## Plant Injury by Air Pollutants: Influence of Humidity on Stomatal Apertures and Plant Response to Ozone

Abstract. Ozone injury to Bel W3 tobacco and pinto bean plants increases with increasing humidity. The degree of plant injury sustained correlates well with porometer measurements; this indicates that the size of stomatal apertures increases with increasing humidity. Humidity may therefore influence plant response to all pollutants and may account in part for the greater sensitivity of plants to ozone-type injury in the eastern United States compared with the same species of plants grown in the Southwest.

Plant injury by sulfur dioxide may vary with atmospheric humidity (1, 2). We noted that, although ozone decay was more rapid, more plant injury occurred during fumigations with a single injection of ozone when the chamber was filled with plants than when the chamber contained fewer plants. Thus we suspected that plant injury by ozone also varies with atmospheric humidity.

Bel W3 tobacco and pinto bean plants were exposed to serial concentrations of ozone at various humidities in an aluminum chamber  $(1.13 \text{ m}^3)$  (3) at constant temperature  $(31^\circ \pm 1^\circ C)$  and constant light intensity (8800 lu/m<sup>2</sup> from fluorescent lamps). Humidity (water vapor from plant transpiration) was maintained by metering charcoalpurified, dried air into the chamber. Ozone was produced (Welsbach Ozone Generator model T-23) from tank oxygen, and ozone concentration was maintained by diluting and metering a second stream of purified, dried air into the chamber. Total air flow was monitored, and the amount injected from each stream was varied to maintain humidity.

The plants were grown in a greenhouse in charcoal-filtered air, in 26cm plastic pots containing soil adjusted to pH 6.5 after heat sterilization. The soil consisted of one part compost soil and three parts sand. Tobacco plants were used when eight full-sized leaves per plant had developed; the bottom three leaves were not included in the injury estimates. The tobacco plants may have been low in nitrogen as the lower leaves were light green. Bean plants were used when the second trifoliate leaf was just reaching maturity, and the oldest trifoliate leaf was used in order to estimate the damage to the surface area.

The plants were kept in darkness about 12 hours prior to fumigation. They were then watered, placed on the rotating table in the chamber, and equilibrated for 2 hours at the light intensity and temperature used during fumigation. During equilibration the humidity was rapidly adjusted as desired. The leaf permeability index was determined from porometer readings made just prior to ozone fumigation. The ozone concentration remained constant within  $\pm .015$  ppm throughout the 90-minute exposures.

Humidity greatly influences plant response to ozone (Table 1). Tobacco plants fumigated at high humidity (95 percent) with 0.10 ppm ozone developed flecking equivalent to those fumigated at 0.30 ppm in air of low relative humidity (26 percent). At high humidity, injury developed not only at lower ozone levels but was also more intense for any given ozone concentration. Porometer readings suggest that the variation in plant response at various humidities is related to the size of stomatal apertures (Table 1, Fig. 1). At high humidity, the presence or absence of water droplets on the leaves of tobacco and bean plants did not alter the degree of injury.

Humidity influences the amount of injury that develops on pinto bean plants (Table 1). However, the spread between effective concentrations at the various humidities is less, and the amount of injury recorded at effective concentrations is greater than for the



Fig. 1. Relation between stomatal activity and humidity. Permeability index (PI) of tobacco and bean foliage is taken as a measure of stomatal activity; PI = 1n $(P_0/P_E)$ ;  $P_0 = 200$  mm-Hg,  $P_E = final$ pressure after 10 seconds.

Table	1. 7	The effe	ct of	humidi	ty c	on plar	it injur	y b	y ozoi	ne. Pe	rcentage	of	foliage	injury	to	Bel	W3	tobacco	was	estimated	on	five	mature
leaves	per	plant;	perc	entage	of f	oliage	injury	to	pinto	bean	plants	was	estimate	ed on	the	oldest	t trif	oliate lea	af of	each plan	t.		

Relative	Permeability	Percentage foliage injury at ozone concentration (ppm)*								
(% at 31°C)	index†	0.05	0.10	0.15	0.20	0.30	0.35	0.40	0.45	
			Bel W3 to	bacco						
26	0.56	0	0	0	0	9	23	34	51	
51	0.73	0	0	2	Õ	39	70±	94±	51	
95	0.84	0	8	34‡	47:	50±	77±	2.17		
95-wet leaves		0	6	33	53‡	50‡	86‡			
			Pinto be	ean .						
26	0.44	0	0	0	0	0	6	0	21	
51	0.54	0	0	Ō	Õ	75	95	95	21	
95	0.65	0	10	0	55	80	88	100		
95-wet leaves	-	0	8	0	80	75	90	100		

\* Measured continuously by a Mast 725-6 equipped with a modified strip chart recorder.  $\dagger$  Permeability index [ln ( $P_0/P_E$ )];  $P_0$  = initial pressure (200 mm-Hg);  $P_E$  = final pressure (after 10 seconds).  $\ddagger$  These plants exhibited fleck and spot necrosis on the most susceptible leaves, as well as tip injury to immature leaves; the others exhibited ozone flecks.

Bel W3 tobacco plants. The injury estimates for beans involve only one sensitive leaf, whereas the tobacco data represent the average estimates of five leaves, some of which were not at maximum sensitivity.

The literature contains reports of varying threshold concentrations producing plant injury (4). In these reports humidity records are usually not included. Variability may be due to differences in atmospheric humidity at the time of plant conditioning and exposure, and humidity records should be included with each study. Toxicant injury to apple fruit at lenticels in which humidity was found to have no influence has been reported (5). This is understandable since the lenticels on mature apples are permanently open. Irrigation (sprinkling) is also reported to increase plant response (weather fleck) to ozone (6). While no explanation for this response is given, it is clear that irrigation would greatly affect humidity around the sprinkled plants, thus increasing susceptibility to ozone. In addition the marked effect of humidity on stomatal apertures should influence plant response to all pollutants. This may ex-

plain the greater sensitivity (7) to ozone injury obtained with plants grown in the eastern United States as compared with those grown in the Southwest.

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## **Pulmonary Gas Transport Time: Larynx to Alveolus**

Abstract. The transport time of gas from the larynx to pulmonary alveolar capillary blood was directly measured by injecting a bolus of carbon monoxide into the inspired airstream of dogs and photoelectrically monitoring the formation of carboxyhemoglobin in the capillaries on the surface of the lung. The rapidity of transit (0.3 second) implies that gas transport during inspiration is facilitated by an interaction between bulk flow and diffusion.

It is currently accepted that inspired gas moves from the nose to alveolar surfaces by combinations of turbulent and laminar flow in the large airways (bulk flow where movement is caused by pressure gradients) and finally by still gas diffusion (where gas transfer is caused by concentration gradients only) near the alveoli. Because of the anatomical complexity of the bronchial tree, the undetermined interactions of the diffusion and bulk flow, and the unknown transit times for inspired gas to reach the alveoli, realistic modeling in any detail of normal gas transport mechanisms and their disruption by disease has been virtually impossible. We now present results of a technique that measures pulmonary gas transport time.

Spectrograms of reduced and oxyhe-

moglobin (HbO<sub>2</sub>) have identical absorption coefficients at a wavelength of 805 nm, whereas carboxyhemoglobin (HbCO) has a significantly lower absorption at that wavelength (1). Thus a photodetector observing blood at 805 nm is insensitive to changes in the level of HbO2, but is very sensitive to the concentration of HbCO. This principle has been used to measure transit time of gas from larynx to alveolus. A bolus of carbon monoxide is injected into the inspired airstream, and the formation of HbCO in the capillaries on the surface of the lung is photoelectrically monitored.

Mongrel dogs (23 to 30 kg), anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg), are tracheotomized and then artificially ventilated (by intermittent positive pressure breathing) throughout the procedure. A transparent thoracic window is implanted in place of a resected portion of the eighth rib (2). A box from which light is excluded containing an RCA-925 phototube is attached to the external surface of the window such that the photocathode is directed toward the surface of the lung. Illumination appropriately filtered to 805 nm from a General Electric-EJV quartz halide lamp is directed through the box onto the surface of the lung under the window. The phototube output is recorded by an Electronics for Medicine DR-8 oscillograph at an amplification sufficient to produce full-screen deflections at the peak of HbCO formation (Fig. 1a). Under our experimental conditions there is approximately a 1 percent increase in reflected light at the peak of the response.

A spring-driven 10-ml syringe is loaded with CO (100 percent). A signal at a predetermined phase of respiration from the DR-8 trigger channel electrically releases the spring which drives the CO rapidly (35 msec) into the base of a T-tube connected to the tracheal cannula at a point equivalent to the larynx. The base of the T-tube, having a dead space of 4.5 ml, reduces the amount of CO which enters the inspired airstream as a bolus to 5.5 ml. The point of injection is demonstrated by a rapid deflection in the signal from a pneumotograph connected to the respiratory side of the T-tube. The animals are respired by a Harvard piston respirator (model 607D) in which piston speeds during inspiration and expiration can be varied independently. Tidal volume has been held constant at 350 ml for all experiments.

We have studied two variables. First, the length of time for a complete inspiration is varied to cover either 0.60 second (designated fast inspiration) or 0.98 second (designated slow inspiration). The inspiratory flows closely approximate the positive half of a sine wave with peak flows of 45.3 liter/min and 25.4 liter/min for fast and slow inspiration, respectively. Second, the CO was injected either before the beginning of inspiration (designated negative injection time) or when inspiratory flows reach half of their maximum rate (designated positive injection time). Transport times for negative injection times are measured from the beginning of inspiration; measurements are made from the point of CO injection for