calibrated with SO_2 and NO calibration tank gases supplied by the Speciality Gas Division of Liquid Carbonic Ltd. The NO was used in a Thermoelectron Series 101 calibrator (Thermo Electron, Hopkinton, Mass.) to calibrate for NO_2.

Net carbon dioxide exchange rates and water loss rates. Inlet and outlet CO_2 concentrations for each CSTR chamber were continuously monitored by a Beckman Model 865 infrared analyzer to permit calculation of net CO_2 exchange rates. A General Eastern 1100 dew point hygrometer (General Eastern Instruments, Watertown, Mass.) continuously monitored inlet and outlet dew point temperatures of each chamber to permit calculation of water loss rates.

Leaf areas for determining net CO_2 exchange rate and water loss rates within each CSTR chamber at 0300, 1100, and 1900 HR were estimated in the following manner. Before placement into the CSTR chamber, a covariate, PLA (Ormrod et al., 1983), was determined for each plant. Several extra plants were harvested to determine the linear relationship between PLA and leaf area. Leaf area was measured using a LI-COR LI-3100 leaf area meter. By substituting the PLA for each plant into the linear regression equation calculated from extra plants, an approximation of the initial leaf area for each experimental plant could be calculated.

Throughout the 10-day exposure, following measurements for stomatal conductance and water potential, two plants from each treatment were harvested (one at ≈ 1100 HR and the second at 1700 HR) every 2 days to measure leaf area and leaf, stem, and root fresh and dry weights. After each plant was harvested, the difference between the actual measured leaf area and the estimated initial leaf area was calculated. The changes in leaf area for plants in each CSTR chamber were regressed over the 10-day exposure. The regression equation for each treatment was used to calculate the approximate photosynthetically active leaf area remaining in the CSTR chamber at 0300, 1100, and 1900 HR for each day of the exposure. The approximate photosynthetically active leaf area was then used to calculate net CO_2 exchange water loss rates for each time (Petitte, 1986).

Pots containing soil at field capacity were placed into each CSTR chamber to determine net CO_2 exchange and water loss not due to the plant. These values were incorporated into the daily CO_2 exchange rates and water loss rates for each treatment in each block.

Stomatal conductance. Stomatal conductance was measured using a LI-COR model LI-700 transient porometer. Conductance measurements were made on days 0, 1, 2, 4, 6, 8, and 10 at ≈ 4 and 10 h after the beginning of the 16-h photoperiod. The terminal leaflets of the fifth, sixth, or seventh leaf from the soil line (mature, lower canopy) and the first or second newly unfurled leaf from the shoot apex (expanding, upper canopy) were measured. The CSTR chamber door was opened wide enough to move the cuvette of the porometer through the opening and clamp it onto a terminal leaflet. To avoid a buildup of CO_2 in the CSTR chambers, the person making the measurements wore a mask connected to an external vacuum pump over the nose and mouth. A single but different plant was used for each day's morning and afternoon readings.

Water potential. Water potential measurements were taken on the same terminal leaflets of mature and expanding leaves as used for the stomatal conductance readings every 2 days up to and including day 10 beginning on day 2. Preliminary statistical analysis indicated significant changes on the morning of day 2 in water potential measurements between the control and the plants that had been exposed to the mixture. Additional replications were conducted to permit measurements to be made at 6 h (afternoon of day O), 24 h (morning of day 1), 30 h (afternoon of day 1),48 h (morning of day 2), and 52 h (afternoon of day 2) after the initiation of the exposure.

For measurement of ψ_{leaf} the leaflet was loosely wrapped with a moist paper tissue. Then the leaflet with ≈ 1 cm of intact petiole was excised from the leaf with a sharp razor blade. The ψ_{leaf} was measured in terms of xylem water potential using a pressure bomb that was lined with moist paper towels.

Table 1. Growth of nontuberizing 'Russet Burbank' potato plants exposed to filtered air or SO_2 and NO_2 for 10 days.

Characteristics	Pollutant concn ($\mu l \cdot liter^{-1}$) SO_2	NO_2	Days of exposure 2	4	6	8	10	r^2	Response equation
Leaf area (cm^2/plant)	0.0	0.0	908	1130	1560	1930	2370	0.99	$y^z = 624 + 86x^y + 8x^2$
	0.11	0.11	788*	897*	1070*	1090*	1210*	0.84	$y = 643 + 55x$
Leaf dry wt (g/plant)	0.0	0.0	2.17	2.84	4.18	5.26	6.83	0.95	$y = 1.43 + 0.20x + 0.032x^2$
	0.11	0.11	2.06	2.62	3.44*	3.53*	4.60	0.90	$y = 1.28 + 0.3x$
Stem dry wt (g/plant)	0.0	0.0	0.760	1.20	1.92	2.57	3.52	0.95	$y = 0.90 - 0.036x + 0.028x^2$
	0.11	0.11	0.724	1.15	1.50*	1.77*	2.26*	0.96	$y = 0.28 + 0.19x$
Root dry wt (g/plant)	0.0	0.0	0.658	0.818	0.945	1.24	1.57	0.92	$y = 0.70 + 0.034x + 0.01x^2$
	0.11	0.11	0.558	0.629*	0.770*	0.728*	0.886*	0.68	$y = 0.46 + 0.039x$

[z]y: Response variable.
[y]x: Days.
*Significantly less than the control (0.0) in the same column for the same criterion ($P \leq 0.05$).

Table2. Net CO_2 exchange rates ($mg \cdot m^{-2} \cdot s^{-1}$) of nontuberizing 'Russet Burbank' potato plants exposed to SO_2 and/or NO_2 during 10 days.

Day	Time[z] (HR)	Pollutant concn ($\mu l \cdot liter^{-1}$) SO_2 / NO_2 0.0 / 0.0	0.11 / 0.0	0.0 / 0.11	0.11 / 0.11
0	1900	0.129	0.106*	0.128	0.124
1	0300	−0.006	−0.000	−0.008	−0.008
	1100	0.158	0.126	0.151	0.138
	1900	0.128	0.112	0.128	0.125
2	0300	−0.005	−0.002	−0.004	−0.009
	1100	0.148	0.127	0.143	0.122
	1900	0.133	0.117	0.135	0.128
3	0300	−0.012	−0.007	−0.009	−0.015
	1100	0.148	0.120*	0.149	0.125
	1900	0.129	0.110	0.120	0.116
4	0300	−0.010	−0.008	−0.010	−0.014
	1100	0.154	0.127	0.152	0.126
	1900	0.140	0.118	0.135	0.123
5	0300	−0.015	−0.012	−0.012	−0.014
	1100	0.157	0.123*	0.153	0.128*
	1900	0.132	0.116	0.139	0.113
6	0300	−0.014	−0.013	−0.014	−0.017
	1100	0.161	0.124*	0.162	0.130
	1900	0.143	0.117	0.150	0.115
7	0300	−0.021	−0.014	−0.018	−0.020
	1100	0.162	0.119*	0.166	0.133
	1900	0.145	0.114	0.145	0.118
8	0300	−0.017	−0.013	−0.019	−0.016
	1100	0.181	0.116**	0.165	0.123**
	1900	0.146	0.108**	0.151	0.121*
9	0300	−0.020	−0.013	−0.026	−0.025
	1100	0.149	0.111	0.190	0.112
	1900	0.140	0.108	0.148	0.106*
10	0300	−0.020	−0.016	−0.028*	−0.022
	1100	0.177	0.109*	0.183	0.127

[z]Photoperiod = 0600 to 2200 HR.
*,** Significantly different from the control in the same row at $P \leq 0.05$ or 0.01, respectively.

Table 3. Water loss rates ($mg \cdot m^{-2} \cdot s^{-1}$) of nontuberizing 'Russet Burbank' potato plants exposed to SO_2 and/or NO_2 during 10 days.

Day	Time[z] (HR)	Pollutant concn ($\mu l \cdot liter^{-1}$) SO_2 / NO_2 0.0 / 0.0	0.11 / 0.0	0.0 / 0.11	0.11 / 0.11
0	1900	15.6	14.6	15.2	16.6
1	0300	4.5	3.8	4.6	4.7
	1100	15.0	14.4	15.2	15.1
	1900	16.3	15.7	15.2	16.6
2	0300	4.1	4.4	4.1	4.6
	1100	17.2	15.9	13.2	17.4
	1900	16.6	16.1	14.0	19.4
3	0300	3.7	3.4	7.2	5.2
	1100	16.8	16.3	14.3	17.8
	1900	14.8	14.8	16.5	25.0*
4	0300	3.4	3.9	4.4	5.1*
	1100	15.3	13.6	10.5	18.5
	1900	14.8	15.9	13.8	23.8**
5	0300	6.3	7.6	7.4	8.2
	1100	13.4	14.7	12.6	23.5**
	1900	14.8	14.3	13.4	27.5*
6	0300	4.8	5.4	7.2	7.4
	1100	16.1	18.2	18.6	25.8**
	1900	19.6	24.1	18.8	31.1*
7	0300	5.2	6.1	9.1	8.4
	1100	17.4	20.2	15.1	29.8**
	1900	19.0	19.6	16.0	29.5**
8	0300	5.0	5.2	9.3	8.5
	1100	23.3	23.2	21.1	37.6**
	1900	22.1	32.4	33.7	39.5
9	0300	6.4	5.4	15.2*	6.1
	1100	28.8	32.2	24.9	36.5
	1900	26.7	27.3	25.8	43.3
10	0300	8.7	8.6	10.6	12.0

[z]Photoperiod = 0600-2200 HR.
*,**Significantly different from the control in the same row at $P \leq 0.05$ or 0.01, respectively.

After the leaflet was removed from the pressure bomb and the paper tissue was removed, the leaflet was sealed in a plastic bag and frozen until IT was determined with a Wescor Model HR-33T microvoltmeter (Wescor, Logan, Utah) operating in a dew point hygrometer mode. For this determination two 5-mm-

Table 4. Water, osmotic, and pressure potentials (kPa) in expanding and mature leaflets of nontuberizing 'Russet Burbank' plants exposed to SO_2 and NO_2 during 10 days.

			Morning leaflets				Afternoon leaflets			
			Expanding		Mature		Expanding		Mature	
			Pollutant concn ($\mu l \cdot liter^{-1}$)							
Day	Potential (kPa)	SO_2 / NO_2	0.0 / 0.0	0.11 / 0.11	0.0 / 0.0	0.11 / 0.11	0.0 / 0.0	0.11 / 0.11	0.0 / 0.0	0.11 / 0.11
0	ψ_{leaf}						−295	−393	−310	−360
	π						−709	−689	−780	−753
	P[z]						399	303	482	359*
1	ψ_{leaf}		−315	−342	−288	−335	−320	−388	−265	−348*
	π		−693	−838**	−655	−769**	−680	−722**	−628	−757**
	P		377	526	377	443	360	385	363	409
2	ψ_{leaf}		−252	−352**	−212	−264	−272	−412**	−242	−320
	π		−664	−772**	−628	−792*	−655	−788*	−668	−814**
	P		427	395	438	504	383	376	426	494
4	ψ_{leaf}		−285	−373*	−233	−317	−267	−392*	−197	−375**
	π		−718	−803	−659	−826**	−725	−826	−647	−923**
	P		466	414	442	521	457	433	449	548
6	ψ_{leaf}		−310	−405*	−255	−313	−280	−433**	−207	−490**
	π		−680	−767**	−588	−814**	−737	−827**	−628	−857**
	P		386	365	340	478*	457	394	421	367
8	ψ_{leaf}		−282	−286	−198	−296*	−290	−447**	−217	−612**
	π		−750	−848**	−667	−887**	−792	−943**	−629	−987**
	P		475	533	469	584	502	495	411	374
10	ψ_{leaf}		−336	−536*	−230	−430*	−288	−424**	−284	−428*
	π		−789	−880	−660	−894**	−770	−961**	−622	−911**
	P		447	397	437	557	465	534	347	541

[z]P = pressure potential.
*,**Significantly different from the control during the same time period at $P \leq 0.05$ or 0.01, respectively.

diameter filter paper disks were quickly placed inside the plastic bag beside the frozen leaflet. The bag was resealed and the leaflet was then allowed to thaw. A smooth rounded object was moved firmly over the leaflet in the bag to break up the tissue and allow the liquid to saturate the paper disks. Each disk was quickly removed with clean forceps from the bag and placed into a Wescor C-52 sample chamber. Following a 15-min equilibration, a reading was taken 30 sec after a 10-sec cooling current (Prange and Ormrod, 1983). The chamber was cleaned with deionized water after each measurement and then dried. The π values of two sap samples from each leaflet sample were measured and their mean was used in the ANOVA. Salt solutions of known concentrations were used to calibrate each sample chamber before each replication of the experiment.

Leaflet pressure potential (P) was calculated according to the equation: $\psi_{leaf} = \pi + m + P$, assuming the matric potential (m) to be negligible (Beringer et al., 1983, Wiebe and Al-Saadi, 1976).

Reducing sugars. The second study was conducted to observe reducing sugar concentration during the morning and afternoon in one of the first primary leaflets of mature and expanding leaves during the first 5 days of exposure. Samples of equal area were removed with a cork borer from each lateral half of the leaflet and used for dry weight determination (W) and sugar analyses (S). Total area for the W or S sample was 77 mm^2. The W samples were dried at 70C for 5 days and weighed. The S samples were wrapped in aluminum foil, placed in a plastic bag, and frozen until the time of extraction.

A modification of the technique of Madore (1984) was used to extract the reducing sugars from the leaf disks. The S samples were placed in a test tube with 2 ml of 80% ethanol. The test tube was placed in boiling water for ≈ 45 sec and then removed. After decanting the liquid from the tissue into a test tube, more ethanol was added, heated, and decanted. The extraction process was repeated two additional times. Preliminary analyses demonstrated that ≈ 95% of the reducing sugars were removed from the leaf tissue with four extractions. The test tube containing the decanted ethanol extract was covered with two layers of parafilm and placed in a freezer until assayed.

The anthrone assay of Pesez and Bartos (1974) was used for the measurement of reducing sugars in the ethanol extracts. Anthrone (0.20 g) was dissolved in a cold 5:2 (v/v) mixture of concentrated sulphuric acid and distilled water. Five milliliters of the anthrone reagent was chilled for 5 min on ice in a test tube. The cold ethanol extract was diluted to 10 ml with cold 80% ethanol and thoroughly mixed. One milliliter of the extract was layered onto the anthrone reagent and chilled on ice for 5 min. After mixing the extract and the anthrone, the mixture was heated at 100C in a shaking water bath for 10 rein, cooled for 5 min in an ice water bath, and the absorbance read at 620 nm on a Beckman DU-8 spectrophotometer (Beckman Instruments). The standard curve was based on glucose dissolved in 80% ethanol. Reducing sugar concentration in the first primary leaflets was expressed as milliequivalents of glucose per square millimeter of leaf area.

Precision was defined as the amount of variation within and between assays (Rodbard, 1971). The within- and between-assay cv were calculated using the ethanol-extracted reducing sugar samples from the control plants. Sensitivity was defined as the amount of glucose that would give an optical reading significantly different from zero, which was calculated as the optical density at zero dose plus twice the SD of the optical density at

Table 5. Reducing sugars (millequivalent glucose per square millimeter of leaf area) in expanding and mature first primary leaflets of nontuberizing 'Russet Burbank' plants during the first 5 days of a 10-day exposure to SO_2 and/or NO_2.

			Pollutant concn ($\mu l \cdot liter^{-1}$)			
		SO_2	0.0	0.11	0.0	0.11
Day	Leaf age	Time[z]	NO_2 0.0	0.0	0.11	0.11
0	Expanding	A	1.93	1.41	1.69	2.39
	Mature	A	1.26	1.13	1.00	0.90
1	Expanding	M	2.18	2.60	2.44	3.20**
		A	2.48	3.14	2.74	3.27
	Mature	M	1.33	1.54	0.87	1.57
		A	1.20	2.10	1.23	1.85
2	Expanding	M	2.43	3.20	2.98	4.03**
		A	3.40	3.80	3.07	4.36
	Mature	M	1.07	1.57	1.59	1.98*
		A	1.34	1.70	1.62	2.31
3	Expanding	M	3.19	2.98	3.40	4.28*
		A	2.42	2.90	2.50	3.89
	Mature	M	1.53	1.69	1.87	2.65**
		A	0.66	1.07	1.36	1.54*
4	Expanding	M	2.66	1.90	2.45	3.64
		A	3.06	3.40	2.73	3.66
	Mature	M	0.94	1.20	1.04	1.49
		A	1.53	0.99	1.98	1.99
5	Expanding	M	2.91	3.54	2.76	3.07
		A	3.88	4.32	3.30	3.01
	Mature	M	1.14	1.71	2.07	3.25**
		A	1.43	1.72	3.19*	3.04

[z]A: afternoon: M: morning.
*,**Significantly different from the control in the same row at $P \leq 0.05$ or 0.01, respectively.

zero dose. Fourteen runs were used to calculate precision and sensitivity of the reducing sugar assay. The within- and between-assay cv were 8.7% and 19%, respectively. The sensitivity of the assays was *5.0* meq glucose.

Results

Leaf injury. On day 4, injury was visible on those plants treated with the combination of SO_2 + NO_2. The injury was observed as a glazing of the abaxial surface of the middle and upper (which included the expanding leaflets) canopy leaflets. Visible injury consisting of interveinal abaxial necrotic areas was present. on day 10 in the middle canopy leaflets of the plants treated with SO_2. Visible injury was absent on plants treated with NO_2 alone.

Growth. Over the 10 days of exposure, significant differences in leaf, stem, and root growth were observed only between the control plants and those treated with SO_2 + NO_2. Leaf area and leaf, stem, and root dry weights of the control plants followed significant quadratic growth patterns over time, while linear responses were obtained for the mixture of gases (Table 1). SO_2 + NO_2 reduced leaf area and leaf dry weight. Leaf area was significantly reduced by day 2, before significant decreases were observed in any of the other growth measurements. By day 6, leaf and stem dry weights were significantly reduced by the SO_2 + NO_2 exposure. There was a significant reduction in root dry weight on day 4 of the exposure. The decrease in root dry weight was accompanied by a significant increase in leaf : root dry weight compared with control plants.

Net carbon dioxide exchange rates. Neither SO_2 nor the mixture had an effect on net CO_2 exchange rates at 0300 HR (dark respiration period) (Table 2). However, dark respiration increased significantly in the NO_2-treated plants, but only on day 10.

The net CO_2 exchange rate of the plants in SO_2 + NO_2 decreased significantly compared to the control at 1100 HR on days 5 and 8 (Table 2). A significant reduction in net CO_2 exchange rate at 1900 HR was observed on days 8 and 9 of plants in SO_2 + NO_2 and on days O and 8 in SO_2 alone (Table 2).

Water loss rates. Significant differences in net water loss rates were observed in the NO_2 and the SO_2 + NO_2 treatments (Table *3).* The NO_2-fumigated plants had a significantly higher water loss rate than the control plants during the 0300 HR measurement on day 9. No significant effects of NO_2 were measured at 1100 HR or 1900 HR. Plants exposed to SO_2 + NO_2 began to lose water significantly faster than the control plants by 1900 HR on day 3. Increased net water loss rates continued until day 8, although they were not always significantly different from the control.

Stomatal conductance. No significant differences in stomatal conductance between plants treated with pollutants and control plants were detected during the experiment (data not presented).

Leaf water potential. During the exposure period, significant responses were observed in the Ψ_{leaf} of the SO_2 + NO_2-treated plants (Table 4). No significant differences from control plants were detected for the SO_2 or NO_2 single gas treatments. About 6 h after beginning the exposure to the combination of SO_2 + NO_2 (afternoon of day 0), P was significantly reduced in the mature leaflets (Table 4), but this difference from the control did not continue.

ψ_{leaf} of the mature leaflets in the mixture treatment was first observed to be significantly less than that of the control on the afternoon of day 1 (Table 4). This significant reduction continued, except for day 2, in the afternoon and was noted for morning periods on days 8 and 10. The expanding leaflets also had a significant reduction in ψ_{leaf} beginning on day 2 except for the morning of day 8.

In the mature and expanding leaflets of the mixture-treated plants, π was first observed to be significantly lower than that of the controls on the morning of day 1 (Table 4), and the difference was more consistent in mature than in expanding leaflets. This difference was observed on most of the measurement days in the morning and afternoon.

Reducing sugars. The concentration of reducing sugars in the expanding leaflets during the afternoons in any of the pollutant treatments did not differ significantly from the control leaflets (Table 5). Significantly higher reducing sugar concentrations in the mornings of days 2, 3, and 5 were observed in the mature leaflets exposed to SO_2 + NO_2. During the morning observation, significant increases in reducing sugars over the control were observed only in the leaflets exposed to the combination of SO_2 + NO_2. Increases in reducing sugar concentration were measured in the expanding leaflets on days 1, 2, and 3 and in the mature leaflets on days 3 and 5 (Table 5). In general, there was an increase in the concentration of reducing sugars in the leaflets that had been exposed to SO_2 + NO_2 relative to control plants throughout the first half of the exposure.

Discussion

Sequential harvests of plants during an exposure to SO_2 and/or NO_2 air pollution for 10 days permitted the identification of

a series of events occurring in the plants. On day 1, leaf π was significantly lower in the plants treated with $SO_2 + NO_2$ than in the control plants. This reduction was accompanied by a significant increase in the concentration of reducing sugars on a leaf-area basis in the expanding leaflets. Sulphur dioxide alone at low concentrations (<3.06 $\mu l \cdot liter^{-1}$) has been shown to cause an increase in free sugar levels and starch levels in *Phaseolus vulgaris* L. (Koziol and Jordan, 1978).

A significant decrease in ψ_{leaf} was not evident until day 2 when leaf area also was significantly reduced. Potato plants undergoing drought stress also exhibit decreases in leaf area growth (Gandar and Tanner, 1976). Leaf expansion of 'Russet Burbank' plants grown under greenhouse conditions was reduced at a ψ_{leaf} of about – 300 kPa, with the cessation of expansion occurring at approximately – 500 kpa. Hsiao (1973) noted that cell expansion is one of the plant processes most sensitive to drought stress. McCree et al. (1984) showed that leaf expansion in water-stressed sorghum plants was not closely related to pressure (turgor) potential in the exposed lamina. Cell enlargement may not always be closely correlated with turgor because it depends on metabolic processes affecting cell wall extensibility and a supply of solutes, as well as on the physical processes affecting the water supply and cell turgor (Kramer, 1988). In our study, it is unlikely that P had much of a role in allowing for the reduction in leaf area, because P did not decrease significantly throughout much of the exposure period. The reduction in leaf expansion rate may have been the result of pollutant by-products affecting cell wall synthesis or the lack of available organic substances to maintain growth (Munns and Weir, 1981). As a consequence of decreased leaf area, leaf area index (leaf area per unit land area) will be reduced, limiting the crop's assimilation of CO_2 and ultimately affecting dry-matter yield of the tuber (Scott and Wilcockson 1978).

Some species have an endogenous ability to control intracellular solute contents by increasing the number of osmotically active particles as water becomes less available to the cell (a decrease in total ψ_{leaf}) (Shackel and Hall, 1983). Osmotic adjustment is the term used to describe this action (Reed, 1984), and it usually occurs under slowly developing drought stress conditions (Kramer, 1983). As π decreases (becomes more negative), an inwardly directed force is established that creates a tendency for water to enter the cell (Reed, 1984) and allows for the maintenance of turgor and turgor-dependent processes, i.e., cell expansion, stomatal opening, and photosynthesis (Turner and Jones, 1980) at a significantly lower water potential (Kramer, 1983). Whether osmotic adjustment was occurring in the pollutant-stressed potato leaflets is uncertain. Three characteristics of the process were evident: turgor was maintained, π decreased, and the concentration of reducing sugars (solutes) increased. More research must be performed on potato plants to determine if the reducing sugars are a direct result of photosynthesis or the products of starch degradation. Vos and Groenwold (1988) have reported in 'Bintje' potato that leaves appearing during a drought stress period have higher π values at full turgidity than those leaves already present during the stress period. The effects of $SO_2 + NO_2$ and drought on the potato plant appear to be similar.

A significant decline in photosynthesis rate was measured by day 5 of the $SO_2 + NO_2$ exposure. Boyer (1970) demonstrated in soybean and Hsiao (1973) stated that, under drought stress, leaf enlargement is often reduced or stopped before photosynthesis is greatly reduced. Leaf area was significantly reduced by day 2 in the potato plants. By day 3 there was a significant increase in rate of water loss. Since stomatal conductance during the exposure did not change significantly in the treatments with pollutants compared with the control, and the rate of water loss rose, the stomates probably had very little or no influence on the reduction in the rate of photosynthesis. Another possible cause for the decrease in net CO_2 exchange rate may have been an increase in respiration rate (Reinert, 1984). Since no significant mixture-induced increases were observed in the dark respiration rates, they were probably not the cause for the reduction in the photosynthesis rates. Thus, reduction of photosynthesis could have been caused by direct inhibition at the metabolic level (Amundson and MacLean, 1982; Carlson, 1983).

A decrease in net CO_2 exchange rate was observed not only in the plants exposed to the mixture but also, and with greater magnitude, in the SO_2-treated plants. The injurious effects of SO_2 may have been the major contributor to the measured reduction in photosynthesis rates of the plants treated with $SO_2 + NO_2$.

The significant increase in water loss rate of the plants under $SO_2 + NO_2$ was observed without a detectable increase in stomatal conductance. This result has also been observed in *Betula* sp. by Neighbour et al. (1988) who stated that the increase in water loss rate probably was not due to abnormal functioning of the stomata but may have been due to areas of gaping stomata or to cuticular injury. Pande and Oates (1986) found that after 4 days of exposure to 0.10 µl $SO_2 + NO_2$/liter, injury in *Commelina communis* L. occurred abaxially in the form of cuticular erosion probably accompanied by an increase in cuticular transpiration. By day 4 in our experiment, leaf injury was observed as abaxial leaf glazing. Since only the abaxial leaf surface was measured for stomatal resistance, it may have reflected injury to the adaxial cuticular surface and may have accounted for the increase in water loss rate. Further studies should consider observing stomatal resistances on both leaf surfaces. In addition, histological studies should be conducted to determine the extent of leaf injury.

By day 4, leaf injury was visible and root fresh and dry weights were significantly reduced by the $SO_2 + NO_2$ treatment. The combination of 0.11 µl SO_2 + 0.11 µl NO_2/liter for 2 weeks on tomato also caused a significant decrease in root growth without leaf fresh and dry weights diminishing at the same rate (Marie and Ormrod, 1984). Whitmore and Mansfield (1983) also observed that the combination of $SO_2 + NO_2$ decreased the partitioning of dry matter to the roots in *Poa pratensis* L. 'Monopoly'. Gould and Mansfield (1988) demonstrated in winter wheat that $SO_2 + NO_2$ caused greater reductions in root dry weight than in shoot dry weight. Less ^{14}C assimilate was received by the roots than the shoots, suggesting an inhibition of translocation either in the stem or in the source leaves. Significant increases in leaf : hypocotyl dry weight ratios were observed in six radish cultivars exposed to 0.15 µl NO_2/liter, indicating a significant alteration of assimilate partitioning between source and sink areas of the plant (Godzik et al., 1985).

Tingey (1978) demonstrated that O_3 altered root growth and root processes by first reducing photosynthesis and changing metabolic pathways. In our experiment with $SO_2 + NO_2$, no significant reductions in photosynthesis rate were observed before reduced root growth. Tingey (1978) stated that reductions in root growth eventually should be expressed as reduced shoot growth. We observed significant reductions in shoot fresh and dry weights after reduced root growth was recorded.

The effects of $SO_2 + NO_2$ in combination on potato plants involved a sequence of two types of injury: hidden and vis-

ible. Heath (1980) defined hidden injury as biochemical or physiological alterations resulting in lowered plant productivity without visible injury. Malhotra and Khan (1984) state that the progression of hidden or invisible injury begins at the biochemical level (interference with photosynthesis, respiration, lipid and protein synthesis, etc.), subsequently progresses to the ultrastructural level (disorganization of cellular membranes), and then to the cellular level (cell-wall, mesophyll, and nuclear breakdown). In our experiment, hidden injury began with the decrease in π, which was at least partially associated with an increase in reducing-sugar concentration. This change was followed by reduced leaf area and then decreased net CO_2 exchange. Reductions in root fresh and dry weights were evident after $\approx$ 4 days of exposure, slightly before or about the same time as injury became visible. Under normal circumstances, the increased reducing sugars that were found in the leaves of treated plants, would be translocated to the roots and used for growth. However, sugars retained by the leaves were probably not available for growth even by the leaves (Munns and Weir, 1981).

Combinations of SO_2 and NO_2 occur in the natural environment (Ashenden, 1979a; Tingey et al., 1971). Our results demonstrate that their interaction can cause reductions in growth and dry-matter partitioning not observed with one gas. In the potato, the osmotic component of water potential appears to be one of the first factors detrimentally involved. The causes of build-up of reducing sugar and its relationship to water potential and growth effects should be an objective for further research to better understand the mechanism of joint 'action of SO_2 and NO_2 in plants.

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