Water Deficits and Environmental Factors Affect Photosynthesis in Leaves of Cucumber (Cucumis sativus)

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Abstract. Cucumber plants were cultured in a greenhouse and subjected to either well-watered or water deficit conditions that reduced leaf water potential to -0.6 MPa. Leaf gas exchange measurements were conducted using an open gas exchange system. Carbon dioxide assimilation (A) attained saturation at a photon flux density (PFD) of 1000 μmol·m⁻²·s⁻¹ (400-700 nm). There were no significant differences in A at ambient temperatures between 16 and 34°C. Water use efficiency decreased rapidly with increasing vapor-pressure deficits to 2.5 kPa. Water stressed plants had lower stomatal conductances and CO₂ assimilation rates. The decrease in A was only partially due to stomatal closure. The A vs. intercellular CO₂ (Ca) relationship for stressed leaves revealed a change in the CO₂ compensation point, and that nonstomatal factors were contributing to the decrease in A in stressed plants. Thus, feedback inhibition of A may have occurred through photoassimilate accumulation. The concentrations of sucrose and raffinose were higher, and the concentration of stachyose was lower in leaves of stressed than of well-watered plants.

Plant water deficits result in low photosynthetic rates in leaves (Ackerson and Hebert, 1981; Huck et al., 1983; Karamanos et al., 1982). The cause of the decrease in photosynthesis with the onset of water stress is still not completely understood. Under water stress conditions, low CO₂ fixation rates have been attributed to low intercellular CO₂ levels (Raschke and Hedrich, 1985; Sharkey and Seemann, 1989), accumulation of assimilates (Ackerson, 1980; Azcon-Bieto, 1983), localized low-water potentials in the mesophyll (Schulze and Kuppers, 1979), and decreased ribulose-1,5-bisphosphate carboxylase activity (Vu and Yelenosky, 1988).

Stomatal closure is in part responsible for reductions in the photosynthetic rate. Stomatal conductance decreases in response to turgor loss upon exposure to low soil-water potentials (Blackman and Davies, 1985) and low humidity (Schulze and Kuppers, 1979). An increase in abscisic acid (ABA) concentration within leaf tissue under water-deficit conditions stimulates rapid ion efflux from guard cells, thus leading to stomatal closure (Ackerson, 1980; Raschke and Hedrich, 1985).

Cucumber plants have a high water requirement (Loomis and Crandall, 1977) and are considered to be sensitive to drought stress. Prolonged exposure to low soil moisture, due to a lack of rainfall or irrigation, has been shown to reduce significantly fruit yield and quality (Doss et al., 1977; Elkner, 1985). Transient water deficits are also observed in cucumber plants when transpiration rates exceed the rate of water uptake by the root system, such as at midday. Plant water deficits are evidenced by leaf wilting, closure of stomates, and, ultimately, a reduction in photosynthetic rate (Ackerson and Herbert, 1981; Gentry et al., 1987).

The objective of this study was to investigate the effects of plant water status on gas exchange rates in cucumber leaves over a range of light, temperature, and vapor-pressure deficit regimes. We also evaluated the recovery of photosynthetic activity following relief of water stress.

Materials and Methods

Plant material. Greenhouse experiments were conducted from June to Aug. 1986 and 1987. Seeds of the pickling cucumber inbred lines Gy 14 and M 21 were sown in a 1:1 peat (Baccto Professional Mix, Michigan Peat Co., Houston) to sandy loam soil mixture in 11-liter plastic containers. Plants were drip-irrigated daily to saturation during vegetative development and fertilized twice weekly using Peter’s 20N-8.8P-16.6K soluble fertilizer at 0.2 g·liter⁻¹. Pistillate flowers were hand-pollinated between 10 AM and noon on the days the flowers opened. Day/night temperatures were maintained at ±30/20 ± 5°C, and no supplemental lighting was provided.

Cucumber plants were also cultured in a field environment during June through Aug. 1987. Seeds were planted into 11-liter plastic containers filled with a fertile Brookston loam (fine-loamy, mixed, mesic, typic Argiaquolls) soil. The containers were buried in the soil at a field cucumber production site at the Michigan State Univ. Horticulture Research and Teaching Center in East Lansing. The containers were arranged in four rows, 90 cm apart. Only one plant was allowed to develop per container. Two irrigations during the vegetative stage supplemented natural rainfall.

Water regime. Water deficit treatments were induced by withholding water from container-grown plants bearing two to three fruits for 3 or 4 days until the predawn leaf water potential had reached -0.6 MPa (measured by Scholander pressure chamber). Plants used in studying recovery of photosynthetic activity were rewatered 12 h before gas exchange measurements were made. Control plants were watered daily.

Leaf gas exchange measurements. Gas exchange responses to light, temperature, and CO₂ concentration on the fifth leaf from the shoot apex were determined using an open gas exchange system previously described by Sams and Flore (1982). Each leaf was
enclosed in a 20 × 10 × 10 cm deep controlled environment chamber. Leaves were allowed to equilibrate with the microenvironment of the chamber for 2 h before gas exchange measurements were initiated.

To determine the light response curve, gas exchange measurements were made in a growth chamber at several levels of photosynthetic photon flux density (PFD) beginning at a flux density of 1800 µmol·m⁻²·s⁻¹ and incrementally decreasing to total darkness. Ambient CO₂ concentration was maintained at 345 ± 5 µl·liter⁻¹ at 25 ± 0.5°C. The temperature response curve was determined by raising leaf temperature from 10 to 40°C in increments of 3 to 5°C. Vapor-pressure deficit (VPD) was maintained at <1.5 kPa up to raising leaf temperature from 25 ± 0.5°C. The temperature response curve was determined by exposing leaves to ambient CO₂ (A), PFD, relative humidity, and leaf temperature in the greenhouse or field. The analyzer was operated in a differential mode with an air flow rate of 600 cm³·min⁻¹. Stomatal conductance (gₛ), transpiration rate (E), and VPD were calculated using a computer program developed by Moon and Flore (1986). All measurements were made under sunlight between 10:30 AM and 12:30 PM. Ambient CO₂ levels were between 325 and 348 µl·liter⁻¹. Measurements were made on the fourth and sixth leaves from the shoot apex of each plant. The experiment design was a randomized complete block with three replications and two plants per plot.

Leaf sugar determinations. Lamina tissue from the fourth or fifth leaf from the shoot apex was freeze-dried for 24 h and ground finely with a mortar and pestle. Soluble sugars were extracted from tissue (0.2 g) with 80% ethanol at 70°C for 1 h. The extract was filtered through Whatman no. 1 filter paper and the ethanol evaporated. The residue was redisolved in 25 ml of deionized water and an aliquot of the resultant solution filtered through a 0.45 µm Millex-HA filter unit (Millipore; Bedford, Mass.). Sugars and sugar alcohols were assayed using a Dionex Carbopac PA 1 anion exchange separation column (Dionex; Sunnyvale, Calif.) with a Dionex series 4000i high performance ion chromatography module and a pulsed amperometric detector with a gold electrode. A 0.1 M NaOH solution was used as the eluant.

### Results

The A rate reached saturation at a PFD of ≈1000 µmol·m⁻²·s⁻¹ (Fig. 1). Subsequent measurements of assimilation rates in the field, greenhouse, and laboratory were conducted at PFD levels >1000 µmol·m⁻²·s⁻¹ to ensure light-saturating conditions. Maximum A rates were measured at gₛ >256 µmol·m⁻²·s⁻¹, whereas transpiration rate continued to increase until gₛ reached 380 µmol·m⁻²·s⁻¹ (Fig. 2). Temperature also influenced CO₂ assimilation at <16°C and >34°C (Fig. 3). Within the range from 18 to 32°C, assimilation rates did not fluctuate significantly. High temperatures, >34°C, resulted in a decline in assimilation rate concomitant with an increase in VPD. Subsequent gas exchange measurements in the field and greenhouse were made at ambient temperatures from 22 to 32°C.

In greenhouse-cultured plants, water use efficiency (WUE) decreased rapidly as VPD increased >1 kPa (Fig. 4a), but stabilized at a low level of WUE (0.5 mmol·mol⁻¹) when VPD was 1.5 kPa or higher. The WUE of field-grown plants decreased less rapidly as a function of increasing VPD than did greenhouse plants, and did not reach 0.5 mmol·mol⁻¹ until VPD exceeded 3.5 kPa (Fig. 4b). At all experimentally induced intercellular CO₂ concentrations, assimilation rates in watered (nonstressed) plants were higher than those in stressed plants (Fig. 5 A and B). Maximum photosynthetic rate in nonstressed plants was reached at a CO₂ of ≈150 µmol·mol⁻¹. In stressed plants, the CO₂ compensation point was ≈100 µmol·mol⁻¹, twice that of nonstressed plants (Fig. 5 A and B).

Drought-stressed greenhouse and field plants had 63% to 73% lower CO₂ assimilation rates than well-watered plants. Stomatal conductances of drought-stressed plants averaged ≈85 µmol·m⁻²·s⁻¹, which was ≈80% lower than gₛ in control plants (data not shown).

Plant water potential recovered rapidly and completely within 12 h after rewatering (Table 1). Recovery of photosynthesis from a water deficit condition following rewatering was rapid in cucumbers. The A rate increased from 3.5 to 11.7 µmol·m⁻²·s⁻¹ at 350 ppm CO₂ within only 12 h after rewatering (Table 1). Increasing ambient CO₂ concentration from 150 to 350 µl·liter⁻¹ caused significant increases in A and in the calculated intercellular CO₂ concentration.

![Fig. 1. Effects of increasing photon flux density on the photosynthetic rate of the fifth leaf from the shoot apex of greenhouse-grown cucumber plants. Ambient temperature and CO₂ concentration were maintained at 25°C and 350 ppm, respectively. Data points represent individual measurements on leaves from different plants. The curve was fitted to data according to the equation: Y = B₁ × Exp (B₃ × X) + B₄, where B₃ are estimated variables; r² = 0.96.](image1)

![Fig. 2. Relationship between stomatal conductance and CO₂ assimilation (O) and transpiration rates (Δ) in well-watered greenhouse-grown cucumber plants. Data points represent individual measurements on cucumber leaves at the fifth node from the shoot apex. The curves were fitted according to the equation: Y = B₁ × Exp (B₃ × X) + B₄, where B₃ are estimated variables. The values of r² for the assimilation and transpiration curves were 0.86 and 0.97, respectively.](image2)
The levels of sugars in young, fully expanded cucumber leaves were affected by water deficits. The concentration of sucrose in leaves of stressed fruiting plants (1.03 mg·g\(^{-1}\) fresh weight) was ≈300% higher than measured in leaves of well-watered plants (Table 2). Stachyose concentration in leaves of stressed fruiting plants was 30% lower than in leaves of nonstressed plants. The concentrations of raffinose and reducing sugars were similar in leaves of both types of plants.

**Discussion**

Studies on the effects of water deficits on photosynthesis have often reached different conclusions regarding the importance of low \(C_i\) in limiting photosynthesis in drought-stressed plants (Ackerson and Hebert, 1981; Krieg and Hutmacher, 1986; Raschke and Hedrich, 1985; Thorne and Koller, 1974). However, it is generally accepted that a \(CO_2\) limitation is at least partially responsible for decreases in the \(CO_2\) assimilation rate in water-stressed plants.

In cucumber plants, we found that water deficits resulted in significant decreases in \(A\) and \(g_s\). Our results indicated that \(g_s < 256\ \mu\text{mol·m}^{-2}·\text{s}^{-1}\) apparently limited \(CO_2\) availability and resulted in lower photosynthetic rates. However, the decrease in \(A\) in water-stressed plants does not appear to be due solely to a decrease in \(CO_2\) availability. The initial slope of the \(A/C_i\) relationship was altered by an internal plant water deficit that resulted in an apparent change in the \(CO_2\) compensation point for photosynthesis in drought-stressed plants (Fig. 5). The shift in \(CO_2\) compensation point to a higher value in stressed plants indicates that nonstomatal factors, possibly involving a change in the carboxylation efficiency of ribulosebisphosphate carboxylase (EC 4.1.1.39; RuBPCase), are contributing to the decrease in \(A\) under water-limiting conditions.

These responses are consistent with the effects of ABA on leaf photosynthesis (Raschke and Hedrich, 1985); ABA is known to accumulate in leaves of drought-stressed plants and to directly affect stomatal aperture and photosynthetic \(CO_2\) fixation (Raschke and Hedrich, 1985). Vu and Yelenosky (1988) reported that the activity of extractable RuBPCase was relatively low in water-stressed orange \(\text{[Citrus sinensis (L.) Osb.]}\) plants. In contrast, Sharkey and Seemann (1989) found no evidence of damage to chloroplastic biochemical reactions and concluded that the decrease in photosynthetic rates of drought-stressed plants was the result of
stomatal closure, which limited CO₂ availability. However, an effect of the chloroplast environment, in situ, on enzyme activity cannot be precluded because both Vu and Yelenosky (1988) and Sharkey and Seemann (1989) assayed only extractable enzyme activities. Berkowitz and Gibbs (1983) reported that photosynthesis was inhibited at low osmotic potentials associated with plant-water deficit conditions due to stromal acidification, which inhibited the activity of fructose-1,6-biphosphatase.

Higher concentrations of sucrose and were detected in leaves of water-stressed cucumber plants than in nonstressed plants. Although gas exchange measurements and sugar determinations were made on different leaves, these observations are in agreement with the findings of Azcon-Bieto (1983) and Thorne and Koller (1974), who attributed the decrease in photosynthesis in drought-stressed plants to feedback inhibition resulting from photoassimilate accumulation.

The adverse effects of water deficits on photosynthesis were reversible. When water stress was relieved, recovery of photosynthetic activity was rapid, occurring within 12 h of rewatering. For complete and rapid recovery of photosynthesis, the adverse effects of water stress on both stomatal and nonstomatal factors must also be reversible within a short period after stress is relieved. In our study, photosynthesis recovered, although stomatal conductance did not recover completely following rewatering (data not shown). Incomplete stomatal recovery may have resulted from the accumulation of ABA in leaves of water-stressed plants (Ackerson, 1980; Eze et al., 1983; Raschke and Hedrich, 1985) at levels sufficiently high to prevent complete stomatal opening. ABA sensitizes stomata to CO₂, leading to stomatal closure at lower Cᵢ levels (Raschke and Hedrich, 1985). RuBPcase activity has been reported to recover partially within 1 day after stress is relieved (Vu and Yelenosky, 1988), suggesting that enzymatic activity might be regained within hours after plant rehydration.

In this study, stachyose, the predominant translocate sugar in cucurbits (Pharr et al., 1985; Richardson et al., 1982;), was present at relatively low concentrations in leaves of stressed plants as compared to well-watered plants. Low stachyose accumulation could be attributed to either low rates of stachyose biosynthesis or rapid export from leaves. Phloem export rates of stachyose, however, would be expected to be low under water deficit conditions, due to an inhibitory effect of low-water potentials on turгор development within the sieve tube-companion cell complex (Sung and Krieg, 1979), even though stachyose synthase activity is localized within intermediary cells in minor veins (Holthaus and Schmitz, 1991). We postulate that a reduction in sink activity may have a feedback inhibitory effect on stachyose synthesis within source leaf tissue. Defruiting of cucumber plants was found to result in significantly (P < 0.05) lower stachyose concentrations in leaf tissue (1.03 mg·g⁻¹ fresh weight) as compared to that in fruiting plants of similar age (1.65 mg·g⁻¹ fresh weight) under well-watered conditions (Widders, unpublished data). This difference occurred in spite of the fact that defruiting also lowers assimilate export from leaves (Barrett and Amling, 1978). The cause of a decrease in stachyose synthesis under low intercellular water potentials is not evident, but apparently was not due to a limitation in availability of precursors, sucrose, and raffinose (Table 2).

The decreases that we observed in CO₂ assimilation rates in stressed plants were probably not a result of a high temperature effect on photosynthesis. Temperatures in the range of 16 to 34°C had no apparent effect on A in cucumber plants. However, leaf temperature, under water deficit conditions in the field, might exceed 34°C if transpiration rates were low. The specific effect of high temperatures (>34°C) on leaf photosynthesis could not be determined because of the associated increase in VPD, which probably induced stomatal closure. Thus, stomatal closure may have contributed to the decline in photosynthetic rate at high temperature.

Water use efficiency was influenced by environmental factors and growing conditions. WUE decreased rapidly as the leaf-air VPD increased, which is in agreement with other reports (Cock et al., 1985). An increase in VPD would induce stomatal closure (Schulze and Hall, 1982), thus limiting CO₂ availability and ultimately reducing photosynthesis (Raschke and Hedrich, 1985). Concurrently, the increase in VPD leads to an increase in transpiration, which reduces the WUE. Comparison of the WUE-to-VPD relationship between greenhouse- and field-grown cucumber plants

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**Table 1.** Effects of water regime and ambient CO₂ concentration on photosynthesis, water use efficiency (WUE), and water potential of cucumber leaves.

| Water regime    | CO₂ concn' (µmol·m⁻²·s⁻¹ ±SE) | WUE (mmol CO₂/mol H₂O) | Water potential (MPa) |
|-----------------|-------------------------------|------------------------|-----------------------|
|                 | 150                           | 250                    | 350                   |                   |
| Water withheld' | 1.5 ± 0.4                     | 2.3 ± 0.3              | 3.5 ± 0.8             | 4.3                | -0.77               |
| Rewatered'      | 8.0 ± 0.4                     | 11.0 ± 1.2             | 11.7 ± 1.1            | 5.1                | -0.10               |
| Watered         | 9.6 ± 0.7                     | 11.2 ± 1.2             | 12.9 ± 1.1            | 4.1                | -0.08               |

' Ambient CO₂ concentration, ppm.
' Water withheld until predawn leaf water potentials reached -0.6 MPa.
' Gas exchange measured 12 h after rewatering of drought-stressed plants.

**Table 2.** Soluble sugar concentrations in recently fully expanded leaves from drought-stressed and well-watered cucumber plants during fruit development.

| Water regime     | Sucrose (mg·g⁻¹ fresh wt ±SE) | Stachyose (mg·g⁻¹ fresh wt ±SE) | Raffinose (mg·g⁻¹ fresh wt ±SE) | Reducing sugars (mg·g⁻¹ fresh wt ±SE) |
|------------------|------------------------------|---------------------------------|---------------------------------|--------------------------------------|
| Stressed         | 1.03 ± 0.14                  | 1.15 ± 0.03                     | 0.12 ± 0.03                     | 0.97 ± 0.03                          |
| Well-watered     | 0.33 ± 0.03                  | 1.65 ± 0.12                     | 0.13 ± 0.07                     | 1.03 ± 0.14                          |

' Water withheld from plants for 3 or 4 days until leaf water potential declined to -0.6 MPa.
(Fig. 4) indicates that, at VPD between 1.5 and 3.0 kPa, field-grown plants had higher WUE than greenhouse-grown plants. Leaves that develop on plants under stress-inducing conditions of high temperature, low soil moisture or high irradiance, characteristic of environmental conditions in the field, have smaller cells (Yegappan et al., 1982) due to less expansive growth. Such leaves typically will have a higher WUE (Nobel, 1980). The same reasoning can be used to explain the higher WUE observed in drought-stressed, greenhouse-grown cucumber plants as compared to irrigated plants.

In summary, our results indicate that the effects of water deficits on photosynthesis in cucumber plants cannot be explained solely by the observed decrease in stomatal conductance and intercellular CO₂ concentrations in drought-stressed cucumber plants. The results of this study also indicate that photosynthesis in cucumber plants is capable of rapid recovery from transient mild water deficits without apparent long-term adverse effects.

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