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Effect of Relative Humidity Prior to and during Exposure on Response of Peas to Ozone and Sulfur Dioxide

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Abstract. **Pea (*Pisum sativum* L. 'Alsweet') plants were grown in a controlled environment and exposed to ozone, sulfur dioxide, or a mixture of the 2 pollutants. Plants were preconditioned with low (36%) or high (77%) relative humidity (RH) for 6 days and then exposed to the pollutants for 2 hr in low (31%) or high (67%) RH. Injury was evaluated as both necrosis and chlorophyll concentrations. A high RH prior to exposure to air pollutants greatly increased subsequent plant injury from pollutants. The increased injury with high RH prior to exposure to pollutants was associated with increased stomatal conductance. Relative humidity during exposure to air pollutants had little effect on plant injury.**

Relative humidity appears to be a significant factor regulating plant sensitivity to gaseous pollutants (5). High RH generally has increased injury to plants; however, in some exposures it has decreased injury.

Most investigators have varied RH only during the period of pollutant exposure. With exposure to ozone, increases in RH always increased injury in *Phaseolus vulgaris* and *Begonia semperflorens*. In *Nicotiana tabacum*, no changes and increases in injury were reported with RH increase (1, 6, 10). With exposure to sulfur dioxide, increases in RH increased injury in *Betula papyrifera* and *P. vulgaris*, but decreased injury in *Avena sativa* (3, 9, 12). Thus, effects of RH during pollutant exposures have varied with species.

Effects of RH, prior to exposure, on sensitivity of plants to pollutants have been studied in controlled environments with ozone treatments. In *P. vulgaris* grown under low light levels, ozone injury increased when plants were preconditioned at high (80%) compared to low (60%) RH but under high light injury was similar at both RH levels (1). In *N. tabacum* grown under low light, ozone injury was similar at both RH levels, but under high light, injury was less at high than at low RH (1).

High RH presumably increases injury in part because it induces stomatal opening (5, 8, 10, 13). However, seldom have the extent of injury and stomatal conductance been measured in the same study. Under carefully controlled environmental conditions, stomata of plants exposed to sulfur dioxide were open more at high than at low RH (9, 12). These stomatal responses were associated with greater injury at high than at low RH.

The objective of this study was to determine the effect of RH prior to and during exposure on sensitivity of peas to pollutants alone and in combination. Pea plants were grown at a high or low RH for 6 days prior to pollutant exposure. Plants from each RH then were divided and subjected to a high or a low RH during 2-hr exposure periods with ozone, sulfur dioxide, or a mixture of the 2 pollutants. Stomatal conductance and the extent of injury were measured to determine is stomatal responses were associated with injury.

Materials and Methods

Plant culture. Pisum sativum L. 'Alsweet' seeds treated with Captan were planted 1.25 cm deep in a peat-vermiculite medium in 10 cm plastic pots. Plants were grown in controlled environment rooms in the Biotron, a controlled environment facility at the Univ. of Wisconsin. Plants were watered to excess with about 35 ml of ASHS baseline nutrient solution (2) 4 times per day. For the 1st 7 days, plants were grown in a 9.5 m^2 room, which will be referred to as the large room. After 7 days plants were moved into 2 small rooms (3.1 m^2), each with a different RH. Plants were kept in these small rooms for 6 days, and then moved to a separate room for pollutant exposure. After fumigation, the plants were returned to the large room.

Light was provided with cool-white fluorescent plus incandescent lamps for 16 hr per day. Photosynthetic photon flux density (PPFD for 400–700 nm) was 285 ± 20 μmol $s^{-1}m^{-2}$ in the large room, and 268 ± 28 μmol $s^{-1}m^{-2}$ in the small rooms, as measured at pot height once a week with a Lambda LI-185 quantum sensor. Temperature during both day and night was 19.5° ± 0.4°C in all rooms, as measured daily with thermocouples at plant height. Relative humidity during both day and night was 75 ± 3% in the large room, 36 ± 4% in one small room, and 77 ± 5% in the other small room as measured daily with an aspirated thermocouple psychrometer.

Plant exposure. Plants were treated in two 0.3 m^3 controlled environment plexiglas chambers located in a room adjacent to the large growing room. One chamber was an exposure chamber with pollutants and the other was a control chamber with no pollutants. Pollutant concentrations were measured occasionally in the control chamber to be sure no pollutants were present. Environment was similar in both chambers. Radiation, provided with cool-white fluorescent plus incandescent lamps, was 289 ± 9 μmol $s^{-1}m^{-2}$ at pot height. Temperature was 20.7° ± 0.8°C. Air was drawn from the room and passed through a dry potassium permanganate (Purafil) filter before entering the chambers. Air exchanges in chambers were 9 per minute. Carbon dioxide was maintained at 325–350 ppm. Carbon dioxide

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Table 1. Effect of relative humidity and pollutant treatment on leaf necrosis in pea leaves.

Relative humidity (%)		Necrosis (%)		
Growing room (prior to exposure)	Pollutant chamber (during exposure)	Ozone	SO_2	Ozone plus SO_2
36	31	34 a[z]	1 a	4 a
	67	22 a	6 a	4 a
77	31	58 b	40 b	75 b
	67	73 b	57 b	65 b

[z]Mean separation within columns based on Duncan's multiple range, 5% level (average of 6 plants). When percentage data were transformed to arcsin prior to analysis, the same statistical results were obtained.

Table 2. Effect of relative humidity and pollutant treatment on chlorophyll concentration in pea leaves.

Relative humidity (%)		Chlorophyll concentration[z] ($\mu g\ mg^{-1}$ dry wt)		
Growing room (prior to exposure)	Pollutant chamber (during exposure)	Ozone	SO_2	Ozone plus SO_2
36	31	12.6 a[y]	15.7 a	15.6 a
	67	13.5 a	16.0 a	15.5 a
77	31	10.7 b	9.4 b	6.7 b
	67	8.4 c	7.5 b	7.0 b

[z]Control chlorophyll concentration was 16.4 ± 1.3 $\mu g\ mg^{-1}$ dry wt and significantly different from all values except SO_2 and ozone plus SO_2 for 36% RH in growing room.
[y]Mean separation within columns based on Duncan's multiple range, 5% level (average of 6 plants).

build-up from humans was prevented by wearing a mask. Carbon dioxide depletion by plants was prevented due to the rapid air exchange rate and the small amount of plant material.

Plants were exposed to pollutants 13 days after seeding when the 4th trifoliolate leaf was in the cup phase. This plant age was chosen because the 2nd leaf was expanded at this time, and all measurements were taken on the 2nd leaf.

Exposure periods were 2 hr long to simulate typical sulfur dioxide fumigation periods in rural areas near sulfur dioxide point sources (14). Sulfur dioxide exposures at these locations rarely exceeded a duration of 2 hr because of changing wind directions and varying vertical mixing patterns. Ozone generally showed an increase in concentration during each day with a maximum around midday, and a decrease at night with a minimum just before sunrise.

Three separate exposures were undertaken each day with one of ozone, one of sulfur dioxide,and one of a mixture of ozone plus sulfur dioxide. Exposures were carried out for 2 hr periods between 2 ½ and 9 hr after the start of the 16 hr light period. In preliminary trials, plant injury was similar in 2 hr fumigations at any time during this period.

Plants were exposed to pollutants at low (31% ± 3%) or high (67% ± 1%) RH. Since plants were grown at 2 humidity levels prior to exposure and exposed at 2 humidity levels, 4 RH treatments were made: 1) low RH both prior to and during exposure, 2) low RH prior to and high RH during exposure, 3) high RH prior to and low RH during exposure, and 4) high RH both prior to and during exposure. Relative humidity was maintained during exposures by controlling RH in the room where chambers were located, and was measured with an aspirated thermocouple psychrometer. Each RH treatment was repeated twice for each pollutant treatment (i.e., ozone alone, sulfur dioxide alone, mixture of ozone plus sulfur dioxide). A total of 6 plants was used for each treatment. On any given day, treatments were undertaken at only one RH exposure level. Different sets of plants were exposed to the 3 pollutant treatments in successive exposures with half the plants in each chamber from the room at high RH prior to exposure and the other half from the room at low RH prior to exposure.

Pollutants were added to the 10 cm diameter air intake duct, which was located on one side of the chamber at the top center. Just below the air intake within the chamber, a fan mixed and dispersed the pollutants and air. Pollutants were monitored in the center of the chamber through teflon sample lines leading to analyzers located immediately outside of the treatment room. Upon leaving the chamber, sample lines were enclosed in a black tube to promote radiation absorption and thus warming to avoid moisture condensation. Pollutant concentrations were 0.20 $\mu l\ l^{-1}$ ozone alone, 1.35 $\mu l\ l^{-1}$ sulfur dioxide alone, or 0.09 $\mu l\ l^{-1}$ ozone plus 0.60 $\mu l\ l^{-1}$ sulfur dioxide as a mixture. These concentrations were chosen because they all produced moderate injury (60% to 70%) in preliminary tests. By having moderate injury with all combinations of pollutants (ozone, sulfur dioxide, mixture), RH effects with different combinationsof pollutants could be evaluated. Pollutant concentrations generally stabilized within 10–15 min after plants were put into the exposure chamber. Ozone was provided by passing air over ultraviolet lamps and monitored with a Monitor Labs 8410 chemiluminescent ozone analyzer. Sulfur dioxide was provided

Table 3. Effect of RH and pollutant treatment on stomatal conductance of pea leaves.

RH in growing room (%)	Stomatal conductance prior to exposure ($cm\ sec^{-1}$)[z]	RH in exposure chamber (%)	Stomatal conductance at end of exposure ($cm\ sec^{-1}$)[z]			
			Control	Ozone	SO_2	Ozone plus SO_2
36	0.43 a[y]	31	0.32 b[x]	0.21 b[x]	0.11 a[x]	0.15 a[x]
		67	0.38 c	0.30 c	0.14 a	0.12 a
77	0.72 b	31	0.20 a	0.14 a	0.19 ab	0.23 a
		67	0.39 c	0.16 ab	0.27 b	0.25 a

[z]Average of upper and lower surface measurements.
[y]Mean separation within column based on *t* test, 5% level (average of 36 plants).
[x]Mean separation within column based on Duncan's multiple range, 5% level (average of 18 plants in control and 6 plants in pollutant exposures).

from a cylinder containing 0.1% sulfur dioxide in N and monitored with a ThermoElectron model 43 pulsed fluorescent SO_2 analyzer. Both analyzers were calibrated with a Bendix 8861DA gas phase titration calibrator.

Stomatal conductance. Measurements of stomatal conductance to water vapor were made with a LI-COR model LI-65 automatic diffusive resistance meter equipped with a horizontal sensor, calibrated at 20°C. Calibrations were checked before and after the study.

Leaf conductances were measured on upper and lower surfaces of the 2nd trifoliolate leaves with one measurement per surface. The response on the 2 surfaces was similar; therefore, data are reported as the average of the 2 surfaces. Measurements were made on 3 sets of plants: 1) plants remaining in the small growing rooms just after the start of an exposure period, 2) plants in the control chamber 1 to 1½ hr after the start of an exposure period, and 3) plants in the exposure chamber with pollutants 1½ to 2 hr after the start of an exposure period. Measurements in chambers were taken through portals utilizing plastic gloves.

Injury evaluation. Injury was evaluated as necrosis and chlorophyll concentration of the 2nd trifoliolate leaf blade 7 days after exposure to pollutants. Necrosis was estimated visually as the percentage of leaf surface showing necrosis. Percentage was estimated in 5% intervals. Chlorophyll was measured by ethanol extraction (4).

Statistical analyses. Necrosis, chlorophyll, and stomatal conductances of plants were analyzed with one way analysis of variance followed by Duncan's multiple range test or with a *t* test.

Results

Ozone and SO_2 injuries were greatly increased by preconditioning plants with high RH prior to exposure to pollutants (Tables 1 and 2). The increased injury with preconditioning at high (77%) compared to low (36%) RH was evident both as increased leaf necrosis and decreased chlorophyll concentration. The magnitude of the increased injury with high RH was greatest with the mixture of ozone plus SO_2, intermediate with SO_2 alone, and least with ozone alone.

In contrast, injury was only slightly altered by differences in RH during the pollutant exposures (Tables 1 and 2). The small differences in pollutant injury with high (67%) and low (31%) RH during the exposure period were not consistent for the different pollutants, and varied with the preconditioning RH. Only for chlorophyll concentrations of plants exposed to ozone were the differences between high and low RH during exposure statistically significant.

Stomatal conductances of plants were lower when preconditioned at low (36%) than at high (77%) RH prior to exposure to pollutants, indicating that stomata were more closed at low than at high RH (Table 3).

The RH in the control chambers during exposures influenced stomatal conductance of plants. Conductances were consistently lower for control plants in the chamber at low (31%) than at high (67%) RH (Table 3). Stomatal conductance apparently was also altered by moving plants from growing rooms to chambers. This effect was shown by a decrease, rather than increase, in stomatal conductance of plants moved from the growing room at 36% RH to a control chamber at 67% RH (Table 3).

During the period of exposure, closure of stomata was observed on the plants for most treatments (Table 3). There was greater closure with exposures of SO_2 and the mixture of pollutants than with exposures of ozone, but this increased closure occurred only when plants were pretreated at low RH. When plants were pretreated at high RH, there were indications of less closure with these pollutants than with ozone.

Discussion

This study emphasizes that humidity conditions prior to exposure of plants to air pollutants affect the extent of injury from pollution. The significant effect that preconditioning RH has on pollutant injury demonstrates the importance of characterizing RH under which plants are growing, as well as the amount of time plants are allowed to equilibrate at different humidities in exposure chambers prior to the addition of pollutants. In many previous studies with air pollutants, RH in which plants were growing prior to exposure has neither been controlled nor reported (6, 7, 8, 10, 11). Air pollution studies undertaken outside or in greenhouses generally will have RH fluctuations from day to day, and thus pollution sensitivity of the plants will usually be constantly changing.

The marked increase in injury of plants preconditioned at high compared to low RH is in contrast to work by Dunning and Heck with *P. vulgaris* and *N. tabacum* (1), where variable humidity responses were found. However, Dunning and Heck altered RH only during light periods with humidity at night being 80% in all treatments. In our study, different RH levels were maintained during both light and dark periods. In addition, Dunning and Heck compared only a 20% difference (60% to 80%) in RH while in this study comparisons were made with a 41% difference in RH.

Stomatal conductance of plants in growing rooms prior to exposure was directly related to injury from the different pollution treatments. Plants grown at high RH had higher stomatal conductances than plants at low RH, reflecting increased opening of stomata which would allow greater pollutant uptake and the potential for greater injury. Norby and Kozlowski (9) showed that increasing conductance with increasing RH was associated with more pollutant uptake and greater injury. McLaughlin and Taylor (8) also found more injury at high than low RH due to increased pollutant uptake, but they concluded that internal leaf resistances rather than stomatal conductances were important in determining uptake. Thus, it should be pointed out that the evidence of increased stomatal uptake of pollutants with elevated RH levels can not preclude the possibility that high RH levels also may be producing physiological changes within the plants that predispose the tissues to high sensitivity for injury.

In contrast, stomatal conductances of plants at the end of the exposure to the different pollutants could not be related consistently to the amount of injury. This lack of association may have resulted because of changes in stomatal conductances over the two hour period of exposure or because the RH treatments caused variations in internal leaf resistances to pollutant uptake as proposed by McLaughlin and Taylor (8).

This study also showed that the transfer of plants from growing rooms to exposure chambers may alter the sensitivity of plants to pollutants. This response to transfer was evident by observing stomatal conductances of control plants maintained in chambers without pollutants. Following transfer, the conductance of plants decreased even though the RH level was similar or higher than the RH before transfer. The altered stomatal response of these control plants could have resulted from the changing environmental conditions during movement from

the growing rooms, about 30 m distant, from vibration during movement or from the slightly different environmental conditions in the control chambers.

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Regulation of Ethylene Biosynthesis and Action in Cut Carnation Flower Senescence by Cytokinins

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Abstract. **Cytokinins delay the onset of senescence in cut carnation flowers (*Dianthus caryophyllus*) by affecting the biosynthesis and action of ethylene in the tissue. The onset of senescence is marked by an increase in ethylene sensitivity and production by the tissue. A characteristic rise in 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene, accompanies the initial stages, but the greatest increases in ACC are associated with the decline in ethylene production during the later stages of senescence. Cytokinins delay the onset of senescence and reduce ethylene sensitivity and production. Benzyladenine (BA), a cytokinin, prevents the rise in endogenous ACC levels and reduces the capacity of the tissue to convert ACC to ethylene. The effects of other anti-senescence agents, aminoethoxyvinylglycine (AVG), silver ions and cobalt ions, are compared with those of BA on ethylene sensitivity and production. The mechanism of action of BA in the delay in flower senescence is discussed.**

The onset of senescence in cut carnation flowers is characterized by a large increase in ethylene production (6), an increase in 1-aminocyclopropane-1-carboxylic acid (ACC) levels, (2) and a disruption of water balance (6) which results in petal curl and the other visible signs of senescence of carnation flowers (3, 5, 13).

Aminoethoxyvinylglycine (AVG) and aminooxyacetic acid inhibit ACC synthase and significantly delay senescence (4, 5). Silver ions alone or as silver thiosulfate are thought to delay senescence by blocking the ethylene receptor site and inhibiting ethylene stimulated ethylene synthesis (13). Cobalt ions have been shown to inhibit the conversion of ACC to ethylene in stem tissues (15). Kinetin has been shown to reduce ethylene synthesis and sensitivity of carnation flowers, and endogenous cytokinins may participate in the natural regulation of senescence (3). This hypothesis is supported by more recent evidence that endogenous cytokinin levels decline with age in carnation petals (12).

Cytokinin treatment appears to reduce ethylene synthetase activity, since pretreatment with benzyladenine (BA) resulted in a 90% reduction in the capacity of carnation flowers to convert exogenously supplied ACC to ethylene (4). Mor et al. (9) have reported similar results with detached carnation petals. They further reported that BA pretreatment prevented the normal rise in endogenous ACC levels associated with the onset of senescence (9). Ethylene and BA must be acting directly on the petals themselves, since isolated petals are sensitive to ethylene and BA (4).

Working with intact cut carnation flowers, we have investigated the effects of BA on ethylene biosynthesis and compared its action with those of other inhibitors of ethylene synthesis

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