

*Short communication*

## **Nonstomatal inhibition of photosynthesis by water stress. Reduction in photosynthesis at high transpiration rate without stomatal closure in field-grown tomato**

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**Abstract.** Large underestimates of the limitation to photosynthesis imposed by stomata can occur because of an error in the standard method of calculating average substomatal pressures of carbon dioxide when heterogeneity of those pressures occurs across a leaf surface. Most gas exchange data supposedly indicating nonstomatal inhibition of photosynthesis by water stress could have this error. However, if no stomatal closure occurs, any reduction in photosynthesis must be due to nonstomatal inhibition of photosynthesis. Net carbon dioxide exchange rates and conductances to water vapor were measured under field conditions in upper canopy leaves of tomato plants during two summers in Beltsville, Maryland, USA. Comparisons were made near midday at high irradiance between leaflets in air with the ambient water vapor content and in air with a higher water content. The higher water content, which lowered the leaf to air water vapor pressure difference (VPD), was imposed either one half hour or several hours before measurements of gas exchange. In both seasons, and irrespective of the timing of the imposition of different VPDs, net photosynthesis increased 60% after decreasing the VPD from 3 to 1 kPa. There were no differences in leaf conductance between leaves at different VPDs, thus transpiration rates were threefold higher at 3 than at 1 kPa VPD. It is concluded that nonstomatal inhibition of photosynthesis did occur in these leaves at high transpiration rate.

### **Introduction**

Calculation of the substomatal pressure of carbon dioxide from photosynthetic rate and stomatal conductance in conventional gas exchange analysis is done by assuming homogeneity of substomatal carbon dioxide pressure across the leaf surface. If there are substantial differences in substomatal carbon dioxide pressure in different parts of a leaf than the calculated average substomatal carbon dioxide pressure is incorrect, and can lead to an underestimate of the limitation to photosynthesis imposed by diffusion

through the stomata. Downton et al (1988) have suggested that the appearance of nonstomatal inhibition of photosynthesis during water stress (Sharkey and Farquhar 1982) may be caused by this error in calculated average substomatal carbon dioxide pressure. While not discounting this possibility, the data presented here are not subject to this error, yet indicate an inhibition of photosynthesis by water deficit in intact leaves.

The error in calculating an average substomatal carbon dioxide pressure when that pressure is not uniform across a leaf arises from taking averages over area for net carbon dioxide exchange rate and leaf conductances and using them to calculate an 'average' substomatal pressure of carbon dioxide. The error comes from the algebra of the calculation. Consider two parts of a leaf of equal area with net carbon dioxide exchange rates of  $A_1$  and  $A_2$  and conductances of  $C_1$  and  $C_2$ . The reduction in substomatal carbon dioxide pressure ( $D$ ) of each part of the leaf compared to the external air can be calculated from the carbon dioxide exchange rate and conductance:  $D_1 = A_1/C_1$  and  $D_2 = A_2/C_2$ . If the two parts of the leaf were in the same gas exchange cuvette, the carbon dioxide exchange rates and conductances of the two parts of the leaf would be correctly averaged by the measurement process. An "average" pressure drop would then be calculated:  $D_3 = (A_1 + A_2)/(C_1 + C_2)$ . The problem is that  $D_3$  is not always equal to the mean of  $D_1$  and  $D_2$ . Downton et al. (1988) considered the special case where  $A_1 \gg A_2$  and  $C_1 \gg C_2$ . In this case  $D_3$  equals  $D_1$ , not the mean of  $D_1$  and  $D_2$ , which is much higher. The error was clearly recognised by Laisk (1983), although it was described as an error in calculated mesophyll conductance rather than as an error in substomatal carbon dioxide pressure, upon which calculations of mesophyll conductance are based. The magnitude of the error depends on how large the differences are in the substomatal pressures for different parts of the leaf surface. This depends on the degree of variation in stomatal aperture, the spatial distribution of this variation, and the internal diffusive characteristics of the leaf (Laisk 1983). Farquhar et al. (1987) found patchy stomatal closure in sunflower leaves after application of abscisic acid, and suggested that some of the leaf area was effectively removed from gas exchange. Downton et al. (1988) showed that application of abscisic acid to grape and sunflower leaves induced complete stomatal closure only in patches of the leaves. They showed that gas exchange analysis overestimated the average substomatal carbon dioxide pressure, and falsely indicated nonstomatal inhibition of photosynthesis. They suggested from preliminary work with water stressed leaves, and from the similarity of gas exchange responses to water stress and applied abscisic acid (Bunce 1987) that nonstomatal inhibition of photosynthesis during water stress may also be incorrect. Laisk (1983) pointed out that at

present the best way to show nonstomatal inhibition of photosynthesis is to demonstrate effects which occur at constant stomatal conductance. This paper presents data on reductions in photosynthetic rates at constant stomatal conductance in leaves of field-grown tomato plants at high transpiration rates.

### Materials and methods

*Lycopersicon esculentum* Mill. cv. Large Red (Burpee Seed Co., Warminster PA, USA) were grown initially from seed in controlled environments. Plants were grown in 10 cm diameter pots filled with vermiculite and flushed daily with a complete nutrient solution. The day/night air temperatures were 28/22 °C, and the dew point temperature was 18 °C. There was 14 h of light per day at 1.0 mmol m<sup>-2</sup>s<sup>-1</sup> photosynthetic photon flux density (PPFD). Plants at the third leaf stage were transplanted into a field plot with a Codorus silt loam soil at Beltsville, MD, USA in late May in 1982 and 1984. There were 10 plants per m<sup>2</sup> in a hexagonal equidistant pattern.

Rates of net carbon dioxide exchange and conductances to water vapor were measured on attached leaves in an open gas exchange system (Bunce 1984). Cuvette air temperatures were kept near the ambient air temperature by circulating water at a controlled temperature through heat exchangers in the cuvettes. Leaves were exposed to PPFDs of at least 1.5 mmol m<sup>-2</sup> s<sup>-1</sup> from sunlight. Air speed in the cuvettes was 3 m s<sup>-1</sup>. In both years gas exchange was measured on terminal leaflets of comparable leaves on two plants each measurement day. Leaves chosen were fully exposed to direct sunlight and recently fully expanded. Measurements were made in midafternoon on several days scattered through June and July of both years. Completely overcast days were avoided, otherwise days were chosen without regard to weather. On each day one leaflet was exposed to air taken from 3 m above the crop, and one leaflet was exposed to the same air after it was bubbled through water at a controlled temperature to increase the water content. Leaflets in the wetter air had leaf to air water vapor pressure differences (VPD) of 1 to 1.5 kPa. Temperatures of the two leaflets, measured with 0.1 mm diameter thermocouples pressed against the undersides of the leaves, were within 1 °C of each other, and were between 30 and 35 °C. The carbon dioxide pressure in the air around the leaflets was 33 to 34 Pa. Conductances to water vapor were calculated assuming air inside the leaflets was saturated with water vapor at the measured leaf temperature. In 1982 leaflets were placed in the cuvettes about 30 minutes before steady-state gas exchange was measured. The leaflet measured at low VPD had been at

the ambient air conditions earlier in the day, and was exposed to low VPD for only this relatively brief time. To determine if the insensitivity of conductance to changes in VPD resulted from a long lag in reopening of stomata, in 1984 leaflets were placed in the cuvettes in early morning, and the leaflet measured at low VPD had not been exposed to high VPD the day of measurement. In all cases the remaining leaf area of the plant was at about the same VPD as the leaflet measured at high VPD.

Linear regressions of net photosynthetic rate and leaf conductance on VPD were developed separately for the two years of data. Correlation coefficients were tested for significance at the 0.05 level of probability. Differences in slope of the regression equations were tested at the 0.05 level of probability using analysis of covariance.

### Results and discussion

Rates of net photosynthesis were negatively correlated with VPD in both years (Fig. 1; Table 1). The slopes of the response (Table 1) were not significantly different between years. The correlations between leaf conductance and VPD were not significant in either year (Fig. 2; Table 1). For leaves at the low VPD, the correlation coefficient between the net photosynthetic rate and the VPD to which the rest of the shoot was exposed was 0.173 for  $n = 10$ , which is not significant at the 0.05 probability level.

The similarity of the responses of net photosynthetic rate to VPD ob-

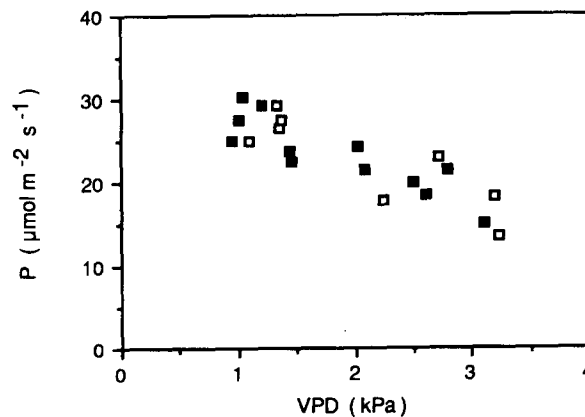


Fig. 1. Relationships between net photosynthetic rates (P) and leaf to air water vapor pressure differences (VPD) in leaves of field-grown tomato plants measured at high light at 30 to 35 °C. Each point is from a different leaflet, each on a different plant. Closed symbols are for 1982; open symbols are for 1984. The slopes of the responses were not significantly different (0.05 probability level) between years.

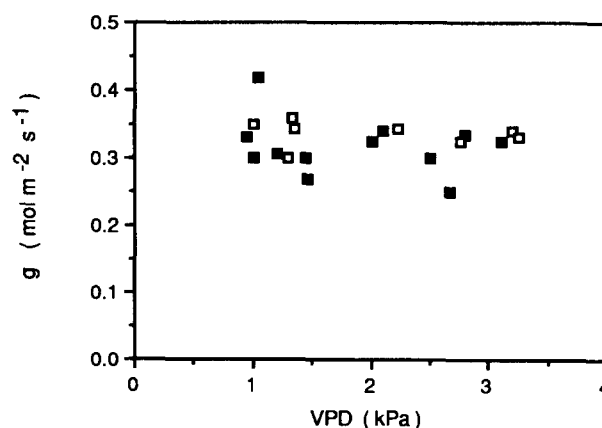


Fig. 2. Relationships between leaf conductances to water vapor ( $g$ ) and leaf to air water vapor pressure differences (VPD) in leaves of field-grown tomato plants measured at high light at 30 to 35 °C. Each point is from a different leaflet, each on a different plant. Closed symbols are for 1982; open symbols are for 1984. The correlation coefficients were not significant (0.05 probability level) in either year.

tained in the two years indicates that the effect of high VPD on photosynthesis was reversible within one half hour. Similarly, the insensitivity of conductance to VPD cannot be attributed to a slow recovery of stomatal opening after return to low VPD. The data indicate that the net photosynthetic rate of a leaflet was responsive to the water content of the air just around that leaflet. The lack of significant correlation between the photosynthetic rate at low VPD and the saturation deficit around the rest of the shoot suggests that the response was localized in the individual leaflets. Because leaf conductance to water vapor was the same at high and low VPD, transpiration rates increased directly with increasing VPD. Sharkey (1984) also found that high transpiration rate decreased photosynthesis in several species, presumably by causing a localized leaf water deficit.

Table 1. Regression equations for relationships between net photosynthetic rates ( $P$ ) and leaf to air water vapor pressure differences (VPD), and between leaf conductances to water vapor ( $g$ ) and VPD. Separate equations were developed for data for two years for leaves of field grown tomato plants. Leaves were in full sunlight, at 30 to 35 °C.  $P$ :  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ;  $g$ :  $\text{mol m}^{-2} \text{s}^{-1}$ ; VPD: kPa.

Year	Variable	Intercept	Slope	Correlation coefficient
1982	$P$	30.4	-4.25	-0.736*
1984	$P$	33.3	-5.18	-0.853*
1982	$g$	0.335	-0.009	-0.177
1984	$g$	0.350	-0.006	-0.371

\* Indicates significance at the 0.05 level of probability.

Because the inhibition of photosynthesis observed at high VPD and high transpiration rate in these tomato plants occurred without change in stomatal conductance, the inhibition can not be attributed to stomatal effects. Boyer (1965) also reported decreased net photosynthesis without stomatal closure in cotton plants in response to osmotic stress.

It is clear that nonstomatal inhibition of photosynthesis at air levels of carbon dioxide can occur in response to water deficits. However, in cases where decreased average conductance also occurs, the possibility of heterogeneity of substomatal carbon dioxide pressures makes separation of stomatal and nonstomatal effects uncertain. Obtaining a correct average for substomatal carbon dioxide pressure would solve the problem of separating stomatal and nonstomatal effects only in cases where photosynthesis responded either linearly or not at all to the range of substomatal pressures which exist. In the cases studied by Downton et al (1988), the assumption of a linear response seemed to fit the data, assuming that it was correct that no significant heterogeneity existed in the control leaves. Clearly, heterogeneity in substomatal carbon dioxide pressure could pose severe problems to the analysis of gas exchange and it will be very important to document its existence.

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