Recovery of Photosynthesis in Sunflower after a Period of Low Leaf Water Potential

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ABSTRACT

Photosynthesis was studied in sunflower plants subjected to 1 to 2 days of desiccation and then permitted to recover. The leaf water potential to which leaves returned after rewatering was dependent on the severity of desiccation and the evaporative conditions. Under moderately evaporative conditions, leaf water potential returned to predesiccation levels after 3 to 5 hours when desiccation was slight. Leaf water potentials remained below predesiccation levels for several days after rewatering when leaf water potentials decreased to −13 to −19 bars during desiccation. Leaf water potential showed no sign of recovery when leaf water potentials decreased to −20 bars or below during desiccation. The lack of full recovery of leaf water potential was attributable to increased resistance to water transport in the roots and stems. The resistance ultimately became large enough to result in death of the leaves because net water loss continued even after the soil had been rewatered.

Measurements of photosynthesis were made at high light intensities, where stomatal aperture often affects photosynthesis, and at low light intensities, where the photochemical activity of the leaves limits photosynthesis. Providing leaf water potentials remained above −12 bars during the desiccation period and returned to predesiccation levels during recovery, photosynthesis under both low and high light paralleled the recovery in leaf water potential after rewatering. After desiccation to leaf water potentials below −12 bars, recovery was incomplete under high light and could be attributed to lack of full stomatal opening. Lack of full opening persisted for 3 days and showed no sign of eventual recovery even though leaf water potentials recovered fully. Under low light, however, recovery in photochemical activity was complete within 15 hours after desiccation to leaf water potentials as low as −17 bars.

Plants often recover only partially from desiccation after they have been rewatered. There is evidence that following a period of desiccation, rates of photosynthesis under high light (2, 11, 21, 25) and transpiration (11, 17, 21) may remain lower, resistance to water movement through the root system may be larger (17, 18), and stomata may open less in light than do those of nondesiccated control plants (12). If these reactions are not simply expressions of accelerated senescence, they could be caused by leaf water potentials that do not rise to the levels present before desiccation. For example, increased root resistance could result in low leaf water potentials, and incomplete stomatal opening might then be attributable to low water potentials rather than to an after-effect of desiccation acting on the stomatal mechanism itself. There are few measurements (7) of the degree to which leaf water potential recovers after a period of desiccation. The present work describes experiments which test whether the incomplete recovery of photosynthesis is associated with leaf water potentials that remain low after rewatering or whether some aspect of the photosynthetic process is inhibited irreversibly by desiccation.

MATERIALS AND METHODS

Sunflower (Helianthus annuus L. var. Russian Mammoth) plants were grown from seed in a controlled environment chamber (day temperature, 29 ± 0.5 C; night temperature, 23 ± 0.5 C; relative humidity, 70 ± 5%; light intensity, 0.19 cal cm⁻² min⁻¹ (fluorescent); photoperiod, 14 hr). In one experiment, soybean (Glycine max L. [Merr.] var. Harosoy) plants were also used and were grown under the same conditions. When the sunflower and soybean plants were approximately 45 cm tall, low leaf water potentials were produced by withholding water from the soil for varying lengths of time. In some instances, soil was gently washed from roots, and low leaf water potentials were produced by placing the root system in water at 10 C. Except when stated otherwise, recovery was initiated by rewatering the soil or, when roots were chilled, by warming the root system.

Leaf water potential was measured by the isopiestic technique with thermocouple psychrometers which could accommodate either intact leaves (6) or excised leaf tissue (4, 10). Several hours before measurements were made, the experimental tissue was rinsed in distilled water and permitted to dry in order to remove surface contaminants. The walls of the psychrometer chambers were coated with melted and resolidified petrolatum to reduce adsorption of water vapor (5), and all measurements were corrected for heat of respiration (3). In intact leaf experiments which required continuous determination of leaf water potentials, an initial isopiestic measurement (4, 10) was made but water was placed on the thermocouple junction for subsequent determinations. The data from the isopiestic measurement provided a correction factor for the diffusive resistance of the tissue which remained the same for any particular tissue (6) and could be used to correct the determinations with water on the thermocouple.

The rates of net photosynthesis and transpiration were measured by a method previously described (9) in shoots of intact plants in an assimilation chamber (air temperature, 25 ± 0.25 C; leaf temperature, within 0.6 C of air temperature; relative humidity, 77 ± 2%; light intensity, 1.6 cal cm⁻² min⁻¹ [incandescent, filtered through 10 cm of water] unless otherwise specified; net radiation, approximately 0.28 of incident light.

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intensity; wind speed, 1.7 m sec⁻¹; CO₂ concentration, 300 ± 1 μl/liter except during a measurement of photosynthetic rate). During measurements of photosynthesis, recovery of desiccated plants was initiated by watering the soil while the shoot was in the assimilation chamber, which provided a moderately evaporative environment around the shoot.

Immediately after measurements of photosynthesis and transpiration, the assimilation chamber was opened, and a disc was removed from the interveinal area of a leaf for a measurement of leaf water potential. The relative diffusive resistance of the leaves was then measured twice on each leaf in the light with a viscous flow porometer (9). The cube root of the time required for the pressure in the instrument to change a standard amount provided a relative diffusive resistance for the stomata (9, 15), which was used to detect changes in stomatal aperture.

The resistances to CO₂ diffusion through the boundary layer and internal gas phase of the leaf (rₛ + rₛ), and from the surface of the mesophyll cells to the site of CO₂ fixation in the chloroplasts (rₛ) were calculated from simultaneous measurements of rates of transpiration and photosynthesis (8, 13). Resistance rₛ was calculated from the thermal resistance of the leaf boundary layer which in turn was calculated from leaf and air temperatures and the rate of sensible heat loss by the leaf (24).

Chlorophyll content was determined by the method of Arnon (1).

RESULTS

Early experiments showed that, in the moderately evaporative environment of the assimilation chamber (transpiration in well watered sunflower was 3 to 4 g hr⁻¹ 100 cm⁻²), leaf water potential (Ψₑ) returned to predesiccation levels of −3 to −5 bars if desiccation had not been severe (Fig. 1). Recovery of Ψₑ was complete in 6 hr in plants desiccated to Ψₑ as low as −13 to −14 bars, but was incomplete for 36 hr in plants desiccated to Ψₑ of −17 to −18 bars. In plants that were desiccated to −20 bars, recovery often was absent and desiccation ultimately became lethal (photosynthesis and respiration became undetectable) if plants were not placed in a humid, dark environment. In these severely desiccated plants, however, rapid recovery could be initiated by removing both the roots and stem to permit the leaf to absorb degassed water through the cut petiole. Recovery was also rapid if the stem was left attached to the leaf and the cut end of the stem was momentarily exposed to a vacuum which was then released with the cut surface of the stem under degassed water.

![Fig. 1. Effect of degree of desiccation on subsequent recovery of sunflower from low leaf water potential measured with the excised leaf thermocouple psychrometer.](image1)

![Fig. 2. Effect of removal of soil and root system on recovery of sunflower from low leaf water potential measured with the intact leaf thermocouple psychrometer.](image2)

Detailed measurements of leaf water potential during recovery were made with a thermocouple psychrometer for intact leaves. Figure 2 shows that the recovery of sunflower desiccated to −9 bars exhibited a short lag, which could be eliminated by removing the roots. In soybean, desiccation to −15 bars also induced a lag and the subsequent rise in leaf water potential was somewhat irregular (Fig. 3B). Leaf water potentials were not completely recovered after 10 hr, just as in sunflower (Fig. 1), but they gradually returned to predesiccation levels (−1.5 to −2.5 bars) after a period of several days.

When leaf water potentials are below about −8 bars, photosynthesis and transpiration are inhibited in sunflower (9). Under low light, the inhibition of photosynthesis appears to be due to a decline in the photochemical activity of the leaves (9) whereas under high light, the inhibition is often attributed to stomatal closure (2, 8, 11, 13, 21). In the present study, desiccation to −10 to −12 bars permitted full recovery of photosynthesis 6 hr after rewetting under both high and low light, so that rates of photosynthesis roughly paralleled leaf water potential over this range. After desiccation to −16 bars, net photosynthesis under high light, transpiration, and the relative
diffusive resistance of the leaf did not return to predesiccation levels, even though leaf water potential did (Fig. 4). For this experiment, the plant was removed from the assimilation chamber and kept in the dark for 2 hr to promote recovery of leaf water potential. Fourteen hours after the plant was rewatered, photosynthesis under high light was 84%, transpiration was 70%, and relative diffusive resistance was larger than before desiccation, suggesting that stomata did not fully recover from the period of desiccation. There were no significant changes in leaf chlorophyll content in the desiccated plants, and well watered control plants showed no change in rates of photosynthesis or transpiration.

The degree to which photosynthesis was affected by stomatal behavior was determined by calculating leaf diffusive resistances to CO₂. Figure 5 shows that resistances to CO₂ diffusion were generally greater after the plant had been subjected to a period of desiccation (resistance \( r_w \) was 46% larger and \( r_m \) was 9% larger). Resistance \( r_w \) was 0.11 sec cm⁻² or less than 15% of \( r_w + r_m \). Since \( r_w \) was constant because of the similar arrangement of the plant in the chamber for each measurement, most of the increase in \( r_w + r_m \) was associated with \( r_m \).

Because of rapid new growth, it was not possible to follow the recovery of photosynthesis in previously desiccated plants for more than a few hours following rewatering. However, stomatal behavior could be followed with the porometer for several days in the leaves that had been desiccated. Figure 6 shows that a slow decrease in relative diffusive resistance occurred, but relative diffusive resistance had not returned to the level of nondesiccated controls after 3 days, although \( \Psi_w \) had completely recovered (−3 to −5 bars) during that time. Resistance tended to decrease fastest in the leaves which were young during the desiccation period (12).

Figure 7 shows the reduction in photochemical activity during desiccation measured in low light in young, recently expanded leaves and indicates that photochemical activity returns to predesiccation levels within 15 hr after rewatering.

Recovery was essentially complete from leaf water potentials as low as −17 bars. If petioles of desiccated sunflower leaves were cut under degassed water, the recovery in leaf water potential was rapid (half-times of 4–6 min) and, after a lag, photochemical activity also began to rise (Fig. 8). In this experiment, the assimilation chamber had to be opened in order to sample the leaf for a measurement of leaf water potential during recovery. Since no measurements of photosynthesis could be made while the chamber was open, the duration of the lag period may be inaccurate. Nevertheless, it appears that recovery in photochemical activity begins soon after leaf water potential begins to rise.
DISCUSSION

The data clearly show two factors that inhibit recovery of photosynthesis after a period of low leaf water potential: incomplete recovery of leaf water potential and incomplete return to full stomatal opening in the light. Providing \( \psi_w \) recovered fully after desiccation below -12 bars, the recovery of photosynthesis was of two types, depending on what was rate-limiting to the process. Under low light, where photochemical activity was limiting (16, 20), photosynthesis responded within a few minutes after rewetting, and recovery was essentially complete after 15 hr. Under high light, where stomatal aperture often affects photosynthetic rate (8, 13), photosynthesis remained below the level for well watered plants primarily because of lack of full stomatal opening. Since exposure to desiccation was short (1–2 days) and the older leaves showed no sign of a reduction in chlorophyll content, senescence probably was not a factor in these experiments. Thus, if it is assumed that \( \psi_w \) recovered completely, the reduced rates of photosynthesis previously reported (2, 11, 21, 25) after a period of desiccation can probably be explained by lack of full stomatal opening, since the measurements were made under high light. On the other hand, if \( \psi_w \) did not recover completely, stomatal aperture may have been reduced because of low turgor.

The lack of rapid recovery of leaf water potential under evaporative conditions following a period of severe desiccation suggests that there was higher resistance in the plant pathway for water transport after desiccation before this idea. This is also supported by the lag in recovery of leaf water potential, which could be largely eliminated by removal of the root system (11, 17), and by the lack of recovery after severe desiccation (-20 bars), which could be changed to normal recovery by removing stems and roots to allow the leaves to absorb degassed water, or by removing only the roots and briefly exposing the cut end of the stem to vacuum under degassed water.

Since the stem resistance returned to normal after a brief exposure to vacuum, the increased stem resistance was probably due to breaks in the water columns of the vascular system (14, 19, 22, 23). The reason for increased root resistance is not apparent (17, 18), although the tendency for leaf water potentials to return to predesiccation levels after several days suggests that the breaks or other sources of resistance were gradually repaired after the plant was rewetted. Breaks in the water columns of the vascular system appear gradually with increasing desiccation (19) and are generally attributed to the large negative pressures which occur in the xylem (19). Since the magnitude of these pressures increases as desiccation becomes more severe, this mechanism would require that an increase in the degree of desiccation be associated with increased breakage and increased resistance to water transport, as reported here. Since the resistance did not increase in the vascular system of the petiole, some factor apparently made the water columns in the petiole less susceptible to breakage than those in the stem. Measurements of vessel diameters in petioles and stems of a sunflower plant showed a range of diameters from 30 to 60 \( \mu \) in the petiole and from 60 to 132 \( \mu \) in the stem. It therefore appears that breakage occurred in the larger vessels first (19).

Since photosynthesis and respiration are appreciable in sunflower at \( \psi_w \) of -19 to -20 bars (9), increases in resistance to water transport precede tissue death in leaves that are being desiccated. The sequence of events appears to be: when \( \psi_w \) decreases to as low as -8 bars, photosynthesis and resistance to water transport are unaffected, but as potentials decrease below -8 bars to about -15 bars, photosynthesis declines. At these potentials, root and possibly stem resistances increase and may not permit full recovery of \( \psi_w \) after rewetting for as long as several days. At -19 to -20 bars, photosynthesis has declined to about 10% of the rate for the well watered plant (9), and stem and root resistances have increased enough to result in continued net loss of water after the plant has been rewetted, if the plant remains in an evaporative environment similar to that used in the present work. Continued exposure of the severely desiccated but rewetted plant to these evaporative conditions permits \( \psi_w \) to decrease below -20 to -25 bars, and tissue death begins.

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Fig. 7. Effect of low leaf water potential on rates of total photosynthesis measured at low light in sunflower. The low light conditions represent the region close to the light compensation point over which photosynthetic response to light was linear. The sunflower plant was desiccated to a leaf water potential of -11 bars before recovery and then was desiccated again to -17 bars before recovery. Photosynthesis was measured during each period of desiccation and 15 hr after each rewet. Leaf water potential was -3 bars in the well watered plant before desiccation, -4 bars after recovery from desiccation to -11 bars, and -5 bars after recovery from desiccation to -17 bars. Recovery took place in a dark humid chamber for 8 hr after rewetting.

Fig. 8. Recovery of rates of total photosynthesis measured in low light (0.18 cal cm\(^{-2}\) min\(^{-1}\)) after a sunflower leaf had been desiccated to a water potential of -17 bars. Leaf was excised from plant and permitted to absorb degassed water through the petiole at time indicated. Assimilation chamber was open for 20 min following excision to permit sampling of excised leaf for psychrometer.
LITERATURE CITED