Effect of Temperature on Stomatal Conductance and Ozone Injury of Pinto Bean Leaves

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ABSTRACT

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Phaseolus vulgaris 'Pinto 111' unifoliolate leaves exposed to 0.10 ppm (196 $\mu g/m^3$) of ozone for 3 hr at 15, 24, and 32 C developed 25, 3, and 24% foliar injury, respectively. Stomatal conductance of both exposed and unexposed leaves was lowest at 15 C, intermediate at 24 C, and greatest at 32 C. Exposure to ozone significantly reduced stomatal diffusion at 32 C but not at 24 or 15 C.

Unifoliolate leaves of *Phaseolus vulgaris* L. 'Pinto 111' are less sensitive to ozone when exposed at 21 C than at 16, 27, or 32 C (3). A preliminary study in our lab also revealed a depression in sensitivity near 24 C. In this study, we sought to determine whether the reduction in foliar injury of pinto bean unifoliolates was related to decreased stomatal conductance at 24 C.

MATERIALS AND METHODS

Pinto bean seeds were germinated in trays of vermiculite within growth chambers maintained at 24 C and 75% RH. Two days after cotyledons emerged, we transplanted 90 seedlings at a uniform stage of development into 950-cc plastic pots (one seedling per pot) containing a 1:1:1 (v/v) peat:perlite:soil mixture. The transplanted seedlings were grown for 5 days in growth chambers at 24 C and 75% RH, with a 12-hr photoperiod of 25 klux at canopy height beginning at 0600 hr. The plants were fertilized every other day with 16.5 g of 20-19-18 (N-P-K) water-soluble fertilizer in 7.6 L of water.

We subdivided the seedlings into three subsets of 30 plants each and used an electrical diffusion porometer (9) to measure abaxial stomatal conductance before exposure to ozone. Conductance of both unifoliolate leaves on each of three randomly selected plants from each subset were measured at 1000 and 1055 hr at 24 C and 75% RH. The three subsets of plants were then placed in the exposure chamber (11) at 1100 hr and subjected to 0.10 ppm (196 $\mu g/m^3$) of ozone at 75% RH and 15, 24, or 32 C, respectively.

During each exposure (1100 to 1400 hr), 15 plants were chosen at random from each subset and removed three plants at a time at 1130, 1200, 1230, 1300, and 1330 hr. The conductance of the six unifoliolate leaves of each group of three plants was measured immediately after removal from the exposure chamber. The plants were then returned to the growth chamber at 24 C. The 15 remaining plants were removed from the exposure chamber at 1400 hr and also placed in the growth chamber. Conductance rates for these plants were measured immediately after removal (at 1405 hr) and again at 1430 and 1530 hr.

We estimated visible foliar injury 3–5 days after exposure on the 15 plants exposed to ozone for 3 consecutive hours and on the three plants removed at each half-hour interval during exposure at each temperature. Injury was evaluated in 10% increments, with values of 0, 1, 5, 95, 99, and 100 included at the extremes. A similar study was conducted using 90 unexposed control plants maintained in charcoal-filtered air in the growth and exposure chambers. Only one exposure chamber was used; controls and exposed plants were treated on separate days.

A square root transformation was performed on the conductance data and an arc sin of the square root transformation was performed on the foliar injury data to stabilize the variance in each case. Data were analyzed using analysis of variance; Fisher's LSD test ($P = 0.05$) was used to separate control and treatment means (8). The study was replicated three times, resulting in a total of 270 plants exposed to ozone and 270 control plants.

RESULTS

Macroscopic symptoms. Ozone induced 3% visible foliar injury (adaxial stipple) at 24 C. This value was significantly less than the 25 and 23% injury induced at 15 and 32 C, respectively. At 15 and 32 C, foliar injury values were not significantly different.

Stomatal conductance. At 15 C, exposure to ozone significantly reduced the stomatal conductance rate at most time periods; however, at 1000 and 1055 hr plants that were to be exposed to ozone already had significantly lower conductance values than their control counterparts before exposure (Fig. 1). Therefore, the conductance rates of exposed and control plants at 15 C were not significantly different during exposure.

Conductance rates of both groups of plants decreased by about 50% by 1130 hr, a half hour after the plants had been moved from the growth chamber at 24 C to the exposure chamber at 15 C. After exposure (at 1405 hr), plants were returned to the growth chamber at 24 C. By 1430 hr, conductance rates of control plants increased to the level observed before exposure, whereas the conductance of plants exposed to ozone remained at the level observed during the exposure.

At 24 C, control plants had a significantly greater conductance rate at 1230 hr than did plants exposed to ozone. At all other times, the conductance rates of controls and plants exposed to ozone were not significantly different before, during, or after exposure at 24 C (Fig. 1).

The stomatal conductance rates of controls and plants to be exposed to ozone at 32 C were not significantly different before exposure (1000 and 1055 hr) when still at conditions of 24 C and 75% RH. When controls and plants to be exposed to ozone were placed in the exposure chamber at 32 C at 1100 hr, stomatal conductance increased and continued to do so until 1330 hr. By that time (2.5 hr after the start of exposure), the conductance rate of controls had approximately tripled and that of plants exposed to ozone had doubled. During the last half hour of exposure (between 1330 and 1400 hr), stomatal conductance declined for both controls and plants exposed to ozone.

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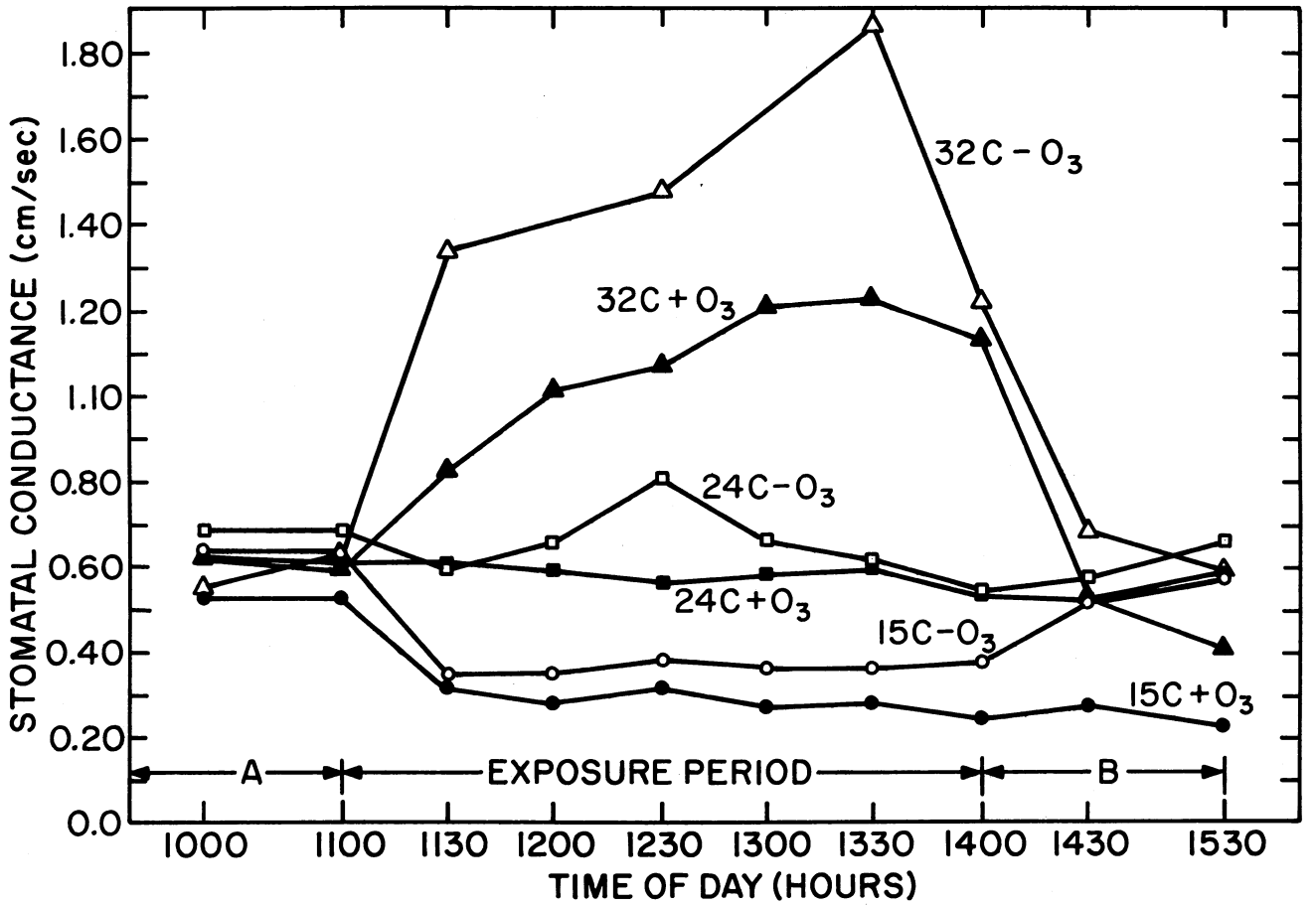

Fig. 1. Stomatal conductance rates of pinto bean unifoliolate leaves at 24 C before exposure (A), during exposure at 15, 24, or 32 C, and at 24 C after exposure (B). Plants were exposed to 0 or 0.10 ppm of ozone between 1100 and 1400 hr.

After exposure, plants were returned to the growth chamber at 24 C. The conductance rate of control plants returned to the value observed before exposure, whereas the conductance of plants exposed to ozone continued to decline. The conductance of plants exposed to ozone was significantly less than that of the controls at each time interval during and after exposure.

DISCUSSION

The stomatal conductance rate during exposure was not related to the severity of ozone injury induced over a range of exposure temperatures. Conductance rates increased with increasing temperatures; in contrast, ozone induced greater foliar injury at 15 and 32 C than at 24 C, indicating that physiologic factors other than decreased gaseous uptake were responsible for the decreased foliar injury at 24 C.

Our observation that the stomatal conductance rate of unexposed plants increased with increasing temperature (Fig. 1) is consistent with previous reports (6,7). Decreased stomatal conductance of plants exposed to ozone was demonstrated statistically only at the highest temperature, where stomatal opening and conductance rates were greatest. In contrast, petunia (*Petunia hybrida* Vilm.) cultivars exhibit stomatal closure when exposed to ozone at 50% RH, but not at 90% RH (4). Stomatal opening is generally considered to be greater at higher humidities (9). In the tests with petunia cultivars, the stomatal conductance of control plants maintained at 50% RH was slightly greater than the conductance of comparable plants kept at 90% RH (4). No explanation was given for this apparent anomaly.

Vargo et al (10) reported that soybean (*Glycine max* (L.) Merr. 'Chippewa 64') plants exposed to ozone at 24 C and 75% RH exhibit stomatal closure. Beckerson and Hofstra (1) also reported that ozone generally decreases stomatal conductance of *P. vulgaris* 'Sanilac' unifoliolates and that stomatal conductance changes during exposure are not related to the degree of visible injury induced by ozone.

We do not understand the mechanism of stomatal closure in response to ozone. Ozone reduces photosynthesis (2,5), perhaps enough to elevate starch levels and decrease the turgor of guard cells, resulting in stomatal closure. The influence of ozone on conductance was significant only at 32 C. More ozone may have entered the plant at 32 C than at 24 or 15 C. This could cause a rapid decrease in the rate of photosynthesis, an increase in the starch content of guard cells, and a subsequent partial closure of stomata when compared with unexposed controls.

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