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Nonstomatal Inhibition of Photosynthesis at Low Water Potentials in Intact Leaves of Species from a Variety of Habitats[1]

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ABSTRACT

Mesophyll resistance to CO_2 uptake was calculated from gas exchange data on intact leaves of 12 species of woody plants. Plants studied were native to habitats ranging from streamsides to deserts. Gas exchange measurements were made at light saturation and constant temperature to eliminate possible effects of light and temperature on estimates of mesophyll resistance. Cuticular transpiration was measured and used in calculation of stomatal resistances from whole leaf transpiration rates. In all species examined, an increase in mesophyll resistance was observed as leaves dried. The increase in mesophyll resistance in all cases occurred at the same water potential as the initial decline in net photosynthesis, and was accompanied by an increase in stomatal resistance.

The occurrence of nonstomatal inhibition of photosynthesis due to low leaf water potentials in intact leaves is still debated. Hsiao (7) reviews the evidence for a nonstomatal inhibition of photosynthesis. The most common type of evidence has been observation of an increase in the calculated mesophyll or residual resistance to CO_2 uptake at low leaf water potentials (*e.g.* 15 17). This evidence has been supplemented by evidence of disruption of photosystem activities, carboxylation activity, and electron transport in intact leaves or isolated systems at reduced water potentials (1, 5, 8, 9, 14). Recently, Mederski *et al.* (11) and Moldau (12) have suggested that procedural errors in gas exchange analysis could account for the observed increase in mesophyll resistance at low water potentials in intact leaves. Moldau (12) suggests that failure to take cuticular transpiration into account may result in a progressive overestimation of mesophyll resistance as leaves dry. Mederski *et al.* (11) suggest that as leaves dry, leaf temperature increases, and that the higher temperature rather than low water potential may cause the increase in mesophyll resistance. Slatyer (17) observed increases in mesophyll resistance at low water potentials in two species (corn and cotton) but not in four more drought-adapted species (wheat, millett, and two *Atriplex* species). This suggests that the occurrence of water stress-induced changes in mesophyll resistance may be species-specific, and related to the aridity of the natural habitat.

Results show that when leaf temperature is carefully controlled, and when cuticular resistance is measured and taken into account, an increase in mesophyll resistance at low leaf water potentials is observed in a variety of woody plant species native to habitats ranging from wet streamsides to deserts. The increase in mesophyll resistance is observed in all cases at the same water potential as the first reduction in net photosynthesis and increase in stomatal resistance.

MATERIALS AND METHODS

Species studied were *Alnus oblongifolia*, *Fraxinus pennsylvanica* ssp. *velutina*, *Pinus ponderosa*, *Juniperus deppeana*, *Vauquelinia californica*, *Simmondsia chinensis*, *Acacia greggii*, and *Larrea divaricata* from Arizona, and *Alnus rugosa*, *Fraxinus pennsylvanica*, *Acer saccharum*, and *Pinus rigida* from New York. The Arizona species were collected in the Santa Catalina Mountains, near Tucson. The New York species were collected near Ithaca. Plants were grown from seed, seedlings, or cuttings under controlled conditions in Ithaca. The plants were grown in 15- or 12.5-cm pots with native soil (*Larrea*, *Acacia*, *Simmondsia*, and the New York species), or in a 2:1:1 loam-peat-sand mixture. Before physiological measurements were made, plants were grown under controlled conditions until new leaves had been produced and had fully expanded. Growth conditions were a 16-hr day with an air temperature of 25 C, 75% relative humidity, and 680 μeinsteins m^{-2} sec^{-1} from 400 to 700 nm, and an 8-hr night with an air temperature of 18.3 C, and 70% relative humidity for the Arizona species. The New York species were grown with a 16-hr day at 20 C, 60% relative humidity, and 440 μeinsteins m^{-2} sec^{-1}, and an 8-hr night at 16.5 C, and 60% relative humidity. All plants were fertilized twice a month, and watered every 3 hr except for *Larrea* and *Simmondsia* which were watered every other day.

Net photosynthesis, transpiration, and leaf water potential were measured once a day under controlled conditions on intact leaves as plants dried for 7 to 10 days upon termination of watering. Net photosynthesis was measured using an IR-gas analyzer as a differential instrument to compare the CO_2 content of air before and after passing over leaf material in a cuvette. Transpiration was measured using LiCl sensors to measure the water content of air before and after passing over the leaf material in the cuvette. Leaf water potentials were measured after gas exchange measurements on leaves outside the cuvette using Peltier thermocouple psychrometry (3) on excised discs. Comparisons of leaf water potentials measured in this way with water potentials of leaves inside the cuvette measured with an *in situ* psychrometer (6) showed no differences greater than 1 bar (Bunce, unpublished data).

Net photosynthesis and transpiration were measured under light-saturating conditions as evidenced by the linear response of net photosynthesis to CO_2 concentration (see below). Light was provided by four 500-w GE Quartzline lamps filtered through 11 cm of water. Leaf temperatures were measured with fine wire thermocouples pressed against the underside of the leaf. Air temperature was varied from 26 to 28 C using a radiator inside the cuvette to maintain leaf temperature at 28.3 ± 0.5 C for all measurements. Relative humidity in the cuvette was controlled

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between 70 and 80%. The boundary layer over the leaf was reduced by internal recirculation which produced a wind speed of about 2 m sec^{-1}. At this wind speed, the largest leaves studied had a boundary layer resistance (to water vapor) of about 1 sec cm^{-1} as estimated by a blotter paper model. As the boundary layer resistance was small, and constant in repeated measurements on the same leaf, it was ignored in the calculations.

Net photosynthesis, transpiration, and leaf temperature measurements were made at at least three CO_2 concentrations from 340 to 200 μl l^{-1} at each measurement date. In all species and at all water potentials, a linear response of net photosynthesis to ambient CO_2 concentration was observed. The reciprocal of the slope of the response of net photosynthesis to ambient CO_2 was taken as the sum of stomatal and mesophyll resistance to CO_2 uptake (16). Leaf temperature, ambient vapor pressure of water, and transpiration rate were used to calculate leaf resistance to water loss. Resistance to water loss was also measured in the dark, after 3 hr of darkness, to estimate cuticular resistance to water loss. This is a minimal estimate of cuticular resistance since I have not verified that all stomates were completely closed. Cuticular and leaf resistances to water loss were combined according to the equation:

$$1/r_{\text{leaf}} = 1/r_{\text{cuticle}} + 1/r_{\text{stomata}}$$

to yield a stomatal resistance to water vapor loss. Stomatal resistance to water vapor times 1.6, the ratio of diffusivities of H_2O and CO_2 in air (16), was subtracted from the sum of resistances to CO_2 uptake to yield mesophyll resistance to CO_2 uptake.

RESULTS AND DISCUSSION

In all of the species studied, there was an increase in the calculated mesophyll resistance to CO_2 uptake as the plants dried (Table I). From three to seven individuals of each species were examined, and increased resistance was found in each case. The water potential at which an increase in mesophyll resistance was first measured was also the water potential at which an increase in stomatal resistance was first observed (Table II). The increases in resistance were accompanied by a decrease in net photosynthesis (4). The increases in mesophyll resistance were observed at 28.3 ± 0.5 C, so that changes in leaf temperature can be ruled out as causes of the change in mesophyll resistance.

These species are found in a variety of habitats (10, 18). The two *Alnus* and *Fraxinus* taxa are from wet habitats (riparian). *Larrea*, *Acacia*, and *Simmondsia* are desert shrubs. The other species cover the range of habitats between these extremes. Species differences in sensitivity to water potential are apparent in the data in Tables I and II, and are related to ecological distributions (4) in ways beyond the scope of this paper.

If cuticular transpiration is a large part of whole leaf transpiration, then a large error could occur in the calculation of mesophyll resistance because it was thought that the cuticle is more permeable to water vapor than to CO_2 (12). Hence, there would be an error in using whole leaf transpiration to estimate stomatal resistance to CO_2. The recent data of Ogawa (13) suggest that leaf cuticles may be substantially permeable to CO_2. If this is the case, then one would need to know the relative resistances to CO_2 and water vapor to calculate an exact mesophyll resistance. Among the species studied here, there was no case in which the estimated cuticular transpiration was a large enough part of whole leaf transpiration to cause as much as a 10% error in the calculation of mesophyll resistance. Among these species, errors in the calculation of mesophyll resistance would arise only in the event that the cuticular resistance was much lower for CO_2 than for water vapor. In this case, I would have consistently underestimated mesophyll resistance.

Table I. Mesophyll resistance to CO_2 uptake (r_m) as a function of leaf water potential (Ψ_l).

species	r_m (sec cm^{-1})							
at Ψ_l (bars):	-5	-10	-15	-20	-25	-30	-35	-40
Alnus oblongifolia	4	14	24	..	..	..	..	..
Alnus rugosa	10	10	35	..	..	..	..	..
Fraxinus pennsylvanica ssp. velutina	5	10	18	25	33	40	50	..
Fraxinus pennsylvanica	10	10	20	30	40	50	..	..
Acer saccharum	..	..	60	90	120	..	..	..
Pinus rigida	..	60	90	120	..	..	..	..
Pinus ponderosa	..	55	65	75	..	..	..	..
Juniperus deppeana	..	..	18	24	32	38	45	52
Vauquelinia californica	..	11	12	14	16	18	20	22
Simmondsia chinensis	..	12	14	16	18	20	22	24
Acacia greggii	..	..	..	..	14	19	23	..
Larrea divaricata	..	..	2	6	10	14	18	22

Table II. Stomatal resistance to water vapor loss (r_s) as a function of leaf water potential (Ψ_l).

species	r_s (sec cm^{-1})							
at Ψ_l (bars):	-5	-10	-15	-20	-25	-30	-35	-40
Alnus oblongifolia	4.4	7.9	18.2	...	...	...	...	...
Alnus rugosa	6.7	6.7	52	...	...	...	...	...
Fraxinus pennsylvanica ssp. velutina	5.6	8.0	11.2	18.7	22.4	25.5	27.9	...
Fraxinus pennsylvanica	10	10	19	40	55	67	...	...
Acer saccharum	...	...	20	31	67	...	...	...
Pinus rigida	...	7.2	18	48	...	...	...	...
Pinus ponderosa	...	9.5	11	50	...	...	...	...
Juniperus deppeana	...	...	10	13.3	25	50	50	50
Vauquelinia californica	...	1.5	1.6	1.8	2.5	4.1	8.8	15
Simmondsia chinensis	...	6.7	7.0	7.7	10.5	16.7	23	33
Acacia greggii	...	...	...	...	10	15	30	...
Larrea divaricata	...	...	4.0	4.2	5.7	6.9	20	27

The use of the slope of the response of net photosynthesis to ambient CO_2 to estimate mesophyll and stomatal resistance is equivalent to using the CO_2 compensation point as the sink CO_2 concentration. The results presented are not affected by this choice of model. The CO_2 compensation point increased as leaves dried in all of the species studied. Using zero as the sink CO_2 concentration would have resulted in progressively higher estimates of mesophyll resistance as plants dried compared with the estimates presented here. The use of the CO_2 compensation point model to calculate the mesophyll resistance may possibly eliminate the influence of respiration on the calculated mesophyll resistance (2), but until this is established we cannot be certain that the cause of the increased mesophyll resistance is decreased fixation capacity (1, 5, 8, 9, 14), and not increased respiration.

Under the experimental conditions used, slowly developing water stress (7-10 days to 0 net photosynthesis) caused an increase in mesophyll resistance to CO_2 uptake in intact leaves of the species studied.

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