Aftereffects of Low and High Temperature Pretreatment on Leaf Resistance, Transpiration, and Leaf Temperature in *Xanthium*[1]

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ABSTRACT

Leaf resistance for water vapor (total diffusion resistance minus boundary layer resistance), transpiration, and leaf temperature were measured in attached leaves of greenhouse-grown *Xanthium strumarium* L. plants that had been pretreated for 72 hours with high (40 C day, 35 C night), or low (10 C day, 5 C night) air temperatures. Measurements were made in a wind tunnel at light intensity of 1.15 cal cm^{-2} min^{-1}, air temperatures between 5 and 45 C, and wind speed of 65 cm sec^{-1}. Leaf resistances in low temperature pretreated plants were higher (8 to 27 sec cm^{-1}) than in controls or high temperature pretreated plants (0.5 to 8 sec cm^{-1}) at leaf temperatures between 5 and 25 C. Thus, the pretreatment influenced stomatal aperture.

The temperature experienced by a plant during its immediate past history produces an effect on the net photosynthesis measured subsequently at any other temperature (3, 5, 6, 10–14). This aftereffect of temperature may be localized within the CO_2 assimilatory apparatus within the leaf (4). It may also be partially due to the stomata (5), since they govern the rate of supply of CO_2 from the air to the carbon fixing tissues. Most studies of aftereffects of temperature upon photosynthesis have neglected this aspect.

The stomata also regulate transpiration, and from simultaneous measurements of transpiration, leaf temperature, and water vapor pressure of the air, it is possible to calculate the total diffusion resistance to water vapor flux (Σr). This resistance can be partitioned into the diffusion resistance across the boundary layer (r_a) of air surrounding the leaf and the diffusion resistance of the leaf (r_l) that is caused by the epidermis, cuticle, and stomatal apparatus. The boundary layer and leaf resistances are in series. When boundary layer resistance is constant and much less than leaf resistance, changes in total diffusion resistance are brought about by the stomata. Since water vapor and carbon dioxide follow similar routes between the moist surfaces within the leaf and the surrounding air (although net movement is in the opposite direction), studies of the transpiration resistances can yield information about the resistances to CO_2 exchange.

In this paper we report results of studies on the aftereffects of 72-hr temperature pretreatment upon the leaf resistance to water vapor loss and the consequent changes in transpiration and leaf temperature in attached leaves of *Xanthium strumarium* L.

MATERIALS AND METHODS

The Wind Tunnel. A wind tunnel in which air temperature, humidity, and velocity could be controlled, with a test section large enough to accomodate two leaves, each up to 12 cm long, was used for all experiments. Air in the wind tunnel could be brought to the desired temperature (± 0.25 C) in the range 3 to 50 C as it passed through a heat exchanger. The temperature of the heat exchanger was in turn determined by water from a thermostatically controlled reservoir. Light was produced by a single, air-cooled, 6.0 kw xenon arc lamp (Osram, series XQO). The area of uniform light produced by this lamp was approximately 40 cm in diameter when the irradiance was 0.80 cal cm^{-2} min^{-1}. The irradiance used in this study was 1.0 to 1.3 cal cm^{-2} min^{-1} net radiation of which 0.80 cal cm^{-2} min^{-1} was from the lamp in the range 0.38 to 2.00 nm. The remaining portion was long wave radiation emitted by the Mylar ceiling and the floor of the wind tunnel. The intensity of the long wave portion of the net radiation increased as the air temperature in the wind tunnel increased. In all experiments, the average wind velocity at the leading edge of the leaf was kept constant at 63 cm sec^{-1}. Experiments with blackened, copper-plated leaf models showed that this wind velocity corresponded to an average boundary layer resistance of 0.8 sec cm^{-1} in leaves that were 10.0 to 11.0 cm across the downwind dimension. Humidity was either high or low. When high humidity was desired, water was allowed to flow through fiberglass filters in the air stream. A degree of control over the water vapor pressure in the air was obtained by regulating the temperature of the water supplied to the filters. Low humidity was attained by stopping the water flow across the filters and passing a portion of the air that was circulating in the wind tunnel through a dryer. As air temperature was varied between 5 and 45 C, the relative humidity of moist air varied between 70 and 55%. Dry air varied between 18 and 5% for the same temperature range.

Measurements. Leaf temperatures were measured with 40-gauge (0.079 mm) copper-constantan soldered thermocouples. Three thermocouples were joined in parallel, and the output from all three was taken to be a measure of the average leaf temperature. Previous experiments (2) had shown that temperature across a *Xanthium* leaf can vary by as much as 5 C at low wind speeds. The thermocouples were inserted into the leaf tissue by threading. The thermal junction was held against the leaf between two of the holes made by threading so that it was not in contact with ruptured leaf tissue. After the thermocouples had been inserted, the leaf temperature they measured was read on a potentiometer and compared with a simultaneous measurement by a Barnes precision radiation thermom-

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eter (Model PRT-10), which was calibrated before and after each reading against an aluminum Leslie cube filled with water at a known temperature. The two measurements agreed within 1.0 C, but the radiation thermometer always gave the higher reading. During the experiments, leaf temperature was recorded on a Leeds and Northrup multipoint recorder. The thermocouples were referenced against a water bath whose temperature was measured with a mercury thermometer. Air temperature was measured by a single 40-gauge soldered copper-constantan thermocouple placed upwind from the leaves and shaded from the direct beam of the xenon arc lamp. Wet bulb temperature was also measured by a 40-gauge thermocouple sewn into a small wick supplied with distilled water. Comparisons of air and wet bulb temperatures were made with those made by an Assman psychrometer.

Transpiration was measured by weight loss from a potted plant. All leaves but one were excised, the pot was covered with aluminum foil and two layers of polyethylene bags, and the stem was wrapped with masking tape. Weight loss for the period of an experiment was divided by the leaf area (upper plus lower surfaces). Transpiration units are g cm^{-2} hr^{-1}.

The leaf resistance was calculated from measurements of transpiration rate made with a diffusion porometer constructed according to the design of van Bavel (15). Morrow and Slatyer (8) discuss the necessary precautions when using this instrument. Calibration of the porometer was carried out in two steps. First, in order to determine the temperature response of the sensor and porometer cup combination, the procedure of van Bavel was followed in which acrylic tubes were used to simulate diffusion resistance by varying the length of the diffusion pathway (15). Second, comparisons were made between values of the leaf resistance determined with the porometer and values calculated from simultaneous measurements of transpiration (V) from a single attached leaf, vapor pressure difference between the leaf and the air (Δe), and boundary layer resistance (r$_a$) according to the relationship

$$r_l = 3.6 \times 10^5 \frac{\Delta e}{V} \frac{c_p \rho}{aL} - r_a \sec \text{cm}^{-1} \qquad (1)$$

where c$_p$ is specific heat at constant pressure, ρ is density of air, L is the latent heat of vaporization, and a is the psychrometric constant. The above relationship (equation 1) results in an erroneous estimate or r$_l$ when the number of stomates on upper and lower surfaces of the leaf is different (7). But this error is small when the number of stomates on the two surfaces is about equal. In our plants, the ratio of stomates on the lower to upper surface was about 1.2.

Pretreatment. Plants were removed from the greenhouse and placed in growth chambers at either 40 C day, 35 C night (high temperature pretreatment); or 10 C day, 5 C night (low temperature pretreatment). Plants remaining in the greenhouse were used as controls. All but the three uppermost leaves were removed before pretreatment was begun. Net radiation at leaf level in the growth chambers was approximately 0.5 cal cm^{-2} min^{-1}. The relative humidity in the high temperature chamber was 21% during the day and 26% at night. In the low temperature chamber it was 65% both day and night. The optimum length of the pretreatment was determined in preliminary experiments in which leaf temperature and diffusion resistances were measured after 24, 48, 72, 144, 336, and 772 hr. The effect of pretreatment could be measured after 24 hr and was nearly fully developed by 72 hr.

The Experiment. After the pretreatment had been completed, plants were removed from the growth chamber, and all but the leaf having a total surface area closest to 160 cm^2 were excised. To keep it from fluttering, a wire loop, just

slightly larger than the leaf was attached with small pieces of masking tape. Two plants were placed in the wind tunnel in the dark. The time from removal of plants from the growth chambers to placing them in the wind tunnel was approximately 45 min. After an equilibration period of 1 to 2 hr to insure that stomata were fully closed at the start of the experiment, the light was turned on, and another equilibration period allowed. This second period continued until the leaf temperature had stabilized and remained stable for 30 min. Stomata in Xanthium take longer to open at low temperature than at high temperatures. At this time, the plant was removed from the test section, weighed on a Mettler Balance and then returned to the wind tunnel. The thermocouple leads in the leaf were attached to copper-constantan leads from the wind tunnel to the recorder by small plugs so that the plant could be removed from the wind tunnel quickly. The temperature in the room outside the test section was within 2 to 3 C of that inside the test section. Upon returning the plants to the test section, a run was started and continued for approximately 1 hr. At the end of the run, the leaf resistance was measured with the porometer, and the plant reweighed. Measurements on any two leaves were made at only one of five different air temperatures (5, 15, 25, 35, and 45 C) but in both moist and dry air. Sets of measurements were thus obtained for each temperature pretreatment at each air temperature and humidity. Results are from 42 separate leaves.

RESULTS

Leaf resistance (r$_l$) is defined in this paper as the total diffusion resistance to water vapor (Σr) minus the diffusion resistance of the boundary layer (r$_a$). Boundary layer resistance is accounted for in the calibration of the diffusion porometer (14). Each data point in Figure 1 is a composite of the resistance measured for each surface and is calculated as follows

$$r_l = \frac{2(r_l^u \cdot r_l^l)}{r_l^u + r_l^l} \sec \text{cm}^{-1} \qquad (2)$$

where u and l refer to upper and lower leaf surfaces. The value of the leaf resistance for each surface was the average of three measurements. Each measurement was made at the location of the three thermocouples in the leaf. Results of measurements of leaf resistance in dry and moist air for the three pretreatments are given in Figure 1. Leaf resistance is higher in the low temperature pretreated plants than in the controls or high temperature pretreated plants. Pretreatment with high temperature resulted in little difference from controls with the exception that the high temperature pretreated plants did not respond to changes in humidity as did the controls or low temperature pretreated plants. The humidity within the high temperature growth chamber was low during pretreatment and this may have affected the ability of the stomata to respond to changes in humidity.

There is considerable scatter in the data for the low temperature pretreated plants. This is due in part to the decreased sensitivity of the porometer at high values of leaf resistance, and in part to the fact that leaf resistance in Xanthium strumarium L. tends to vary widely, especially as it increases (2).

The data in Figure 1 are supported by the results of the transpiration and leaf temperature measurements shown in Figure 2. There was little difference between the transpiration and temperature of plants pretreated with high temperature and the controls, but the low temperature pretreated plants transpired very little below 35 C leaf temperature. Between 25 and 35 C air temperature, leaf temperature in low temperature
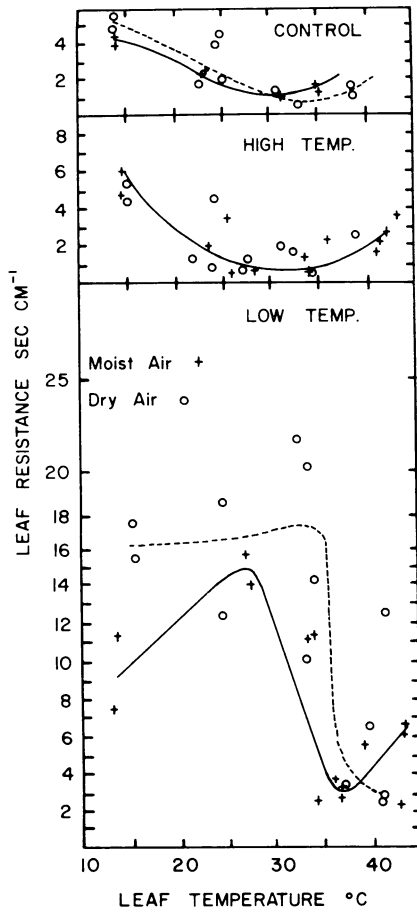
FIG. 1. Leaf resistance at different leaf temperatures in leaves pretreated for 72 hr with low (10 C day, 5 C night), high (40, 35 C), and control (26, 21 C) temperatures. Boundary layer resistance constant for all treatments (0.8 sec cm^{-1}). Light intensity, 1.15 cal cm^{-2} min^{-1}.

pretreated plants was 7 to 10 C higher than in high temperature pretreated plants or controls.

Results of measurements of leaf resistance made in the dark and in the light on high and low temperature pretreated plants are given in Table I. Resistance is lower in the light than the dark for both treatments.

DISCUSSION

We wanted to know whether aftereffects such as those reported for net photosynthesis (3, 5, 6, 10–14) can be seen in the diffusion resistance of the leaf to water vapor. Our results show that after 72 hr of low temperature (10 C day, 5 C night) leaf resistance is increased, compared with controls (Fig. 1). Plants pretreated with high temperature (40 C day, 35 C night) show little change from controls. The consequences of increased leaf resistance in low temperature pretreated plants are reduced transpiration and elevated leaf temperatures. In the air temperature range 20 to 40 C, leaf temperatures of low temperature pretreated plants were between 6 to 10 C higher than controls or high temperature pretreated plants (Fig. 2). These results demonstrate the degree to which leaf resistance affects leaf temperature by its effect upon transpiration.

Are increases in leaf resistance associated with low temperature pretreatments due to changes in the cuticular or stomatal diffusion resistance? Norris and Bukovac (9) found no

change in the penetration of naphthaleneacetic acid through isolated pear leaf cuticle after 48 hr at 5 C. Minimum leaf resistance in the dark (when stomata are presumed to be closed) in low temperature pretreated plants was approxi-
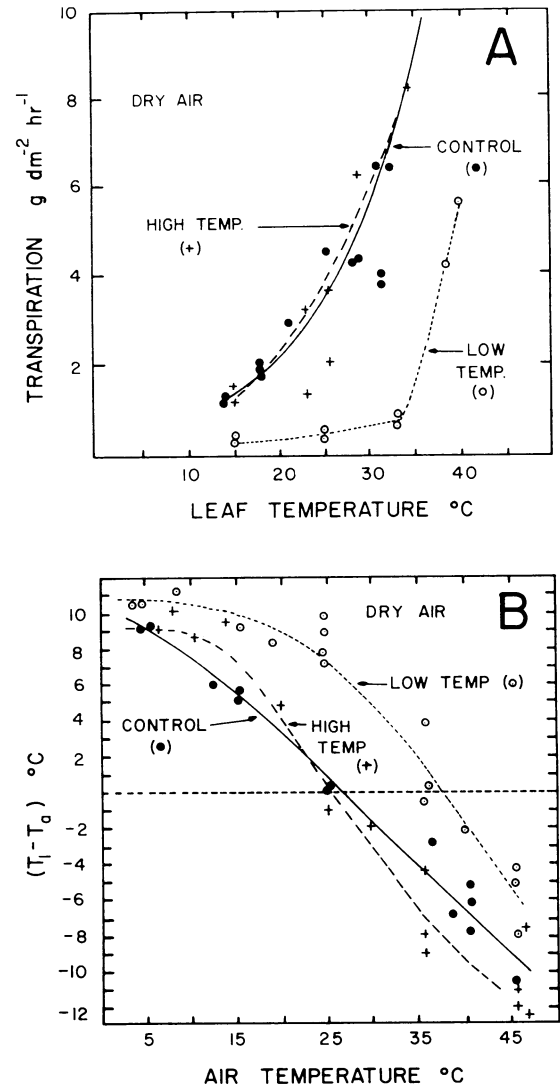

FIG. 2. Transpiration (A) and leaf temperature (B) in leaves pretreated with low, high, and control temperatures. Results are shown for measurements made in dry air. Results in moist air reflected the same differences between treatments, but absolute values were not the same as in dry air. Other conditions are the same as in Figure 1.

Table I. *Leaf Resistance Measured in the Light and Dark for Low and High Temperature Pretreated Plants*

Relative humidity was 20%, light 1.15 cal cm^{-2} min^{-1}, wind 65 cm sec^{-1} (boundary layer resistance 0.85 sec cm^{-1}), and air temperature 30 C. Data are for two experiments.

Pretreatment Temperature	Leaf Resistance (r_1)	
	Light off	Light on
	sec cm^{-1}	
Low: 10 C day, 5 C night	76.17	12.86
	52.24	6.35
High: 40 C day, 35 C night	14.84	0.98
	5.46	1.41

mately an order of magnitude higher than leaf resistance in the light (Table I). Thus, while there is the chance that low or high temperature pretreatment had an effect upon the cuticle of our leaves, there is reason to suppose that it did not, and that aftereffects of temperature pretreatment upon leaf resistance are due to changes in stomatal aperture and not to alterations in the amount or physical characteristics of the surface waxes.

The pretreatment with low or high temperature does not appear to have rendered the stomata inoperative. Results of measurement of resistance in the light and in the dark show that leaf resistance is lower in the light (Table I) for both treatments. Our results show that low temperature pretreatment inhibits stomata and high temperature does not; they do not provide a satisfactory explanation for the reduction in photosynthesis obtained when plants are pretreated with low or high temperature. If leaf resistance to water vapor diffusion is increased it seems reasonable to assume that CO_2 diffusion, and hence net photosynthesis, will be reduced. But the increase in stomatal diffusion resistance may just as well be a result, rather than a cause, of reductions in net photosynthesis.

If the CO_2 assimilatory system within the leaf is inhibited by low temperature pretreatment, then the internal CO_2 concentration would be expected to rise and the stomata would be expected to respond by closing (1).

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