INTERACTION BETWEEN WATER AND NUTRIENT DEFICIENCIES IN *HELIANTHUS ANNUUS*

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INTRODUCTION

The physiological response of plants to water deficits are known to vary according to the conditions of application of drought stress and the rate of development of leaf water deficits. At the whole plant level the effect of the water stress is usually perceived as a decrease in photosynthesis and growth, and is associated with alterations in C and N metabolism (McDonald and Davies, 1996). The decrease in water potential affects transpiration and hence xylem transport of nitrate or reduced N into growing regions. The response of the photosynthetic apparatus either to water stress or rehydration seems to be dependent on leaf age (O'Neill, 1983; Wolfe *et al.*, 1988). Degradation of both thylakoid and stromal N-containing compounds can occur in response to water stress, recovery from which may require more than a week (Chaves, 1991).

While the contribution of damage and down-regulation to decreased photosynthesis with drought has been appreciated for some time, the potential impact of drought-related decreases in leaf N status on photosynthesis has been largely ignored (Kaiser, 1987; Heckathorn *et al.*, 1997), and little attention has been given to post-drought effects.

The primary purpose of the present study was to estimate the importance of alterations in foliar N fractions to the response of the photosynthetic capacity during and especially after drought in sunflower (*Helianthus annuus* L., variety Giant, commercialised by Royalfleur, France) leaves of different ages. Since under field conditions water stress may often be combined with a limited availability of N in the soil, the importance of the N regime to the extent of the water stress response was also studied.

MATERIAL AND METHODS

Plant material, growth conditions and sampling

One month old plants, growing on a mixture of unfertilised peat (Shamrock) and vermiculite (1:1), in the greenhouse, were gradually subjected to water stress during 18 days. Water loss was measured gravimetrically every two days, and plants were then watered with nutrient solution corresponding to a third of the water lost. Nutrient deficiency was combined with water stress by supplying the plants with Hoagland solution (see Sutcliff and Baker, 1974) diluted to a final concentration of total nitrate of 9 mM (N+) or 4.5 mM (N-). Measurements were made on the last day of the water stress period and two days after rewatering, in leaves of two ages: Old – recently expanded leaf at the beginning of the water stress period, and Young – leaf expanded during the water stress period. For the determination of each parameter, at least four plants were used. Treatments were WS – water stressed; WW – well watered; RW – rewatered.

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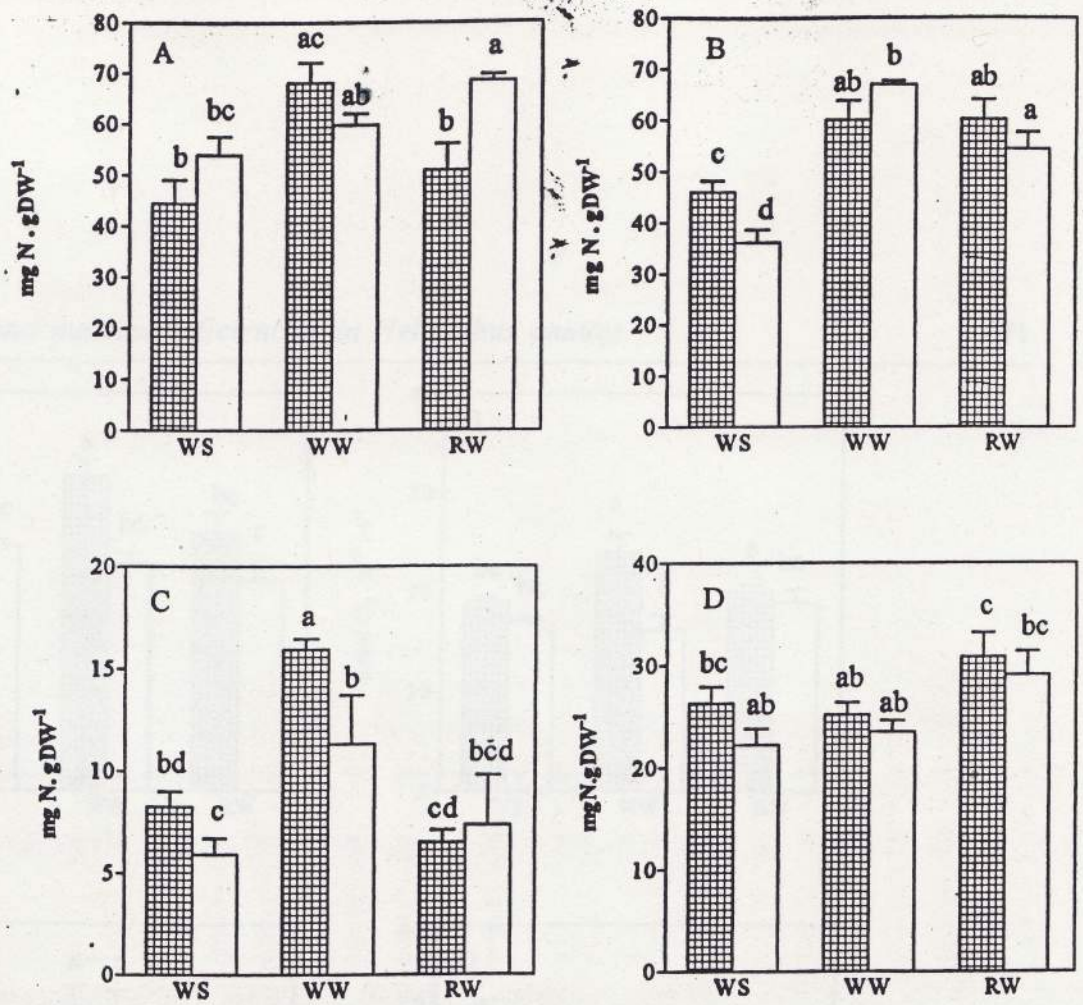

Figure 1. Total nitrogen concentration in young leaves (A), old leaves (B), stems (C) and roots (D) of water stressed (WS), well watered (WW) and re-watered (RW) plants, grown at either high N (chequered bars) or low N (white bars). Results are the mean of four replicates (± S.E.). Different letters indicate significant differences ($p<0.05$) between the means (Ficher's LSD test).

Water potential

Leaf water potential (Ψ) was measured with a Scholander pressure chamber.

Photosynthetic capacity

Amax was measured with an oxygen-electrode (Hansatech), on foliar discs, at 5% of CO_2 and saturating light.

RuBisCO and total protein

Samples for ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) determination were extracted as in Quick *et al.* (1991). RuBisCO protein was assayed by indirect enzyme-linked immunosorbant assay (ELISA) according to protocols described in Ausubel *et al.* (1990). The immunoreactants used were policlonal antibodies against RuBisCO (from wheat) raised in rabbit (courtesy of M. Paul, I.A.C.R. – Rothamsted, UK), and an anti-rabbit IgG peroxidase conjugate (Sigma, St. Louis, USA). The RuBisCO protein used as a standard was purified from sunflower, as in Paech and Dybing (1986). For total protein, samples were extracted with 50mM HEPES with 0.1% Triton X-100, and soluble protein was determined in the supernatant. The pellet was further ressuspended in TCA 10% and dissolved in 0.1M NaOH, for the undissolved membrane protein determination (Fichtner *et al.*, 1993). Both fractions were determined with the BioRad Pro-

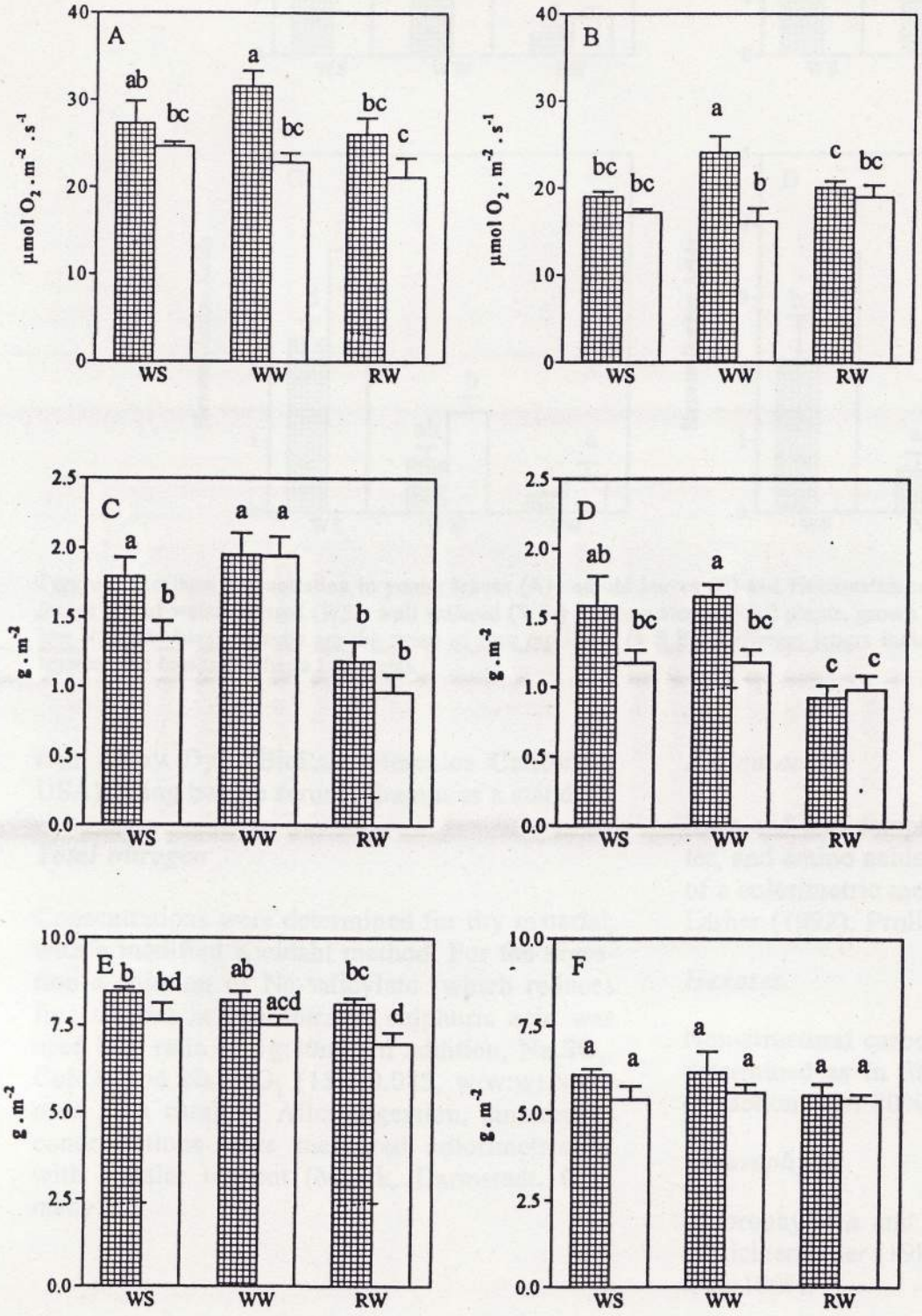

Figure 2. Photosynthetic capacity (Amax) of young leaves (A) and old leaves (B), RuBisCO levels in young leaves (C) and old leaves (D) and total protein levels in young leaves (E) and old leaves (F), of water stressed (WS), well watered (WW) and re-watered (RW) plants, grown at either high N (chequered bars) or low N (white bars). Results are the mean of four replicates (± S.E.). Different letters indicate significant differences ($p<0.05$) between the means (Ficher's LSD test).

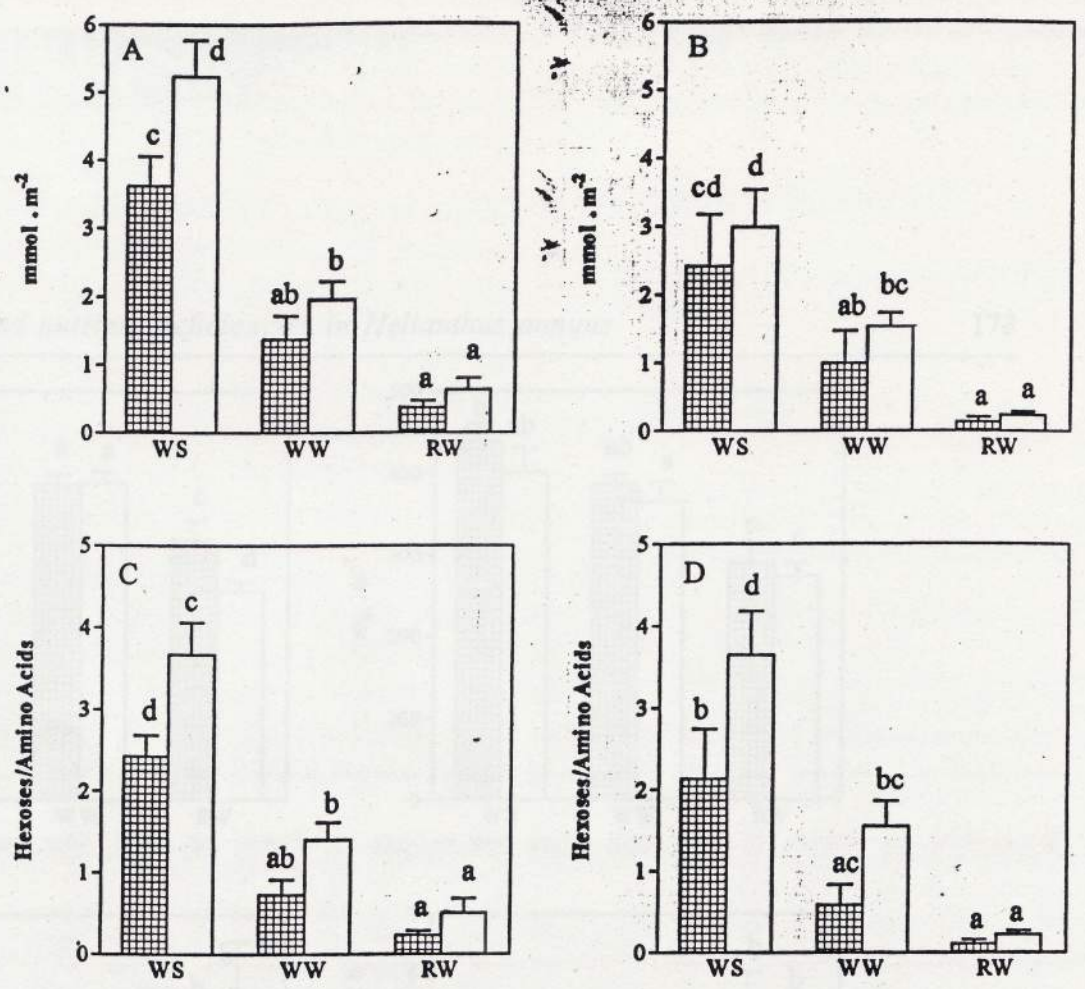

Figure 3. Hexoses concentration in young leaves (A) and old leaves (B) and Hexoses/amino acids in young leaves (C) and old leaves (D) of water stressed (WS), well watered (WW) and re-watered (RW) plants, grown at either high N (chequered bars) or low N (white bars). Results are the mean of four replicates (± S.E.). Different letters indicate significant differences ($P<0.05$) between the means (Ficher's LSD test).

tein Assay Dye (BioRad, Hercules California, USA), using bovine serum albumin as a standard.

Total nitrogen

Concentrations were determined for dry material, with a modified Kjeldahl method. For the digestion a solution of Na-salicylate (which reduces free nitrate) in concentrated sulphuric acid was used in a ratio of 1g:30ml. In addition, Na_2SO_4, $CuSO_4$ and Na_2SeO_3 (15:5:0.085, w:w:w) were used as a catalyst. After digestion, ammonium concentrations were measured colorimetrically with Nessler reagent (Merck, Darmstadt, Germany).

Amino acids

Root and leaf samples were extracted in hot water, and amino acids were quantified with the use of a colorimetric method according to Magné and Larher (1992). Proline was not quantified.

Hexoses

Non-structural carbohydrates were enzymatically determined as in Stitt *et al.* (1978, 1989), after extraction with 80% ethanol, at 80°C .

Chlorophylls

Chlorophylls *a* and *b* were quantified according to Lichtenthaler (1987), after extraction with acetone 100%.

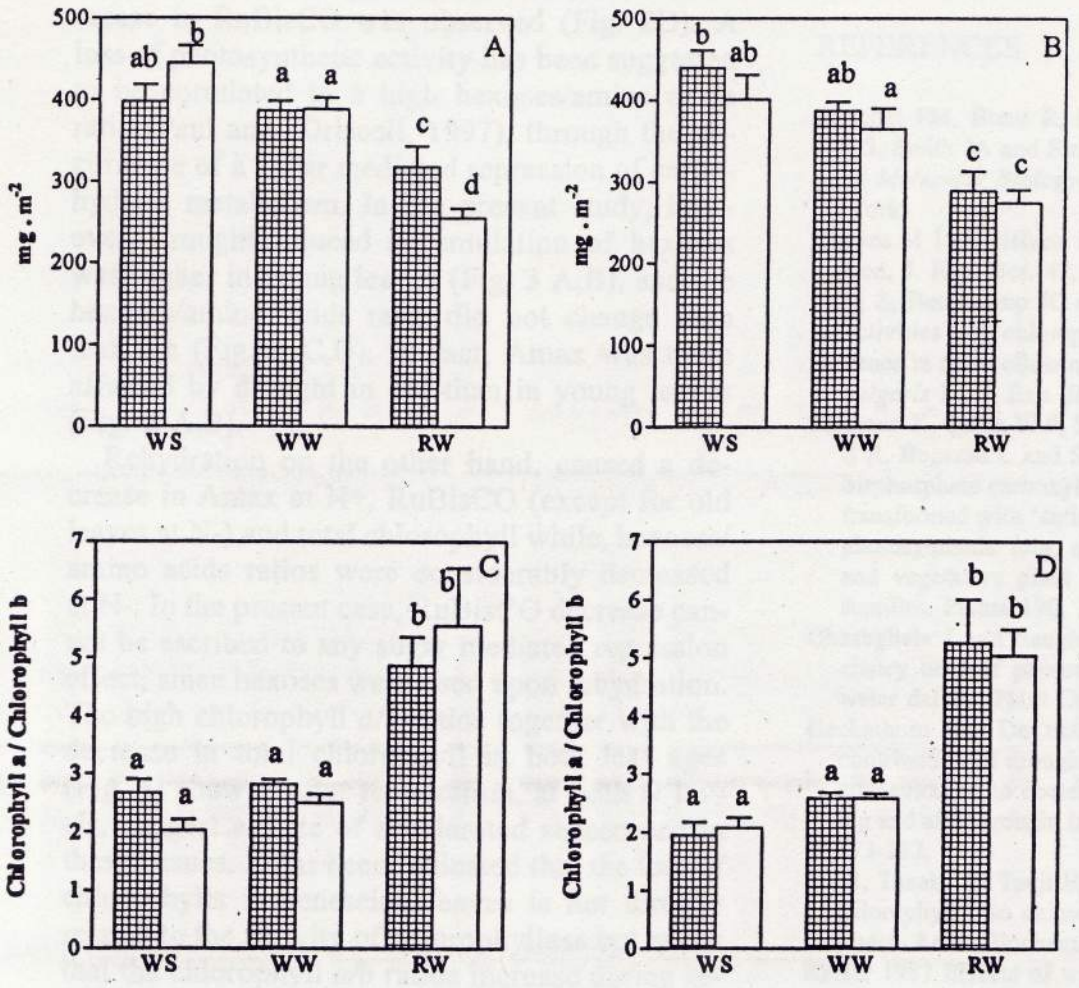

Figure 4. Total chlorophyll concentration in young leaves (A) and old leaves (B) and chlorophyll a/ chlorophyll b in young leaves (C) and old leaves (D) of water stressed (WS), well watered (WW) and re-watered (RW) plants, grown at either high N (chequered bars) or low N (white bars). Results are the mean of four replicates (± S.E.). Different letters indicate significant differences ($P<0.05$) between the means (Ficher's LSD test).

RESULTS AND DISCUSSION

Irrespective of N supply, water potentials of young and old leaves were decreased (by approximately -0.2MPa) by the gradually imposed water stress (data not shown), and upon rehydration did recover to control levels.

Total nitrogen concentration in leaves did not reflect the differences in N supply (Fig.1). Water stress reduced nitrogen concentration more in young leaves at N+ (35%, compared to 10% at N-) and in old leaves at N- (46%, compared to 23% at N+). The decreases in stem nitrogen concentration both during water stress and after rewatering (Fig.1C) might be due to a poor nitrogen uptake and translocation into the stem in water-stressed plants or to the use of the stem as a source of reduced nitrogen. This remobilized nitrogen might have been transported to the roots and leaves, avoiding a greater decrease in these plant parts. In fact, nitrogen levels in the roots were not affected by water stress (Fig. 1D).

Independently of the N level, a significant decrease in Amax related to leaf age was observed (Fig. 2A,B), but the way in which water stress affected the photosynthetic capacity varied with N level. In fact, whereas Amax of the N- plants was not affected by soil drying, N+ plants showed a decrease, mainly in the old leaves, which persisted after rehydration (Fig. 2A,B). These results are in agreement with what has been found for other species (Ghashghaie and Saugier, 1989). The variation in the apparent photosynthetic susceptibility to water stress resulted essentially from a

strong negative effect of N- over the photosynthetic capacity of the control plants. In fact, Amax measured in WWN-plants were similar to those measured in WSN+ plants, both for young and old leaves (Fig. 2A,B).

Decrease in Amax in WWN- young leaves and drought-induced Amax decrease in N+ was not related to a decrease either in RuBisCO, total protein or chlorophyll (Fig. 2 and Fig. 4). In the old leaves of the same plants, however, a 30% decrease in RuBisCO was observed (Fig. 2D). A loss of photosynthetic activity has been suggested to be correlated to a high hexoses/amino acids ratio (Paul and Driscoll, 1997), through the occurrence of a sugar mediated repression of carbohydrate metabolism. In the present study, however, drought-induced accumulation of hexoses was higher in young leaves (Fig. 3 A,B), and the hexoses/amino acids ratio did not change with leaf age (Fig. 3 C,D). In fact, Amax was more affected by drought in old than in young leaves (Fig. 2 A,B).

Rehydration on the other hand, caused a decrease in Amax at N+, RuBisCO (except for old leaves at N-) and total chlorophyll while, hexoses/amino acids ratios were considerably decreased at N-. In the present case, RuBisCO decrease cannot be ascribed to any sugar mediated repression effect, since hexoses were used upon rehydration. The high chlorophyll *a/b* ratios together with the decrease in total chlorophyll in both leaf ages (Fig. 4) showed after rehydration, at both N levels, suggest a state of accelerated senescence in these tissues. It has been indicated that the loss of chlorophylls in senescing leaves is not directly related to the activity of chlorophyllase but rather that the chlorophyll *a/b* ratios increase during senescence (Fang *et al.*, 1998) as chlorophyll *b* is degraded by first being converted to chlorophyll *a* (Scheumann *et al.*, 1996; Ito *et al.*, 1993). Since chlorophyll b is associated to the protein-chlorophyll complexes, it seems that during rehydration the light harvesting complexes might have suffered a preferential degradation in relation to the reaction centres.

In the present study, remobilization of nitrogen after rehydration, irrespectively of the N regime, seems to be preferred to the reduction of newly uptaken nitrate, even if this is done at the expense of the degradation of apparently vital compounds. It is possible that at this stage young and old leaves are both functioning as source leaves, with the plants investing in the formation of new sinks.

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