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# PHYSIOLOGICAL AND PHYTOCHEMICAL PROPERTIES OF TINNEVELLY SENNA, *Cassia angustifolia* Vahl: RESPONSES TO DROUGHT, NITROGEN AND DEFLOWERING

by

**Harish Ratnayaka**

A dissertation submitted to the Graduate Faculty of Biology in partial fulfillment of the requirement for the degree of Doctor of Philosophy,
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## ABSTRACT

**Physiological and phytochemical properties of Tinnevelly Senna, *Cassia angustifolia* Vahl: Responses to drought, nitrogen and deflowering**

by

**Harish Ratnayaka**

**Advisor - Professor Dwight T. Kincaid**

Experiments were conducted to evaluate the promise of Tinnevelly senna as an alternative crop for stressful agroecosystems, and to identify component technologies that would increase leaf sennoside yield. Effects of drought, foliar nitrogen spray, crop type and deflowering on net photosynthesis ($P_{net}$) and sennoside A and B yields were investigated.

Net photosynthesis was completely suppressed at leaf xylem water potential of -2 MPa. Long term drought reduced leaf biomass and number of stomata per plant by 77% and 74%, respectively, mainly due to defoliation. Foliar applied nitrogen spray increased $P_{net}$ in both watered and droughted plants. Leaf yield was 156% increased by foliar nitrogen application, in the watered plants, but was unaltered in the droughted plants. Foliar nitrogen spray decreased stomatal density on the adaxial surface but increased it on the abaxial surface. Trichome density was increased by drought but decreased by foliar nitrogen spray. Among the crop types, $P_{net}$ was highest in seedlings followed by cuttings and ratoon, while leaf yield was highest in seedlings followed by ratoon and cuttings. Deflowering reduced $P_{net}$ by 20% but increased the leaf yield by 63% compared to

the flowering plants. In all the treatments, $P_{net}$ was closely associated with leaf chlorophyll concentration.

Short term drought increased sennoside A+B concentration (% dw) in the dried leaves. Long term drought, after the drought-induced morphological changes occured, did not influence sennoside A+B concentration. Foliar nitrogen spray decreased sennoside A+B concentration in both droughted and watered plants but increased sennoside yield per plant by 142%, only in the watered plants. Ratoon had the highest sennoside A+B concentration followed by seedlings and cuttings but the sennoside yield per plant was highest in seedlings followed by ratoon and cuttings. Deflowering increased the sennoside A+B concentration by 25%, and doubled the sennoside yield per plant. Youngest leaves had the highest sennoside concentration (% dw). Sennoside yield per plant was more a function of leaf biomass than the sennoside concentration. Short term drought, foliar nitrogen spray, ratooning, deflowering and picking of young leaves are promising component technologies for field-research toward increasing sennoside yields.

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# INTRODUCTION

Environmental stresses such as moisture deficit and limited availability of soil nitrogen constrain the productivity of a wide range of agroecosystems. A cost-effective and environmentally sound way of managing this situation is adoption of crop species capable of producing acceptable yields under these conditions. Crops that are agriculturally underutilized and bear great industrial potential in a global context, deserve top priority in this regard.

Senna, *Cassia angustifolia* Vahl (Caesalpiniaceae, syn. *Senna alexandrina* Mill. as per revision by Irwin and Barneby, 1982), is included in pharmacopeias of US, UK, India and many other countries mainly for its cathartic properties (Husain *et al.*, 1984; Lemli, 1986; Folkard, 1995). Primary active constituents of senna are two rheindianthrone-8, 8' diglucosides, sennoside A and sennoside B (hereafter referred to as sennosides, Figure 4. 1), accumulated in industrially extractable quantities mainly in dry leaflets and pods. Despite the availability of some synthetic laxatives, sennoside-based formulations are increasingly being used as safe, common laxatives (Farnsworth, 1973; Atzorn *et al.*, 1981; Al-Dakan *et al.*, 1995), and as colon cleansing pre-treatments in special medical procedures (Ziegenhagen *et al.*, 1991; Cameron, 1992; Pahor *et al.*, 1995). Senna leaflets also contain cassic acid (rhein) which is antibiotic to *Staphylococcus aureus* (Perry and Metzger, 1980), and effective against other bacterial and fungal infections (Folkard, 1995).

Commercial production of senna is restricted mainly to India where it is grown in tropical marginal drylands (Gupta, 1980; Pareek *et al.*, 1983). My preliminary on-farm observations in the dry zone of Sri Lanka proved its promise as a rainfed

crop in the dry season using residual moisture from the preceding wet season for early crop establishment. A senna crop can be established by seedlings, cuttings and ratooning, as well. Furthermore, senna responds to nitrogen fertilization substantially, partly due to its inability to fix atmospheric nitrogen (Kalyanasundaram *et al.*, 1980; Gupta *et al.*, 1982; Pareek *et al.*, 1983).

Yields of secondary metabolites are influenced by the rate of primary metabolism, relative proportions of photosynthate allocation and partitioning into different organs, and patterns of photosynthate conversion to secondary products (Bernath, 1986; Geiger and Servaites, 1991). Likewise, environmental stresses such as moisture and nitrogen deficiency inextricably interact with these phytophysico-chemical properties (Chapin *et al.*, 1987). However, in senna, the nature of functional relationships between primary metabolic processes and the sennoside yields as affected by different plant water and nitrogen status in different developmental phases are yet to be established.

The main objective of this study was therefore, to investigate the effects of the plant moisture and nitrogen status, crop type (seedlings, cuttings and ratoon) and developmental phases (age and flowering) on primary metabolism, and sennoside accumulation in the leaves, the major harvest of senna. Furthermore, assessments on leaf ultrastructure, leaf elemental composition and whole plant biomass partitioning were undertaken for a more comprehensive understanding of the relationships among the physiological, phytochemical and developmental properties.

The knowledge generated by the study will help evaluate senna as a potential new crop to agroecosystems that are faced with moisture and nitrogen stresses. The

treatment variables that show promise for increasing sennoside productivity would be identified for investigation in field research. The results will also reveal the physiological and morphological mechanisms that confer tolerance to drought and nitrogen stress.

# CHAPTER 1

## EFFECTS OF DROUGHT AND FOLIAR NITROGEN APPLICATION ON GAS EXCHANGE AND LEAF CHARACTERISTICS

### SUMMARY

Four treatment combinations with drought and foliar application of 1% urea (w/v) were assigned to five week old ratooned, deflowered senna plants in a glasshouse. Xylem water potential, leaf gas exchange and chlorophyll concentration, and yield components were assessed using leaves that developed during the treatments.

A leaf xylem water potential of -2.1 MPa completely suppressed $P_{net}$ without causing permanent wilting. Foliar application of urea increased $P_{net}$ in both droughted and watered plants. There was no effect of drought on $P_{net}$ and other gas exchange characteristics during the first four days of a 10 day long drought that followed four one week long drought cycles. In severe drought, foliar nitrogen application increased WUE, although it increased transpiration, as well. With an interaction with foliar nitrogen, drought increased leaf chlorophyll concentration.

Individual leaflet area was 22% reduced by drought. Foliar nitrogen application increased the leaflet area more in watered plants than in droughted plants. Drought increased leaf specific mass (LSM) by 7%. Foliar nitrogen application increased LSM by 9% and 15% in watered and droughted plants, respectively. Drought-induced defoliation and reduced leaflet size each decreased the leaf area and leaf weight per plant by 78%. Foliar nitrogen spray increased the leaf area and leaf weight (by 156%) in watered plants, with no effect in the droughted plants. Although there were no effects of drought on $P_{net}$, the nitrogen effects still persisted nine weeks after the drought and foliar nitrogen treatments were discontinued and plants were allowed to flower and to set pods.

## INTRODUCTION

Plant primary metabolism is tightly linked to the acquisition and use of resources such as nitrogen and moisture. For instance, energy from photosynthesis is necessary for nitrogen acquisition, and photosynthesis itself is strongly influenced by leaf nitrogen status. Similarly, maintenance of moist mesophyll cell surfaces is essential to facilitate $CO_2$ absorption to the leaf (Chapin *et al.*, 1987; Robert *et al.*, 1987; Henry and Raper, 1991), in addition to the role of water as a reactant in photophosphorylation. Furthermore, in most species the photosynthetic capacity is highly plastic, being closely influenced by resource availability (Chapin *et al.*, 1987).

Experiments were conducted to evaluate the effects of plant nitrogen and moisture status on gas exchange and leaf characteristics of senna using deflowered ratoon plants in the glasshouse. Foliar spraying of a 1% urea solution as a means of nitrogen application was tested. This was considered an alternative to the traditional soil-application of nitrogen which requires high quantities of expensive fertilizer that are unaffordable especially to the subsistence farmers of developing countries. Furthermore, the fraction of soil-applied fertilizer that is absorbed by the crops is considerably less due to volatilization and leaching losses.

Xylem water potential and gas exchange were investigated concurrently, in both droughted and watered plants with and without foliar urea spray, to establish the lower threshold of leaf xylem water potential that significantly suppresses the gas exchange. During these measurements, leaf chlorophyll concentration, as an intimate determinant of the photosynthetic capacity, in response to drought and nitrogen was investigated. Leaflet area and leaf specific mass were measured, as

well, to study their relationship to the above plant physiological indicators. Furthermore, recovery capacity of the plants and the patterns of persistence of the treatment effects were assessed by monitoring $P_{net}$, leaflet area and leaf specific mass during pod development, eight weeks after the drought and nitrogen treatments were discontinued.

## MATERIALS AND METHODS

### *Plant material and growth conditions*

A group of 12 approximately one-year-old seedling plants growing in 25 cm X 25 cm pots with a common soil mixture was pruned to obtain new shoot growth in early spring 1995. The soil mixture contained top soil: sand: peat, 2:1:1 by volume. A fertilizer mixture of urea (46% N) - 1.73 g, muriate of potash (62% $K_2O$) - 1.29 g and triple super phosphate (46 % $P_2O_5$) - 1.73 g was soil-applied to each plant two weeks before pruning. All plants were deflowered by nipping the flower buds at emergence, and adequately watered until the new shoots were five weeks old at which time experimental treatments were started.

### *Treatments and experimental design*

Treatments included two levels of nitrogen foliar application (N0 - no foliar nitrogen, and N1 - foliar nitrogen sprayed) and two levels of experimental drought stress (D0 - adequately watered, and D1 - drought-stressed). A 1% (w/v) urea:water solution with a trace of detergent spreader-sticker was used as the foliar nitrogen spray. Plants receiving this treatment were separated from the other plants, sprayed and replaced, weekly for three weeks. Control plants were sprayed with the same solution without urea. For water stress, plants were subjected to a one week drought starting a week prior to the beginning of nitrogen application.

Thus, plants receiving water stress and nitrogen were already one week droughted when they received the first urea spray. Four-one-week long drought cycles and three weekly foliar nitrogen applications were completed prior to all measurements that were taken during the fifth drought cycle of 10 days. This allowed for the development of sufficient numbers of new leaves, to be used for all measurements, while plants were being subjected to the treatments.

The four treatment combinations (D0N0, D0N1, D1N0 an D1N1) were assigned to the 12 plants in a Randomized Complete Block design with three replications. Plants were randomized within blocks twice a week.

## *Measurements*

### Gas exchange

An infrared gas analyzer-based photosynthetic system (Model LI 6200, LI-COR Inc., Lincoln, Nebraska, USA) was used to assess the gas exchange characteristics. Measurements were taken from the two most distal leaflet pairs of the second and third fully expanded young leaves from the apex of two equally developed branches in each plant. Readings were obtainable for nine consecutive days in the fifth drought cycle until the droughted plants reached $CO_2$ compensation point. A 1000 W metal halide lamp was used to secure the desirable intensity of full-sun PAR (approximately 1500 µmol $m^{-2}$ $s^{-1}$, 400-700 nm) during the measurements. Gas exchange variables measured were as follows.

| Variable | Units |
|---|---|
| Net photosynthesis ($P_{net}$) | (µmol $m^{-2}$ $s^{-1}$) |
| Stomatal conductance to water vapor ($g_{wv}$) | (mol $m^{-2}$ $s^{-1}$) |
| Transpiration (E) | (mol $m^{-2}$ $s^{-1}$) |

| | |
|---|---|
| Intercellular $CO_2$ ($C_i$) | (ppm) |
| Relative humidity (RH) | (0-100%) |
| Air temperature | (°C) |
| Photosynthetically active radiation (PAR) | (μmol $m^{-2}$ $s^{-1}$) |
| Vapor pressure deficit (VPD) | (mb) |

Vapor pressure deficit, a measure of air dryness, was computed as water vapor pressure of bulk air at a specific temperature and at 100% RH, minus the vapor pressure of air at its current humidity and at the same temperature. Water use efficiency (μmol $mol^{-1}$) was computed as the ratio of $P_{net}$ to transpiration rate. Due to the difficulty experienced in securing adequate contact between the leaflets and the thermocouple of the cuvette the three gas exchange parameters, $g_{wv}$, E and $C_i$ should be considered approximate rather than absolute, as per the technical advice of LICOR Inc.. Daily maximum and minimum temperatures were recorded during the measurements using a maximum-minimum mercury thermometer in the glasshouse.

**Xylem water potential**

Midday leaf xylem water potential was measured using the fifth or sixth leaf from the apex of a well developed branch using a Scholander pressure chamber every other day during the $P_{net}$ measurements.

**Leaf chlorophyll concentration**

During the course of gas exchange measurements, leaf chlorophyll concentration of each plant was measured thrice using disks removed from fully developed young leaflets. These leaflets were on the leaves at the closest lower node than the leaves that were used for the $P_{net}$ measurements. Extraction was performed in

96% ethanol for three days in dark at room temperature and absorbance of the supernatant was read using a spectrophotometer (Model, Perkin-Elmer Lambda 2, UV/VIS) at 649, 654 and 665 nm. Chlorophyll concentration (μg $cm^{-2}$) was computed as described by Wintermans and Mots (1965). Furthermore, a Minolta leaf greenness/chlorophyll meter (SPAD 501) was used to assess the leaf chlorophyll content nondestructively, based on SPAD units (Marquard and Tipton, 1987). A total of 110 SPAD readings per treatment combination was taken on three days during the series of $P_{net}$ measurements.

**Leaf area and leaf specific mass**

The eight leaflets used for $P_{net}$ measurements of each plant were used to determine the leaf area and leaf specific mass as well (LSM). Leaflets were photocopied and the copies of the images were weighed using a digital scale (OHAUS Analytical Plus). Leaf area (LA, $cm^2$) was calculated based on the average weight of five circles with known area removed from the same paper that was used to obtain the copies of the leaf images. The same leaflets used for LA measurements were oven dried at 70°C to a constant weight, and LSM was calculated as the ratio of dry weight of the total leaflet (μg) to the area of the same leaflet ($mm^2$). Total leaf area per plant was estimated by multiplying the total leaflet number of each plant by each of four leaflets to generate a sample size of 12 per treatment combination. Total leaf weight per plant was estimated based on LSM and total leaf area.

**Post-treatment performance during late reproductive phase**

***Plant material and growth conditions***

Upon completion of the above experiment, the 12 plants in the pots were all

adequately watered and placed outside the glasshouse in the early summer of 1995. They were again maintained with uniform growth conditions and the four plants in each block were randomized at least twice a week. No fertilizers were applied. All plants were allowed to flower and set pods. Active insect-pollination was observed.

## *Measurements*

### Gas exchange

Photosynthetic measurements were taken twice during pod development (seed filling). The two most distal pairs of the leaflets of two leaves on two branches in each plant were used.

### Leaf specific mass, leaflet area and total leaf area

The leaflets used to assess $P_{net}$ were detached for measuring leaf area and LSM by photocopy and oven-dry methods, respectively, as described earlier. Thus, these two parameters were measured based on 24 leaflets from each of the 4 treatments combinations.

### Biomass partitioning

Plants were uprooted when the pods turned dark brown. Oven-dried (70°C to constant weight) roots, stems, leaves and pods were separately weighed.

### Statistical analyses

StatView (ver. 1.01, Feldman and Gagnon, 1986) and JMP (ver. 2.0.1; SAS Inst. Inc.) were used on Macintosh computers to test the hypotheses that there is no effect of drought or foliar nitrogen application or interaction between these main treatments on the response variables investigated. R-square was computed as the

ratio of sum of squares of respective treatment to the total sum of squares. Statistical power analysis (Cohen, 1977) was performed using JMP, specifying a type one error of 0.05, sample size, root mean square error and effect size of the raw data.

## RESULTS

### Xylem water potential

Water-stressed plants were clearly drier than the watered plants as shown in Figure 1. 1. Foliar nitrogen application exhibited no significant effect on leaf xylem water potential in either watered or droughted plants throughout the measurements.[1] Droughted plants experienced a range of 1.6 MPa of xylem water potential with a minimum of -2.1 MPa on the $10^{th}$ day of the experimental drought. The range of leaf xylem water potential observed in watered plants was only 0.7 MPa with a minimum -1 MPa observed on the $10^{th}$ day of the measurements. Maximum air temperature fluctuated through a range of 23 °C as it became warmer toward the last several days of the experimental drought. Wilting did not occur at the levels of xylem water potential measured in this experiment.

[1] F=0.08, P=0.77, R-sq=0.00

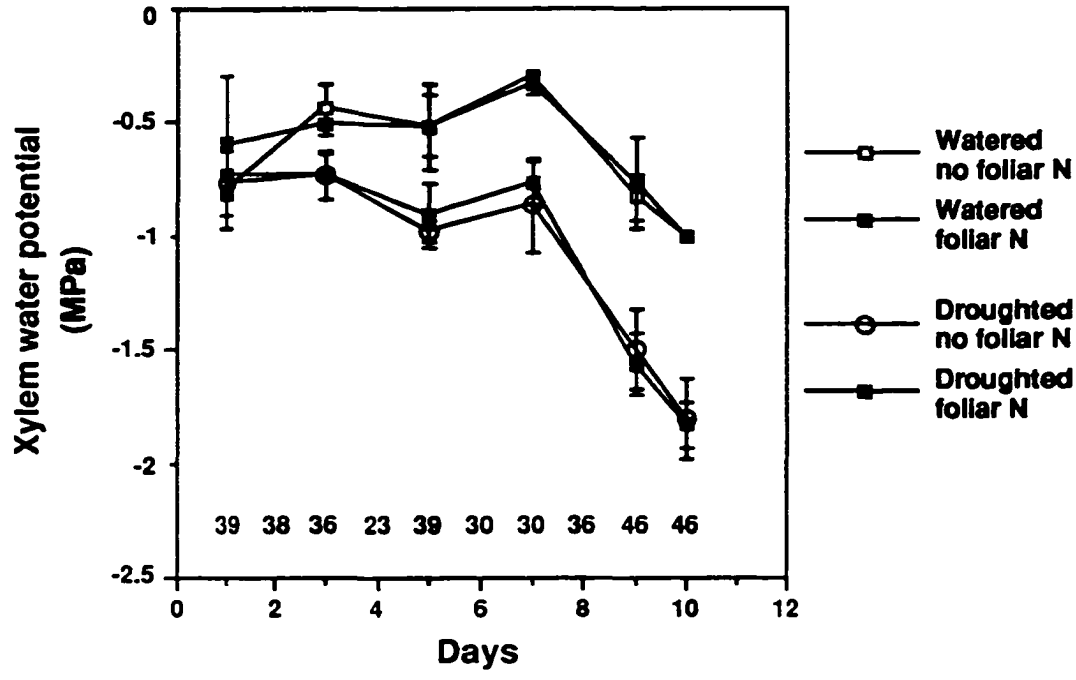


**Figure 1. 1** Midday leaf xylem water potential during the fifth drought cycle. Water potential of the droughted plants on the 10$^{th}$ day represents the water stress that completely suppressed the net photosynthesis without causing permanent wilting. Each value is an average of three measurements, with 95% CI. Numbers above the days indicate the maximum glasshouse temperature (°C) of each day.

**Net photosynthesis**

Experimental drought showed no effect on net photosynthesis when the data of the first four days were pooled as illustrated by Figure 1. 2.[1] However, foliar nitrogen application showed a highly statistically significant effect on $P_{net}$ during this time (F=22.3, P<0.001, R-sq=0.06). Foliar nitrogen application increased $P_{net}$ by 18% and 22% in the watered and droughted plants, respectively, during this period. There was no interaction effect between nitrogen and drought.[2] The whole model test for $P_{net}$ was associated with a statistical power of ca 0.94 with the least significant number (LSN), the sample size for realizing a significant effect at P<0.05, of 127. Statistical power of nitrogen effect for this period was ca 0.97 with a LSN of only 57.

Effect of drought on $P_{net}$ was, however, highly significant when the results of the last five days were pooled (F=45.72, P<0.001, R-sq=0.08). Foliar nitrogen application showed a highly significant effect as well (F=54.27, P<0.001, R-sq=0.10) with again no significant interaction with drought.[3] Water stress decreased the $P_{net}$ by 32% and 20% in plants without and with foliar nitrogen application, respectively. Foliar nitrogen application increased $P_{net}$ by 40% and 67% in watered and water-stressed plants, respectively. Whole model and main effects were each associated with a statistical power of nearly one. The LSN was only 37 for the whole model test, and 33 and 31 for drought and nitrogen, respectively.

Tables 1. 1 and 1. 2 present the daily photosynthetic performances of the watered and water-stressed plants with one way ANOVA. Plants that received foliar

---

[1] F=1.19, P=0.27, R-sq=0.00
[2] F=1.27, P=0.26, R-sq=0.00
[3] F=1.28, P=0.26, R-sq=0.00

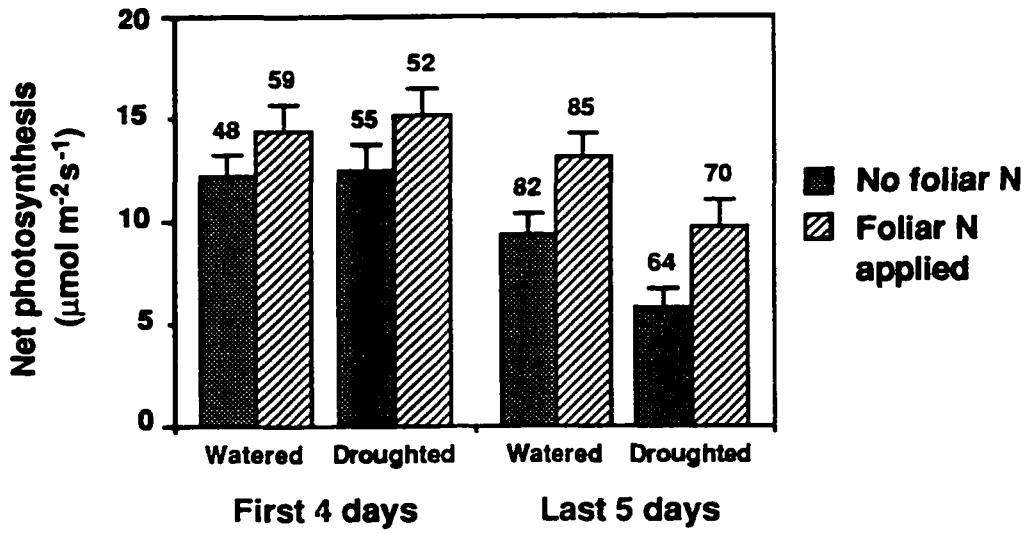


**Figure 1. 2** Net photosynthesis of droughted and watered plants in response to foliar nitrogen application. Data were grouped into first four days and next five days in the 10 day drought cycle ($P_{net}$ was not detectable in droughted plants on $10^{th}$ day) to show the effect of the length of drought. Error bars represent 95% CI with number of measurements (multiple daily readings on three plants in each group) on top.

**Table 1. 1** Net photosynthesis (μmol $m^{-2}$ $s^{-1}$) of the watered plants with and without foliar nitrogen (N) application, over nine consecutive days (mean with SE). Column N, number of observations, corresponds to the mean $P_{net}$ on its left.

| Days | Foliar N applied | N | No foliar N applied | N | F | P | R-sq |
|---|---|---|---|---|---|---|---|
| **Day 1** | 14.26 (1.41) | 18 | 12.23 (0.60) | 12 | 1.26 | 0.27 | 0.04 |
| **Day 2** | 13.46 (14.48) | 17 | 11.05 (1.17) | 12 | 2.88 | 0.10 | 0.09 |
| **Day 3** | 14.48 (1.21) | 14 | 13.33 (1.09) | 12 | 0.47 | 0.49 | 0.00 |
| **Day 4** | 16.10 (1.89) | 10 | 12.26 (1.06) | 12 | 3.39 | 0.08 | 0.14 |
| **Day 5** | 17.18 (1.77) | 14 | 9.97 (0.89) | 18 | 15.00 | < 0.001 | 0.33 |
| **Day 6** | 16.45 (0.68) | 24 | 13.27 (0.97) | 16 | 7.54 | < 0.01 | 0.16 |
| **Day 7** | 8.72 (1.09) | 14 | 5.30 (0.52) | 20 | 9.60 | < 0.01 | 0.23 |
| **Day 8** | 13.34 (1.27) | 15 | 10.70 (1.24) | 15 | 2.10 | 0.15 | 0.07 |
| **Day 9** | 8.55 (0.97) | 18 | 8.66 (1.06) | 16 | 0.00 | 0.94 | 0.00 |
| **Total** | 13.62 (0.45) | 144 | 10.39 (0.38) | 130 | 28.26 | < 0.001 | 0.09 |

**Table 1. 2** Net photosynthesis (μmol $m^{-2}$ $s^{-1}$) of the water-stressed plants with and without foliar nitrogen (N) application, over nine consecutive days (mean with SE). Column N, number of measurements, corresponds to the mean $P_{net}$ on its left.

| Days | Foliar N applied | N | No foliar N applied | N | F | P | R-sq |
|---|---|---|---|---|---|---|---|
| **Day 1** | 12.99 (0.36) | 12 | 10.62 (0.77) | 16 | 6.20 | 0.02 | 0.19 |
| **Day 2** | 15.28 (2.08) | 14 | 10.26 (1.19) | 14 | 4.36 | 0.04 | 0.14 |
| **Day 3** | 15.91 (0.64) | 10 | 13.98 (1.47) | 12 | 1.25 | 0.27 | 0.06 |
| **Day 4** | 16.40 (0.74) | 16 | 15.58 (1.41) | 13 | 0.30 | 0.59 | 0.01 |
| **Day 5** | 9.36 (1.43) | 15 | 6.58 (0.37) | 15 | 3.52 | 0.07 | 0.11 |
| **Day 6** | 12.25 (1.02) | 16 | 8.97 (1.38) | 14 | 3.77 | 0.06 | 0.12 |
| **Day 7** | 8.56 (0.46) | 19 | 5.07 (0.53) | 15 | 24.67 | < 0.001 | 0.43 |
| **Day 8** | 15.35 (1.27) | 14 | 7.92 (0.93) | 12 | 21.44 | < 0.001 | 0.47 |
| **Day 9** | 3.60 (1.36) | 6 | 0.96 (0.36) | 8 | 4.52 | 0.05 | 0.27 |
| **Total** | 12.52 (0.49) | 122 | 9.13 (0.48) | 119 | 23.83 | < 0.001 | 0.09 |

nitrogen application maintained a higher $P_{net}$ compared to the plants without foliar nitrogen application, with an overall increase of 31% and 37% in watered and water-stressed plants, respectively. There was no significant effect of the interaction between drought and foliar nitrogen application for the whole dataset.[1] Furthermore, as Figure 1. 3 illustrates, net photosynthesis was positively correlated to VPD<45, across all four treatment combinations, and negatively correlated when the data for the whole period were pooled.

**Stomatal conductance to water vapor**

There was no effect of drought on stomatal conductance over the first four days as shown in Figure 1. 4.[2] However, with a significant interaction with drought (F=9.59, P<0.01, R-sq=0.03), foliar nitrogen spray increased $g_{wv}$ insignificantly in the watered plants[3] and highly significantly in the droughted plants (for nitrogen - overall ANOVA, F=17.68, P<0.001, R-sq=0.05; droughted plants, F=23.2, P<0.001, R-sq=0.14). The whole model test showed a statistical power of ca 0.98 with a LSN of only 63.

When the data of the last five days were pooled, a highly significant interaction effect, although with a low R-sq of 0.02, was observed (F=13.24, P<0.001). Both drought and foliar nitrogen application showed very highly significant effects on stomatal conductance (drought, F=68.2, P<0.001, R-sq=0.13; nitrogen application, F=18.0, P<0.001, R-sq=0.02). The pattern of increase in stomatal conductance by foliar nitrogen application in the watered and water-stressed plants during this period was similar to that of the first four days. Statistical power values were

---

[1] F=0.90, P=0.34, R-sq=0.00
[2] F =0.16, P=0.68, R-sq=0.00
[3] F = 0.71, P = 0.39, R-sq = 0.00

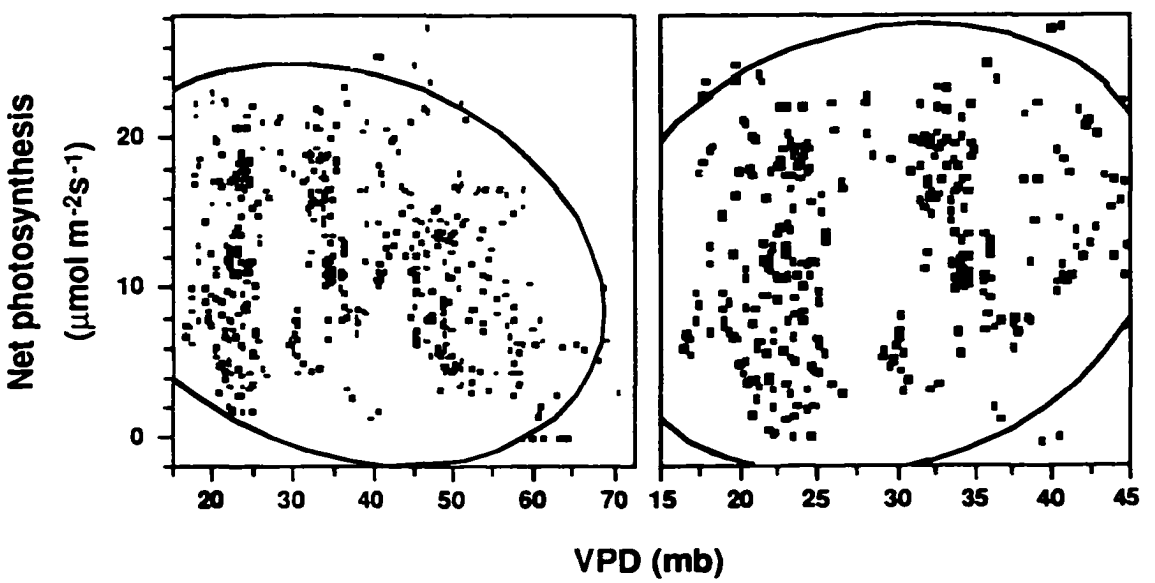


**Figure 1. 3** Scatter plots and correlation analyses for $P_{net}$ versus atmospheric VPD, with 95% prediction ellipses. Left; all $P_{net}$ measurements ($r = -0.22$, $P < 0.001$, $N = 515$). Right; $P_{net}$ values associated with VPD<45 ($r = 0.17$, $P < 0.01$, $N = 338$). Both plots represent data pooled across all four treatment combinations.

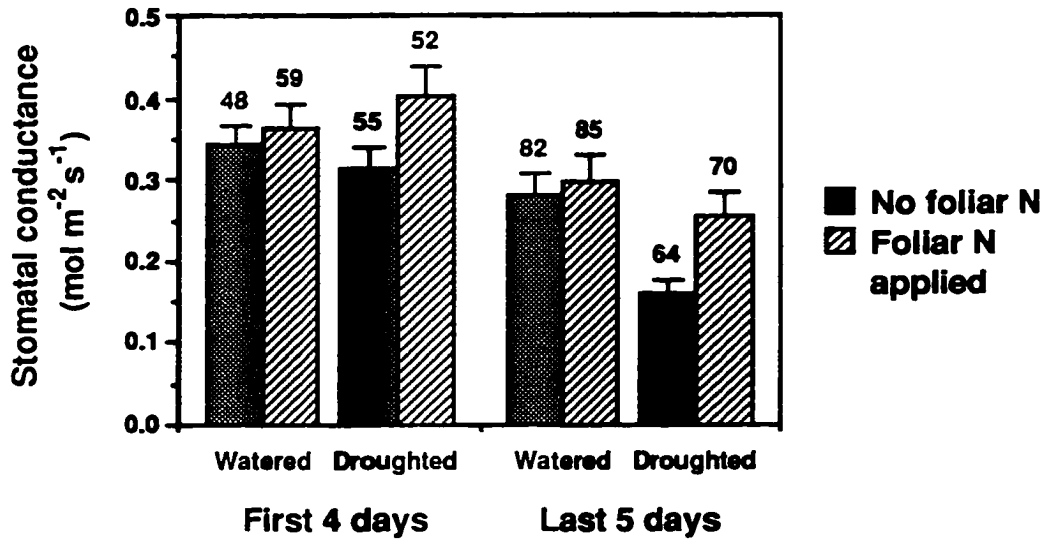


**Figure 1. 4** Stomatal conductance of watered and droughted plants, with and without foliar nitrogen application, during the fifth drought cycle. Data are grouped into first four and last five days to show the response of each treatment combination to the length of drought. Values on error bars (95% CI) represent number of measurements.

nearly one, nearly one, and ca 0.98 with LSN of only 37, 28 and 71 for the whole model test, drought, and foliar nitrogen application, respectively, during the last five days of the measurements.

Intercellular $CO_2$ concentration showed no or weak correlation to stomatal conductance when data were pooled in each nitrogen treatment (Figure 1. 5). However, the plants that received foliar nitrogen maintained 11% less intercellular $CO_2$ concentration than the plants that were deprived of foliar nitrogen ($F=18.68$, $P<0.001$, R-sq=0.03). This test was associated with ca 0.99 of power and a LSN of only 145.

**Transpiration rate**

Figure 1. 6 illustrates the patterns of transpiration rate during early and late stages of the fifth drought cycle. Experimental drought showed no effect on transpiration during the first four days.[1] Furthermore, the increase in transpiration rate by foliar nitrogen application was significant only in the droughted plants[2] ($F=5.02$, $P<0.05$, R-sq=0.04). During the later spell of the drought as well, only the droughted plants exhibited a highly significantly greater transpiration rate when foliar nitrogen was applied, compared to the plants that were deprived of the foliar nitrogen supplement ($F=21.2$, $P<0.001$, R-sq=0.12). The interaction effect between drought and foliar nitrogen application on the transpiration rate during this time, was significant ($F=5.34$, $P<0.05$, R-sq=0.02). Furthermore, water-stressed plants experienced an overall 40% drop in transpiration rate from the early four days to the later five days (stomatal resistance during the same time increased 100%, data not presented).

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[1] $F=0.26$, $P=0.60$, R-sq=0.00

[2] watered plants - $F=0.11$, $P=0.73$, R-sq=0.02

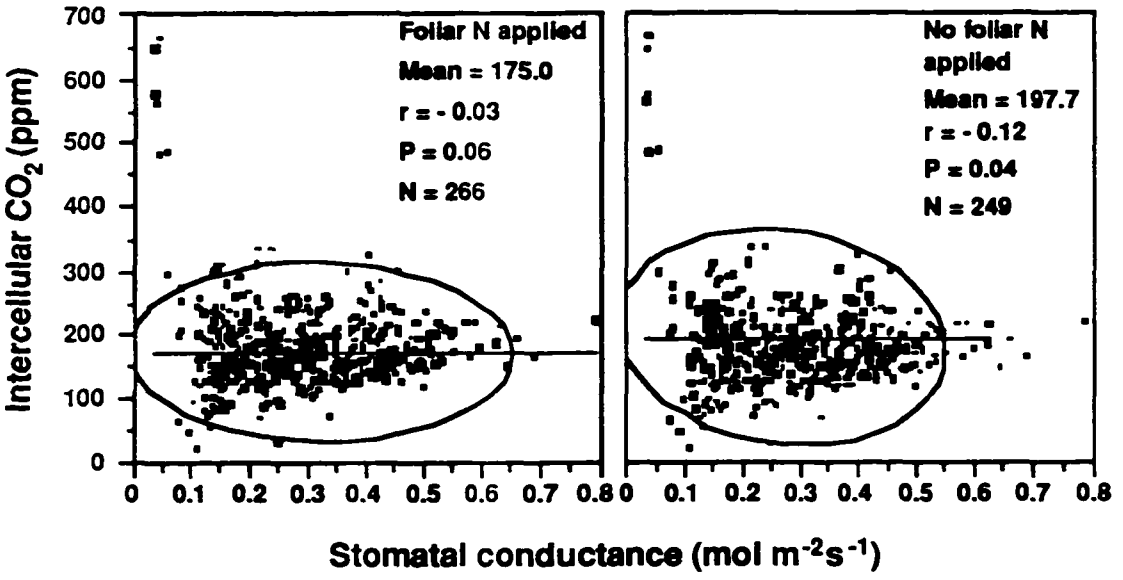


**Figure 1. 5** Scatter plots with 95% prediction ellipses of intercellular $CO_2$ concentration versus stomatal conductance for plants with and without foliar nitrogen application. Droughted and watered plants were pooled in each plot. Line through each scatter represents the mean.

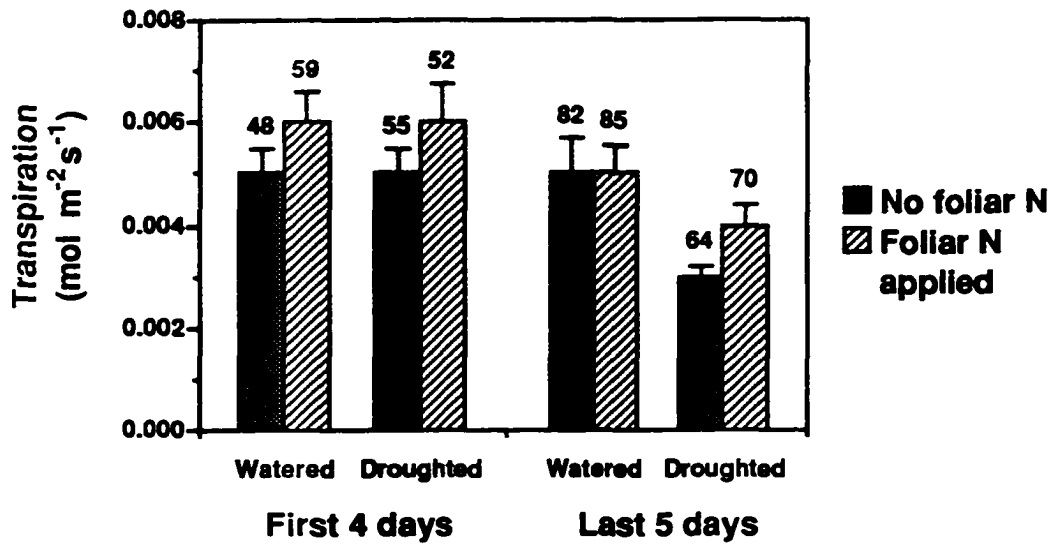


**Figure 1. 6** Transpiration rate of watered and droughted plants as influenced by foliar nitrogen application. Data are grouped into early (first four days) and late (last five days) periods of the drought to show the changes in transpiration pattern among the treatment combinations at varying severities of drought. Value on error bar (95% CI) represents the number of measurements.

**Water use efficiency**

All four treatment combinations showed statistically similar water use efficiencies (WUE) during the early four days of the measurements (Figure 1. 7). However, either watered or water-stressed plants that received the foliar nitrogen application showed a 25% higher WUE toward the later stage compared to the plants without foliar applied nitrogen (watered plants, $F=5.1$, $P<0.05$, R-sq=0.03; water-stressed plants, $F=7.0$, $P<0.01$, R-sq=0.05). The statistical power of the whole model test for the last five days was ca 0.87 with a LSN of 219, and ca 0.97 with LSN of 83 for nitrogen application.

**Chlorophyll concentration**

Foliar nitrogen application increased the leaf chlorophyll concentration by 47% in the watered plants, and by only 10% (nonsignificantly) in the droughted plants as shown in Figure 1. 8. This interaction effect between drought and foliar nitrogen application ($F=6.7$, $P<0.05$, R-sq=0.03) was confirmed by the leaf greenness Minolta SPAD units ($F=30.1$, $P<0.001$, R-sq=0.02). Both main effects resulted in very highly significant increases in extractable chlorophyll concentration (drought, $F=64.4$, $P<0.001$, R-sq=0.32; foliar nitrogen, $F=28.3$, $P<0.001$, R-sq=0.14), and in leaf greenness SPAD units (drought, $F=391.5$, $P<0.001$, R-sq=0.30; foliar nitrogen, $F=175.7$, $P<0.001$, R-sq=0.13). Furthermore, SPAD readings showed that the increase in leaf chlorophyll concentration by foliar nitrogen application was highly significant in the droughted plants, as well ($F=24.9$, $P<0.001$, R-sq=0.07). Leaf chlorophyll concentration was not correlated to LSM (Figure 1. 9).

**Leaflet area and LSM**

As presented in Table 1. 3, the leaflet area was 38% decreased by the water stress. Foliar nitrogen application resulted in 15% more leaflet area in the watered plants,

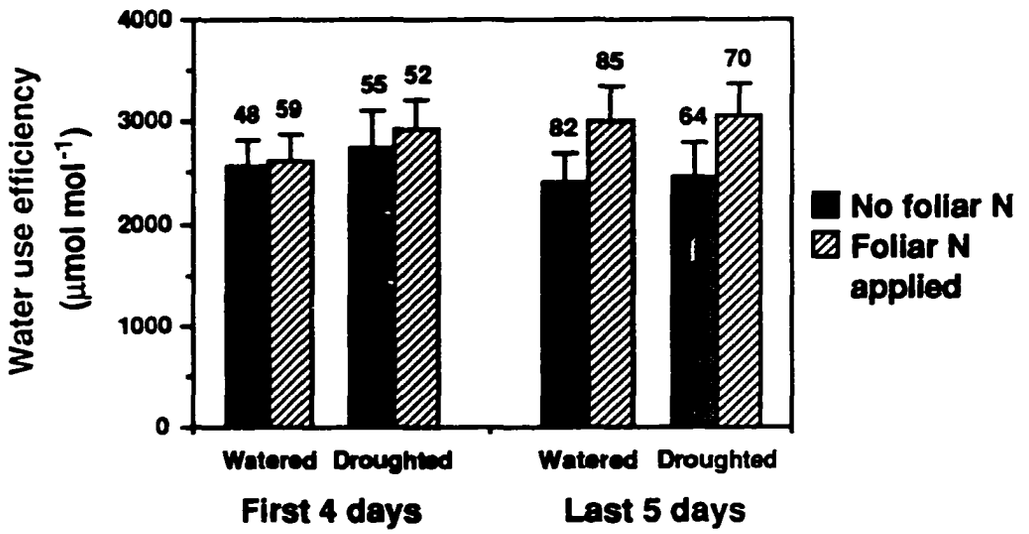


**Figure 1. 7** Effect of drought and foliar nitrogen application on water use efficiency during the fifth drought cycle. Results are shown separately for early and later stages of drought to show the effect of intensity of water stress on WUE in each treatment combination. Number on 95% CI error bars indicates the number of observations.

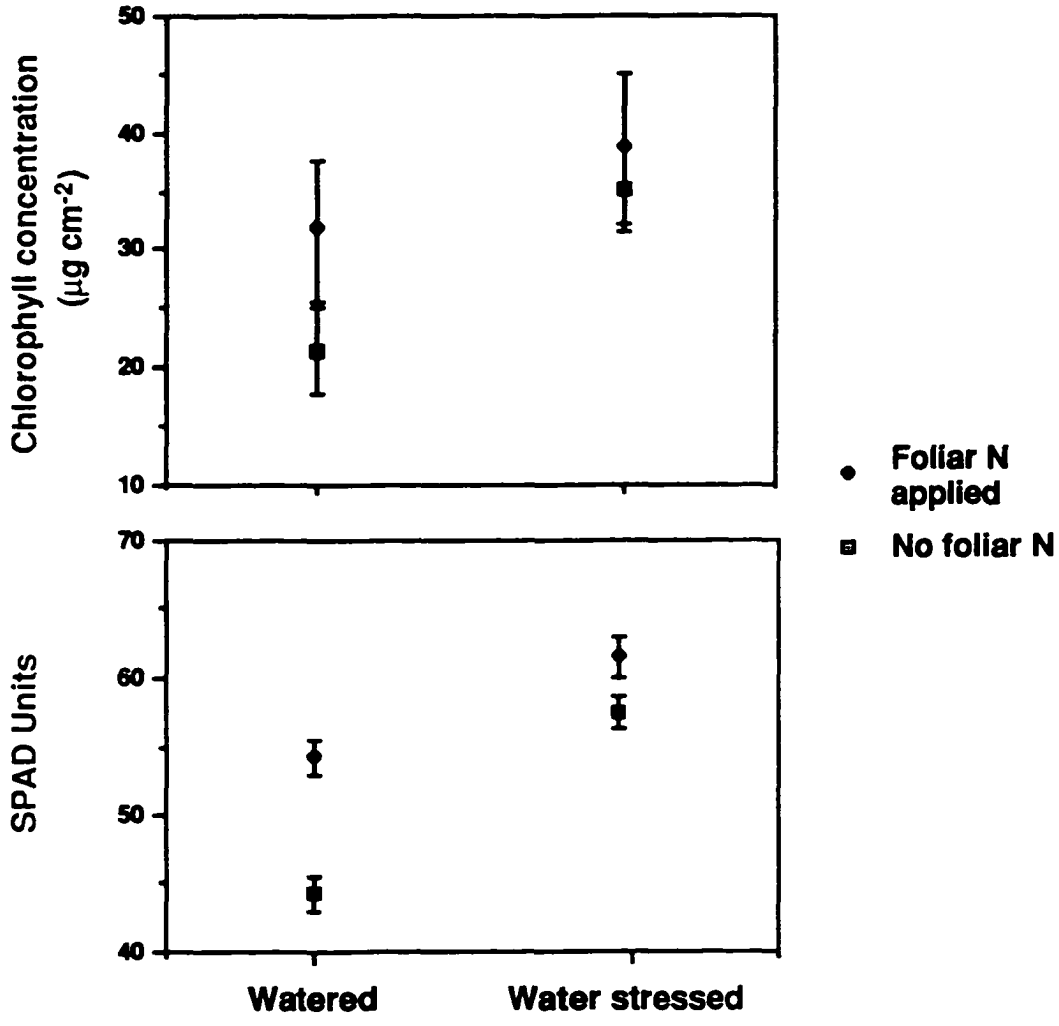


**Figure 1. 8** Effect of foliar nitrogen application and plant water status on chlorophyll concentration. The top panel presents leaf chlorophyll concentration measured destructively by the extraction method (N = 9). The lower panel illustrates the consistency of the same pattern as measured nondestructively by SPAD units (N = 110). Error bars indicate 95% CI of the mean.

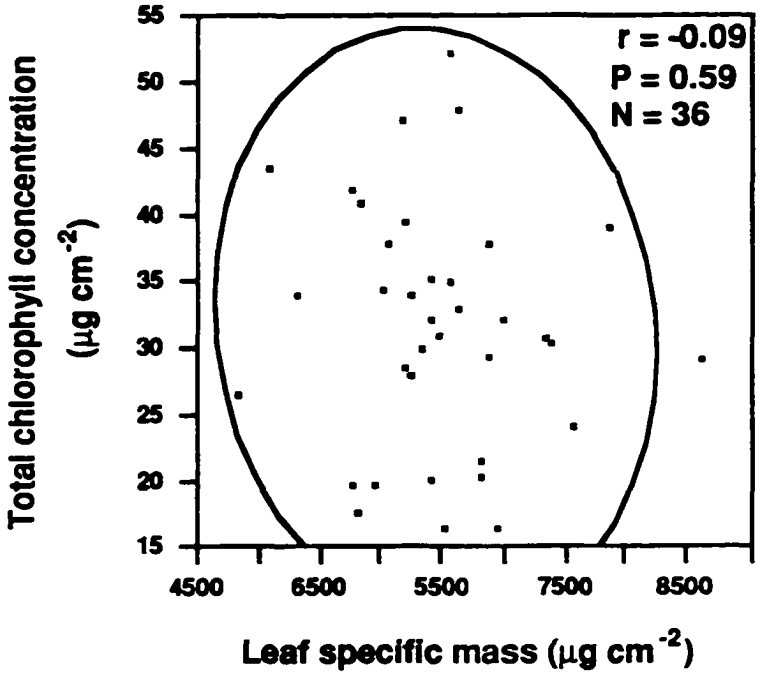


**Figure 1. 9** Scatter plot and correlation analysis of total chlorophyll concentration versus LSM (converted to the same units as of total chlorophyll). Data, pooled across all treatments, with 95% prediction ellipse.

**Table 1. 3** Mean leaflet area ($cm^2$) of watered and droughted plants as affected by foliar nitrogen application. Number in parenthesis is SE. Column N represents the number of observations (leaves) of three plants in each group.

| Treatment | N | Leaflet area ($cm^2$) |
|---|---|---|
| **Watered** | | |
| **No foliar N** | 12 | 1.71 (0.17) |
| **Foliar N applied** | 12 | 1.97 (0.08) * |
| **Total** | 24 | 1.85 (0.09) |
| **Droughted** | | |
| **No foliar N** | 12 | 1.09 (0.14) |
| **Foliar N applied** | 12 | 1.21 (0.13) NS |
| **Total** | 24 | 1.15 (0.10) |

**ANOVA**

| | F | P | R-sq |
|---|---|---|---|
| **Droughted (D)** | **53.82** | **< 0.001** | **0.36** |
| **Nitrogen (N)** | **3.92** | **0.05** | **0.02** |
| **D X N** | **0.54** | **0.46** | **0.00** |

*, significantly greater than ($P<0.05$), and NS, not significant ($P=0.45$) from the other nitrogen treatment within the same water status by PLSD.

and only a nonsignificant 11% increase in droughted plants. The whole model test for leaflet area was associated with a statistical power of ca 0.99 with a LSN of 20. Water-stressed plants acquired 7% more LSM than the watered plants (Table 1. 4). Foliar nitrogen application caused very highly significant 9% and 15% greater LSM in watered and droughted plants, respectively. Statistical power of the whole model test for LSM was ca 0.98 with a LSN of only 23.

### Total leaf area and leaf weight

There was a highly significant interaction effect of the foliar nitrogen application and drought on total leaf area per plant as presented in Table 1. 5. Experimental drought caused 78% reduction in the total leaf area. However, foliar nitrogen application increased the total leaf area (by 156%) only in the watered plants with no significant effect in the droughted plants. The whole model test was associated with a power of nearly one with a LSN of nine. Similarly, leaf weight per plant was 76% decreased by the drought (Table 1. 6). Foliar nitrogen application increased the leaf biomass in watered plants by 185% with no effect in the droughted plants. A statistical power of nearly one and a LSN of six were detected for this whole model test.

## Post-treatment performance during late reproductive phase

### Net photosynthesis

As illustrated in Figure 1. 10, $P_{net}$ during pod development was not influenced by the drought cycles that the plants had undergone eight weeks earlier.[1] However, foliar nitrogen application in watered and water-stressed plants produced

[1] F=0.29, P=0.58, R-sq=0.01

**Table 1. 4** Leaf specific mass (LSM) of watered and water-stressed plants with and without foliar nitrogen application. Number in parenthesis is SE. Column N represents the number of observations.

| Treatment | N | LSM ($\mu g\ mm^{-2}$) |
|---|---|---|
| **Watered** | | |
| **No foliar N** | 12 | 59.32 (1.81) |
| **Foliar N applied** | 12 | 64.49 (1.46) ** |
| **Total** | 24 | 61.91 (2.38) |
| **Droughted** | | |
| **No foliar N** | 12 | 61.85 (2.38) |
| **Foliar N applied** | 12 | 71.06 (1.94) ** |
| **Total** | 24 | 66.45 (1.78) |

**ANOVA**

| | F | P | R-sq |
|---|---|---|---|
| **Drought (D)** | **8.49** | **0.006** | **0.08** |
| **Nitrogen (N)** | **21.23** | **< 0.001** | **0.21** |
| **D X N** | **1.68** | **0.20** | **0.02** |

**, significantly greater than the other nitrogen treatment within the same water status by PLSD ($P<0.01$).

**Table 1. 5** Total estimated leaf area per plant ($cm^2$) of watered and droughted plants as influenced by foliar nitrogen application. Number in parenthesis is SE. Column N represents the number of observations.

| Treatment | N | Leaf area ($cm^2$) |
|---|---|---|
| **Watered** | | |
| **No foliar N** | 12 | 474.39 (37.31) |
| **Foliar N applied** | 12 | 1213.41 (85.92) *** |
| **Total** | 24 | 843.90 (89.63) |
| **Droughted** | | |
| **No foliar N** | 12 | 195.95 (37.91) |
| **Foliar N applied** | 12 | 175.55 (13.88) NS |
| **Total** | 24 | 185.75 (19.86) |

**ANOVA**

| | F | P | R-sq |
|---|---|---|---|
| **Droughted (D)** | **485.41** | **< 0.001** | **0.53** |
| **Nitrogen (N)** | **144.67** | **< 0.001** | **0.16** |
| **D X N** | **161.57** | **< 0.001** | **0.17** |

***, significantly greater than ($P<0.001$), and NS, not significant ($P=0.43$) from the other nitrogen treatment within the same water status by PLSD.

**Table 1. 6** Total estimated leaf biomass of watered and droughted plants as affected by foliar nitrogen application. Number in parenthesis is SE. Column N represents number of measurements.

| Treatment | N | Leaf weight (g) |
|---|---|---|
| **Watered** | | |
| **No foliar N** | 12 | 2.76 (0.14) |
| **Foliar N applied** | 12 | 7.77 (0.49) *** |
| **Total** | 24 | 5.26 (0.58) |
| **Droughted** | | |
| **No foliar N** | 12 | 1.14 (0.19) |
| **Foliar N applied** | 12 | 1.23 (0.08) |
| **Total** | 24 | 1.19 (0.10) |

**ANOVA**

| | F | P | R-sq |
|---|---|---|---|
| **Droughted (D)** | **211.37** | **< 0.001** | **0.50** |
| **Nitrogen (N)** | **83.06** | **< 0.001** | **0.20** |
| **D X N** | **77.21** | **< 0.001** | **0.10** |

*** Significantly greater than the other nitrogen treatment within the same water status by PLSD ($p<0.001$).

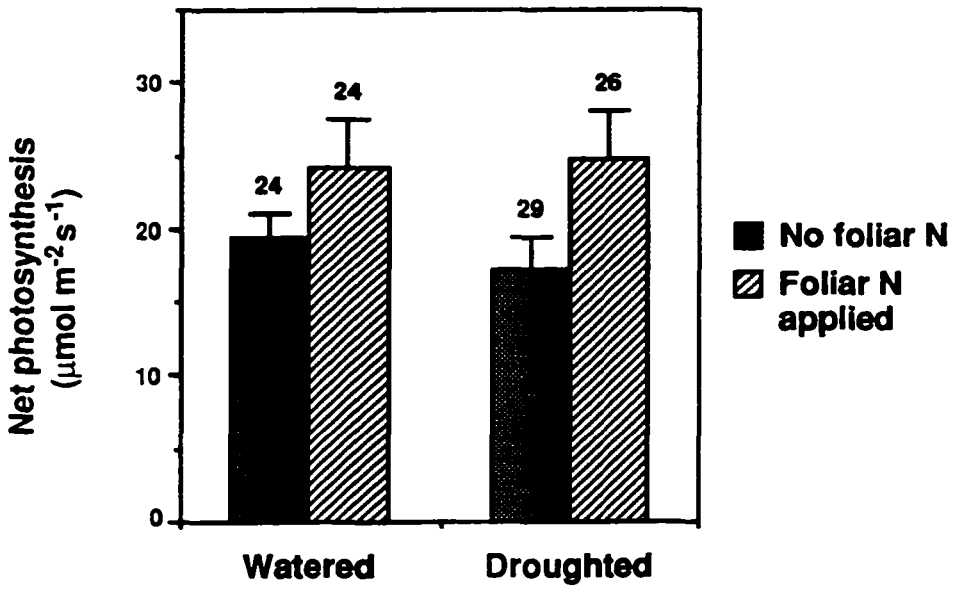


**Figure 1. 10** Net photosynthesis of plants during pod development, eight weeks after nitrogen and drought treatments were discontinued. Error bars, with number of observations above, represent 95% CI of the mean.

24% and 44% greater net photosynthesis, respectively, compared to the plants that were deprived of foliar nitrogen application (F=20.14, P<0.001, R-sq=0.85; statistical power=0.97, LSN=51).

**Leaflet area and LSM**

Plants that underwent drought cycles eight weeks earlier maintained 9% more leaflet area than the continuously watered plants (Table 1. 7). Foliar nitrogen application increased leaflet area by 32% and 28% in watered and droughted plants, respectively, compared to the plants that were not sprayed with foliar nitrogen. Leaf specific mass of droughted plants was 9% greater than watered plants. Watered, foliar nitrogen-applied plants maintained a highly significant 16% greater LSM compared to the watered plants that were deprived of foliar nitrogen application (Table 1. 8). However, in droughted plants, the plants that were deprived of foliar nitrogen application had 5% greater LSM than the plants that were sprayed with foliar nitrogen (F=5.66, P<0.05, R-sq=0.02).

**Biomass partitioning**

Figure 1. 11 presents the biomass of different plant organs, 12 weeks after the treatments were discontinued. All organs show increased biomass accumulation when foliar nitrogen was applied both in watered and droughted plants except for the leaf weight of watered plants. Droughted plants maintained slightly less biomass in all plant parts than the watered plants. However, none of these differences was statistically significant at the sample size of this experiment, except for the 100% increase in pod weight by foliar nitrogen application in watered plants (F=10.24, P<0.05, R-sq=0.46).[1]

[1] For total biomass - drought, F=1.32, P=0.28, R-sq=0.12; nitrogen, F=1.32, P=0.28, R-sq=0.12; interaction, F=0.09, P=0.76, R-sq=0.00

**Table 1. 7** Individual leaflet area ($cm^2$) of plants during pod development, approximately eight weeks after treatments were discontinued and uniform growth conditions were made available. Number in parenthesis is SE. Column N is the number of observations.

| Treatment | N | Leaflet area ($cm^2$) |
|---|---|---|
| **Watered** | | |
| **No foliar N** | 24 | 1.02 (0.04) |
| **Foliar N applied** | 24 | 1.35 (0.05) *** |
| **Total** | 48 | 1.19 (0.04) |
| **Droughted** | | |
| **No foliar N** | 24 | 1.14 (0.07) |
| **Foliar N applied** | 24 | 1.46 (0.06) *** |
| **Total** | 48 | 1.30 (0.05) |

**ANOVA**

| | F | P | R-sq |
|---|---|---|---|
| **Droughted (D)** | **6.94** | **0.01** | **0.02** |
| **Nitrogen (N)** | **58.11** | **< 0.001** | **0.25** |
| **D X N** | **0.00** | **0.98** | **0.00** |

***, significantly greater than the other nitrogen treatment within the same water status by PLSD ($P<0.001$).

**Table 1. 8** Leaf specific mass (LSM) of plants during pod development, approximately eight weeks after treatments were discontinued and uniform growth conditions were made available. Number in parenthesis is SE. Column N represents the number of observations.

| Treatment | N | LSM ($\mu g\ mm^{-2}$) |
|---|---|---|
| **Watered** | | |
| **No foliar N** | 24 | 73.34 (1.44) |
| **Foliar N applied** | 24 | 85.51 (2.99) *** |
| **Total** | 48 | 79.43 (1.86) |
| **Droughted** | | |
| **No foliar N** | 24 | 88.61 (3.77) * |
| **Foliar N applied** | 24 | 84.35 (1.42) |
| **Total** | 48 | 86.48 (2.01) |

**ANOVA**

| | F | P | R-sq |
|---|---|---|---|
| **Droughted (D)** | **38.64** | **< 0.001** | **0.06** |
| **Nitrogen (N)** | **12.12** | **< 0.001** | **0.02** |
| **D X N** | **52.38** | **< 0.001** | **0.08** |

***, *, significantly greater than the other nitrogen treatment within the same water status by PLSD at $P<0.001$ and $P<0.05$, respectively.

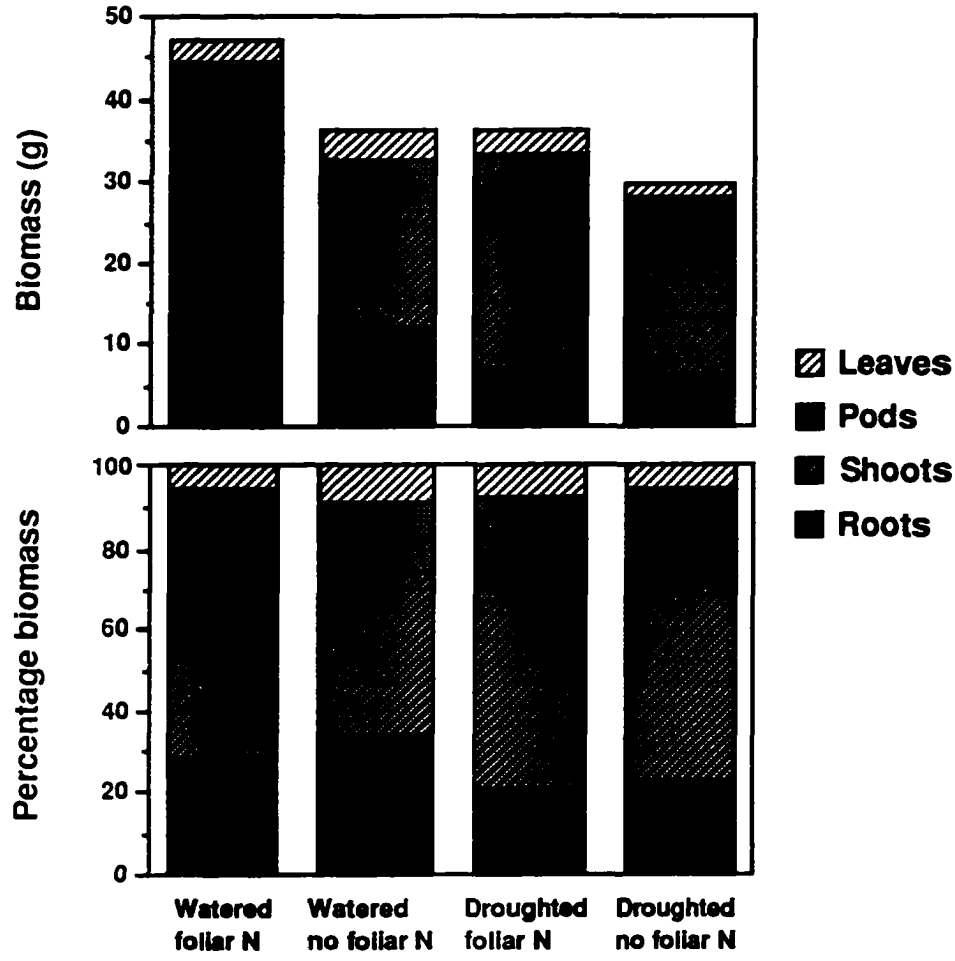


**Figure 1. 11** Biomass partitioning into different plant organs (upper panel), 12 weeks after the treatments were discontinued and uniform outdoor growth conditions were maintained. Lower panel presents the biomass of each organ expressed as a percentage of the whole plant.

## DISCUSSION

Although the leaf xylem water potential was significantly lower in the droughted plants, net photosynthesis was not affected by the drought during the first four days of the fifth drought cycle. During the course of measurements, the droughted plants were first observed to photosynthesize significantly less than the watered plants only on the fifth day at around -0.8 MPa of xylem water potential at a maximum air temperature of 39°C. Again, during the following cooler sixth and seventh days, although xylem water potential of the droughted plants remained stable, net photosynthesis dropped continuously. This, with the similar fluctuations of stomatal conductance and transpiration, suggests the operation of a drought sensitive stomatal control of water loss, considerably independent of the existing plant water status. The patterns of changes in xylem water potential, especially the sharp drop during the very warm final several days of the measurements, of both watered and droughted plants suggest that this strategy is probably influenced by ambient air temperature, as well. The ability to regain primary metabolic activity promptly, upon restoration of water regime following a long drought constitutes an important adaptive advantage in the dry agroecosystems with scanty rainfall.

Water-stressed senna plants carry only a marginal total leaf area with leaves present only around the apical bud of each branch, primarily due to defoliation. Individual leaflet size is reduced in a severe drought. These morphological responses combined with stomatal regulation enabled the droughted plants to maintain xylem water potentials of around -2 MPa without causing permanent wilting even in the exceptionally high maximum air temperature of 46°C. Maximum air temperatures recorded in an open dry farming tract of Sri Lanka

during the dry season were less than 40°C (DASL, 1990). The post-treatment performance in $P_{net}$, leaf area, LSM and biomass partitioning also testify that the photosynthetic apparatus of water-stressed senna plants remains intact being capable of full physiological recovery upon water supply, and allowing for setting of seed.

This drought tolerant capacity is further exemplified by the same WUEs of the droughted plants compared to the watered plants during the fifth drought cycle, and same $P_{net}$ (and the WUEs, data not presented) of the droughted and watered plants upon restoration of plant water status of droughted plants, during pod development. Moreover, although $P_{net}$ shows a weak positive correlation to VPD < 45 mb, $P_{net}$ clearly decreases in the drier atmosphere. This constitutes a physiologically advantageous adaptation that avoids permanent damage to the photosynthetic systems in the dry atmospheric conditions. Although VPDs > 60 mb were recorded in this experiment, Weerasuriya and Kincaid (1998) reported maximum VPDs of only < 30 mb in a dry zone site of Sri Lanka during the dry season where senna is a prospective crop. Furthermore, the net photosynthesis being low in droughted plants is manifested as reduced biomass in different plant organs. This, however, may have been influenced by the biomass of the plants prior to pruning, as well.

Foliar nitrogen application proves to boost net photosynthesis irrespective of the plant water status (even in the droughted, highly defoliated plants) probably through increased chlorophyll concentration indicating very high nitrogen investment for carbon gain. Approximately 75% of the leaf nitrogen in a $C_3$ plant is found in the chloroplasts and most of it is invested in the primary metabolism (Chapin *et al.*, 1987). Droughted plants concentrate chlorophyll in the top

remaining leaves with an increased LSM, without regard to foliar nitrogen application. This indicates effective reabsorption and reallocation of resources from the defoliating leaves ensuring adequate metabolic activity for tissue maintenance during the water stress. Maintenance of increased LSM in the plants that received foliar nitrogen application, even during pod fill, is probably related to the increased photosynthetic capacity, compared to the plants that were deprived of the nitrogen supplement. However, this needs to be confirmed by leaf elemental analyses and cellular ultrastructural (chloroplast development) investigations. Kalyanasundaram *et al.* (1980), Gupta *et al.* (1982) and Pareek *et al.* (1983), as well, reported high nitrogen responsiveness of senna which was manifested through yield increases.

Increased stomatal conductance and transpiration rate by foliar nitrogen application in droughted plants further suggest that plants readily acquire carbon if adequate nitrogen is available even at a reasonable cost of water. Higher $C_i$ in plants without foliar nitrogen application, though uncorrelated to stomatal conductance, may be due to reduced $CO_2$ metabolism compared to plants that received foliar nitrogen application.

Chlorophyll concentration is not correlated to LSM, even though drought or foliar nitrogen application increases both LSM and chlorophyll content. This suggests that the treatment combinations have different magnitudes of effects on these two response variables. Leaf specific mass, however, confounds leaf thickness and density (Witkowski and Lamont, 1991). If the chloroplast density per cell is plastic then density probably contributes more to the LSM than the thickness in senna leaves. Reduced leaf turgor in water-stressed plants presumably suppresses

cell expansion resulting in the same dry mass contained in a smaller leaf of increased leaf density (Smith and Nobel, 1978; Rascio *et al.*, 1990).

Foliar nitrogen application increased the total leaf area and leaf weight per plant, important yield components, only when moisture was not a severe constraint, under the conditions of the experiment. However, the levels of soil nitrogen in the pots of droughted plants without foliar nitrogen in the experiments, are most likely greater than the typical tropical dry farming highlands. Thus, a greater response to foliar nitrogen application can be expected in such agroecosystems. Moreover, the strong interaction effect between drought and foliar nitrogen application on leaf area and leaf weight suggests that level of drought-induced defoliation plays an important role in determining the degree of nitrogen deficiency, in terms of plant productivity, at a given level of drought stress. Thus, site-specific field investigations should determine the optimum plant population densities, depending on the level of defoliation and the resource availability, that will yield sustainable returns to the farmers. However, investigations on leaf sennoside concentrations as influenced by these treatments are essential, for assessing the final economic value of the harvest.

# CHAPTER 2

## GAS EXCHANGE AND LEAF CHARACTERISTICS OF CROP TYPES

### SUMMARY

Crop types established by ratoon, cuttings and seedlings in pots were compared using gas exchange, leaf chlorophyll concentration and leaf yield components. Effects of drought and time of day on gas exchange were assessed, as well. Gas exchange response to varying degrees of photosynthetic photon flux density (PPFD) was evaluated in seedling crop.

A severe drought that totally suppressed $P_{net}$ without permanent wilting caused the maximum suppression of water potential, -2.2 MPa, in ratoon. Upon watering, seedlings and cuttings re-acquired the pre-drought rates of $P_{net}$ overnight. In fully watered conditions, $P_{net}$ was 20% and 64% higher in seedlings than in cuttings and ratoon, respectively. Water use efficiency was 17% and 18% higher in seedlings than in cuttings and ratoon, respectively. Net photosynthesis of seedlings was highest at 1600 $\mu mol\ m^{-2}\ s^{-1}$ of PPFD. Outside the glasshouse, all three crops exhibited the maximum $P_{net}$ in early morning (8:30 am, DST) when air dryness (VPD) was lowest, although the PPFD was maximum around noon.

Mean leaf chlorophyll content of seedlings (82.96 $\mu g\ cm^{-2}$) was 17% and 55% higher than cuttings and ratoon, respectively. Individual leaflet area was 93% and 154% higher, and LSM was 8% and 9% higher in the seedlings than cuttings and ratoon. Leaf weight per plant was 44% and 170% higher in seedlings than ratoon and cuttings, respectively. Ratoon had 91% higher leaf area and 88% higher leaf weight per plant than cuttings. Cuttings developed 57% and 60% less root biomass, and 36% and 37% less total biomass than ratoon and seedlings, respectively.

## INTRODUCTION

Senna plants can be established by direct seeding, by vegetative propagation using cuttings, and by ratooning. Although not exploited so far agriculturally, this flexibility offers a tremendous agronomic advantage that extends the acceptance of senna to a wide range of cropping conditions. However, the specific crop characteristics such as stature, drought tolerance capacity, gas exchange and yield components in these three crop types must be investigated before any extensive field experimentation or production operation with them is conducted. Furthermore, understanding the net photosynthetic response to different light intensities will help assess the adaptability of senna to a variety of agroecological contexts.

Thus, several experiments were conducted to evaluate senna established by seedlings, cuttings, and ratooning using plant water relations and gas exchange characteristics in drought. Individual leaflet area, leaf area per plant, and leaf weight per plant were assessed as yield components and leaf chlorophyll concentration was measured to qualify the photosynthetic capacity of the crop types. Diurnal gas exchange patterns were monitored in all three crop types, and the response to light was recorded in seedling plants. Furthermore, biomass partitioning into leaves, stems and roots was evaluated.

## MATERIALS AND METHODS

Ratoon shoot growth was obtained from approximately one year old seedling plants. A ratoon is the new growth that sprouts from the plant base (stubble) after harvesting. Seedling crop was established by direct seeding in pots.

Cuttings were propagated from a continuously deflowered, 12 month old, seedling mother plant by planting 6 - 8 cm long branch cuttings in vermiculite. A rooting hormone mix (Dip'N Grow, Astoria Pacific Inc., Oregon; IBA - 1%, NAA 0.5%) was applied to the cuttings immediately before planting. The rooted cuttings were transferred to the pots 10 weeks after planting in vermiculite. All plants were raised in similar pots with the same soil mixture, were deflowered as described in Chapter one, and were placed outside the glasshouse when each crop was five weeks old. The crops established by seedlings, cuttings and ratoon will be hereafter referred to as seedlings, cuttings and ratoon. The same N - P - K fertilizer mixture as described in Chapter one was applied at two and half months of crop age. Plants were randomized twice a week in a Completely Randomized design.

Three plants of each crop type (crop type will be hereafter referred to as crop) were used for measurements at three months of age. Diurnal rhythms of leaf gas exchange were recorded on a sunny, late spring day using two fully expanded young leaves in each plant between 8:30 am (DST, day light saving time, one hour earlier than GMT) and continued up to 5:30 pm at one and half hour intervals. These same leaflets were detached for measurements of leaflet area and LSM as described in Chapter one. Leaf chlorophyll extraction and leaf greenness assessment (Minolta SPAD units) were performed using young, fully expanded leaves. Total leaf area per plant was estimated by multiplying the total leaflet number of each plant by its leaflet area, and 20 observations per crop were obtained.

The same plants of the three crop types were placed in the glasshouse and watered every other day for two weeks before subjecting them to two

consecutive drought cycles. Thus, plants were approximately three and half months old at the start of the drought cycles. Using the pressure chamber, midday leaf xylem water potential was measured using two mature leaves of each plant every other day. Both midday and predawn leaf xylem water potentials were measured at the end of the second drought cycle when $P_{net}$ was not detectable. Net photosynthesis was measured daily and plants were watered when $P_{net}$ was completely suppressed by moisture stress. Similar measurements were taken from the day immediately after watering, in the second drought cycle. Upon the completion of the two drought cycles all plants were watered. Two days later they were uprooted and oven-dried (70°C to constant weight). Roots, stems and leaves were separately weighed. Furthermore, another two, ratoon plants were subjected to two cycles of drought at three months of age, and $P_{net}$ and leaf xylem water potential were measured.

Eight three month old seedling plants grown in the glasshouse (soil and pots as in Chapter one) were subjected to varying photosynthetic photon flux densities (PPFD) using a 1000 W metal halide lamp. Plants were placed on a bench in the glasshouse. Another bench without top planks was lifted onto this bench as a frame for mounting gray plastic filter screens (between the plants and the lamp) that were used to allow PPFDs between 250 and 2600 μmol $m^{-2}$ $s^{-2}$ through to the plants. Net photosynthesis was measured, as described in Chapter one, in PPFDs increasing approximately at 250 μmol $m^{-2}$ $s^{-2}$ intervals by changing the number of shading screens.

StatView and JMP computer software (Chapter one) were used to test the hypothesis that there is no effect of the crop type on the response variables

studied. Computation of R-square and statistical power analyses (Sokal and Rohlf, 1994) were performed as stated in Chapter one.

## RESULTS

### Leaf xylem water potential during drought

Cuttings maintained a higher xylem water potential than the other two crops throughout the first and during most of the second drought cycle, as illustrated in Figure 2. 1. All three crops regained the pre-drought water status overnight upon watering at the end of the first drought. Ratoon experienced the highest range of water potential, 1.3 and 1.4 MPa during the two consecutive droughts with minimums of -2.2 MPa being the driest at the end of each drought. Furthermore, the predawn leaf xylem water potential, an indication of soil water potential, at the end of the second drought was 41% and 92% less in the ratoon than in the seedlings and cuttings, respectively (Table 2. 1). However, cuttings lost 22% and 25% higher xylem water potential from predawn to midday compared to the seedlings and ratoon, respectively, at the end of the second drought cycle when net photosynthesis was completely suppressed without reaching permanent wilting.

### Net photosynthesis during drought

Ratoon exhibited consistently lower net photosynthesis throughout the first drought as illustrated in the upper panel of Figure 2. 2. Some data of the second drought cycle (whole of ratoon and after third day of other two crops) were lost due to an error committed during data logging on IRGA. Both seedlings and cuttings re-acquired net photosynthesis to the pre-drought levels overnight upon watering at the end of first drought cycle as shown in Figure 2. 2. Ratoon,

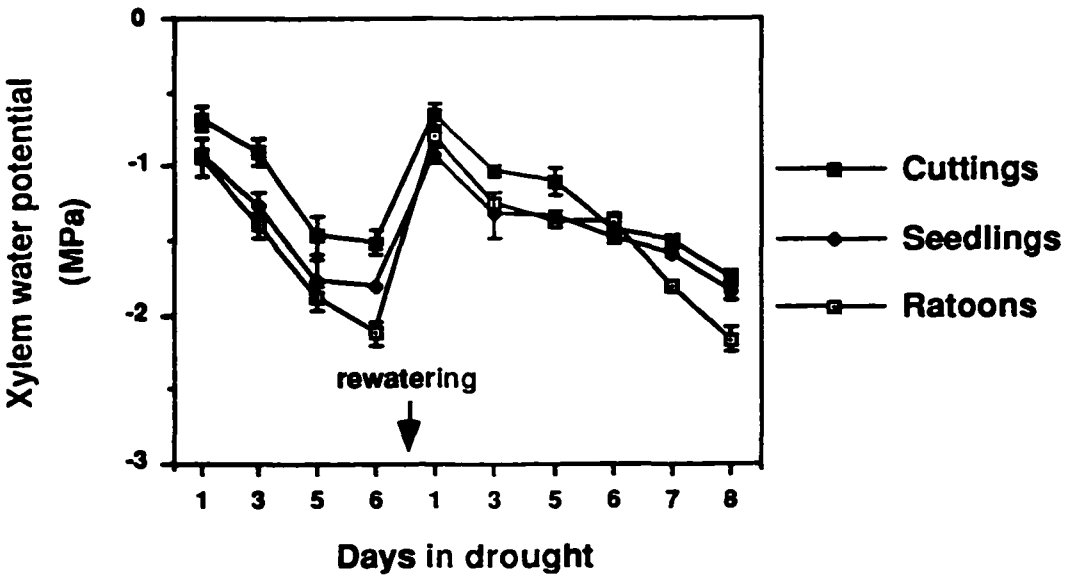


**Figure 2. 1** Midday leaf xylem water potential of the three crops undergoing two consecutive experimental drought cycles. Error bars represent 95% CI of the mean (N=6).

**Table 2. 1** Leaf xylem water potential (MPa) of the crops established by seedlings, cuttings and ratoon at the end of the two drought cycles when $P_{net}$ of each crop was negligible due to water stress. Mean with SE.

| Crop | Predawn | Midday | Predawn - Midday |
|---|---|---|---|
| **Seedling** | -0.87(0.37) b | -1.85(0.22) b | 0.90(0.51) b |
| **Cutting** | -0.64(0.37) c | -1.75(0.22) b | 1.10(0.30) a |
| **Ratoon** | -1.23(0.18) a | -2.16(0.40) a | 0.88(0.30) b |
| **ANOVA** | | | |
| **N** | **7** | **6** | **6** |
| **F** | **79.11** | **55.43** | **8.11** |
| **P** | **< 0.001** | **< 0.001** | **< 0.01** |
| **R-sq** | **0.88** | **0.89** | **0.52** |

Means followed by different letters in a column indicate statistically significant difference by PLSD ($P<0.05$).

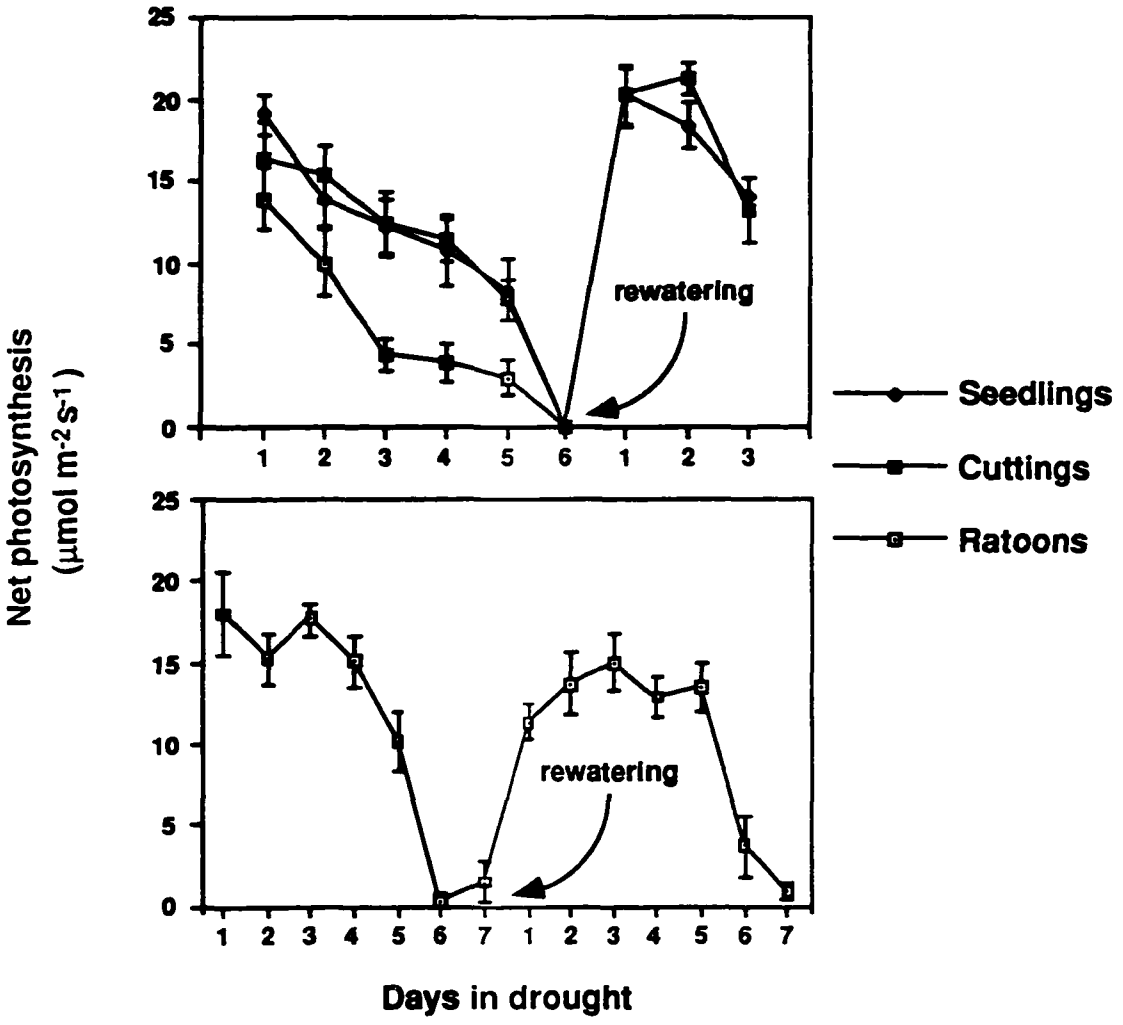


**Figure 2. 2** Fluctuations of $P_{net}$ in the three crops during two consecutive droughts. First drought cycle was followed by restoration of the plant water status to investigate photosynthetic recovery. Upper panel - all three crops in the first drought cycle, seedlings and cuttings in the second cycle (measurements for 9 consecutive days). Lower panel - another group of ratoon plants, at a different time (measurements for 14 consecutive days). Mean of 12 - 24 measurements with 95% CI.

monitored two weeks earlier, however, showed a gradual resumption of $P_{net}$ upon restoration of plant water status after the first drought. Seedlings and cuttings maintained statistically similar net photosynthesis during drought except for the second day of the second drought cycle.

Data pooled from the first day of the first drought cycle and the pre-noon period of the diurnal gas exchange investigations, clearly distinguishes the three crops, seedlings, cuttings and ratoon as comparatively high, moderate and low photosynthesizers ($F=44.5$, $P<0.001$, R-sq=0.31; Figure 2. 3). Seedlings maintained 20% and 64% higher rates of net photosynthesis than cuttings and ratoon, respectively. The whole model test was associated with a statistical power of nearly one with a LSN of only 17.

**Stomatal conductance, transpiration, intercellular $CO_2$ and water use efficiency**

Seedlings exhibited 15% and 60% higher stomatal conductance, respectively, than the cuttings and ratoon as presented in Table 2. 2. Stomatal conductance was 39% greater in the cuttings than in the ratoon. The transpiration rate was 28.5% lower in ratoon than either seedlings or cuttings. The intercellular $CO_2$ concentration of ratoon was 10% and 17% greater than that of the cuttings and seedlings, respectively. Six percent greater intercellular $CO_2$ concentration of cuttings than seedlings was nonsignificant (Table 2. 2). Water use efficiency of seedlings was 17% and 18% greater than cuttings and ratoon, respectively, and was similar in cuttings and ratoon. The statistical power and LSNs for stomatal conductance, transpiration rate, intercellular $CO_2$ and water use efficiency were nearly one, 20; nearly one, 23; ca 0.98, 64, and ca 0.94, 83, respectively.

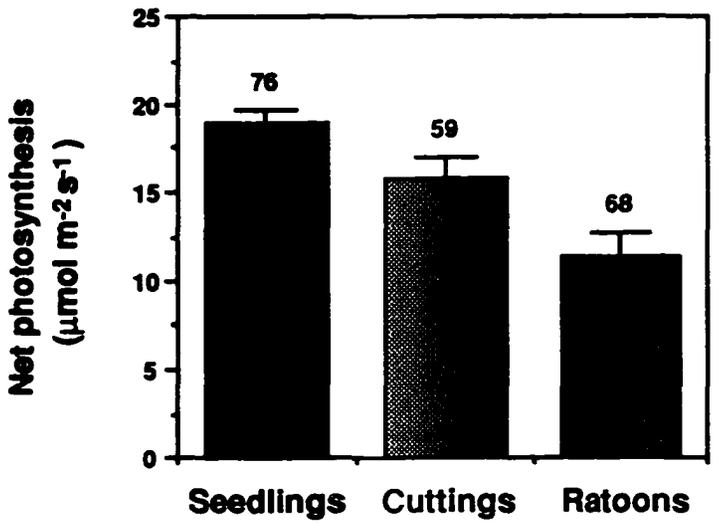


**Figure 2. 3** Net Photosynthesis of the three crops established by seedlings, cuttings and ratoon. Means are based on the pooled IRGA readings from the diurnal gas exchange pattern investigations (between 8.30 am and noon) and from the first day of the droughting experiment. Error bars represent 95% CI of the mean with number of observations on top.

**Table 2. 2** Selected gas exchange characteristics and water use efficiency of the three crops with ANOVA. Means are based on the pooled IRGA readings from the diurnal gas exchange investigations (between 8.30 am and noon) and from the first day of the droughting experiment. N is number of measurements and the number in parentheses is SE.

| Crop | N | Stomatal conductance (mol $m^{-2}$ $s^{-1}$) | Transpiration (mol $m^{-2}$ $s^{-1}$) | Intercellular $CO_2$ (ppm) | Water use efficiency (μmol $mol^{-1}$) |
|---|---|---|---|---|---|
| **Seedlings** | 76 | 0.45 (0.01) a | 6.91 (0.20) a | 152.81 (3.63) a | 2842. 85 (78.80) a |
| **Cuttings** | 59 | 0.39 (0.01) b | 6.79 (0.27) a | 162.63 (5.01) a | 2437.98 (94.66) b |
| **Ratoon** | 68 | 0.28 (0.01) c | 4.69 (0.20) b | 179.29 (4.50) b | 2417.48 (95.81) b |
| **ANOVA** | | | | | |
| **F** | | **36.88** | **31.71** | **10.01** | **7.63** |
| **P** | | **< 0.001** | **< 0.001** | **< 0.001** | **0.001** |
| **R-sq** | | **0.26** | **0.20** | **0.09** | **0.15** |

Means followed by different letters denote statistically significant difference across crops by PLSD ($P<0.05$).

**Photosynthetic response to PPFD**

Net photosynthesis in seedlings grown in the glasshouse increased nearly linearly up to approximately 1100 μmol $m^{-2}$ $s^{-1}$ of PPFD, as illustrated in Figure 2. 4. However, then the photosynthetic rate more slowly increased with PPFD increasing up to a mean maximum of around 1600 μmol $m^{-2}$ $s^{-1}$, and decreased afterwards. Furthermore, net photosynthesis exhibited a greater variance in the higher PPFDs than the low light intensities, e.g. less than 1000 μmol $m^{-2}$ $s^{-1}$. In the "closed" IRGA system of the LI 6200, air and leaf temperature, and VPD are not controlled and change as the level of incident radiation changes (data not presented).

**Diurnal gas exchange in the three crops**

Net photosynthesis was maximum in all three crops in early morning and gradually declined as the day progressed (Figure 2. 5). First photosynthetic measurements (8:30 am) for the seedlings, cuttings and ratoon were 21.47 (SE, 1.12), 17.78 (SE, 2.16) and 13.47 (SE, 1.19), respectively, with ratoon being significantly lower than the other two crops ($F=7.16$, $P<0.01$, R-sq=0.38). The decrease in net photosynthesis as the day progressed was slow in seedlings and cuttings (convex regression curves) while ratoon experienced a sharp drop resulting in a characteristic concave regression curve (Figure 2. 9).

In all three crops, stomatal conductance showed a gradual diurnal drop. Seedlings had the highest stomatal conductance (19% and 100% higher than cuttings and ratoon), with cuttings 72.5% higher than the ratoon in early morning ($F=33.29$, $P<0.001$, R-sq=0.74). Seedlings, with a sharp drop between 8.30 and 10.00 am, converged with cuttings later in the day while ratoon maintained a lower conductance than the other two crops throughout the day (Figures 2. 6 and 2. 9).

The transpiration rate increased from early morning to around 10:00 am and gradually declined afterwards in all three crops, as illustrated in Figure 2. 7. Seedlings and cuttings exhibited similar rates and fluctuation patterns of transpiration with more convex polynomial fits than ratoon (Figure 2. 9). Ratoon, however, showed a lower and more steadily decreasing transpiration rate compared to seedlings and cuttings. Water use efficiency in the early morning (8:30 am) was statistically similar in ratoon and seedlings, 3679.32 (SE, 161.60) and 3496.16 (SE, 177.77), respectively, (Figure 2. 8). However, in ratoon, WUE declined more rapidly toward 11:30 am resulting in a convex polynomial fit (Figure 2. 9) whereas in the other two crops, WUE maintained stable toward 4:00 pm following an early decrease between 8:30 and 10:30 am (Figure 2. 8). Cuttings exhibited a 22% and 18% lower WUE than the seedlings and ratoon in early morning ($F=4.97$, $P<0.05$, $R\text{-sq}=0.30$) with a slower decline over the course of the day (Figure 2. 9).

Mean PPFD at 8.30 am was 1283 $\mu mol\ m^{-2}\ s^{-1}$ which gradually increased to a maximum of 1621 at solar noon (1.00 pm). Although there was a slow decrease in PPFD in the afternoon, the mean PPFD remained high at 1456 $\mu mol\ m^{-2}\ s^{-1}$ even at 4.00 pm. Vapor pressure deficit was lowest in the early morning, increased sharply toward noon and stabilized between approximately 35 and 45 mb during the remainder of the gas exchange measurements (Figure 2. 10).

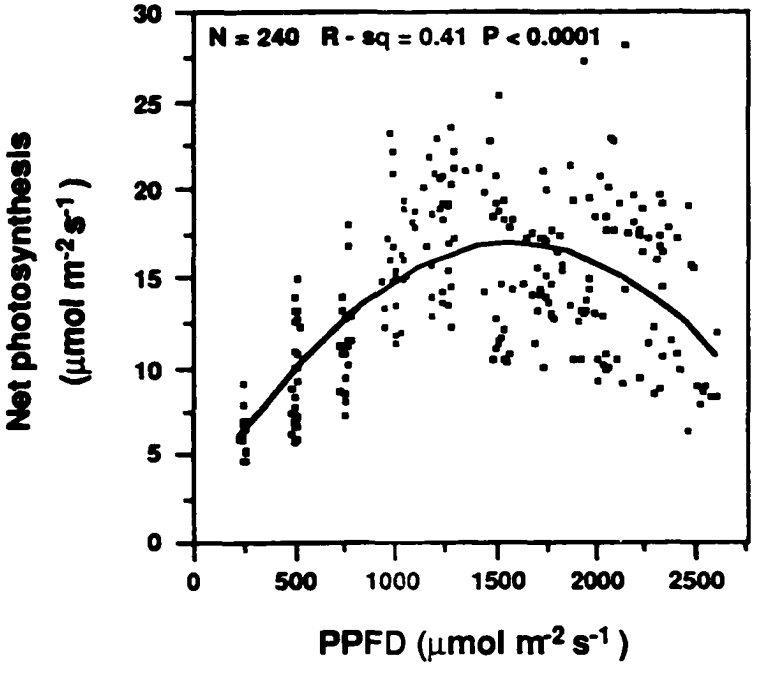


**Figure 2. 4** Net photosynthetic response of seedling plants to light intensity. Scatter plot is superimposed with second degree polynomial regression curve. Eight plants were used for measurements at each level of PPFD.

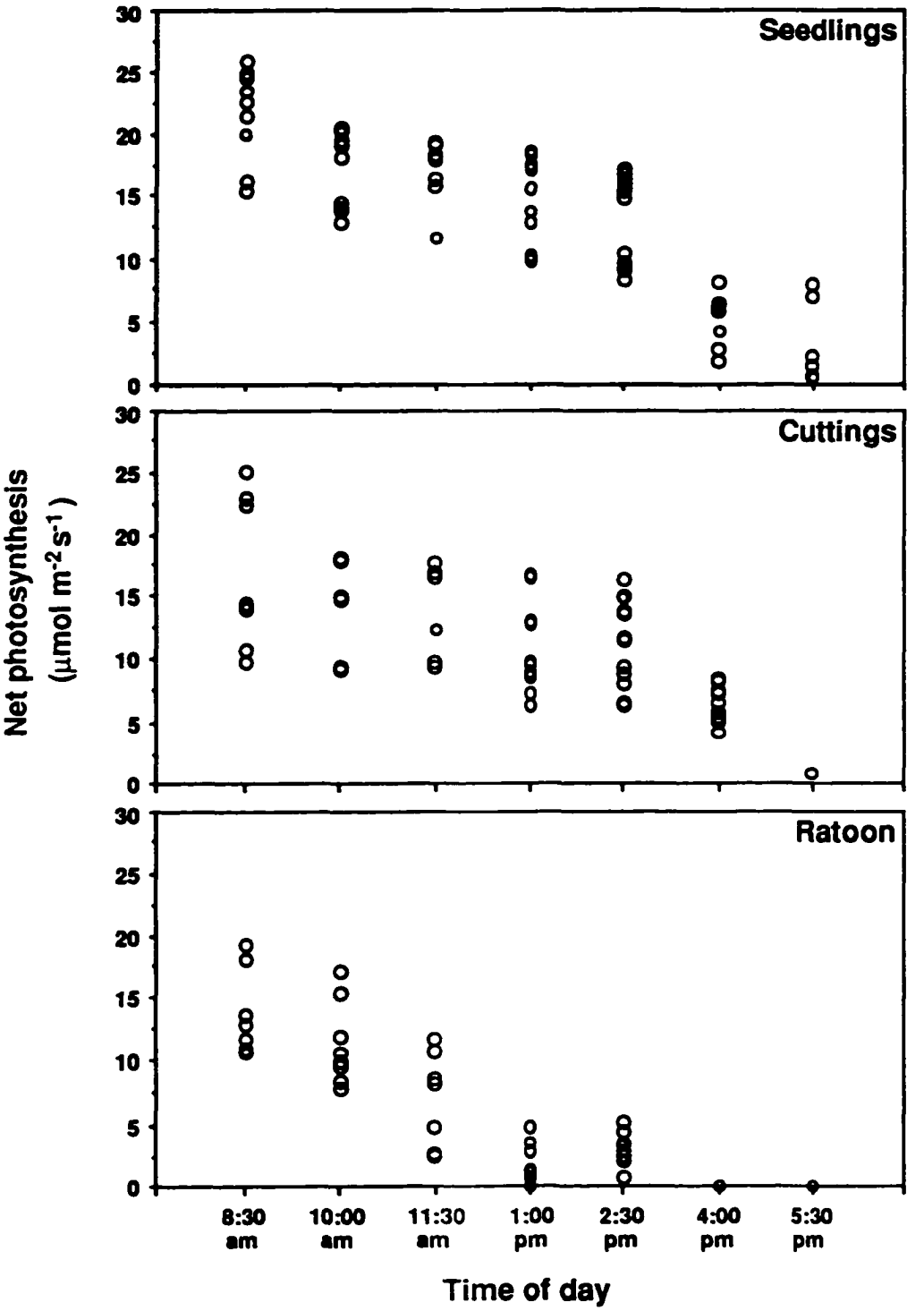


**Figure 2. 5** Diurnal rhythms of net photosynthesis of the three crops. Multiple readings on the same leaves followed across the day.

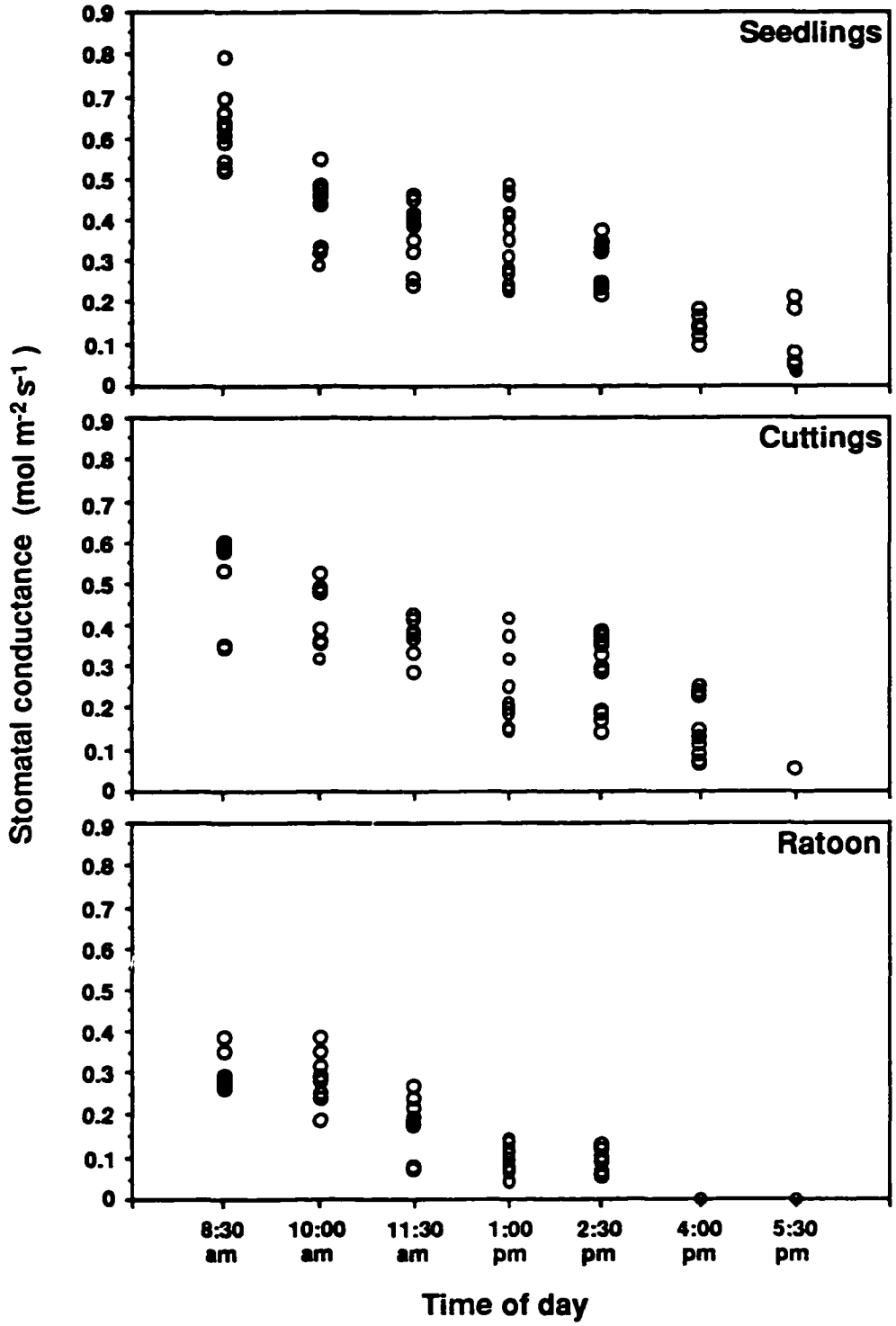


**Figure 2. 6** Diurnal fluctuations of stomatal conductance of the three crops. Multiple readings on the same leaves followed across the day.

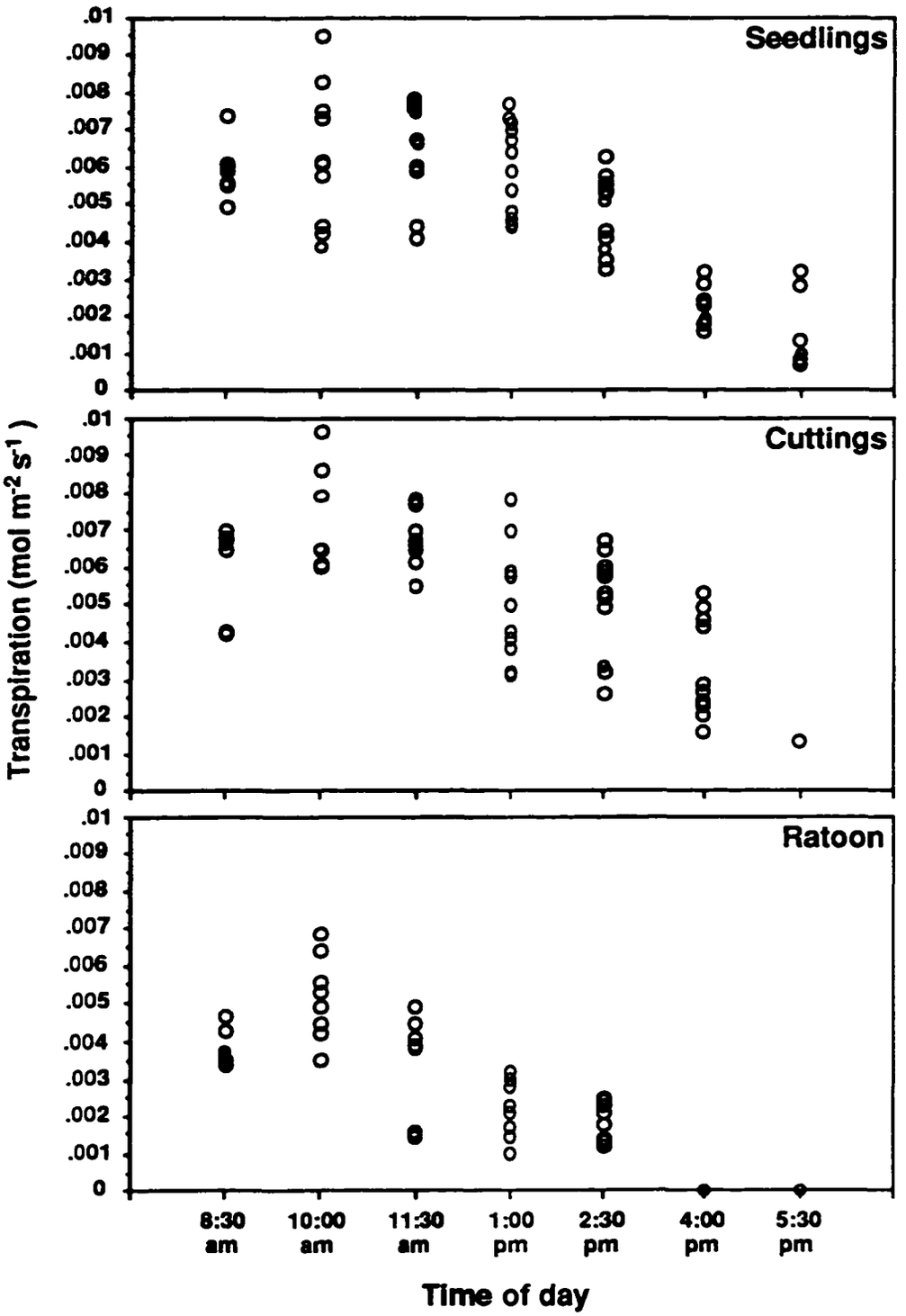


**Figure 2. 7** Diurnal changes in the transpiration rate of the three crops. Multiple readings on the same leaves, followed across the day.

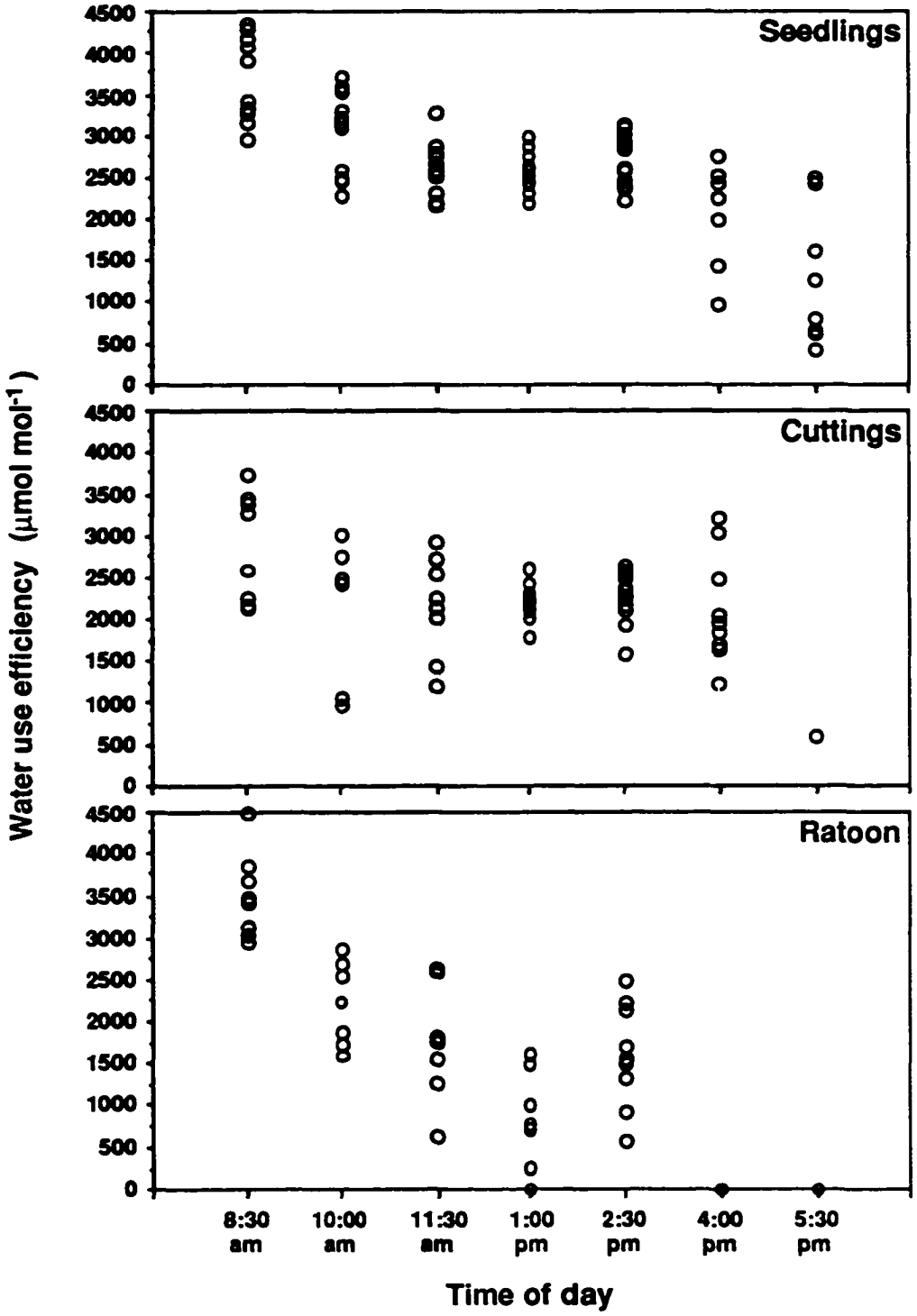


**Figure 2. 8** Diurnal changes in water use efficiency in the three crops. Multiple readings on the same leaves followed across the day.

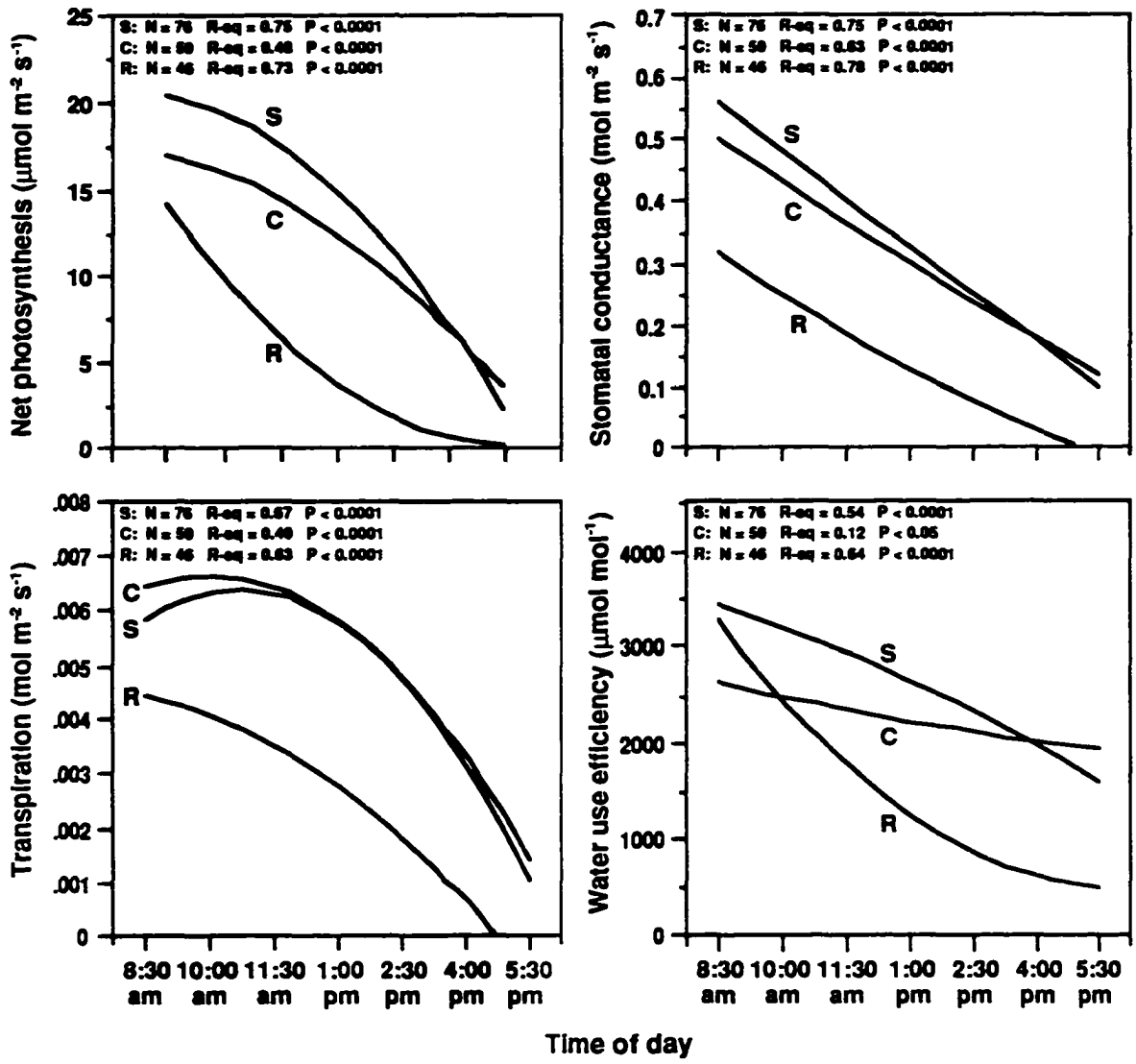


**Figure 2. 9** Second degree polynomial regression curves for diurnal gas exchange characteristics of the three crops (S, seedlings; C, cuttings; R, ratoon).

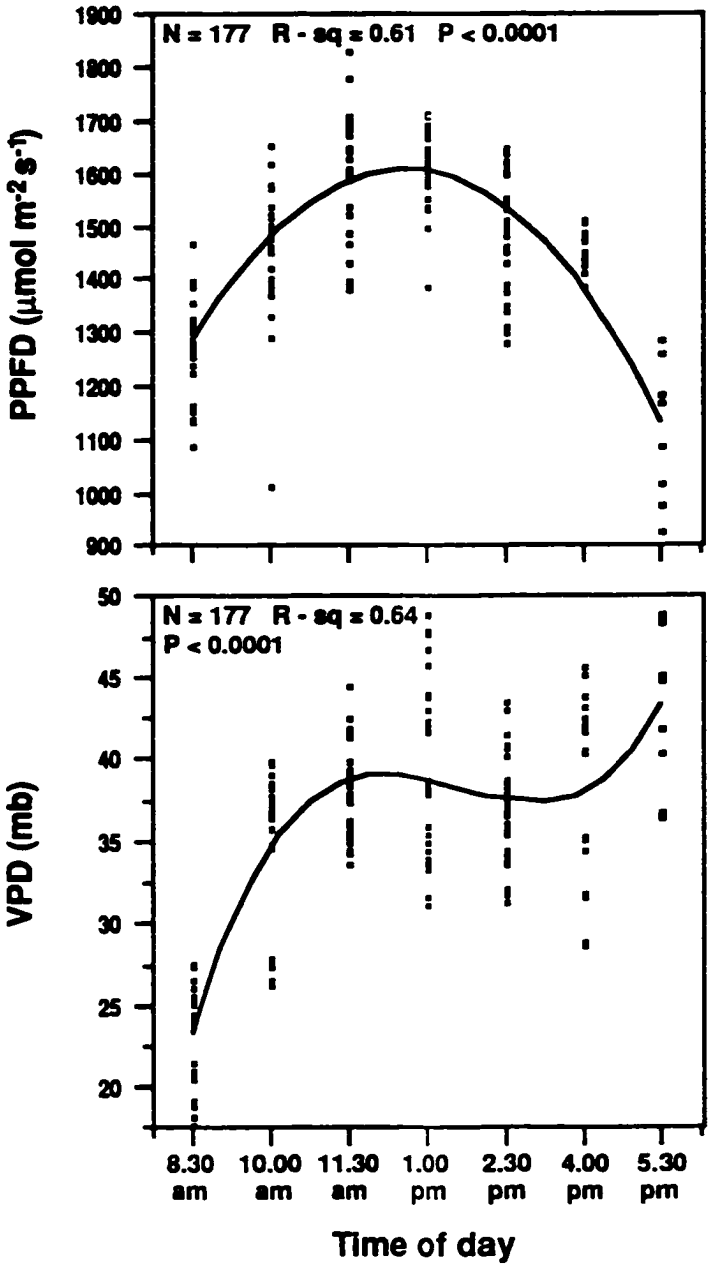


**Figure 2. 10** Diurnal fluctuations of PPFD and VPD during the gas exchange measurements. Scatter plots are superimposed with second degree (PPFD) and 3rd degree (VPD) polynomial regression curves.

**Chlorophyll concentration**

Leaf chlorophyll concentration grouped ratoon, cuttings and seedlings into comparatively low, medium and high chlorophyll crops respectively, as illustrated in the Figure 2. 11. Mean chlorophyll concentration of seedlings (82.96 μg $cm^{-2}$) was 17% and 55% greater than cuttings and ratoon, respectively. Chlorophyll concentration of cuttings was 33% greater than ratoon ($F=62.42$, $P<0.001$, R-sq=0.83). The power of this whole model ANOVA was nearly one with a LSN of only six. In seedlings and cuttings, the leaf greenness SPAD units were numerically more different from the extractable chlorophyll concentration measured in μg $cm^{-2}$ (Figure 2. 11). However, SPAD units were numerically closer to the lower chlorophyll concentration values of ratoon. Similar statistical differences, as observed in extractable chlorophyll concentration, in the three groups were revealed by SPAD units, as well ($F=115.86$, $P<0.001$, R-sq=0.57). Statistical power for SPAD units was nearly one with a LSN of only nine.

**Leaflet area and LSM**

As presented in Table 2. 3, the area of individual leaflets was 93% and 154% greater in seedlings than in cuttings and ratoon, respectively. Cuttings had a 32% greater leaflet area than ratoon. Statistical power of the whole model test for leaflet area was nearly one with a LSN of 10. Leaf specific mass of seedlings was 8% and 9% higher than cuttings and ratoon, respectively, while cuttings and ratoon showed statistically similar LSMs. Statistical power of the whole model test for LSM was ca 0.76 with a LSN of 42.

**Leaf area and leaf weight per plant**

Seedlings possessed 30% and 150% more leaf area per plant than ratoon and

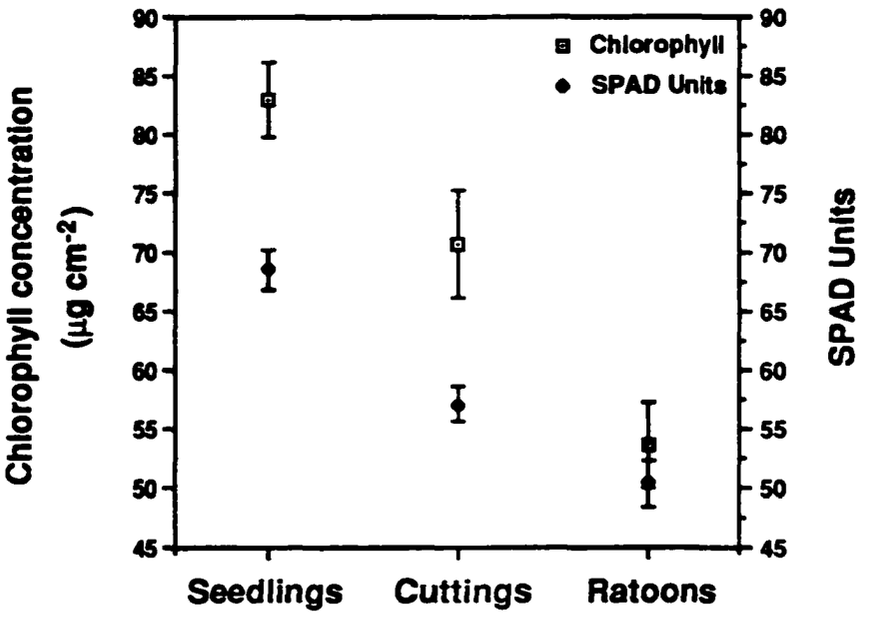


**Figure 2. 11** Leaf chlorophyll concentration measured by extraction method and leaf greenness assessed nondestructively by SPAD units among the three crops. Number of observations, 10 for chlorophyll concentration, and 60 for SPAD units. Error bars indicate 95% CI of the mean.

**Table 2. 3** Individual leaflet area and LSM of the three crops, before drought, with ANOVA. Mean with SE. N is the number of observations on three plants in each group.

| Crop | N | Leaflet area ($cm^2$) | LSM ($\mu g\ mm^{-1}$) |
|---|---|---|---|
| **Seedling** | 20 | 2.95 (0.28) a | 81.33 (2.30) a |
| **Cutting** | 20 | 1.53 (0.06) b | 75.55 (1.15) b |
| **Ratoon** | 20 | 1.16 (0.07) c | 74.79 (1.26) b |
| **ANOVA** | | | |
| **F** | | **30.19** | **4.65** |
| **P** | | **< 0.001** | **< 0.05** |
| **R-sq** | | **0.51** | **0.14** |

Means followed by the different letters in a column indicate statistically significant difference by PLSD ($P<0.05$).

cuttings, respectively, as shown in Table 2. 4. Ratoon maintained 91% greater leaf area than the cuttings. Statistical power of the whole model test for leaf area was nearly one with a LSN of 12. Furthermore, the total leaf weight of seedlings was 44% and 170% greater than ratoon and cuttings, respectively. Ratoon exhibited 88% more total leaf weight than the cuttings. Power of the whole model test for leaf weight was nearly one with a LSN of only 12.

**Biomass partitioning**

Cuttings developed 57% and 60% less root biomass than seedlings and ratoon. Furthermore, their total biomass was 37% and 36% less than ratoon and seedlings, respectively, as presented in Figure 2. 12. No significant differences in the biomass of other plant organs of the three crops were detected by ANOVA at the end of the two drought cycles with the sample size of this experiment.[1]

[1] F=1.25, P=0.35, R-sq=0.28 for shoot; F=3.56, P=0.09, R-sq=0.53 for leaves

**Table 2. 4** Total estimated leaf area and leaf weight per plant of the three crops, before drought, with ANOVA. Mean with SE. N is the total number of observations on three plants in each group.

| Crop | N | Leaf area ($cm^2$) | Leaf weight (g) |
|---|---|---|---|
| **Seedling** | 20 | 1223.58 (116.84) a | 10.07 (1.04) a |
| **Cutting** | 20 | 491.04 (18.98) c | 3.72 (0.17) c |
| **Ratoon** | 20 | 939.96 (59.58) b | 7.01 (0.43) b |
| **ANOVA** | | | |
| **F** | | **23.30** | **23.23** |
| **P** | | **< 0.001** | **< 0.001** |
| **R-sq** | | **0.45** | **0.45** |

Means followed by different letters in a column denote statistically significant difference by PLSD ($P<0.05$).

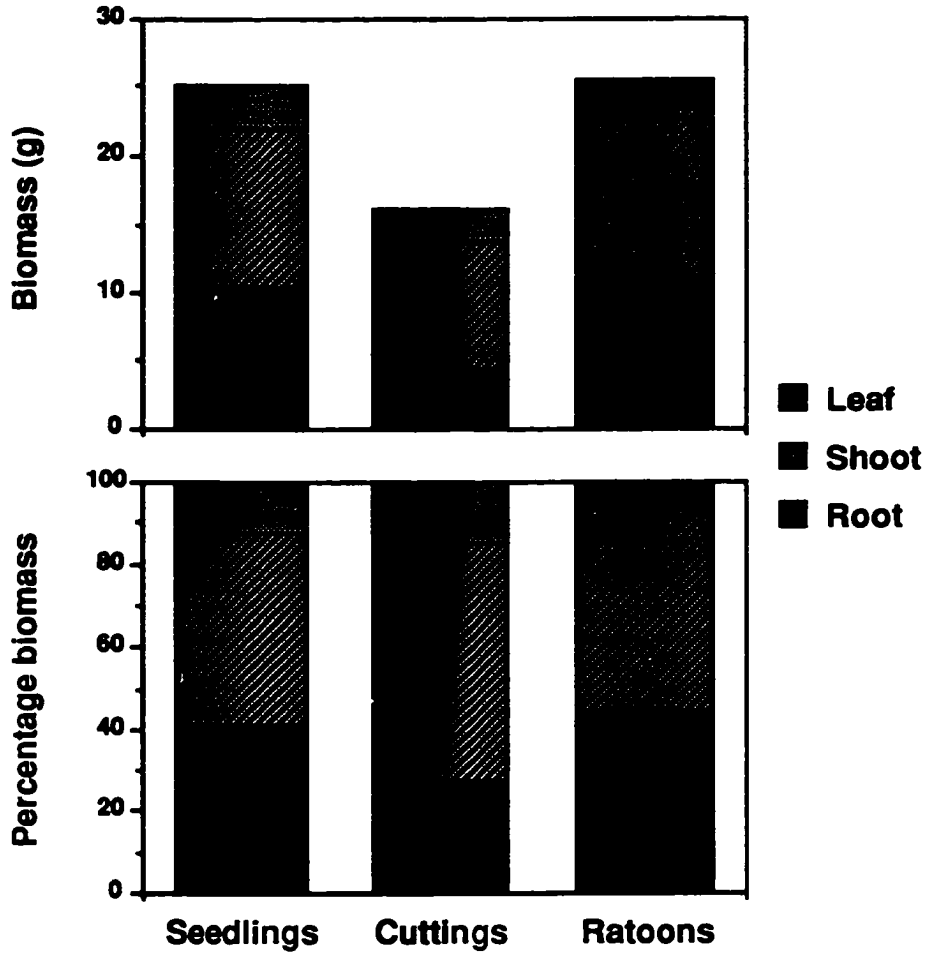


**Figure 2. 12** Biomass partitioning into different plant parts after two consecutive drought cycles (upper panel). Lower panel presents percentage biomass in relation to whole plant.

## DISCUSSION

Net photosynthetic rate of the ratoon was less than the cuttings and seedlings both before and during the drought. This is explained by the lower leaf chlorophyll concentration in ratoon. Although, $P_{net}$ of seedlings was greater than cuttings before the drought, both crops had comparable $P_{net}$ during the drought. Higher leaf xylem water potential of cuttings during drought under the conditions of this experiment, probably enabled them to more efficiently utilize their relatively moderate chlorophyll concentration, compared to seedlings. This suggests that seedlings down-regulate the primary metabolism for managing the water status in a drought even though its chlorophyll-based photosynthetic capacity is greater than cuttings.

The smaller plant stature with less transpirational surface area may enable cuttings to maintain a higher xylem water potential than the other crops in pots. Less water depletion by comparatively smaller statured plants is well documented (Freckman and Virginia, 1989; de Soyza *et al.*, 1996). The lower xylem water potential in ratoon compared to other crops, especially during the drought, could be due to a year older root system and the stubble which are probably less efficient in absorption and conduction of resources. This explains its lower chlorophyll concentration than seedlings and cuttings, although it is still higher than many common annual field crop species (Marquard and Tipton, 1987). Furthermore, even though the composition of the soil and fertilizer mixtures used were same for all three crops, soils of ratoon were exposed to the glasshouse environment and were used by the mother plants in the pots for a year longer than the other two crops. Thus, the soils in the pots of ratoon plants would have depleted fertility more than the soils of seedlings and cuttings. The influence of

nutrient availability on gas exchange is well documented (Wong *et al*, 1979; Koch and Rawlik, 1993).

In the watered plants of each crop, the patterns of other gas exchange characteristics are well correlated with $P_{net}$. Stomatal conductance to water vapor was highest in seedlings (Table 2. 2) with the lowest intercellular $CO_2$ indicating higher stomatal activity (lower stomatal resistance, data not presented). A similar inverse relationship between these two parameters was observed by other authors (Smith *et al*, 1993) and in my earlier experiments with senna (Chapter one). Highest water use efficiency in seedlings is mainly due to its higher rate of primary metabolism supported by the chlorophyll-rich photosynthetic machinery, although seedlings had a higher transpiration rate than ratoon. Ratoon, however, maintains a water use efficiency as high as cuttings by more active stomatal control causing the lowest transpiration rate.

The need to investigate net photosynthetic response to varying light intensities for understanding how annual crops cope with their environment is well recognized (Fay and Knapp, 1993). Senna plants (seedlings) attained light saturation approximately at 1600 $\mu mol\ m^{-2}\ s^{-1}$ under glasshouse conditions. With the increasing PPFDs the temperature and dryness (high VPD and less RH, data not presented) increased in the "closed" system of the LICOR 6200 IRGA. This would have interfered with the actual photosynthetic response to light intensity by the confounding of variables. An "open" photosynthetic system, as opposed to the "closed" system that was used in these experiments, would have more precisely assessed the net photosynthetic responses to increasing PPFDs in the glasshouse.

All three crops investigated, however, attained maximum photosynthesis in light intensities of early morning, most likely less than 1200 μmol $m^{-2}$ $s^{-1}$ and at the time of lowest VPD (Figures 2. 5 and 2. 9). Furthermore, the stomatal conductance and transpiration decreased with increasing VPD. This again clearly alludes to the high stomatal sensitivity of senna to external environmental conditions. Martin *et al.* 1983 reported that the initial stomatal opening in the morning is more rapid in higher light intensities. Ratoon plants grown under less light intensities in the glasshouse, however, showed a weak positive relationship between $P_{net}$ and light intensity, with VPD < 45 mb (Chapter one). This also reflects the physiological capacity of senna to control photosynthetic primary metabolism according to the current surrounding weather conditions that influence the plant water balance, (e.g. $P_{net}$ does not increase with increasing light intensity when plants are grown outside the glasshouse with adequate light). These are important characteristics of plants that are successful in xeric environments (Ehleringer and Björkman, 1978; Mooney, 1980). Again, the contribution by stomatal regulation in this regard is evident by the transpiration rates that began to drop in all three crops well before solar noon with the increasing VPD.

Furthermore, a characteristic paraheliotropic movement in which the pairs of leaflets of the compound leaves met on the adaxial surfaces, occured especially in the droughted seedlings and ratoon. This exposed the abaxial surface, and reduced the total leaf area incident, and the angle of leaflets to beam of the sun. Paraheliotropic leaf movements have been suggested to enhance both water and nitrogen use efficiencies (Ehleringer and Forseth, 1980; Forseth and Ehleringer, 1983; Kao and Forseth, 1991) and could be an important drought tolerance strategy in the field (Fitter and Hay, 1987).

Higher LSM and leaf area per plant result in a significant yield increase in seedlings over ratoon and cuttings. Although ratoon plants exhibited the lowest photosynthesis, they prove a more promising yielder than cuttings. Advantageous plant stature, both higher shoot and root biomass, in seedlings and ratoon is an important factor, in this regard. However, the leaf sennoside yields presented in chapter four will provide the information essential for conclusively comparing the yield potentials of the three crops.

The very high statistical power associated with most of the response variables, e.g. gas exchange, chlorophyll and leaf characteristics, exemplifies the low Type II error, $\beta$ involved in the experimentation. This resulted in much lower LSNs than the number of observations used in these experiments. Thus, fewer number of observations, if similar experimental conditions be ensured, could be considered for future investigations related to above variables. However, a greater sample size is suggested in the assessments of biomass partitioning.

# CHAPTER 3

## EFFECTS OF DEFLOWERING AND NITROGEN ON PHOTOSYNTHESIS AND YIELD COMPONENTS

### SUMMARY

Gas exchange, leaf xylem water potential, leaf chlorophyll content and yield components were investigated in deflowered and flowering plants grown at three nitrogen levels on a garden-plot.

Although, a 28 day long drought started when plants were seven weeks old, the minimum midday xylem water potential was greater than -1 MPa throughout the experiment. Net photosynthesis of flowering plants was higher than deflowered plants at any age during measurements, an overall 26% increase. The unreplicated nitrogen treatments, 40 kg $ha^{-1}$ and 80 kg $ha^{-1}$ showed no effect on $P_{net}$ in the flowering plants but reduced $P_{net}$ in the deflowered plants compared to the control. Leaf chlorophyll concentration was 20% greater in the flowering plants than the deflowered plants. Linear regression of Minolta SPAD meter readings and extractable leaf chlorophyll contents produced a R-sq of 0.89.

Deflowering increased leaflet area by 13%. Nitrogen application increased leaflet area in deflowered plants but decreased in flowering plants growing on the plot intrinsically rich in nitrogen. Leaflet area declined with age more dramatically in the flowering plants than in the deflowered plants. Leaf specific mass was greater in the deflowered plants until the plants were three months old, after which the trend then reversed. The biomass of leaflets and total plant was 63% and 35% greater, respectively, in the deflowered plants than the flowering plants. Nitrogen showed no effect on the biomass of any organ. Deflowering increased the Harvest Index and Leaf Area Index by 21% and 67%, respectively.

## INTRODUCTION

The relationship between net photosynthesis and plant productivity is mediated by sink activity. Flowers, fruits, expanding leaves and roots constitute important sinks in this regard (Radin and Boyer, 1982; Micallef *et al.* 1995). Effects of manipulations of reproductive sinks on the biomass partitioning into harvest organs have been studied for field crops (Hicks and Pendelton, 1969; Wittenbach, 1983; Talwar *et al.* 1992; Marschner, 1995). In senna, however, mechanisms of how flowering influences the source activity and the biomass partitioning into harvest organs is not known.

Nitrogen, the most commonly deficient mineral nutrient in a variety of agroecosystems, correlates well with photosynthesis (Field, 1983; Field and Mooney, 1986) and alters dry matter partitioning into different organs (Olsthoorn *et al.* 1991). Moreover, it influences the plant nutrient composition more than any other mineral nutrient (Marschner, 1995). However, nitrogen uptake, and root extension, depend on the supply of soluble carbohydrate from the leaf primary metabolism (Raper *et al.* 1978; Henry and Raper, 1991, Imsande and Touraine, 1994). Thus, sinks such as expanding leaves (Radin and Boyer, 1982) and reproductive organs that reduce the carbohydrate flux to the roots could suppress nitrogen uptake. Furthermore, the photosynthetic rate is less responsive to changes in shoot nitrogen content than to leaf initiation and expansion that represent sink activity (Henry and Raper, 1986).

In senna the most commonly obtained harvest is leaves. Thus, investigations on the effect of floral bud removal at varying nitrogen levels on photosynthesis and dry matter allocation are an essential prerequisite for planning of both field

experiments and on-farm trials. An experiment was, therefore, conducted to investigate the effect of flowering and nitrogen application on gas exchange characteristics, growth and yield components of senna using plants grown on a garden-plot. Leaf specific mass (LSM), leaflet area, leaf area index (LAI), harvest index (HI), and biomass allocation patterns into organs were assessed. Measurements on gas exchange, leaflet area and LSM were taken at weekly intervals to evaluate the effect of plant maturity on these parameters as well. Leaf xylem water potential, and leaf chlorophyll concentration were measured as variables influencing the primary metabolism.

## MATERIALS AND METHODS

Plants were established by direct seeding on the open garden managed by the Greenhouse staff, Biological Sciences Department, Lehman College. Four to five seeds were dibbled per hill in late spring. Seedlings were thinned out to two plants per hill. One randomly selected plant at each hill was kept deflowered with the other plant allowed to flower during the whole experiment.

As shown in Figure 1, three nitrogen rates were applied (unreplicated, 0 kg $ha^{-1}$, 40 kg $ha^{-1}$ and 80 kg $ha^{-1}$) using urea (46% N) to three groups of plants each on six hills. Each group of plants receiving a nitrogen treatment was bordered by one row of plants (two plants per hill) that was not applied with urea. All plants were fertilized with a mixture of triple super phosphate (46% $P_2O_5$, 2.82 g per hill) and muriate of potash (60% $K_2O$, 2.09 g per hill). Plants receiving nitrogen 40 kg $h^{-1}$ and nitrogen 80 kg $h^{-1}$ were applied with 1.37 g and 2.79 g of urea per hill, respectively (based on approx. 62,000 hills $ha^{-1}$ at the plant spacing of the experiment). Other plants were not treated with urea. All fertilizers were mixed

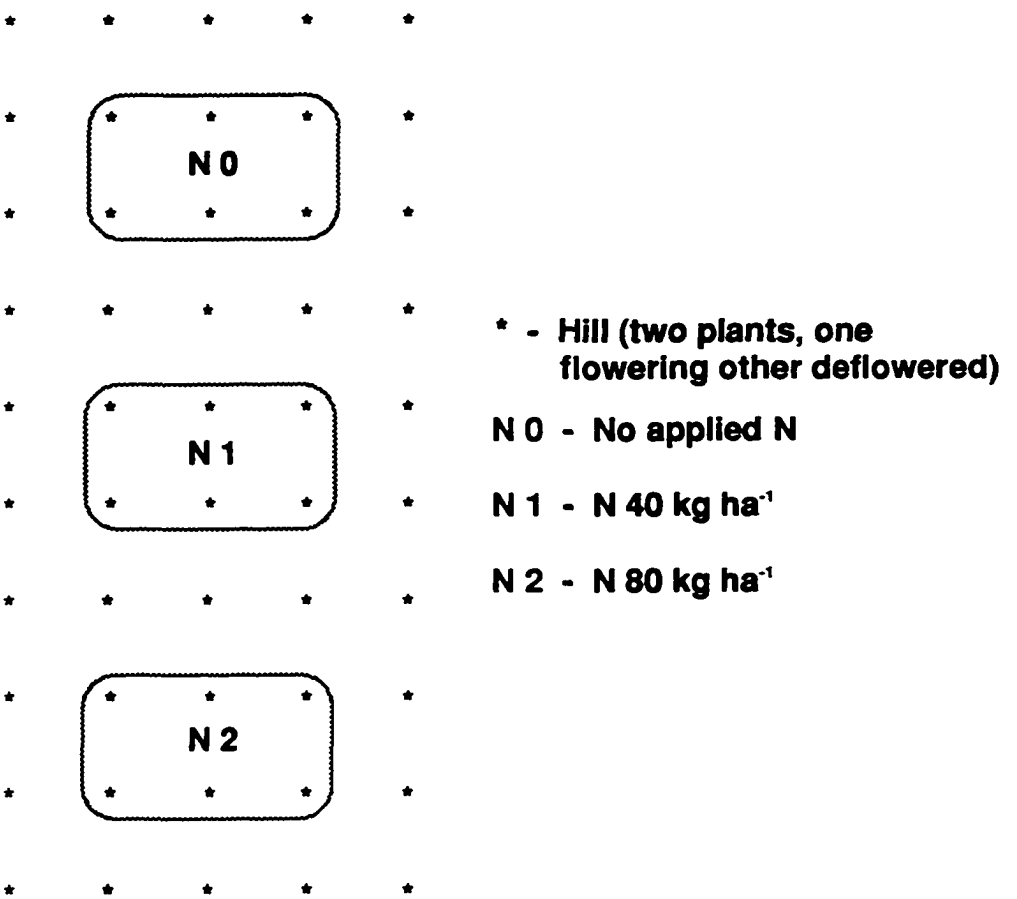


**Figure 3. 1** Garden plot - plants within the rectangles only were used for measurements. Plant spacing, 40 cm X 40 cm.

into the soil around hills in two equally split applications, at three and six weeks after seeding.

The crop was not watered after the seedling emergence until the end of the experiment. The weather outdoor was such that the plants underwent a continuous drought of 28 days starting at six weeks of age. The plot was kept weed-free by spot weeding, and no pesticides were used.

Gas exchange measurements were taken weekly between eight to sixteen weeks of age except for 15$^{th}$ week when inclement weather prevailed. Two terminal leaflets of the second or third fully expanded leaf from the apex were used for measurements on gas exchange, leaflet area and LSM (Chapter 1). Other leaflets of the same leaves mentioned above were used for SPAD units and chlorophyll measurements taken when the plants were 12 weeks old. The SPAD meter was calibrated by taking chlorophyll and SPAD readings on the same leaflets with varying greenness from the plants that were applied with nitrogen 40 kg ha$^{-1}$ at 14 weeks of age. Leaf xylem water potential was measured at the beginning and toward the end of the drought as described in chapter 1. For the investigations on biomass allocation, all the plant parts were dried at 70°C to a constant weight at 16 weeks of age. Harvest index (HI) was computed as total leaflet weight divided by the total shoot weight. Leaf area index (LAI) was determined as total leaf area (cm$^2$, estimated through LSM and total leaf weight) divided by land area occupied by each plant ((40 cm X 40 cm)/2).

## RESULTS

### Xylem water potential

The minimum mean midday leaf xylem water potential was greater than -1 MPa in any nitrogen or flowering treatment even after the 28 day long drought (Table 3. 1). Predawn water potential was more than three times greater than the midday water potential throughout the drought. Flowering or nitrogen application did not significantly influence the leaf xylem water potential.

### Net photosynthesis

Pooled across nitrogen levels and plant ages, flowering plants photosynthesized 26% greater than the deflowered plants as illustrated in Figures 3. 2 and 3. 3 (one way ANOVA, F=195.9, P<0.0001, R-sq=0.12; two way ANOVA with age as a treatment, F=266.3, P<0.0001, R-sq=0.13; two way ANOVA with nitrogen level as a treatment, F=199.9, P<0.0001, R-sq=0.90). Although, flowering showed interactions with both age and nitrogen, the R-squares were very low, 0.01 and 0.04, respectively. In deflowered plants, nitrogen control showed 14% and 9% greater $P_{net}$ than nitrogen 40 kg $ha^{-1}$ and 80 kg $ha^{-1}$, respectively (Figure 3. 2, F=7.9, P<0.001, R-sq=0.01). Net photosynthesis was not affected by nitrogen application in flowering plants.[1] A dramatic drop in $P_{net}$ was observed in both flowering and deflowered plants after 12 weeks of age (Figure 3. 3). The statistical power for the model with nitrogen, flowering and their interaction as treatment variables approximated to one with LSN of only 74. The Power of the model involving flowering, age and their interaction as the treatment variables was nearly one as well, with a LSN of 27. Furthermore, although $P_{net}$ exhibited an overall positive

[1] F=1.5, P=0.22, R-sq=0.00

**Table 3. 1** Leaf xylem water potential (MPa, mean with SE) of 2 1/2 month old plants after 26 days of continuous drought. Column N is the sample size.

| Flowering/N rate | N | Predawn | Midday |
|---|---|---|---|
| **Flowered** | | | |
| **N 0 kg ha$^{-1}$** | 6 | -0.26(0.24) | -0.98(0.16) |
| **N 40 kg ha$^{-1}$** | 6 | -0.27(0.17) | -0.96(0.21) |
| **N 80 kg ha$^{-1}$** | 6 | -0.28(0.16) | -0.96(0.21) |
| **Total** | 18 | -0.27(0.10) | -0.97(0.10) |
| **Deflowered** | | | |
| **N 0 kg ha$^{-1}$** | 6 | -0.26(0.10) | -0.96(0.21) |
| **N 40 kg ha$^{-1}$** | 6 | -0.27(0.11) | -0.98(0.16) |
| **N 80 kg ha$^{-1}$** | 6 | -0.27(0.11) | -0.96(0.21) |
| **Total** | 18 | -0.27(0.06) | -0.97(0.10) |

**ANOVA**

| | Predawn | | | Midday | | |
|---|---|---|---|---|---|---|
| | **F** | **P** | **R-sq** | **F** | **P** | **R-sq** |
| **Nitrogen (N)** | **0.27** | **0.76** | **0.00** | **0.25** | **0.77** | **0.00** |
| **Flowering (F)** | **0.04** | **0.84** | **0.00** | **0.04** | **0.83** | **0.00** |
| **N X F** | **0.04** | **0.96** | **0.00** | **0.28** | **0.97** | **0.00** |

Means followed by the same letter denote statistical similarity in a column by PLSD.

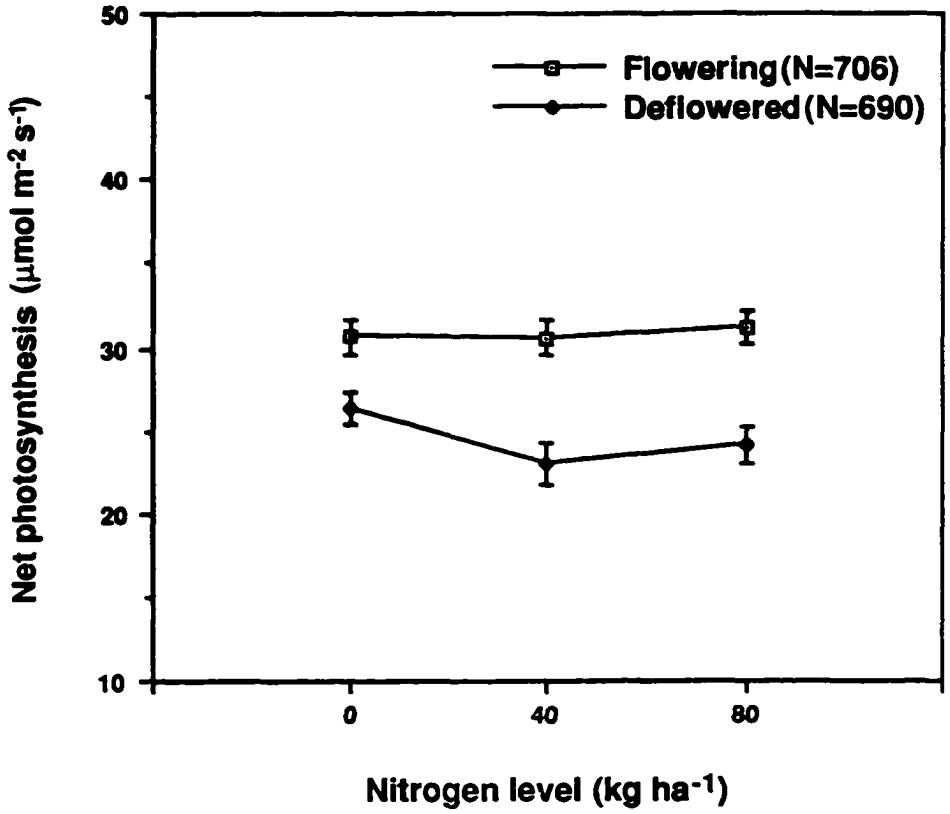


**Figure 3. 2** Effect of nitrogen level and flowering on net photosynthesis. Data collected between eight to 16 weeks of age were pooled for each nitrogen level in either flowering or deflowered plants. N is total number of observations. Error bars represent 95% CI.

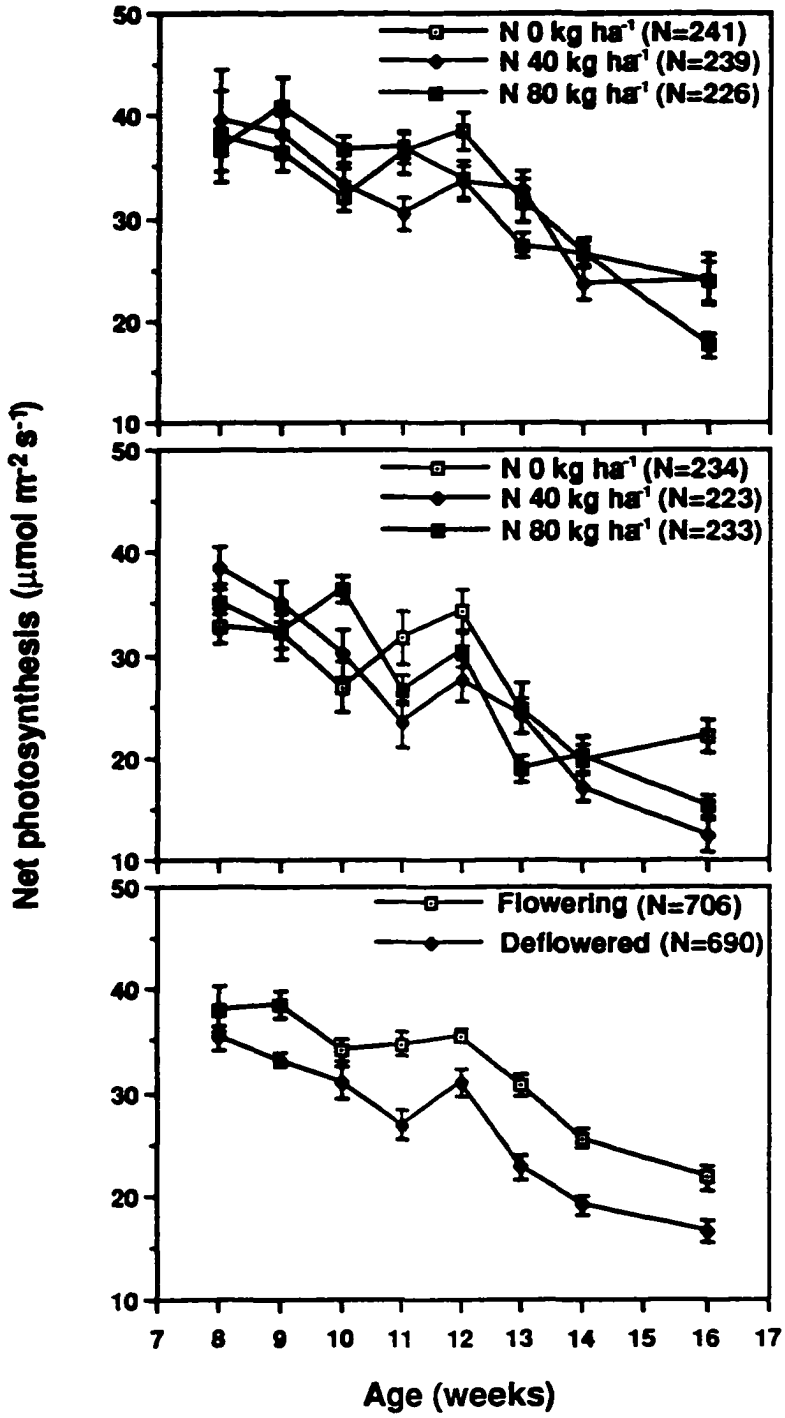


**Figure 3. 3** Net photosynthesis as influenced by nitrogen fertilization and flowering during eight to 16 weeks of age. Upper and middle panels - flowering and deflowered plants, respectively, with three nitrogen rates, lower panel - flowering and deflowered plants, pooled across the nitrogen rates. Mean with 95% CI. N is the total number of measurements.

correlation to VPD, $P_{net}$ declined abruptly at VPDs > 36 mb, in both flowering and deflowered plants (Figure 3. 4).

**Stomatal conductance**

Flowering plants maintained an overall 24% higher stomatal conductance than deflowered plants as shown in the Figure 3.5. Nitrogen application at 40 kg $ha^{-1}$ and 80 kg $ha^{-1}$ reduced stomatal conductance by 12% and 14%, respectively, compared to nitrogen control in the deflowered plants, in contrast to the nonsignificant differences in the flowering plants. Two way ANOVA indicated significant effects of nitrogen and flowering with nonsignificant interaction effect[1] (F=5.5, P<0.01, R-sq=0.09 for nitrogen; F=95.3, P<0.0001, R-sq=0.86 for flowering; power nearly one with the a LSN of 143). Stomatal conductance started dropping sharply from the ninth week of age.

**Transpiration**

As illustrated in Figure 3. 6, transpiration was consistently greater in the flowering plants, except at the eighth and 16$^{th}$ weeks of age, resulting in an overall 30% increase compared to the deflowered plants (F=57.96, P<0.0001, R-sq=0.91; statistical power nearly one with LSN = 250). The main effect of nitrogen or its interaction with flowering was nonsignificant.[2] Statistical power for this whole model test was nearly one with a LSN of 250. Furthermore, a sharp increase in transpiration from the eighth week to the ninth week, and a following gradual drop were observed.

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[1] F=2.79, P=0.06, R-sq=0.05 for interaction

[2] F=1.8, P=0.16, R-sq=0.06 for N; F=0.95, P=0.38, R-sq=0.03 for interaction

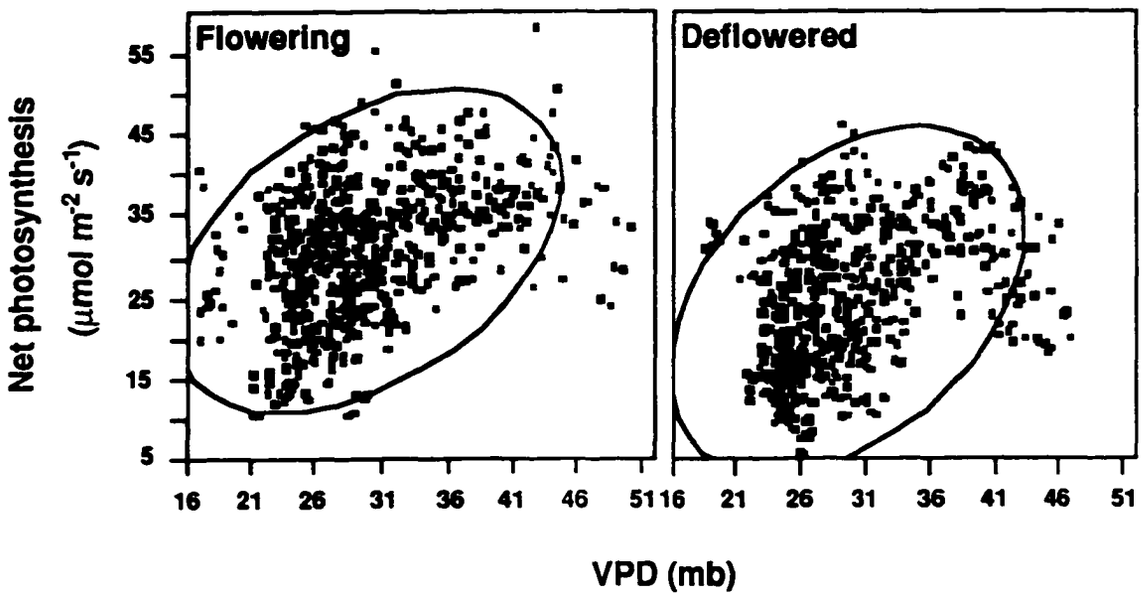


**Figure 3. 4** Scatter plots of $P_{net}$ vs. VPD with 95% density ellipses for flowering and deflowered plants over the whole period of measurements. Flowering; r = 0.43, P < 0.0001. Deflowered; r = 0.41, P < 0.0001. Numbers of observations, 706 and 690 in flowering and deflowered plants, respectively.

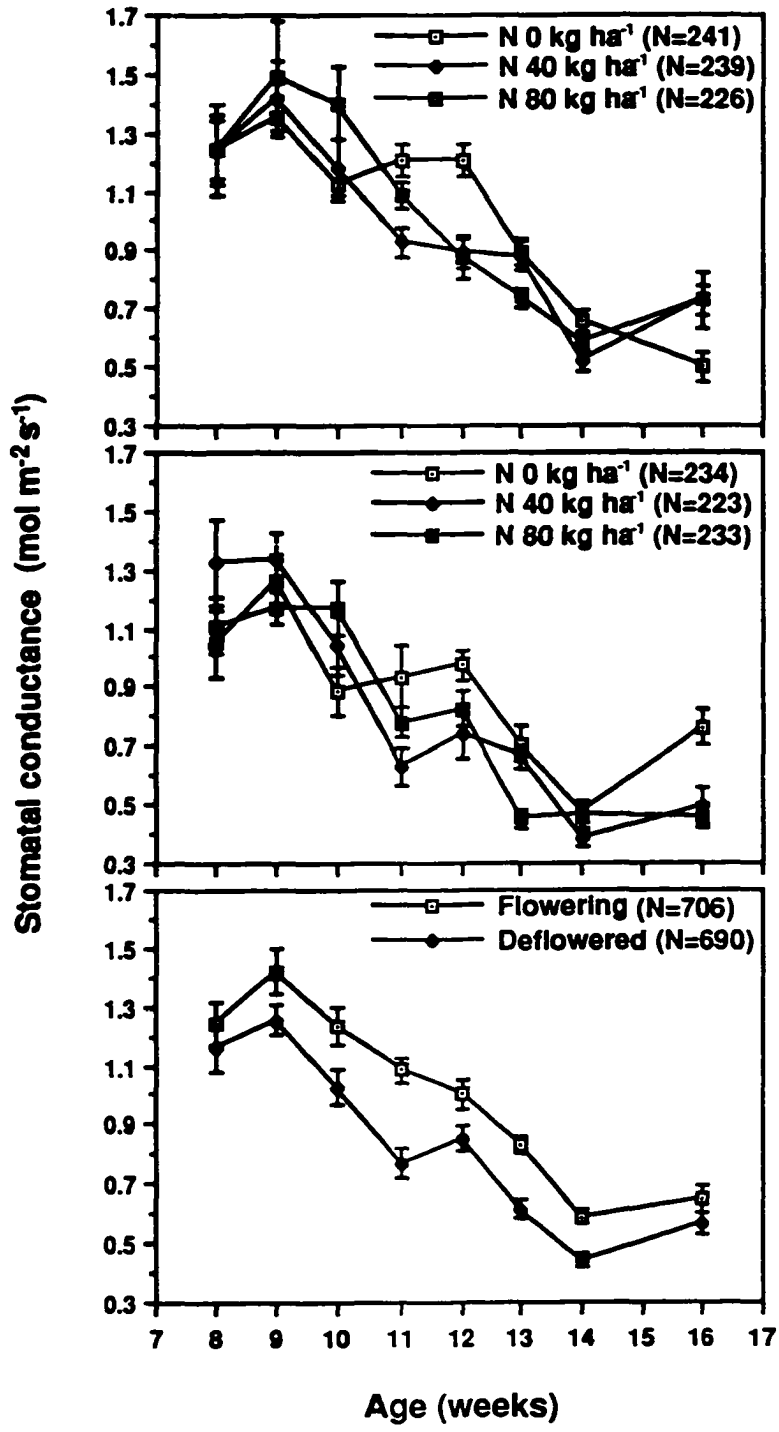


**Figure 3. 5** Effect of nitrogen fertilization and flowering on stomatal conductance. Upper and middle panels - flowering and deflowered plants, respectively, with nitrogen rates, lower panel - flowering and deflowered plants, pooled across the three nitrogen rates. N is the total number of measurements. Means with 95% CI.

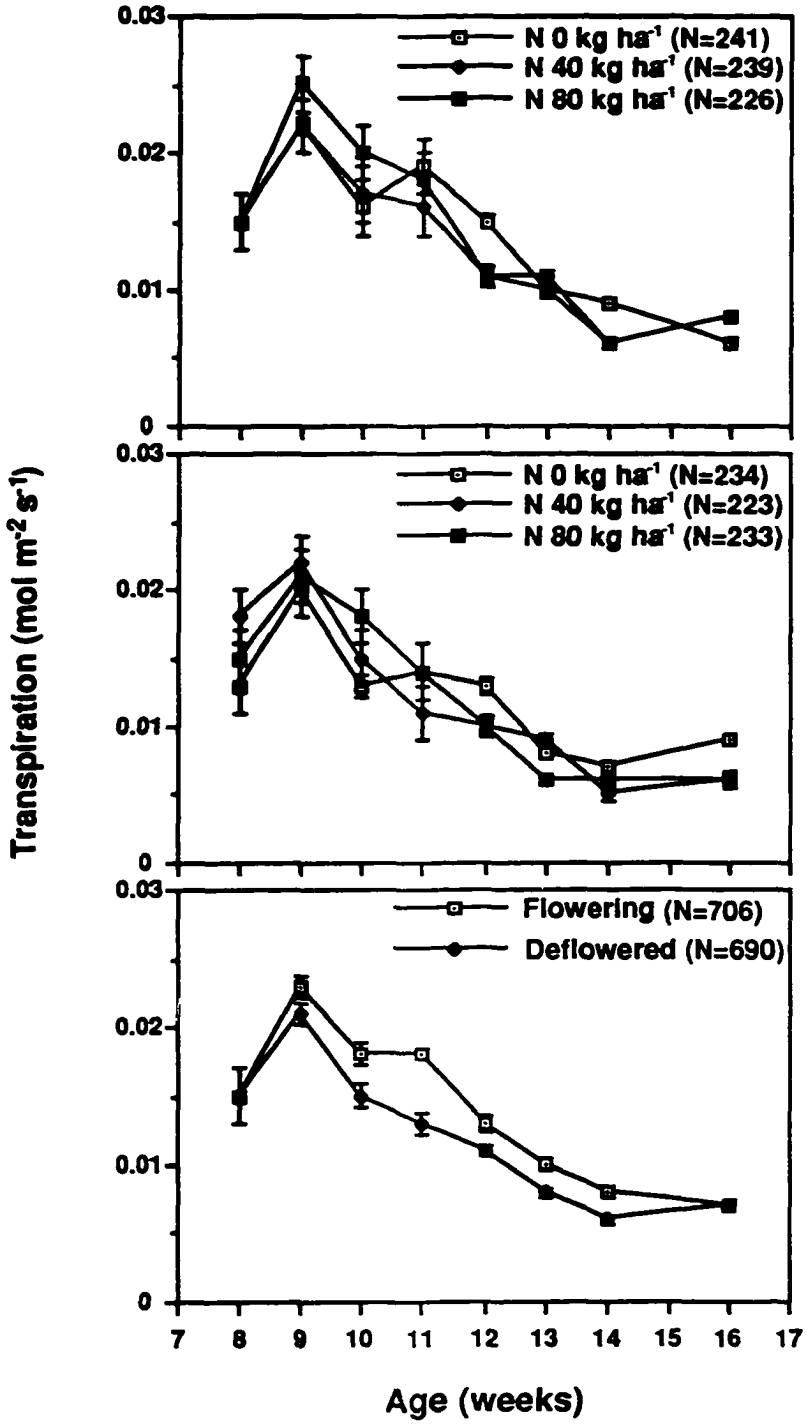


**Figure 3. 6** Transpiration rate as influenced by nitrogen fertilization and flowering. Upper and middle panels - flowering and deflowered plants, respectively, with three nitrogen rates, lower panel - flowering and deflowered plants, pooled across the three nitrogen rates. N is the total number of measurements. Mean with 95% CI.

**Water use efficiency**

Flowering plants showed a marginal 4% greater WUE than the deflowered plants. Two way ANOVA indicated significant effects of nitrogen and flowering, and their interaction, with extremely low R-squares ($F=3.28$, $P<0.05$, R-sq=0.00 for nitrogen; $F=6.30$, $P<0.05$, R-sq=0.00 for flowering; $F=4.22$, $P<0.05$, R-sq=0.00 for interaction). Statistical power for this model was ca 0.96 with a LSN of 730. The only effect of nitrogen was the 5% increase in WUE by nitrogen 40 kg $ha^{-1}$ compared to the control (Figure 3. 7). Water use efficiency dropped sharply from the eighth to the ninth week of age and steadily increased toward the $14^{th}$ week of age.

**Air temperature and PPFD**

Ambient air temperature gradually declined from 34°C to 26.5°C during the experimental period, except for the sharp increase from the eighth to the ninth week. However, the variation in PPFD during the measurements was slight around a mean of 1616 μmol $m^{-2}$ $s^{-1}$ (Figure 3. 8).

**Chlorophyll concentration**

Leaf chlorophyll concentration was closely correlated to SPAD units in the calibration of SPAD meter, as shown in Figure 3. 9. However, the individual SPAD values deviated more from the regression fit as the leaf chlorophyll concentration increased beyond approximately 50 μg $cm^{-2}$ than at the lower chlorophyll concentrations.

Flowering plants showed 20% higher leaf chlorophyll concentration than the deflowered plants. The highest increase in chlorophyll by flowering, 46%, was detected in the plants without applied nitrogen as illustrated in the Figure 3. 10. Plants applied with nitrogen 80 kg $ha^{-1}$ associated with 11% more chlorophyll

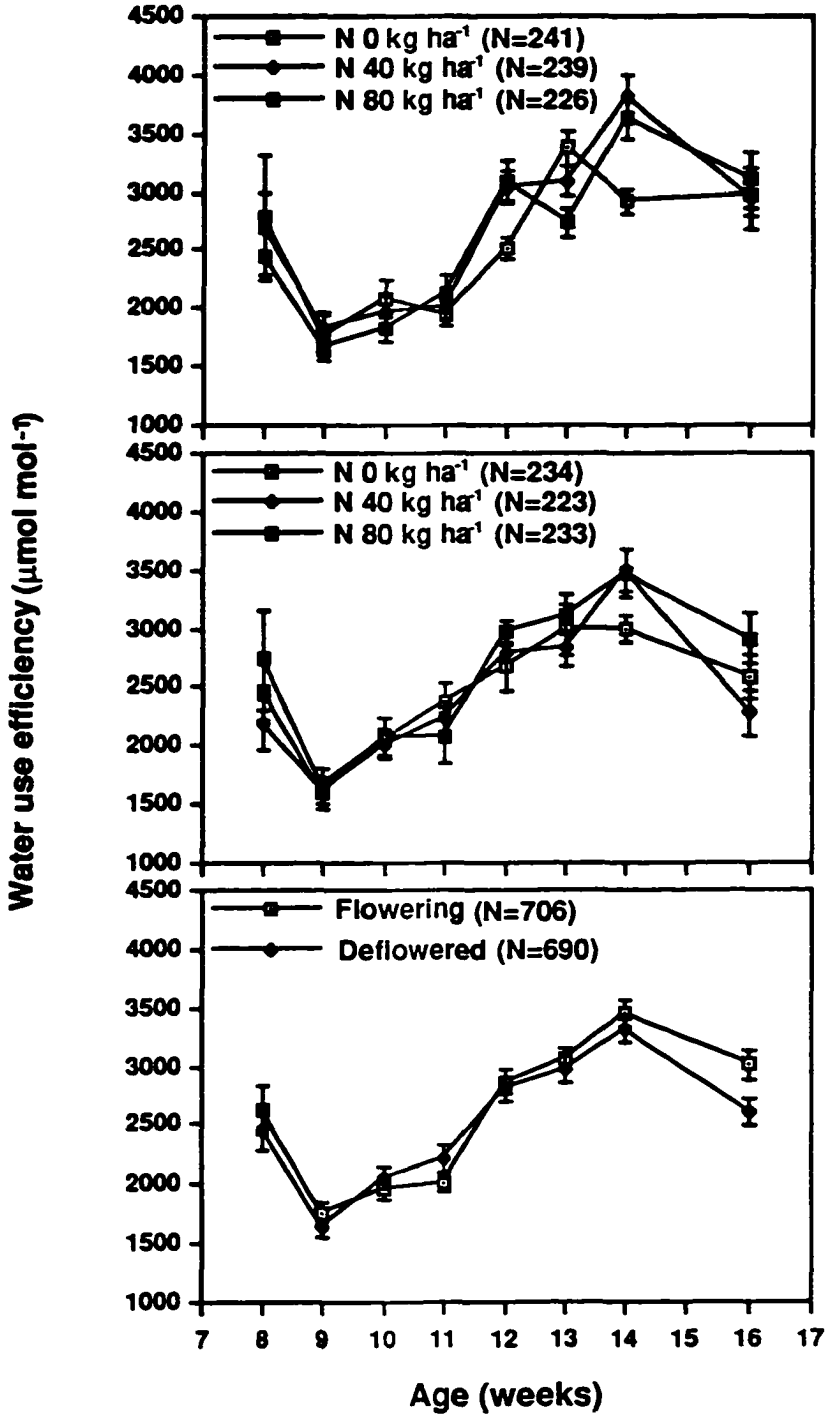


**Figure 3. 7** Water use efficiency as influenced by nitrogen fertilization and flowering during eight to 16 weeks of age. Upper and middle panels - flowering and deflowered plants, respectively, lower panel - flowering and deflowered plants pooled across the three nitrogen rates. N is the total number of measurements. Mean with 95% CI.

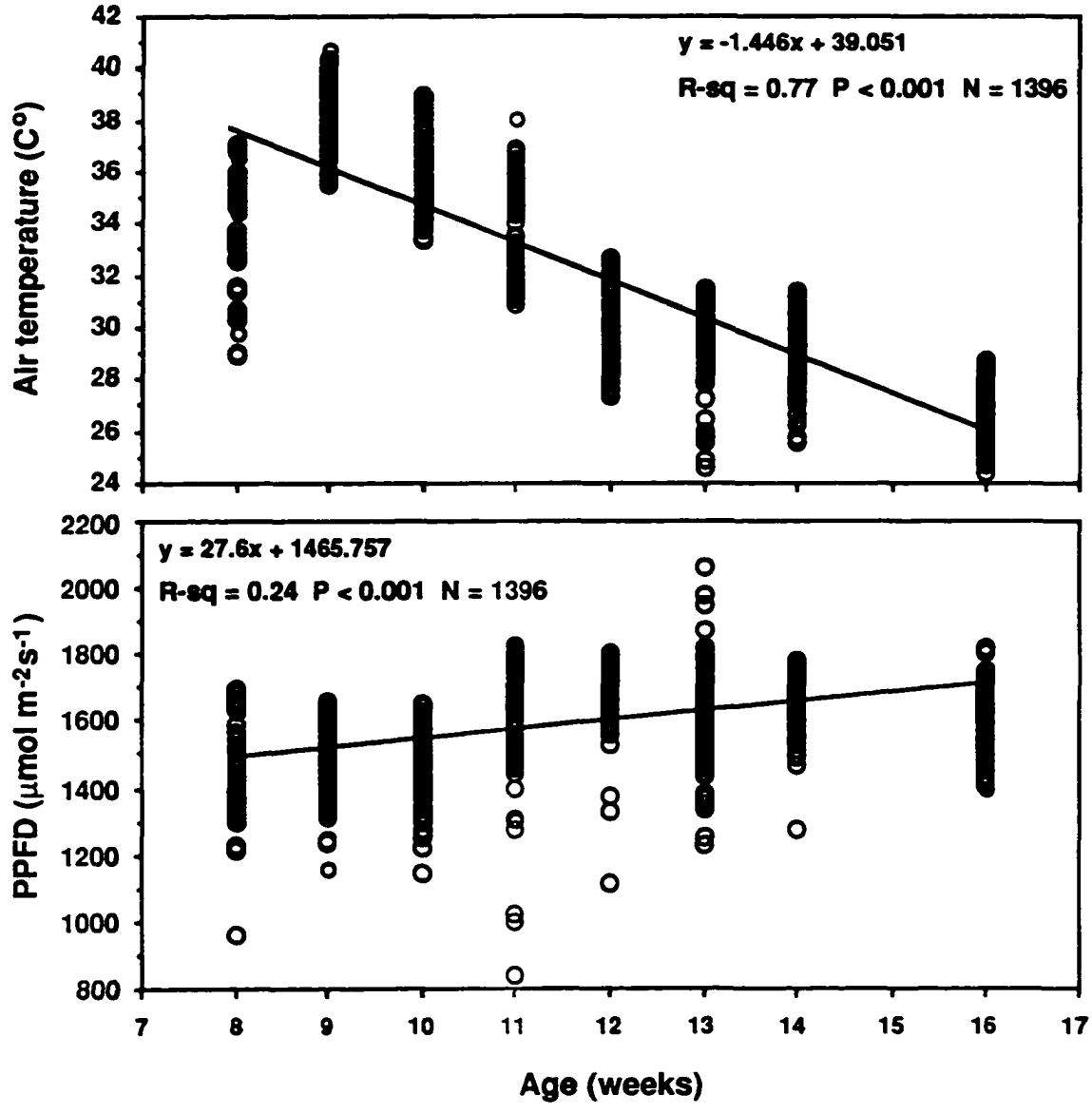


**Figure 3. 8** Fluctuations of ambient air temperature and photosynthetic photon flux density (PPFD) during gas exchange measurements. Scatter plots are superimposed with linear regression lines.

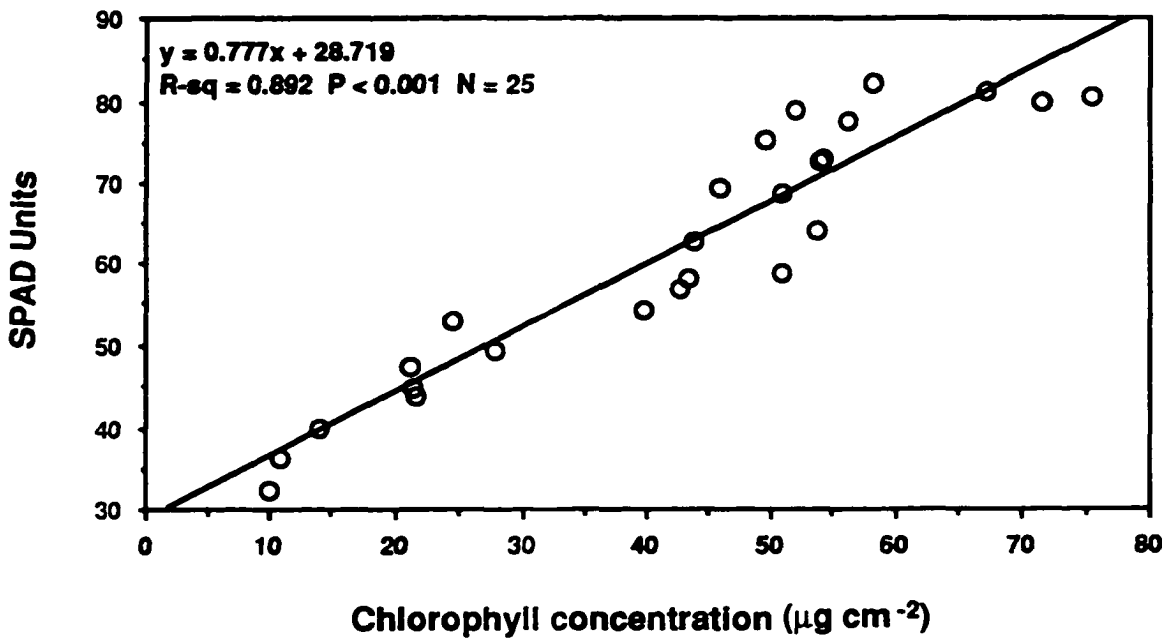


**Figure 3. 9** Linear regression of SPAD units and extractable leaf chlorophyll concentration. Each SPAD value is an average of 12 readings taken on the same leaflet of which the chlorophyll concentration was destructively measured. Twenty five leaflets with visually evident, varying degrees of greenness were selected from the plants with nitrogen 40 kg $ha^{-1}$ treatment.

when allowed to flower. However, the 8% increase in chlorophyll concentration of flowering plants compared to the deflowered plants was insignificant at nitrogen 40 kg $ha^{-1}$ (two way ANOVA, F=6.07, P<0.01, R-sq=0.08 for nitrogen; F=49.67, P<0.001, R-sq=0.34 for flowering; F=13.66, P<0.001, R-sq=0.19 for interaction). Although this interaction effect of nitrogen and flowering on both chlorophyll and SPAD values was significant, R square, especially in SPAD readings with higher sample sizes, was low (F=27.28, P<0.001, R-sq=0.02 for nitrogen; F=1266.59, P<0.001, R-sq=0.60 for flowering; F=20.45, P<0.001, R-sq=0.02 for interaction).

**Leaflet area**

The two way ANOVA shows significant main effects of nitrogen and flowering and their interaction, with statistical power of ca 0.99 and LSN of 152 (F=3.63, P<0.05, R-sq=0.16 for nitrogen; F=2.45, P<0.0001, R-sq=0.46 for flowering; F=7.94, P<0.001, R-sq=0.36 for interaction). The effect of nitrogen application was only detected in the deflowered plants in which nitrogen at 80 kg $ha^{-1}$ produced 9% and 23% greater leaflet area than the control and 40 kg $ha^{-1}$ of nitrogen, respectively. However, deflowered plants without applied nitrogen produced 11% more leaflet area than deflowered plants that were applied with nitrogen 40 kg $ha^{-1}$ (Figure 3. 11). Deflowering increased the leaflet area by 13% (F=19.72, P<0.001, R-sq=0.03). Furthermore, the leaflet area declined with age in both flowering and deflowered plants.

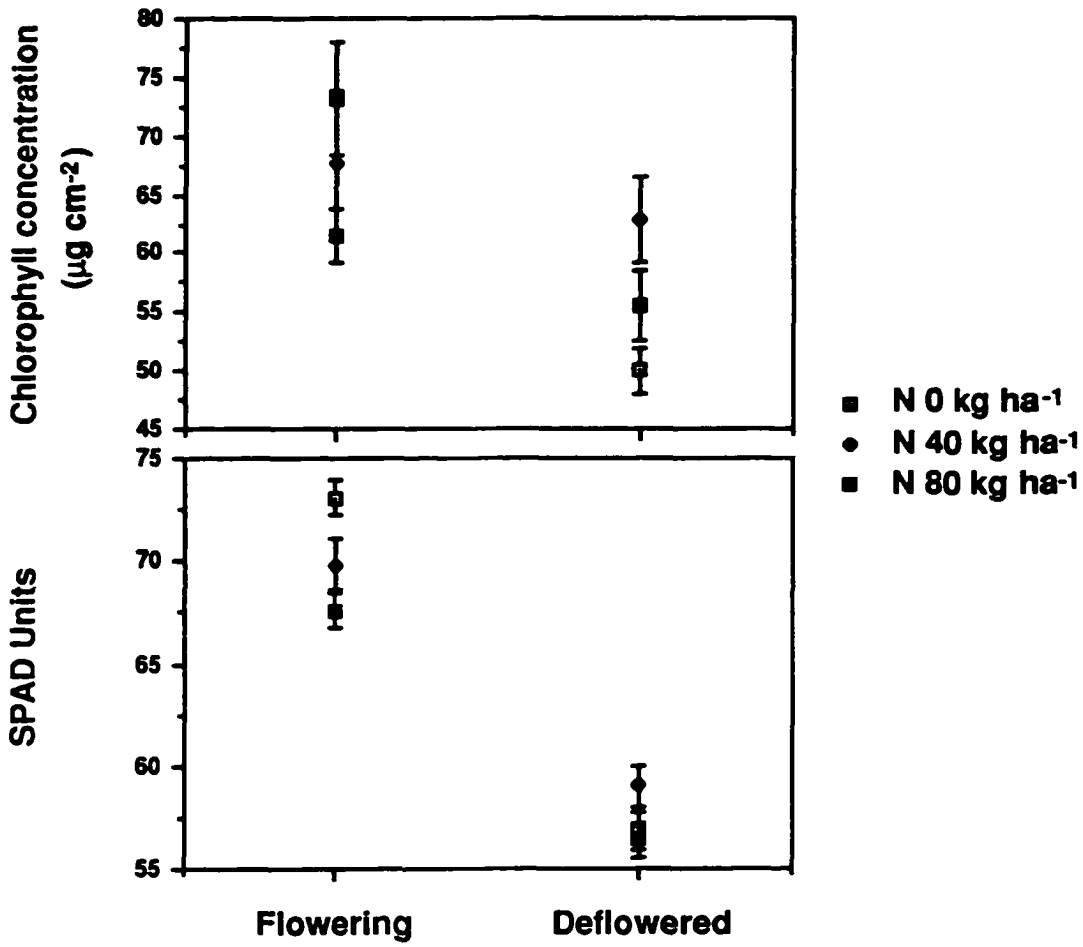


**Figure 3. 10** Effect of flowering and nitrogen fertilization on extractable chlorophyll concentration and non-destructively evaluated SPAD units. Mean of 10 and 121 - 124 observations for chlorophyll concentration and SPAD units, respectively, with 95% CI.

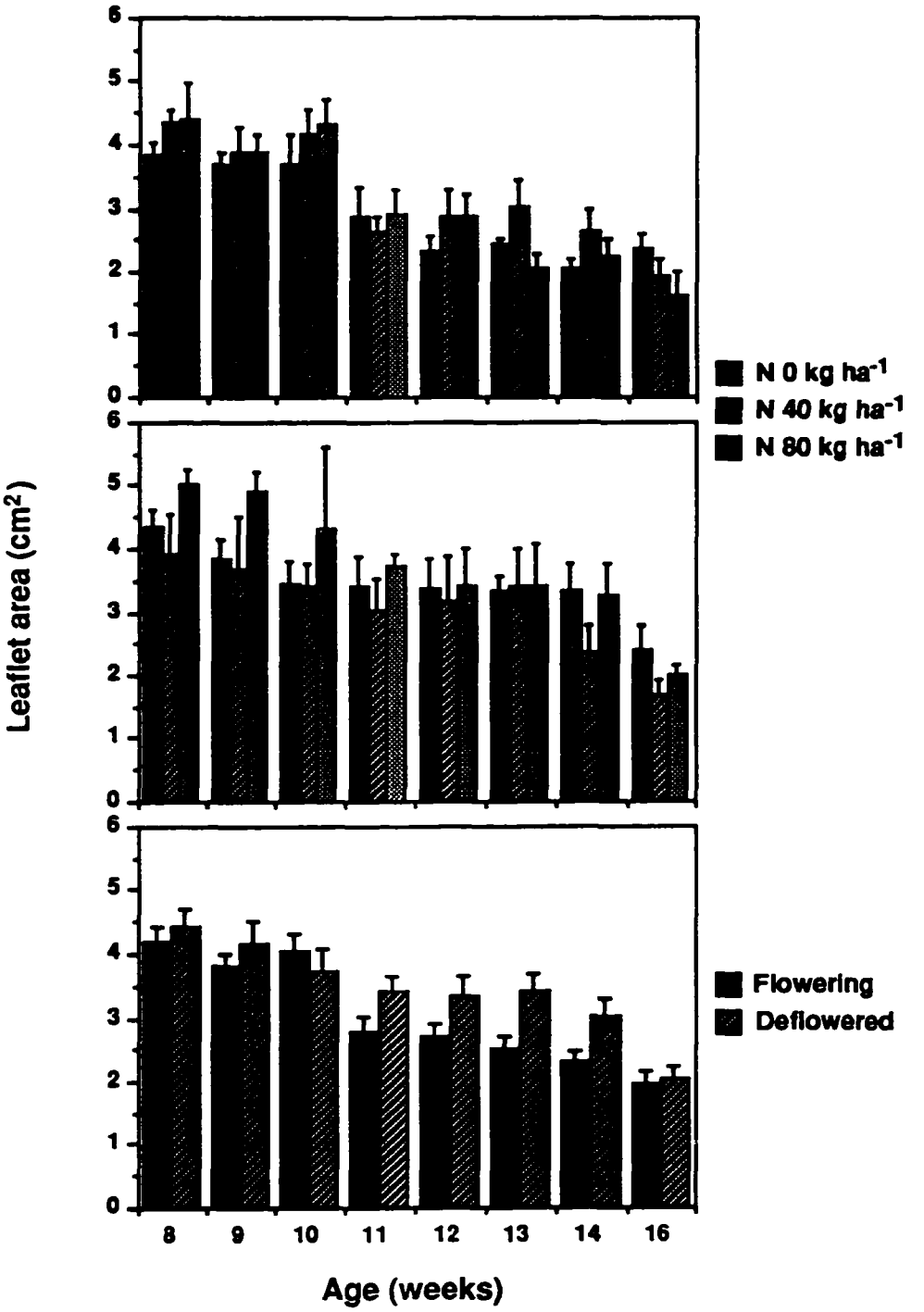


**Figure 3. 11** Individual leaflet area of the flowering and deflowered plants at each nitrogen level from eight to 16 weeks of age. Upper and middle panels, flowering and deflowered plants, respectively (mean of 12). Lower panel, data pooled across all nitrogen levels in either flowering or deflowered plants (mean of 36). Error bars are 95% CI of the mean.

**Leaf specific mass (LSM)**

No significant effect of flowering or nitrogen on LSM was detected when data were pooled across all ages.[1] However, as illustrated in the Figure 3. 12, deflowered plants showed an overall 3% higher LSM than the flowering plants, during the first four weeks of measurements (F=6.72; P<0.05; R-sq=0.02). This response reversed during the measurements taken from 12$^{th}$ week of age when flowering plants acquired 6% higher LSM than the deflowered plants (F=14.82; P<0.001; R-sq=0.05). Although weekly variations were observed, LSM was generally constant over the whole period of measurements.

**Biomass partitioning and root:shoot ratio**

Deflowered plants produced greater biomass of roots, leaflets, leaf rachis and stems than the flowering plants, as illustrated in Figure 3. 13 (F=14.42, P<0.001, R-sq=0.30 for roots; F=21.24, P<0.001, R-sq=0.38 for leaflets; F=24.39, P<0.001, R-sq=0.40 for rachis; F=13.45, P<0.001, R-sq=0.28 for stems). Leaflet weight was 63% greater in the deflowered plants than the flowering plants. Total shoot biomass and total plant biomass were 34% and 35% higher, respectively, in deflowered plants than the flowering plants (F=9.20, P<0.01, R-sq=0.21 for total shoot; F=10.57, P<0.01, R-sq=0.23 for total plant). However, nitrogen effects or the interaction effect of nitrogen and flowering on the biomass of any plant organ or of whole plant were nonsignificant[2], except for the interaction effect on roots (F=5.03, P<0.05, R-sq=0.16). Statistical power for the whole model test with nitrogen and flowering as main effects was ca 0.97 with LSN of 20 for leaflet weight

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[1] F=0.24, P=0.78, R-sq=0.00 for N; F=2.22, P=0.13, R-sq=0.00 for flowering; F=0.05, P=0.94, R-sq=0.00 for interaction

[2] For N - F=1.42, P=0.25, R-sq=0.04 for root; F=0.13, P=0.87, R-sq=0.00 for leaflet; F=0.50, P=0.61, R-sq=0.01 for rachis; F=0.24, P=0.78, R-sq=0.00 for stem. For interaction - F=2.42, P=0.10, R-sq=0.04 for leaflet; F=1.8, P=0.18, R-sq=0.06 for rachis; F=2.40, P=0.10, R-sq=0.10 for stem

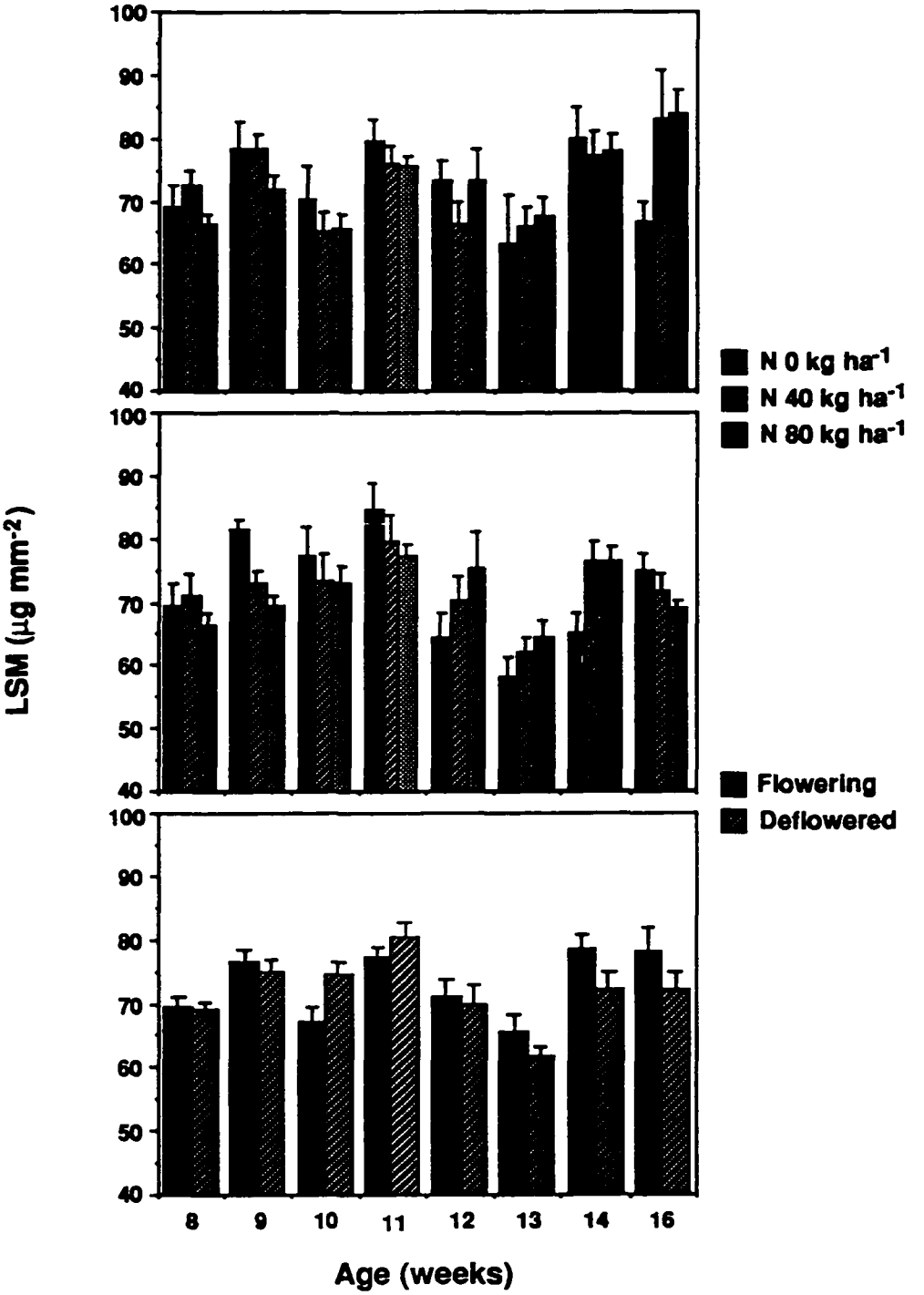



**Figure 3. 12** Leaf specific mass in flowering and deflowered plants at each nitrogen level during eight to 16 weeks of age. Upper and middle panels, flowering and deflowered plants, respectively, with mean of 12. Lower panel, data pooled across all nitrogen levels in either flowering or deflowered plants with mean of 36. Error bars are 95% CI of mean.

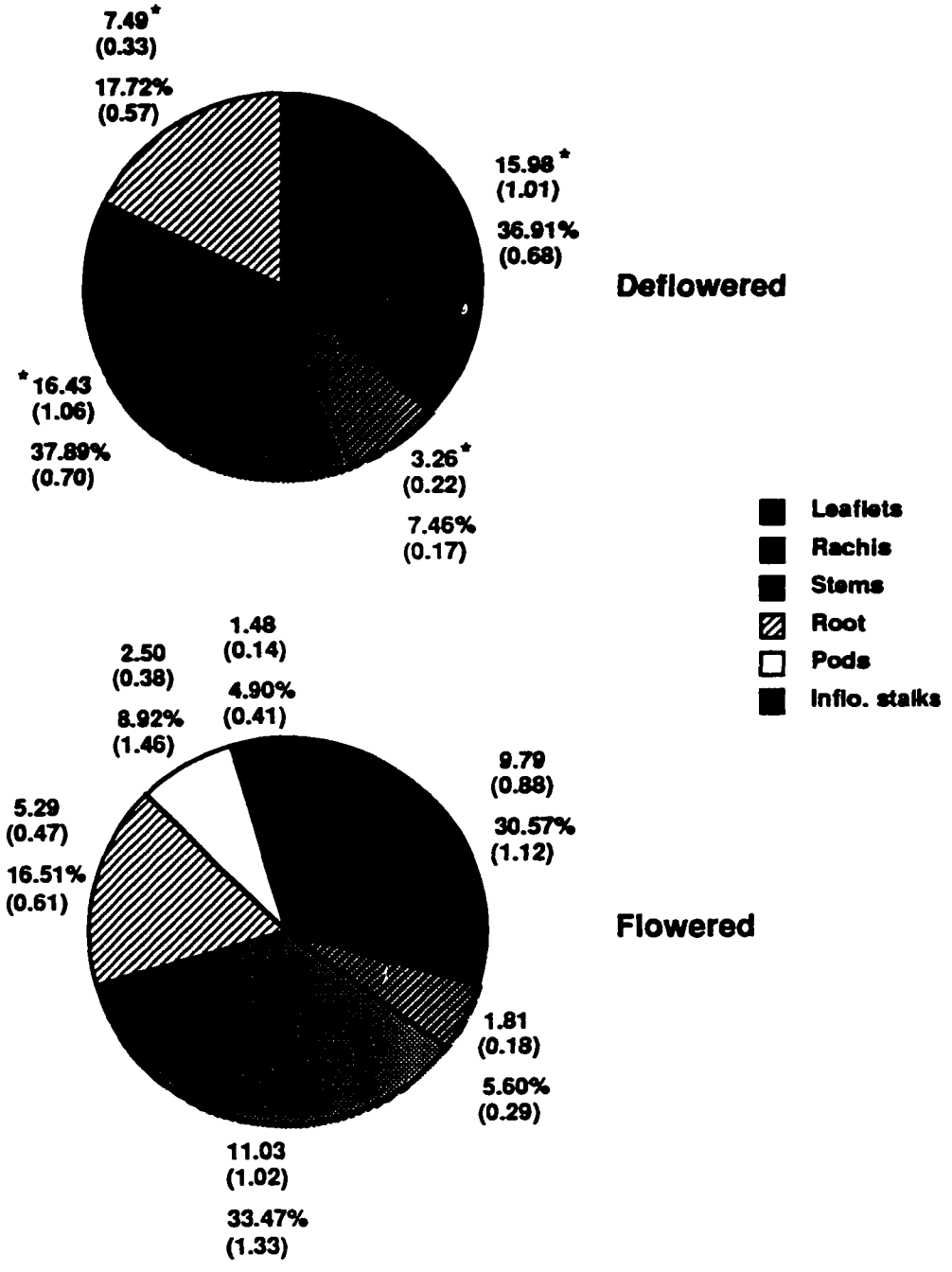



**Figure 3. 13** Biomass partitioning into different organs in flowered and deflowered plants, data pooled across three nitrogen levels. The value above the percentage biomass in each portion of a pie is the biomass (g) of the given organ. Mean of 18 with SE, * indicates significant increase compared to flowering plants at $P<0.001$.

and was 0.81 with LSN of 26 for total plant weight.

Although, the deflowered plants produced higher root:shoot ratio at each nitrogen level (Figure 3. 14), no significant main effect or interaction effect was detected in the two way ANOVA at the sample size of this experiment (36).[1] Statistical power analysis for the whole model indicated a LSN of 61 with the power of ca 0.42 only.

**Harvest index (HI) and leaf area index (LAI)**

As shown in the Table 3. 2, HI was greater in the deflowered plants at all nitrogen levels with an overall increase of 25% compared to the flowering plants. Similarly, deflowered plants produced higher LAI at each nitrogen level with an overall 67% increase over the flowering plants (Table 3. 3). However, nitrogen or interaction effect of nitrogen and flowering on either HI or LAI was nonsignificant. Statistical power of the whole model test for HI was ca 0.98 with a LSN of 20 while LAI was associated with a power of ca 0.97 and LSN of 20.

[1] F=1.99, P=0.15, R-sq=0.10 for N; F=2.08, P=0.15, R-sq=0.06 for flowering

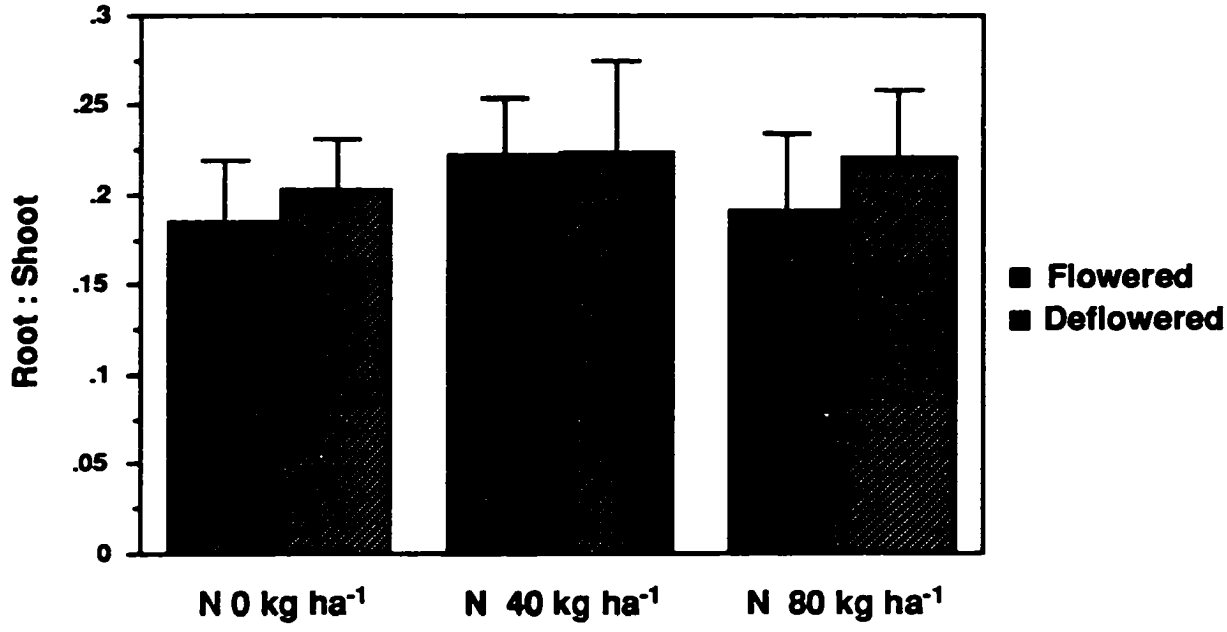



**Figure 3. 14** Root:shoot as affected by flowering at each nitrogen rate. Error bars represent 95% CI of the mean of six.

**Table 3. 2** Harvest index based on biomass of leaflets (without rachis) and shoots at 4 months after seeding. Mean with SE.

| Flowering | Nitrogen Rate | | | |
|---|---|---|---|---|
| | N 0 kg/ha | N 40 kg/ha | N 80 kg/ha | Total |
| Flowered | 0.37(0.02)a | 0.34(0.01)a | 0.37(0.03)a | 0.36(0.01)a |
| Deflowered | 0.45(0.01)b | 0.43(0.01)b | 0.45(0.02)a | 0.45(0.01)b |
| **ONE WAY ANOVA** | | | | |
| N | 6 | 6 | 6 | 18 |
| F | 9.36 | 26.53 | 4.37 | 28.00 |
| P | < 0.05 | < 0.001 | 0.06 | < 0.001 |
| R-sq | 0.48 | 0.71 | 0.31 | 0.45 |

| TWO WAY ANOVA | F | P | R-sq |
|---|---|---|---|
| Nitrogen (N) | 0.84 | 0.44 | 0.04 |
| Flowering (F) | 26.25 | < 0.001 | 0.68 |
| N X F | 0.09 | 0.90 | 0.00 |

Means followed by the same letter in each column denote statistical similarity by PLSD.

**Table 3. 3** Estimated leaf area index as affected by nitrogen application and deflowering. Mean with SE.

| Flowering | Nitrogen Rate | | | |
|---|---|---|---|---|
| | N 0 kg/ha | N 40 kg/ha | N 80 kg/ha | Total |
| Flowered | 1.38(0.12)a | 1.86(0.12)a | 1.78(0.13)a | 1.67(0.07)a |
| Deflowered | 3.14(0.15)b | 2.39(0.10)a | 2.79(0.16)b | 2.77(0.08)b |
| ONE WAY ANOVA | | | | |
| N | 6 | 6 | 6 | 18 |
| F | 19.26 | 2.49 | 5.12 | 22.20 |
| P | < 0.01 | 0.14 | < 0.05 | < 0.001 |
| R-sq | 0.65 | 0.19 | 0.33 | 0.39 |

| TWO WAY ANOVA | F | P | R-sq |
|---|---|---|---|
| Nitrogen (N) | 0.19 | 0.82 | 0.00 |
| Flowering (F) | 23.06 | < 0.001 | 0.40 |
| N X F | 2.46 | 0.10 | 0.08 |

Means followed by the same letter in each column denote statistical similarity by PLSD.

## DISCUSSION

The photosynthetic advantage of flowering senna plants over deflowered plants is consistent with the leaf chlorophyll concentrations. That is, in senna, lack of sink demand limits the rate of primary metabolism by attenuating the photosynthetic machinery. This curtails the light harvesting capacity of the leaves, when other photosynthetic inputs are non-limiting. Foyer (1987, 1988); Layne and Flore (1995); and Micallef *et al.* (1995) have reported the correlation of source-sink balance to $P_{net}$ that results from the effects of sink demand on activities in starch and sucrose biosynthetic pathways. Even though an end-product feed-back inhibition of photosynthesis may underlie the reduced chlorophyll concentration in deflowered plants, both the rate of $P_{net}$ and chlorophyll concentration in the deflowered plants were remarkably higher than common agricultural field crop (Yadava, 1986; Marquard and Tipton, 1987; Stitt and Krapp, 1995; Micallef *et al.* 1995), temperate fruit crop (Layne and Flore 1995) and tropical tree species (Ellsworth and Reich, 1996).

The low ambient air temperatures particularly after the $11^{th}$ week reduced the sink size dramatically by causing mature flower and blossom drop. Deflowered plants, however, possessed a greater sink in the expanding leaves. The demand of the reproductive sink of flowering plants for photosynthate is probably greater than the demand of the vegetative sink of deflowered plants. This needs to be confirmed by quantitative and comparative analyses of carbon-based caloritic values of the two sinks.

In this experiment with no replication for nitrogen treatments, $P_{net}$ of flowering plants was not influenced by nitrogen application. Net photosynthesis dropped

in the deflowered plants when nitrogen was applied. However, the elemental analyses show no marked differences in leaf nitrogen content across nitrogen or flowering treatments (Chapter five). This suggests that senna plants both acquire and mobilize nitrogen effectively in accordance with physiological demand and not strictly with the availability in soil. Furthermore, assimilation of nitrogen by the reproductive sink may cause a greater demand for nitrogen in flowering plants compared to the vegetative sink of the deflowered plants.

The patterns of stomatal conductance and transpiration are consistent with fluctuations in $P_{net}$, which is in agreement with the findings of other authors (Bunce, 1988; Fay and Knapp, 1993; Schierenbeck and Marshall, 1993; Layne and Flore, 1995). The photosynthetic advantage of flowering plants (26% more than deflowered plants) did not directly translate into WUE (only 4% more in the flowering plants) due to disproportionately higher transpiration in the flowering plants. However, this marginal increase in WUE of flowering plants would have enabled them to maintain $P_{net}$ more steadily than the deflowered plants at high VPDs such as > 40 mb. However, greater sensitivity of deflowered plants to very high VPD's, with their greater transpirational surface area, could be adaptive in a xeric environment.

The 28 day-long drought in August did not reduce the xylem water potential lower than -1 MPa in any area of the plot. Photosynthesis of glasshouse-grown senna plants was suppressed by drought only at xylem water potentials less than -1.5 MPa. Furthermore, although glasshouse-grown potted plants exhibited paraheliotropic leaflet movement at water potentials less than -1.5 MPa during midday on sunny days, and defoliation between -1.5 to -2 MPa, these drought tolerance mechanisms were absent in the outside-grown plants. Plants grown

outside, had a long tap root with a negligible proportion of surface-running lateral roots. This explains the high predawn xylem water potentials (higher than -0.3 MPa even after the drought) indicating that the tap root effectively exploited deep zones of soil moisture.

The use of SPAD meter as an in-situ estimation of leaf chlorophyll content has been well justified especially for the species with leaf chlorophyll concentrations less than 50 μg $cm^{-2}$ (Yadava, 1986; Marquard and Tipton, 1987). The manufacturer of the meter recommends its use only for the leaves with chlorophyll concentrations below 50 μg $cm^{-2}$. Chlorophyll concentrations of the garden-grown senna plants in this study averaged 67.4 μg $cm^{-2}$ and 56.1 μg $cm^{-2}$ for the flowering and deflowered plants, respectively, and a maximum of 75.4 μg $cm^{-2}$ was detected while collecting data for the calibration of SPAD meter. Thus, leaf chlorophyll content of senna is remarkably higher than a wide range of $C_3$ and $C_4$ crops as reported by Yadava (1986); Marquard and Tipton (1987). High leaf chlorophyll contents are associated with leaves adapted to high light intensities, e.g. sun leaves (Bolhàr-Nordenkampf and Draxler, 1993). Although the SPAD values clearly deviate from the linear regression fit for the leaf chlorophyll values more than 50 μg $cm^{-2}$, a R-sq of 0.89 was still obtained for the whole range of values. Thus, the SPAD meter could be considered a useful tool especially on-farm, for estimating senna leaf chlorophyll concentrations if a site-specific calibration curve is established. Its ability to generate very high sample sizes of data nondestructively in a short time is an immense advantage especially in experiments involving a series of gas exchange measurements taken on the same plants.

Flowering clearly increased leaf chlorophyll concentration. When the plants were not exogenously supplied with nitrogen, this increase was greater indicating that adequate nitrogen was already available. This is consistent with the leaf nitrogen contents (Chapter 5). The higher chlorophyll concentration in the plants that were applied with nitrogen 40 kg $ha^{-1}$ compared to the plants applied with nitrogen 80 kg $ha^{-1}$ testifies this, as well. Nonsignificant effects of nitrogen application on LSM of both flowering and deflowered plants and on leaflet area of flowering plants further suggest that the available nitrogen content in the soil was adequate for realizing the optimal performance of these response variables.

Deflowering remarkably increased the leaflet area, total leaf area and weight, total shoot weight, plant height (at all nitrogen levels, data not presented), root weight and total biomass. This strongly suggests that deflowering alters the more determinate growth of senna into a more indeterminate growth (although inflorescences emerge from axillary buds, the further apical vegetative growth was hindered by flowering). This results in economically advantageous increases in LAI and HI in deflowered plants even though the $P_{net}$ per unit leaf area is less.

The 63% greater leaf yield in the deflowered plants compared to the flowering plants raises new questions for investigation. This increased transpirational surface area could be disadvantageous in a severe drought. Deflowering increases cost of production and time at farming, thus, economic gains by yield increases need to be assessed against increased costs. The impact of deflowering on the sennoside yields must be studied. Essentially, a method of deflowering appropriate to the field has to be developed should this yield advantage be realized. Von Caemmerer and Farquhar (1984) demonstrated that partial defoliation in *Phaseolus vulgaris* enhanced $P_{net}$ across a range of intercellular $CO_2$

concentrations. Layne and Flore (1995) reported that partial defoliation of *Prunus cerasus* clearly increased $P_{net}$ at low, medium and high PPFDs. This alludes to the possibility of developing a method of leaf harvesting and deflowering performed together while maintaining the $P_{net}$ of the plants comparable to flowering plants. Such investigations on sink manipulations must be planned site-specifically with parallel photosynthetic measurements especially if the sennoside yields are closely correlated to the rate of concurrent $P_{net}$.

The increased assimilate partitioning into the root by the deflowered plants would directly enhance the drought tolerance especially due to the clear dominance of the tap root over laterals in senna. Change in the growth habit (indeterminate growth in the deflowered plants) may increase the economic life-span of the crop. Increased LAI provides more effective ground cover which would help reduce cost of the cropping system in the long run by suppressing weed growth, by controlling erosion, and by conserving soil moisture through increased infiltration. However, quantitative determination of sennoside A and B must be undertaken to more definitively assess the practical value of the yield advantage of deflowered plants as opposed to the flowering plants which is the focus of Chapter four.

# CHAPTER 4

## EFFECTS OF DROUGHT, NITROGEN AND DEFLOWERING ON LEAF SENNOSIDE CONTENT AND ELEMENTAL COMPOSITION

## SUMMARY

A reverse phase HPLC method was developed to separate and quantify sennosides in dry senna leaves. Effects of drought, nitrogen, crop type, deflowering, time of day and leaf maturity on leaf sennoside yields were studied, with simultaneous assessments on $P_{net}$ as presented in the previous Chapters.

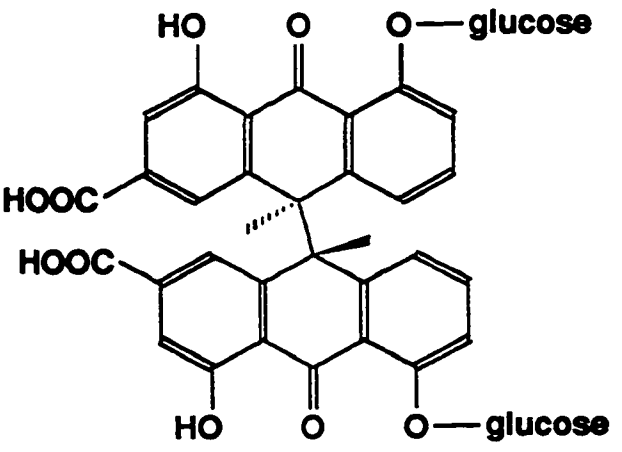
Short term drought, before the plants morphologically adapted to drought, increased sennoside A+B concentration (% dw) due to a relatively greater increase in sennoside A than in B. Long term drought, after the drought-induced morphological changes occured, increased sennoside A:B without changing sennoside A+B concentration. Foliar nitrogen application increased the sennoside A+B yield per plant by 142% in the watered plants, and showed no effect in the droughted plants. However, sennoside A+B concentration was reduced by foliar nitrogen supplement in both droughted and watered plants and the effect was long persistent after the treatments were discontinued. However, the effects of drought on sennoside contents were not persistent. Droughted plants had less leaf K, Ca and Mg but higher leaf N than the watered plants. In a comparison among the crop types, ratoon showed 15% and 32% higher sennoside A:B ratio than seedlings and cuttings. Furthermore, ratoon had the highest sennoside A+B concentration followed by seedlings and cuttings. Sennoside A+B concentration steadily decreased along the nodal position of the leaves from top to the seventh expanded leaf in both seedlings and cuttings. Sennoside A:B ratio remained unchanged with varying leaf maturity.

Deflowered plants produced 25% higher sennoside A+B concentration with no difference in A:B ratio, and 100% higher sennoside yield per plant compared to the flowered plants. Sennoside A:B ratio dramatically decreased in the cold weather in fall, in three and half month old plants. Sennoside A+B concentration increased in deflowered plants diurnally toward evening although decreased in the flowering plants. Deflowered plants accumulated 15% and 20% higher leaf P and K but 10% less leaf Ca than the flowered plants.

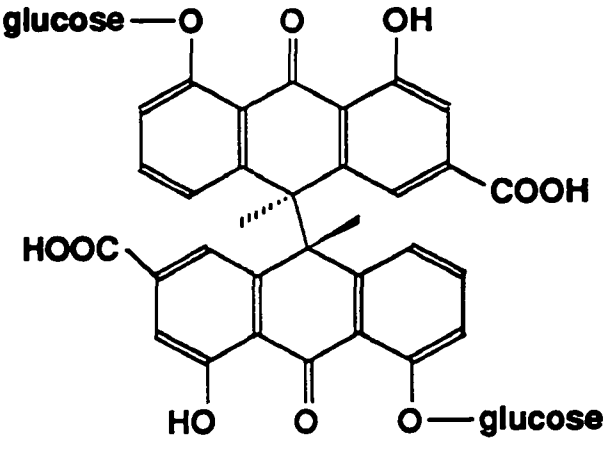
Sennoside yield per plant was more a function of the total leaf biomass than the sennoside concentration, under any treatment studied. Sennoside levels were higher with reduced $P_{net}$ that was associated with drought, nitrogen stress, deflowering, and ratoon compared to other crop types. However, senna has the metabolic capacity to produce higher leaf sennoside yields while maintaining higher $P_{net}$ as well, as exemplified by the seedlings compared to cuttings and the younger leaves compared to mature leaves. Furthermore, sennoside A+B yields could increase through increase or decrease of sennoside A:B ratio under specific environmental cues.

## INTRODUCTION

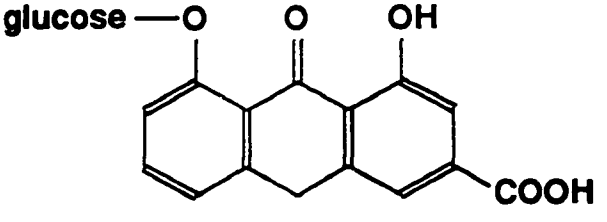
Sennosides A and B, mesomeric forms of rheindianthrone-8, 8' diglucosides (Figure 4. 1), are the most biologically active laxative constituents of senna. Sennoside C, D, D1 (the mesomeric forms rhein-aloe-emodin dianthrone-8,8'-diglucoside), and aloe-emodin dianthrone-diglucosides in senna contribute to only less than 20% of biological activity (Stuppner and Sturm, 1996; Al-Dakan *et al.*, 1995). Crude plant drug, pure sennosides or calcium salts of sennosides are marketed world-wide (Atzorn *et al.*, 1981; Suman *et al.*, 1990). Mainly due to




Sennoside A




Sennoside B




8-Glucosido-rheinanthrone

**Figure 4. 1** Sennoside A and B with their proposed monomeric precursor.

higher sennoside content, leaves are the preferable harvest although pod shells are used as well (Gupta, 1984; Stuppner and Sturm, 1996). Sennoside yields in *Cassia angustifolia* are the highest in all *Cassia* species that have been phytochemically analyzed for these secondary metabolites (Lohar *et al.*, 1979).

Although formation of sennosides (or leaf sennoside yields) is expected to be correlated to changes in the primary metabolism, no investigations have been undertaken to study how photosynthesis is related to sennoside synthesis. However, reports indicate that senna grown in arid, nutrient-poor, sandy soils in India (Sharma *et al.*, 1980; Gupta, 1984), is able to produce higher leaf sennoside yields with lower nitrogen applications such as 50 kg $ha^{-1}$ (Pareek *et al.*, 1983). Furthermore, Lohar *et al.* (1979) observed a decrease in leaf sennoside content during the reproductive phase due to probable translocation of these metabolites to reproductive organs.

Sennosides are absent in the fresh plant material of senna or of other sennoside sources, and form during postharvest water loss from leaves (Labadie, 1970; Atzorn *et al.*, 1981). Furthermore, there is evidence that sennosides, 8-glucosidorheinanthrone (the supposed monomeric precursor of sennosides) and anthraquinones (the oxidized monoanthrones) are all readily inter-convertible (Auterhoff and Scherff, 1960; Atzorn *et al.*, 1981). This seems to rule out the possibility of only one sennoside precursor in senna (Atzorn *et al.*, 1981).

Leaves from the experimental plants that were used to investigate gas exchange responses to drought, nitrogen, flowering, and in different crop types (three previous chapters) were analyzed by high performance liquid chromatography (HPLC) to assess sennosides A and B yields. The main focus of this chapter is to

establish the relationship between the current photosynthesis and sennoside content in dry leaves. The varying environmental and plant physiological/developmental conditions of the experiments provided with the wide spectrum $P_{net}$ values required for this objective. Effects of nodal position and time of day on sennoside contents were investigated, as well, as important considerations of a harvesting schedule. Furthermore, the leaf macro- and microelemental analyses were undertaken to understand the relationship between sennoside yields and the leaf nutrient status, and to assess the effects of the drought, nitrogen and flowering on the leaf elemental analyses.

## MATERIALS AND METHODS

### *Plant materials*

Leaflets from the fourth or fifth leaf from the youngest expanded leaf at the apex were collected for sennoside extraction from the same plants as used in the Chapters, one, two and three. All the leaflets were harvested approximately at 4:00 pm, except for the investigations on the diurnal fluctuations of sennosides. They were air-dried in opaque plastic vials at room temperature (24±1°C) to a constant weight.

In the experiment of Chapter one with foliar nitrogen application and drought as treatments, leaflets were collected from each plant every other day in the course of daily gas exchange measurements during the fifth drought cycle. The uppermost expanded leaves of each plant were stripped and air-dried to a constant weight at room temperature for elemental analyses (undertaken by A & L Eastern Agricultural Laboratories, Inc.,VA, USA) at the end of fifth drought cycle. In the experiments of Chapter two with the three crop types (ratoon, cuttings

and seedlings), leaflets stripped from four plants of each group, on the first day of the drought treatments, were used for sennoside extraction.

In the experiment with nitrogen and deflowering treatments on the garden-plot (Chapter three), the same plants that were used for weekly gas exchange measurements were used for leaf sennoside extractions, as well. Leaflets were harvested every other week between two to four months of age on the same day of the corresponding weekly gas exchange measurement. Furthermore, two flowering and two deflowered, four month old plants on two hills that were applied with 40 Kg $ha^{-1}$ of nitrogen were used for investigations on diurnal variations of sennosides. Composite samples of leaflets from the two plants were stripped to minimize injury to each plant, between 9:00 am to 9:00 pm (day light saving time) at one and half hour intervals on a sunny day. The topmost expanded leaves of two flowering and two deflowered plants in each nitrogen level were collected at four months of age for elemental analyses.

For assessments of the leaf sennoside concentration in relation to nodal position, a separate group of plants, two seedlings and two cuttings, were grown in the glasshouse as described in Chapter 1. They were placed outside the glasshouse one month after establishment, in summer. All the expanded leaves from a branch of each plant were stripped at 110 days of age. These leaves were labeled with their respective nodal position from apex and individually air-dried to a constant weight in opaque plastic vials at room temperature.

### *Extraction of sennosides*

Fifty mg of leaflets from each replicate were cut into approx. 3-5 mm pieces and blended in two ml of 70% methanol using an Ultra - Turrax T 25 blender (Junke &

Kunkel IKA - Labortechnik, Germany). The blender was washed twice using two ml of 70% methanol each time and combined to the previous two ml. Extraction was performed by stirring the mix for 30 min on a magnetic stirrer. This initial extract was centrifuged for 10 min at 7000 rpm and decanted into glass vials, covered and immediately frozen at -20°C. The pellet was extracted twice with two ml 70% methanol each time and centrifuged. Supernatants were combined with the first extract and refrigerated again.

### *Calibration of HPLC and quantitation of sennosides*

Authentic sennoside A and B were obtained from Carl Roth GmbH & Company, Germany and frozen at -20°C in desiccated conditions. One mg of each sennoside was dissolved in five ml of 70% methanol with a trace of ammonium hydroxide. This was subjected to serial dilution to obtain solutions at two µg, four µg, six µg, eight µg and 10 µg/50µl of each sennoside. Each calibration standard was subjected to high pressure liquid chromatography using a Waters HPLC system consisting of a 600 pump/controller, a 717 WISP autosampler, a 996 Photodiode Array detector and Millenium 2. 1 software. Calibration curves are presented in Figure 4. 2.

All samples of leaf extracts were analyzed within less than three weeks of storage. For both calibration and sennoside quantitation a reversed phase Nucleosil $C_{18}$ column (Macherey and Nagel, 5 µm, 250 X 4.6 mm) was used. Two solvent systems, solvent A - 5% phosphoric acid in water, and solvent B - 5% phosphoric acid, 45% water and 50% acetonitrile at the flow rate of one ml $min^{-1}$ were used. A linear gradient elution of A 100% and B 0% at 0 min, A 20% and B 80% at 30 min, A 20% and B% 80 at 34 min, and A 100% with B 0% from 35 min to 55 min was adopted. Both solvent systems were degassed for 30 min with helium before use.

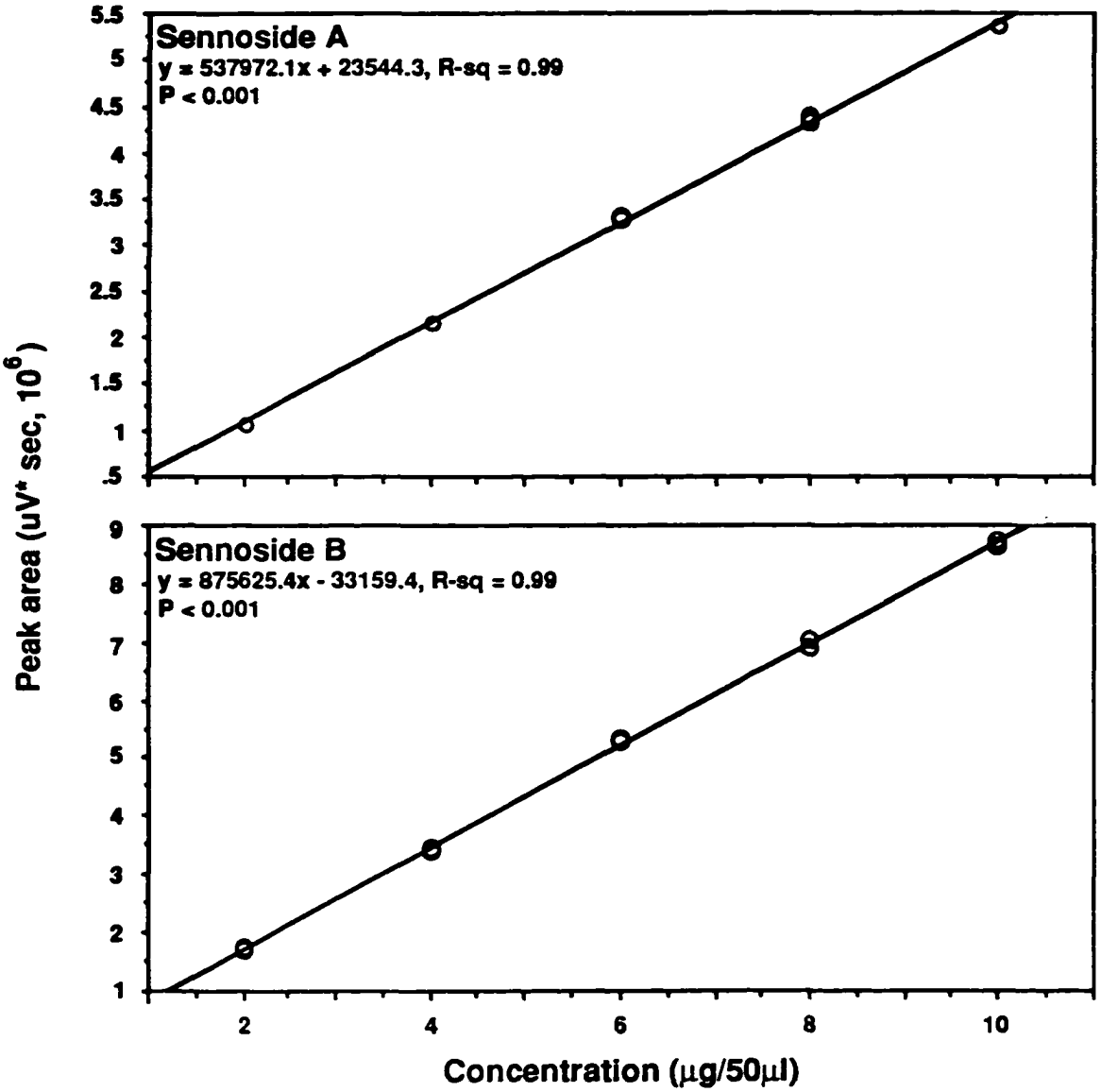



**Figure 4. 2** Calibration curves for sennoside A and B based on the peak areas of HPLC chromatogram (UV, 307 nm) of known concentrations of authentic sennosides in 70% aqueous methanol.

To verify the identity of sennosides in the extracts, a mixture of pure sennoside A and B was used as the last sample to inject in each set of samples. Quantifications were performed by integrating peak areas corresponding to sennoside A and B at 307 nm.

Sennoside yields were computed as a percentage of the leaf dry weight. Per plant yields in each experiment were expressed based on either the leaf biomass measured at the end of the experiments (Chapter one and three) or the estimated leaf weights (measurements of leaflet weights during gas exchange measurements multiplied by the total number of leaflets) per plant (Chapter one and two). These sennoside contents will be hereafter referred to as sennoside concentrations and sennoside yields per plant, respectively.

## RESULTS

### Standardization of quantification method

Base-line separation of peaks corresponding to the sennosides were mandatory for the quantification of these substances. Various gradient and solvent systems were tried, and the one described in the Materials and Methods section was found to be the most effective. A high concentration of phosphoric acid (5%) was found to provide highest resolution and no overlap with potentially co-eluting flavonoids. However, this high acid concentration had a negative effect on the life time of the HPLC columns. Extracts were analyzed by HPLC after various periods of storage at -20°C. No decomposition of sennosides A and B was noted after three weeks.

Integrations were performed at 307 nm which is a minor absorption maximum of

the sennosides. This minimized the interference with flavonoids nearly co-eluting with sennoside B. A sample HPLC trace and a UV absorption spectrum of sennoside A and B are shown in Figure 4. 3.

Although, it has been demonstrated that sennosides are artifacts of the drying process, and are absent in the fresh plant material (Labadie, 1970; Atzorn *et al.*, 1981) I quantified sennosides rather than their proposed, naturally occuring monomeric precursor, 8-glucosidorheinanthrone due to the pharmaceutical significance of sennosides. The drying conditions of the leaflets used for sennoside determinations in each experiment were maintained the same to avoid a possible effect of the drying regime on the sennoside concentration.

**Sennoside concentration in response to long term drought and foliar nitrogen application**

Leaflets harvested from the droughted and watered plants, with and without foliar nitrogen application, during the fifth drought cycle (Chapter one) were analyzed for sennosides. No treatment or interaction effect on sennoside A, B or A+B was found to be significant in early or late phases of the fifth drought cycle, at the sample size of the experiment (Figures, 4. 4 and 4. 5). However, as illustrated in Figure 4. 5, the sennoside A:B ratio was 14% greater in the droughted plants than in the watered plants in the early five days of the fifth drought cycle, with nitrogen and interaction effects insignificant[1] ($F=7.88$, $P<0.01$, R-sq=0.19, statistical power=0.77, LSN=21 for drought in two way ANOVA). No effect of treatment or interaction on the sennoside A:B ratio was detected in the last four days.[2]

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[1] $F=0.02$, $P=0.88$, R-sq=0.00 for N; $F=0.00$, $P=0.92$, R-sq=0.00 for interaction

[2] $F=0.30$, $P=0.58$, R-sq=0.01 for drought; $F=0.44$, $P=0.51$, R-sq=0.02 for nitrogen, $F=0.60$, $P=0.44$, R-sq=0.03 for interaction

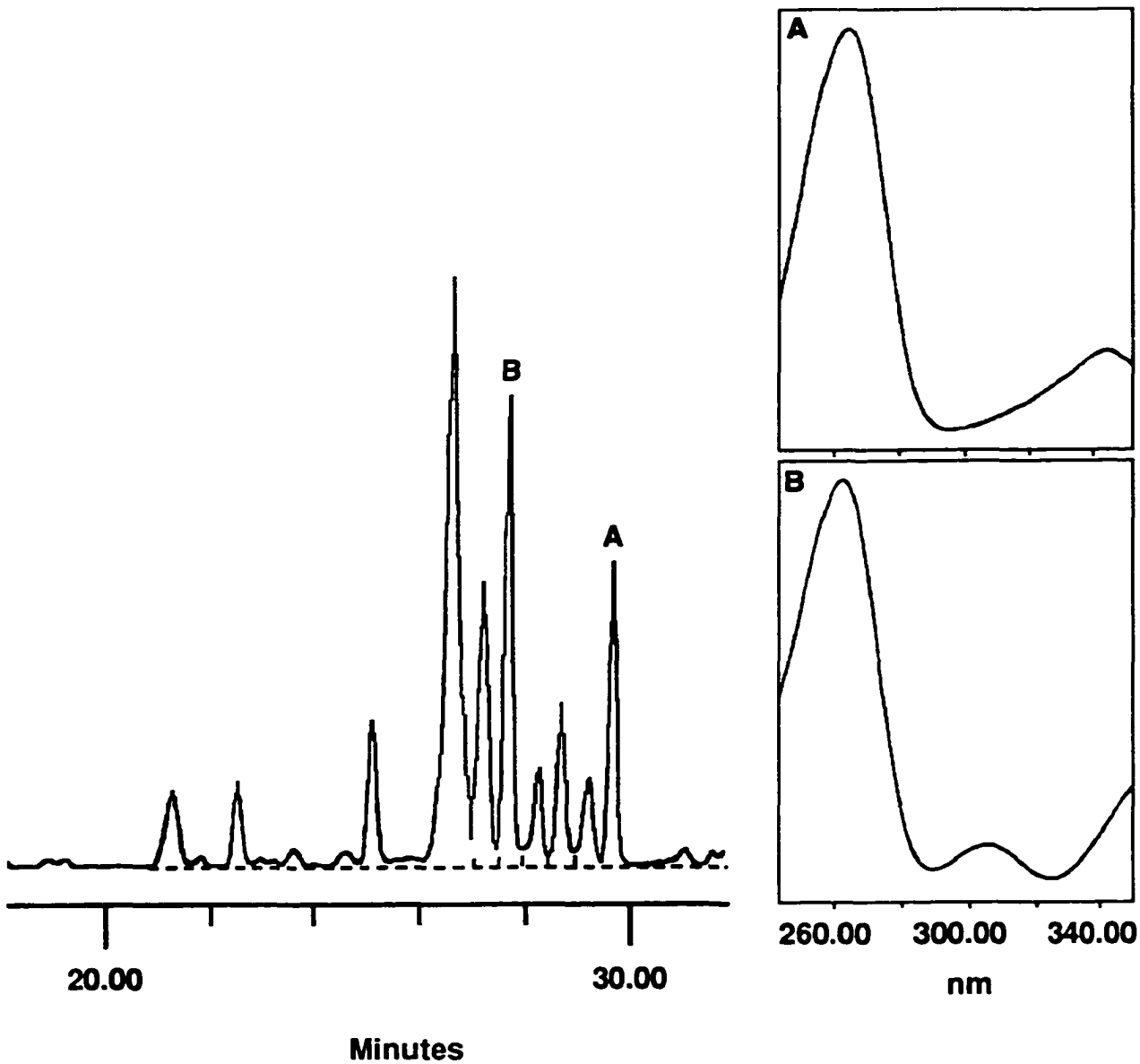


**Figure 4. 3** HPLC analysis (left), and the UV absorption spectra of sennoside A and B (right). A - sennoside A, B - sennoside B.

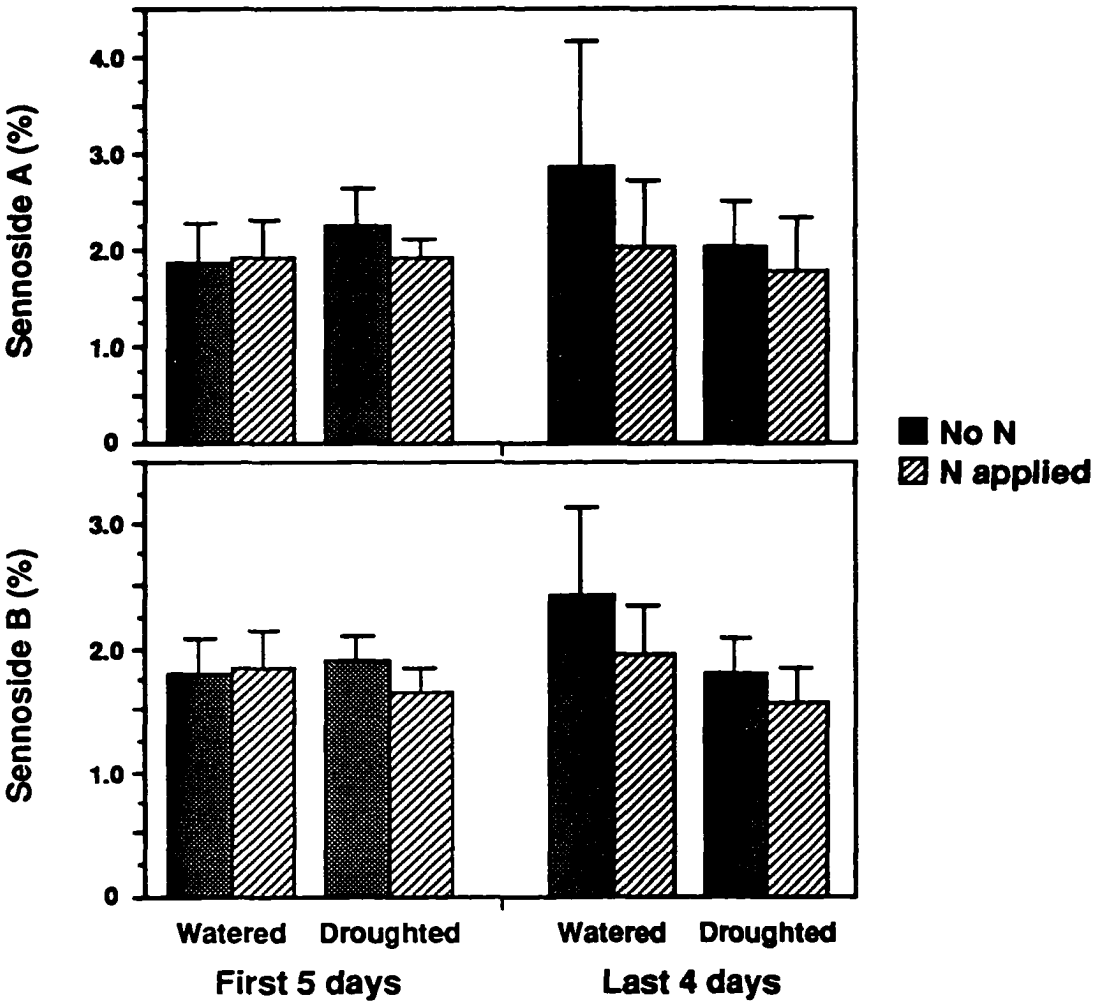


**Figure 4. 4** Leaf sennoside A and B concentrations of droughted and watered plants in response to foliar nitrogen application. Data are grouped into first five days (N = 9) and next four days (N = 6) in the nine day drought cycle to show the effect of severity of drought. Error bars represent 95% CI of the mean.

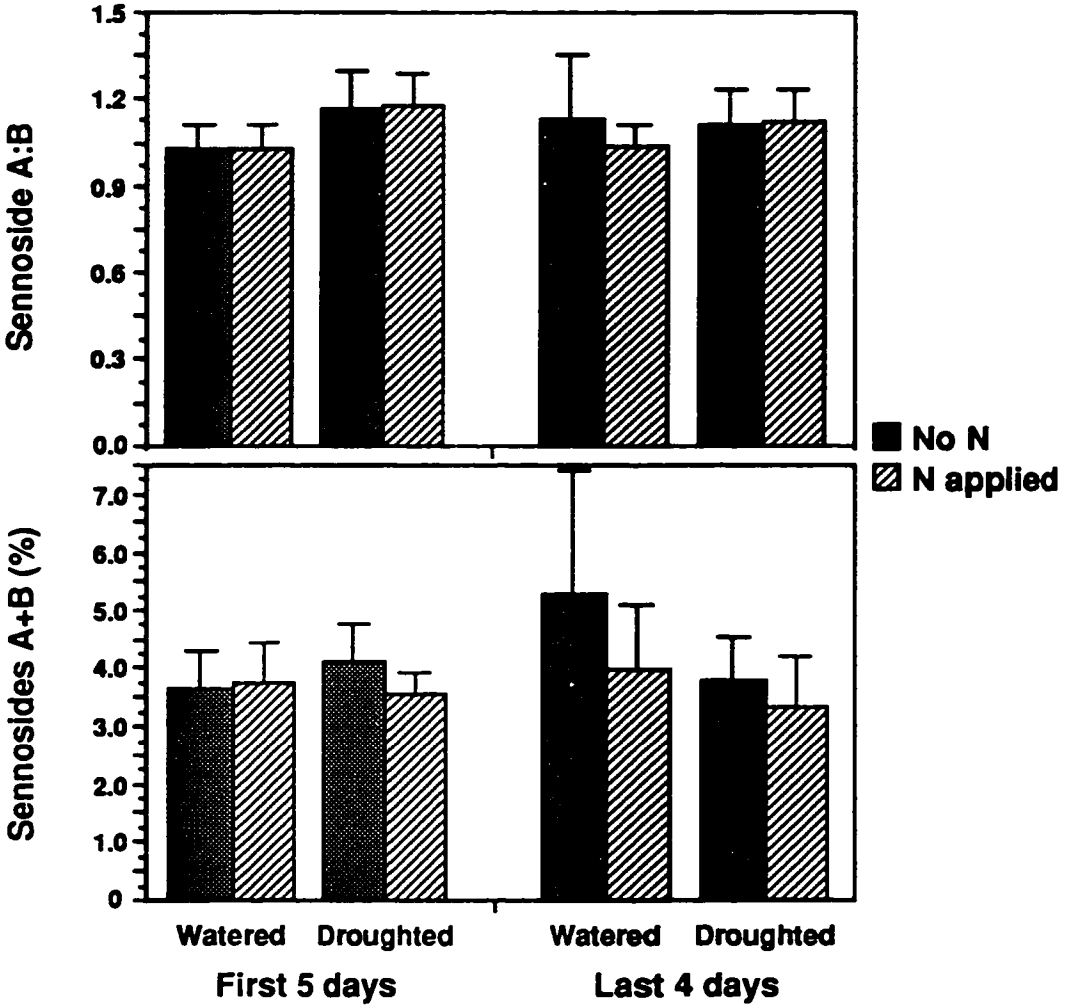


**Figure 4. 5** Leaf sennoside A:B ratio and A+B concentration of droughted and watered plants in response to foliar nitrogen application. Data are grouped into first five days (N = 9) and next four days (N = 6) in the nine day drought cycle to show the effect of severity of drought. Error bars represent 95% CI of the mean.

Droughted plants without foliar nitrogen application exhibited greater sennoside A, B, and A+B concentration than the droughted plants with foliar nitrogen in both early and late drought. This was a 16% nonsignificant increase in sennosides A+B with the statistical power of ca 0.41 and LSN of 38 when the number of total observations was 30 in the experiment.[1] In two way ANOVA for sennosides A+B during the early five days of the final drought cycle, the statistical power was only ca 0.16 with LSN of 153 when number of observations was 36 in the experiment.[2] Similarly a statistical power of ca 0.48 and LSN of 32 were detected for the last four days at the sample size of 24.[3]

Sennoside A, B and A+B yields per plant were estimated by multiplying each mean sennoside content by estimated total leaf weight of each plant. As illustrated in Figure 4. 6, a significant interaction effect between drought and nitrogen application on the estimated sennoside yields per plant was detected . Watered plants that were applied with nitrogen produced 136%, 151% and 142% higher sennoside A, B and A+B yields per plant, respectively, than the watered plants without nitrogen. However, sennoside contents in the droughted plants were not influenced by foliar nitrogen application (two way ANOVA of sennosides A+B - for drought, $F=232.32$, $P<0.001$, R-sq=0.64; for nitrogen, $F=62.58$, $P<0.001$, R-sq=0.18; for interaction, $F=66.35$, $P<0.001$, R-sq=0.18). Statistical power for this whole model test was nearly one with LSN of seven.

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1 $F=3.23$, $P=0.08$, R-sq=0.10 for nitrogen in droughted plants, early and late stages pooled

2 $F=0.20$, $P=0.65$, R-sq=0.00 for drought; $F=0.59$, $P=0.44$, R-sq=0.02 for nitrogen; $F=1.08$, $P=0.30$, R-sq=0.03 for interaction

3 $F=3.64$, $P=0.07$, R-sq=0.14 for drought; $F=2.53$, $P=0.12$, R-sq=0.10 for nitrogen; $F=0.52$, $P=0.47$, R-sq=0.02 for interaction

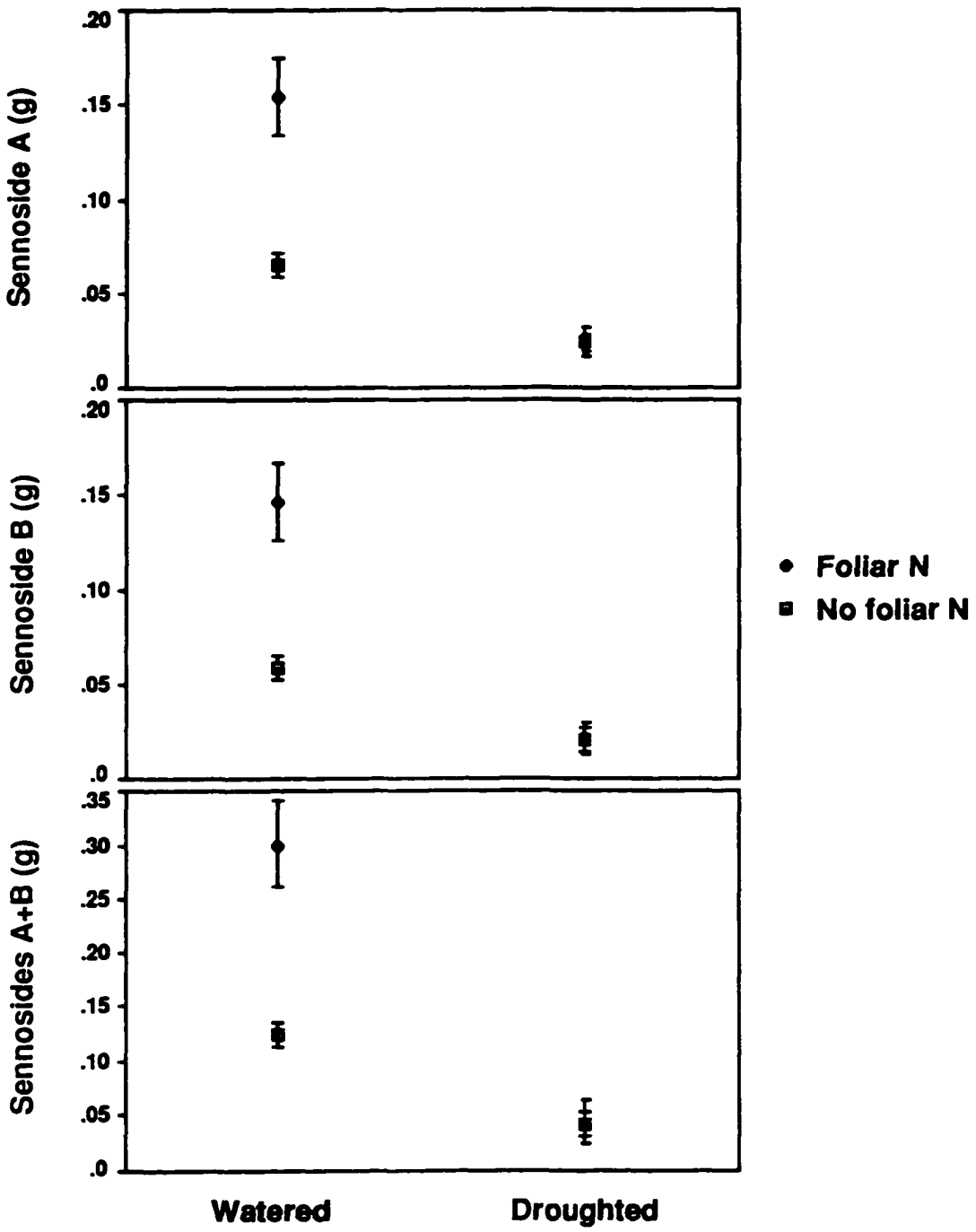


**Figure 4. 6** Estimated sennoside yield per plant in watered and droughted plants as affected by foliar nitrogen application. Means are based on four estimates of total leaf weights for each plant in each group (12 observations) during the fifth drought cycle. Error bars are CI of the mean.

**Sennoside concentration during the late reproductive stage**

Leaf sennosides A and B were assessed when the above plants were in green pods with seeds (podfill stage, nine weeks after the treatments were discontinued), and in mature pods (browning pod, 12 weeks after the treatments were discontinued). As illustrated in Figures 4. 7 and 4. 8, there was no significant difference between the podfill and mature pod stages in sennoside A or sennosides A+B when data were pooled across nitrogen and drought levels.[1] However, sennoside B was 17% less, and sennoside A:B was 12% greater in the mature pod stage than in the podfill stage (F=20.67, P<0,001, R-sq=0.48 for sennoside B; F=5.03, P<0.05, R-sq=0.09 for sennoside A:B). In two way ANOVA, no effect of drought was detected on sennoside A, B and A+B concentrations, and A:B ratio when the data were pooled across late and early stages of pod development.[2] However, plants without foliar nitrogen application yielded 21% and 17% more sennosides A and A+B, respectively (F=6.62, P<0.05, R-sq=0.23 for sennoside A; F=6.44, P<0.05, R-sq=0.72 for sennosides A+B). The 11% higher sennoside B concentration and 10% higher sennoside A:B ratio in the plants without nitrogen application as opposed to the plants with nitrogen were statistically nonsignificant.[3]

As shown in the Figure 4. 9, the effect of drought, nitrogen or their interaction on the estimated sennoside content per plant was nonsignificant at the sample size of the experiment.[4] The statistical power of the whole model test was ca 0.42 with

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[1] F=0.76, P=0.39, R-sq=0.03 for sennoside A; F=3.46, P=0.07, R-sq=0.14 for sennoside A+B

[2] F=2.46, P=0.13, R-sq=0.08 for sennoside A; F=0.80, P=0.38, R-sq=0.03 for sennoside B; F=2.09, P=0.16, R-sq=0.23 for sennoside A+B

[3] F=3.74, P=0.06, R-sq=0.15 for sennoside B; F=3.02, P=0.10, R-sq=0.13 for sennoside A:B

[4] sennoside A+B, F=3.45, P=0.10, R-sq=0.40 for drought, F=0.48, P=0.50, R-sq=0.06 for nitrogen, F=3.78, P=0.08, R-sq=0.53 for interaction

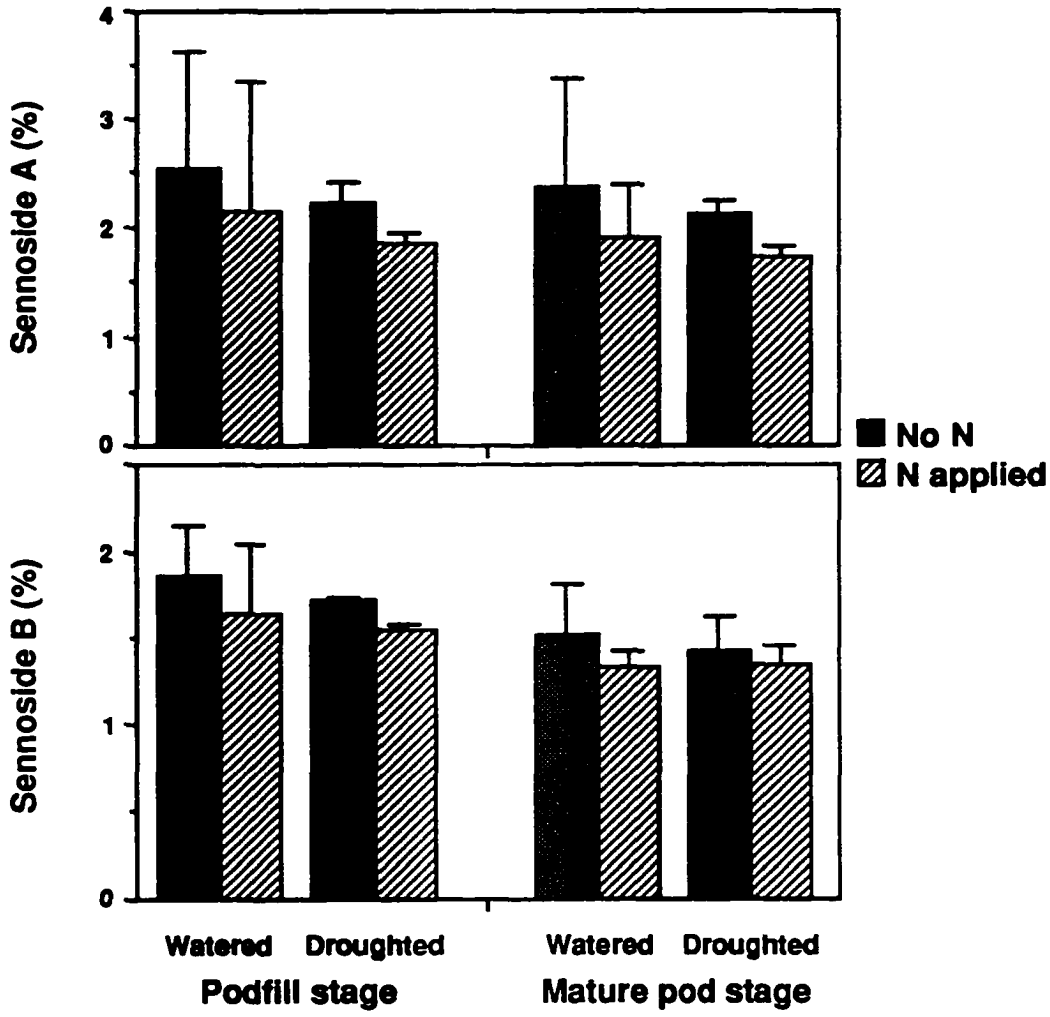


**Figure 4. 7** Leaf sennoside A and B concentrations of the plants during late reproductive stage. Podfill and mature pod stages represent nine and 12 weeks after nitrogen and drought treatments were discontinued, respectively. Mean of three plants with 95% CI error bars.

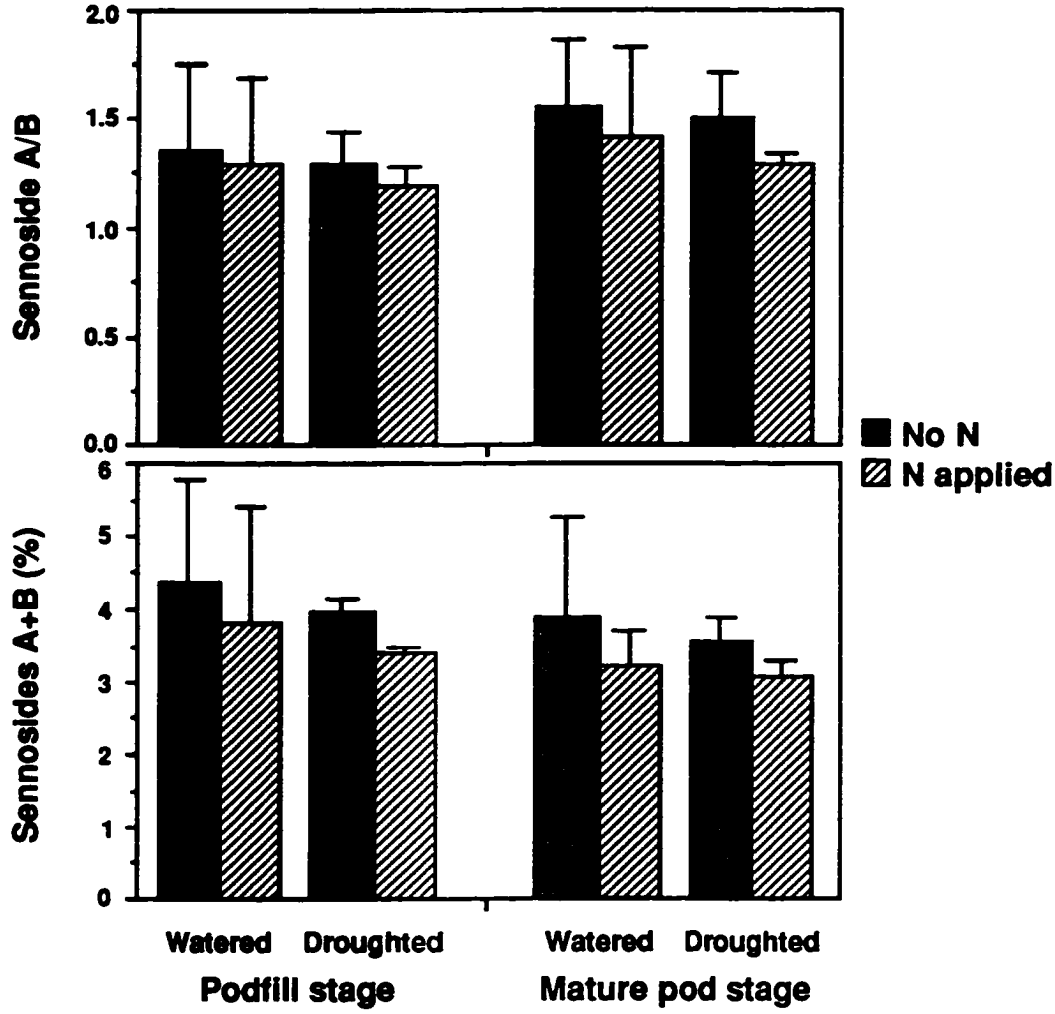


**Figure 4. 8** Leaf sennoside A:B ratio and A+B concentrations of the plants during the late reproductive stage. Podfill and mature pod stages represent nine and 12 weeks after nitrogen and drought treatments were discontinued, respectively. Mean of three plants with 95% CI error bars.

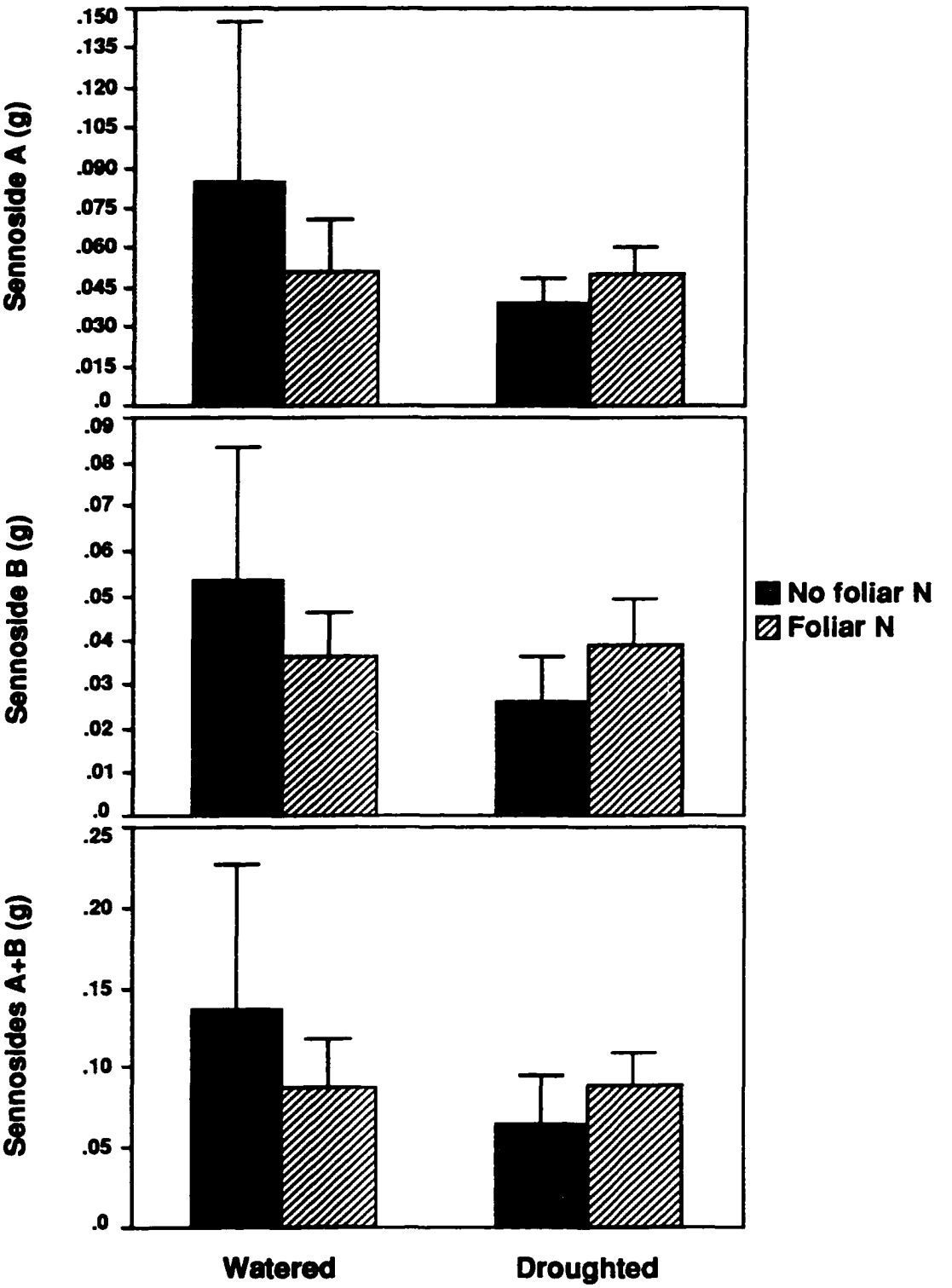


**Figure 4. 9** Leaf sennoside yield per plant while pods were drying 12 weeks after the drought and foliar nitrogen treatments were discontinued. Mean of three plants with CI error bars.

a LSN of 16. However, a similar pattern for all sennoside concentrations emerges: plants with no foliar nitrogen application yield more when watered, and plants with foliar nitrogen application yield more when droughted.

**Sennoside concentration in short term drought**

Leaflets stripped periodically from the two ratoon plants undergoing two consecutive drought cycles were analyzed for sennosides (Chapter two). Sennoside concentrations (A, B and A+B) and sennoside A:B ratio increased, exhibiting a positive slope against intensity of drought, after the third day of first one week long drought, as presented in the Figures 4. 10 and 4. 11. However, after watering, sennoside concentrations and sennoside A:B ratio tended to plateau, and decreased toward the seventh day of the second drought cycle.

**Sennoside yields of crop types**

Leaf sennoside concentrations were assessed in the three crop types established by ratoon, cuttings and seedlings (Chapter two). As illustrated in the Figure 4. 12, ratoon had 61% more sennoside A concentration than cuttings. However, the 27% higher sennoside B concentration in ratoon than in cuttings was nonsignificant.[1] Seedlings had 39% and 33% greater sennoside A, and A+B content, respectively, than the cuttings, again with a nonsignificant 26% increase in sennoside B[1] ($F=9.86$, $P<0.01$, R-sq=0.68 for Sennoside A; $F=6.48$, $P<0.05$, R-sq=0.59 for sennosides A+B), as shown in Figures 4. 12 and 4. 13. Seedlings and ratoon showed statistically similar sennoside A, B and A+ B concentrations at this sample size. The statistical power of ANOVA for sennosides A+B was ca 0.77 with a LSN of 10. The mean sennoside A:B ratio was more than one in all three plant types (Figure 4. 13).

[1] $F=2.84$, $P=0.11$, R-sq=0.38 for sennoside B

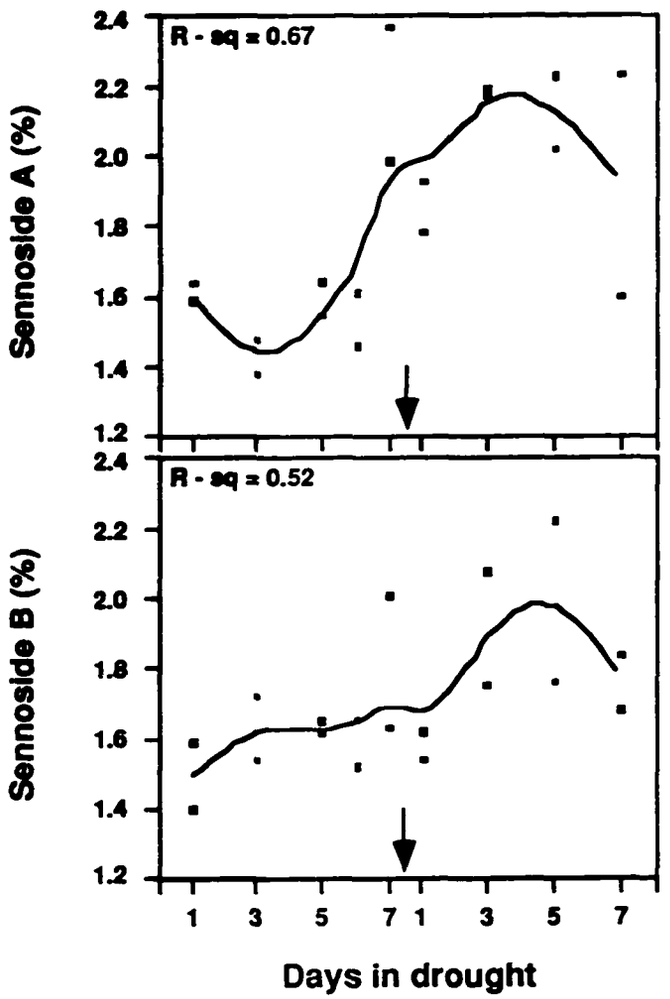


**Figure 4. 10** The fluctuations of sennoside A and B concentrations in two ratoon plants undergoing two consecutive drought cycles. Scatter plots with λ 1 splines. Arrow indicates watering.

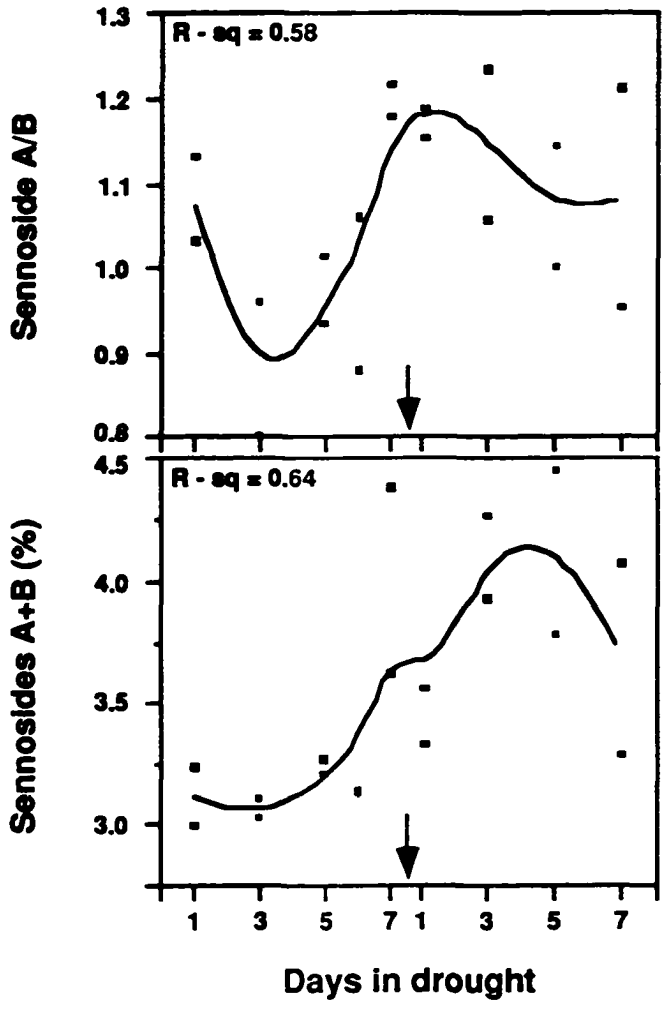


**Figure 4. 11** The variation of sennoside A:B ratio and A+B concentrations in two ratoon plants undergoing two consecutive drought cycles. Scatter plots with λ 1 splines. Arrow indicates watering.

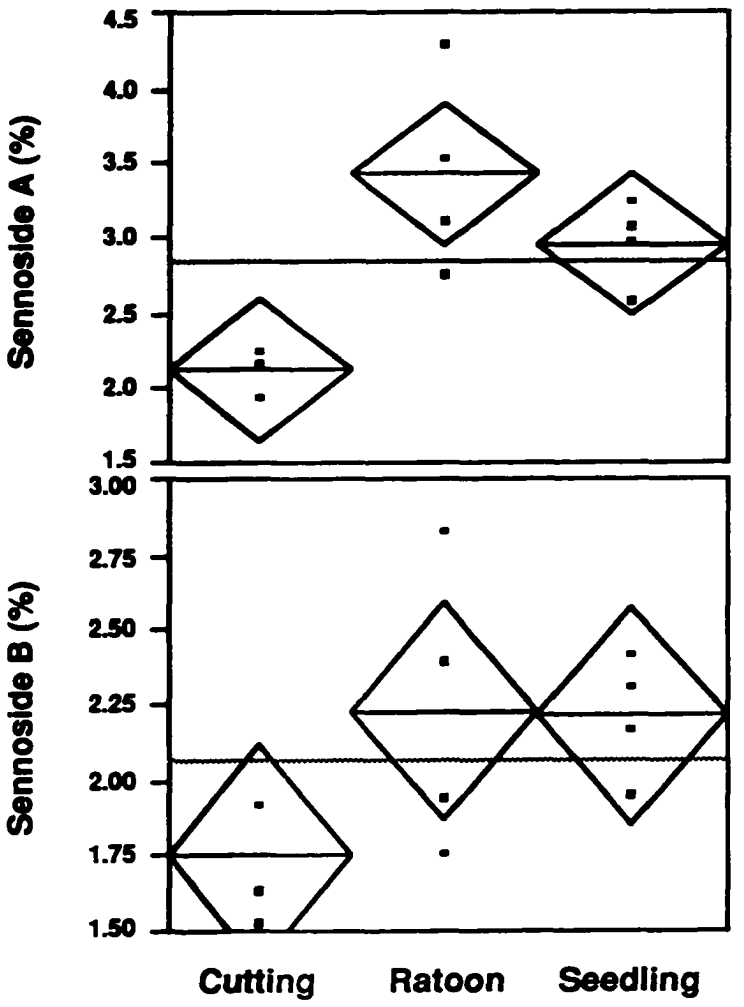


**Figure 4. 12** Leaf sennoside A and B concentrations of the three plant types. Diamonds represent 95% CI of mean. Line across the plots represents grand mean of 12 plants, four plants in each group.

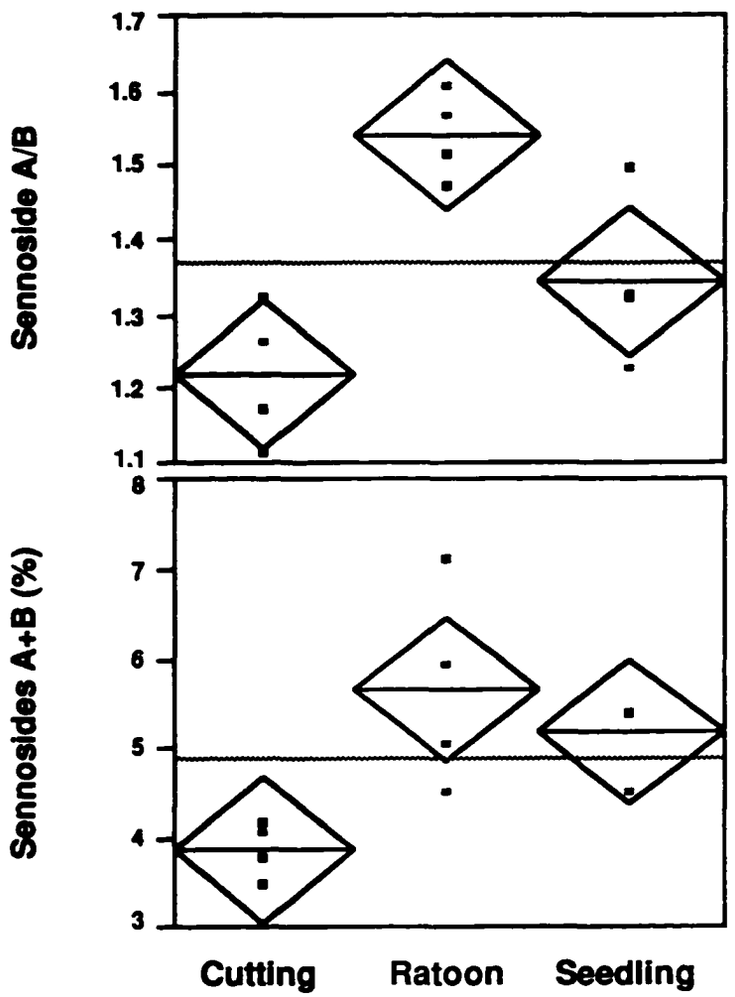


**Figure 4. 13** Sennoside A:B ratio and A+B concentrations of the three crop types. Diamonds represent 95% CI of mean. Line across the plots represents grand mean of 12 plants, four plants in each group.

Ratoon showed 15% and 32% higher sennoside A:B ratio compared to seedlings and cuttings, respectively (F=13.07, P<0.01, R-sq=0.74).

Sennoside A and B yields per plant were greater in seedlings and ratoon than in cuttings, as illustrated in Figure 4. 14. Seedlings and ratoon produced 2.7 times and 2.0 times greater sennoside A+B yields, respectively, than cuttings. Sennoside A+B content was 24% higher in seedlings than in ratoon (F=31.17, P<0.001, R-sq=0.52). The statistical power for this test was nearly one with LSN of 10.

**Effect of deflowering on sennoside concentration**

Leaf sennoside concentrations were determined in deflowered and flowering plants in the outdoor experiment between two and four months of age (Chapter three). As illustrated in Figures 4. 15 and 4. 16, sennoside A, B and A+B, pooled across three, three and half, and four months, were 26%, 24% and 25% greater in the deflowered plants than in the flowering plants (F=7.31, P<0.01, R-sq=0.13 for sennoside A; F=6.04, P<0.05, R-sq=0.11 for sennoside B; F=20.68, P<0.001, R-sq=0.28 for sennosides A+B). Statistical power of the whole model test and of flowering status for sennoside A+B concentration was ca 0.97 with LSN of 28, and ca 0.99 with LSN of 14, respectively. The effect of nitrogen or interaction between nitrogen and flowering on the sennosides A, B, A:B or A+B content at any age of the plants was nonsignificant (Figures 4. 15 and 4. 16).[1] Sennoside A:B ratio was greater than one up to three months but was lower than 0.45 at three and half or four months of age. Flowering, nitrogen or their interaction effect on sennoside

[1] For nitrogen - F= 1.13, P=0.33, R-sq=0.04 for sennoside A; F=0.50, P=0.60, R-sq=0.01 for sennoside B; F=0.01, P=0.98, R-sq=0.00 for sennoside A:B; F=2.22, P=0.11, R-sq=0.06 sennosides A+B; for interaction - F=0.30, P=0.73, R-sq=0.01 for sennoside A; F=0.18, P=0.83, R-sq=0.00 for sennoside B; F=0.08, P=0.92, R-sq=0.00 for sennoside A:B; F=0.46, P=0.62, R-sq=0.01 for sennosides A+B, data pooled across three, three and half, and four months

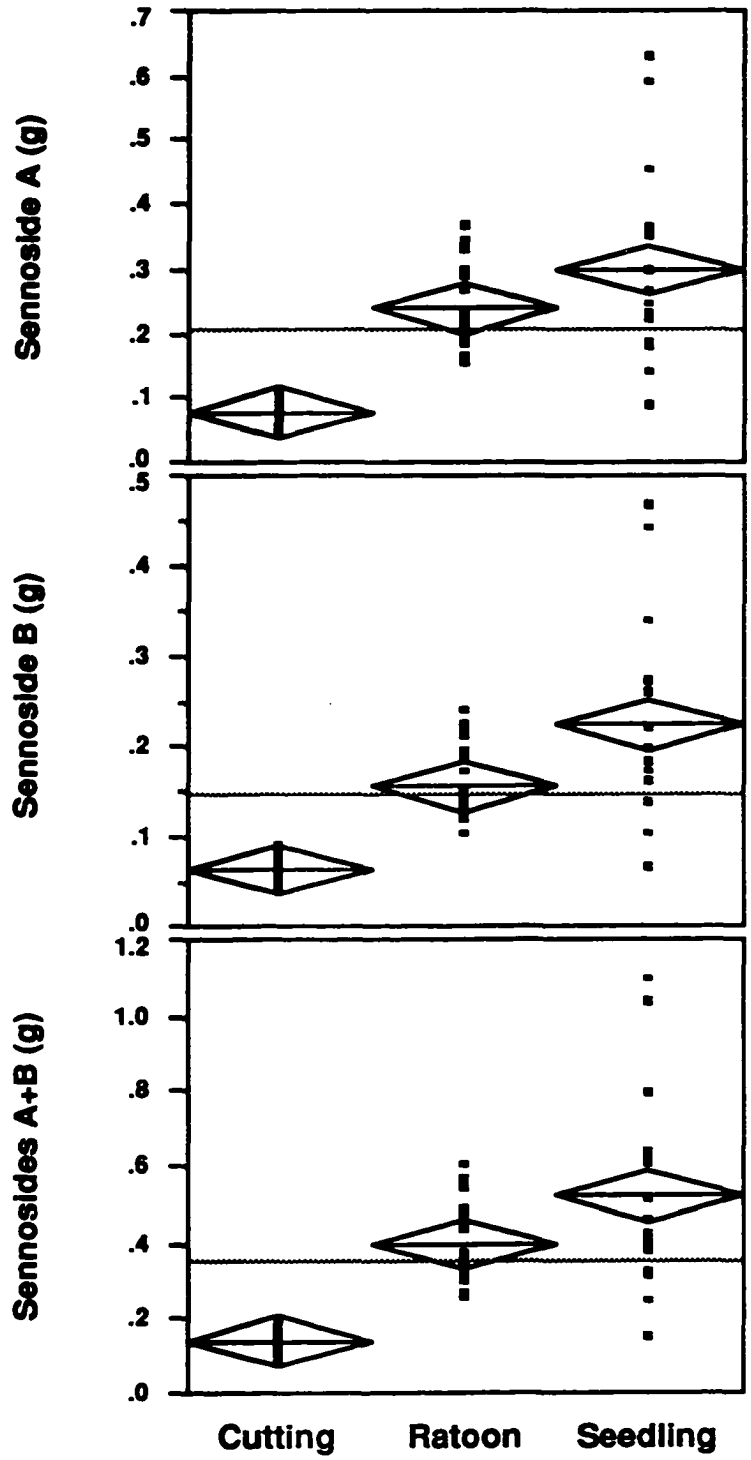


**Figure 4. 14** Estimated sennoside yields per plant of the three crop types. Diamonds represent 95% CI of the mean of 20 observations. Line through the scatter is the grand mean.

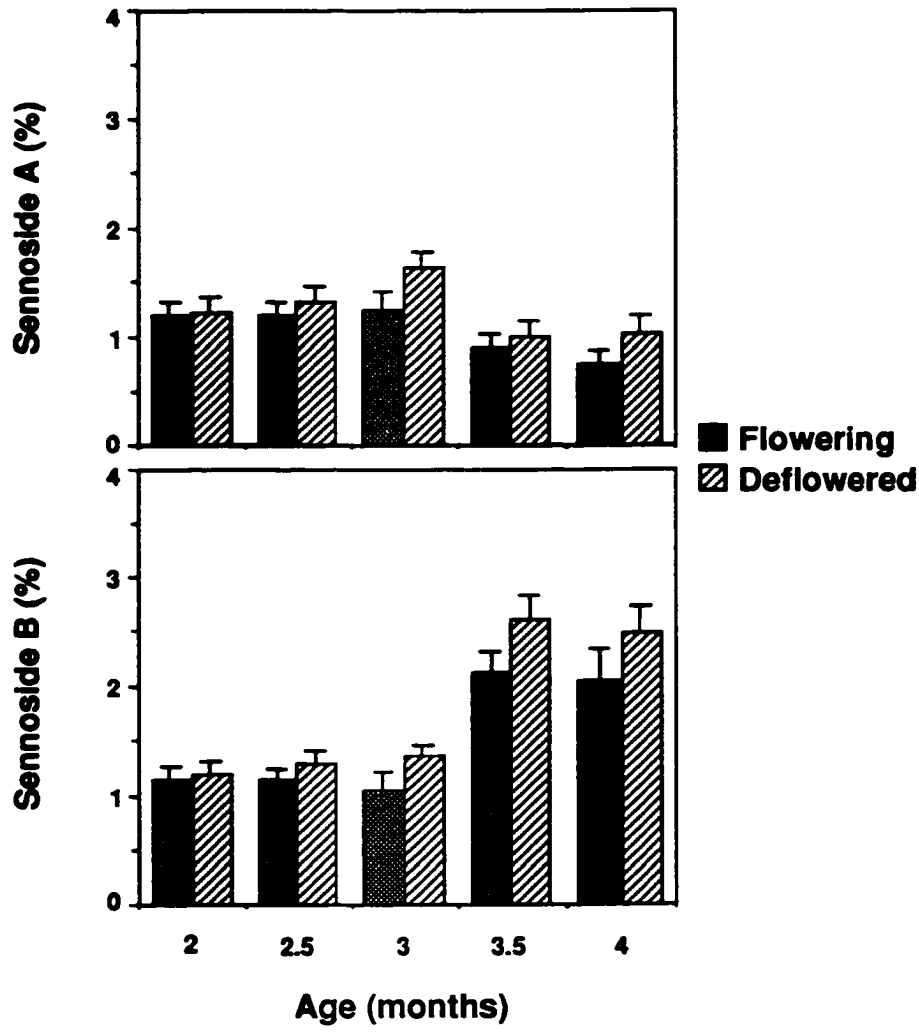


**Figure 4. 15** Sennoside A and B concentrations versus age in the flowering and deflowered plants. Each value is an average of nine plants pooled across three nitrogen levels. Error bars are 95% CI of the mean.

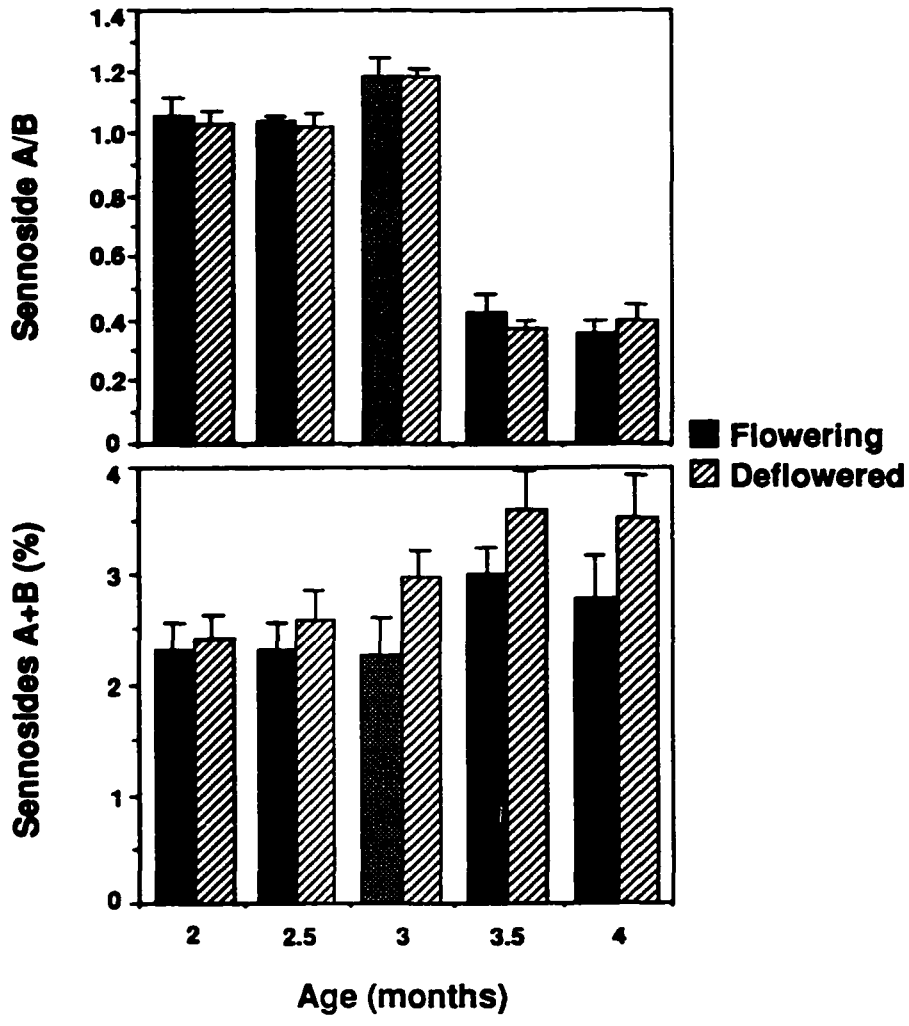


**Figure 4. 16** Sennoside A:B ratio and A+B concentration in flowering and deflowered plants at different ages. Mean of nine plants pooled across three nitrogen levels. Error bars are 95% CI of the mean.

A:B was not significant.[1]

Concentrations of sennoside A, B and A+B remained statistically similar across two, two and half, and three months of age in both flowering and deflowered plants. However, sennoside A concentration decreased after 3 months while sennoside B and A+B concentrations increased and remained same at three and half, and four months of age. Sennoside A+B concentration increased 32% and 21%, respectively, from three months of age to three and half months of age (one way ANOVAs with age as the treatment, $F=5.49$, $P<0.01$, R-sq=0.35 in flowering plants and $F=14.51$, $P<0.001$, R-sq=0.59 in deflowered plants). Statistical power of the whole models of above ANOVAs was ca 0.96 with LSN of 24, and nearly one with LSN of 13, respectively.

The logistic regression of sennoside A+B concentration and sennoside yields per plant in flowering and deflowered plants after three months is shown in Figure 4. 17. Logistic regression establishes that the probability of the sennoside A+B concentration exceeding 3% is 70% in a deflowered plant, while only 30 % in a flowering plant. Furthermore, deflowered plants produced approx. 100% more sennoside A, B or A+B yield per plant than the flowering plants ($F=46.29$, $P<0.0001$, R-sq=0.89). Sennoside yields per plant were not influenced by nitrogen level or interaction between nitrogen and flowering.[2] Statistical power for the above two way ANOVA was ca 0.99 with a LSN of 14.

---

[1] $F=0.01$, $P=0.98$, R-sq=0.00 for nitrogen; $F=0.00$, $P=0.95$, R-sq=0.00 for flowering; $F=0.08$, $P=0.92$, R-sq=0.00 for interaction for data pooled across three, three and half, and four month

[2] $F=0.09$, $P=0.91$, R-sq=0.00 for nitrogen, $F=3.05$, $P=0.06$, R-sq=0.10 for interaction

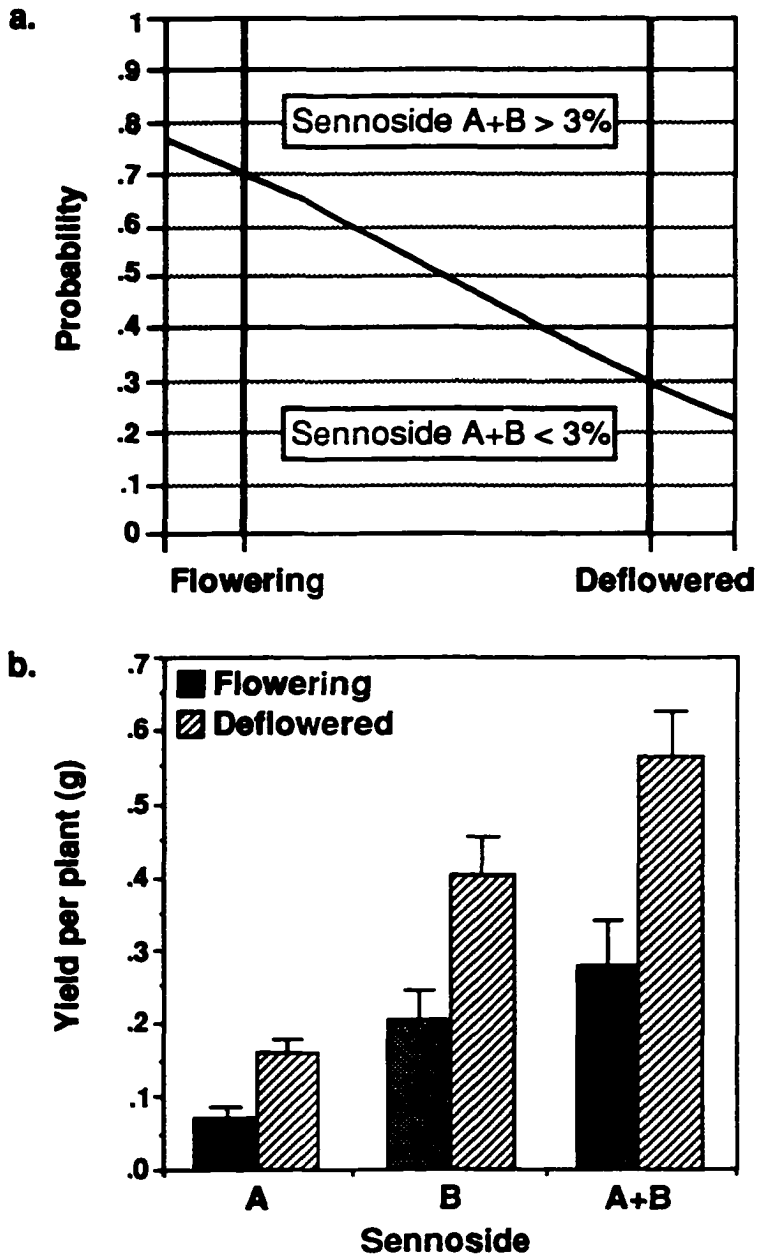


**Figure 4. 17 a.** Logistic regression of sennoside concentration (A+B > 3% or < 3%) as the response variable versus flowering status, deflowered and flowering (N=54, P<0.01). Data are pooled across three, three and half, and four months of age. **b.** Effect of deflowering on sennoside A, B and A+B yields per plant, using the same data as of **a.** Error bars are 95% CI of the mean.

**Diurnal rhythms of sennosides**

Figures 4. 18 and 4. 19 illustrate the diurnal variations of sennoside A, B, and A+B concentrations and A:B ratio of the flowering and deflowered plants. Flowering and deflowered plants exhibited different diurnal patterns of sennoside accumulation. Sennoside A:B ratio and A+B concentrations, showed a slightly downward trend toward evening in the flowering plants but an upward trend in deflowered plants.

**Sennoside content versus leaf maturity**

As shown in Figure 4. 20, sennoside A or B concentration was highest in the youngest leaves, in both seedlings and cuttings. Sennoside A and B concentrations declined with a clear negative slope from the tendermost leaf to the seventh nodal leaf in both crop types. From the seventh nodal position to the lowermost nodal position the leaf sennoside contents were somewhat constant. Seedlings showed a 22.5% higher mean sennoside A and 14% higher sennoside B than cuttings though the difference in sennoside B was statistically nonsignificant[1] (sennoside A, $F=4.76$, $P<0.05$, R-sq=0.11).

Sennoside A:B ratio remained unchanged, to a great extent, with varying leaf maturity, as illustrated in Figure 4. 21. Mean sennoside A:B ratio was 9% greater in seedlings than in cuttings. The 18% higher mean sennoside A+B concentration in seedlings than cuttings was nonsignificant.[2]

[1] $F=1.66$, $P=0.20$, R-sq=0.04 for sennoside B
[2] $F=3.05$, $P=0.08$, R-sq=0.07

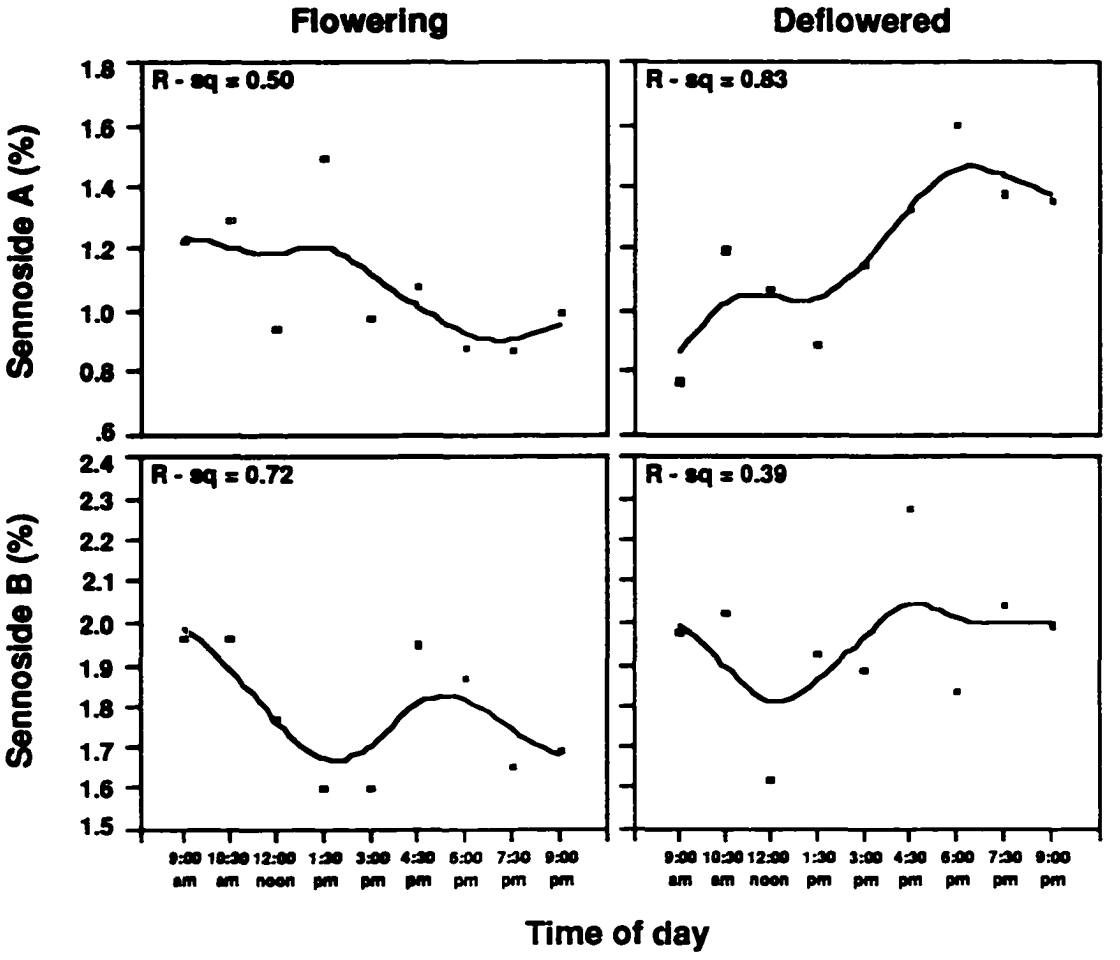


**Figure 4. 18** Diurnal fluctuations of sennoside A and B concentrations in flowering and deflowered 125 day old plants. Each value is based on a composite sample of two plants at each time. Scatter plots are superimposed with the λ 1 splines.

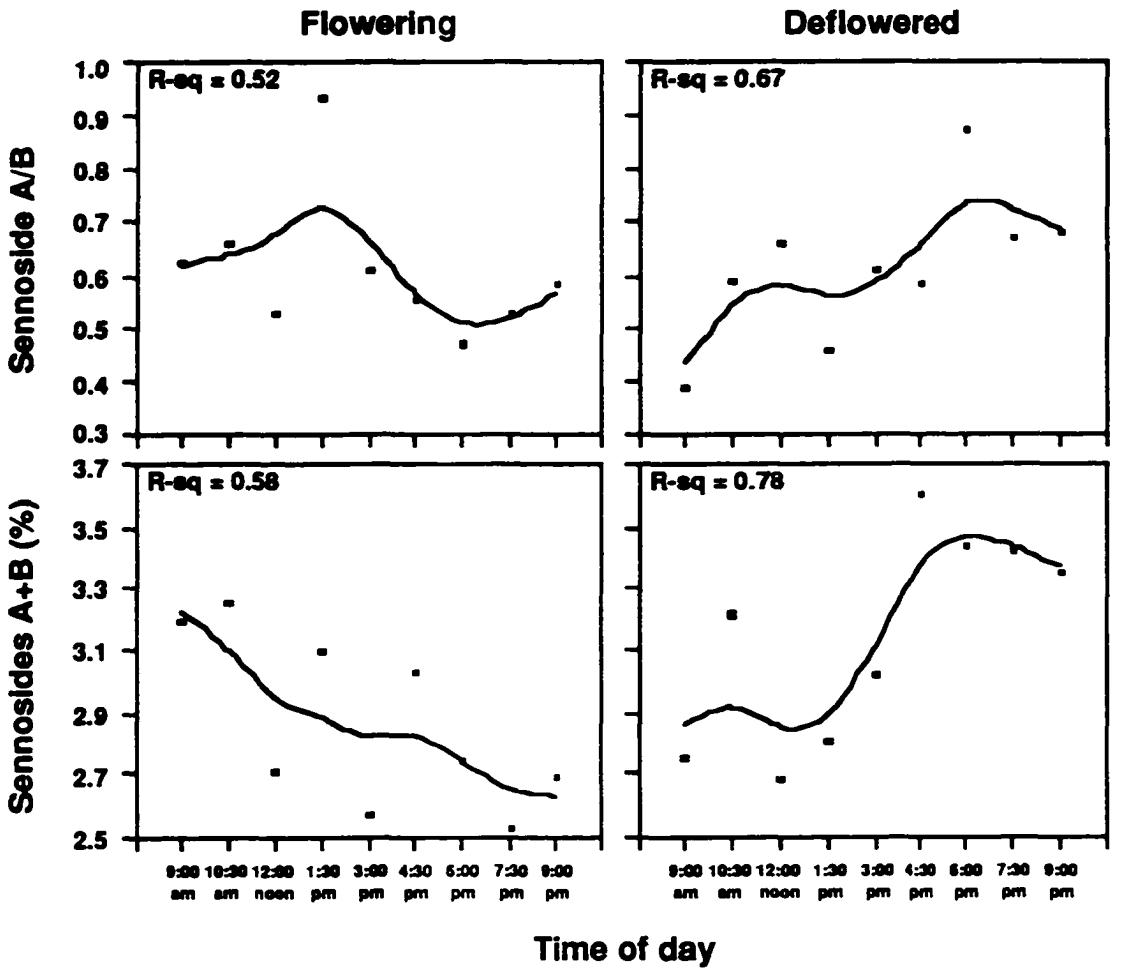


**Figure 4. 19** Diurnal fluctuations of sennoside A:B ratio and A+B concentration in flowering and deflowered 125 day old plants. Each value is based on a composite sample of two plants at each time. Scatter plots are superimposed with the λ 1 splines.

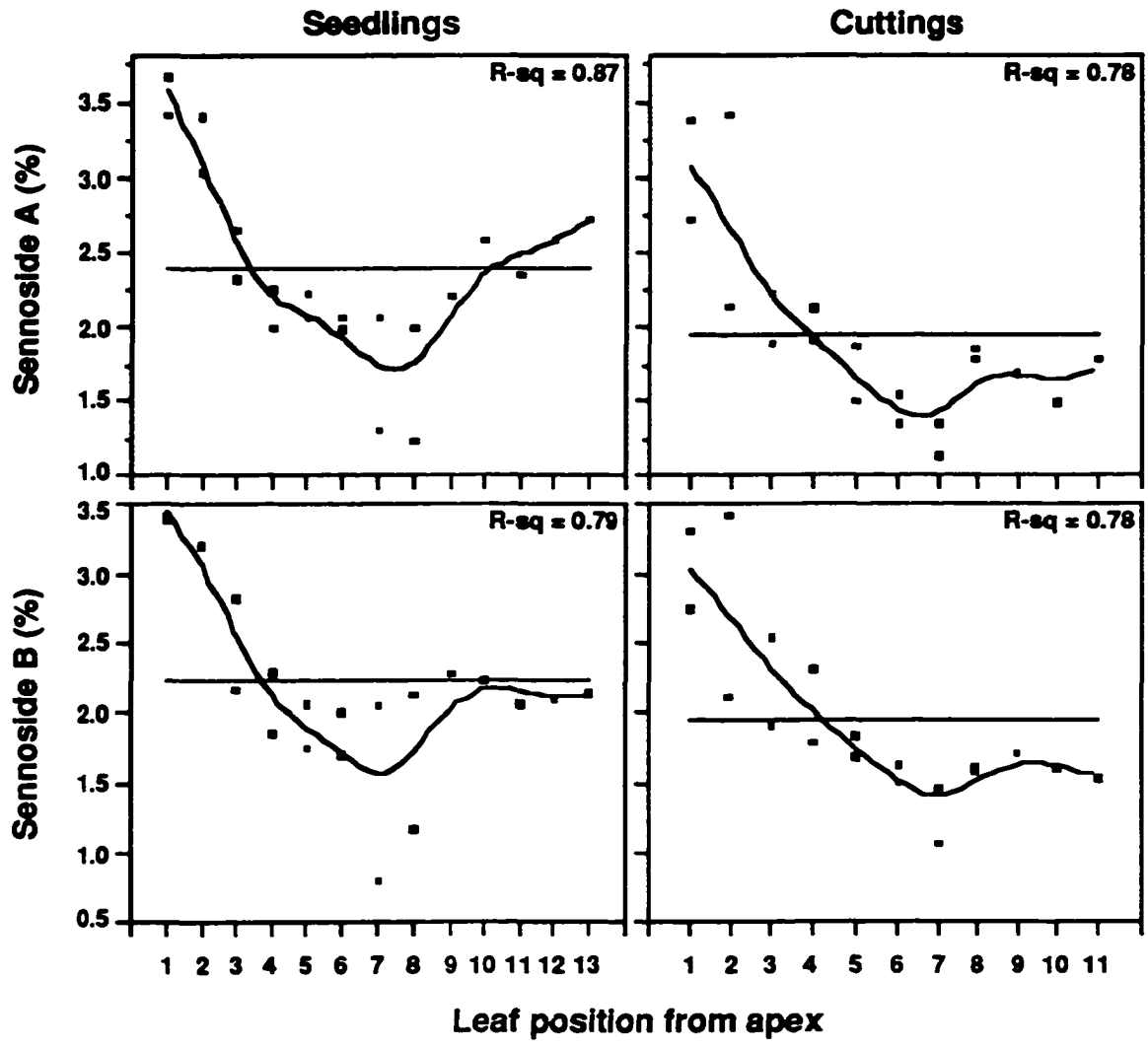


**Figure 4. 20** Sennoside A and B concentrations in the leaves of varying degrees of maturity. All the leaves from one branch each in two seedling and two cutting plants were stripped and individually analyzed. Scatter plots are superimposed with λ 1 splines. Straight line through the scatter indicates the mean.

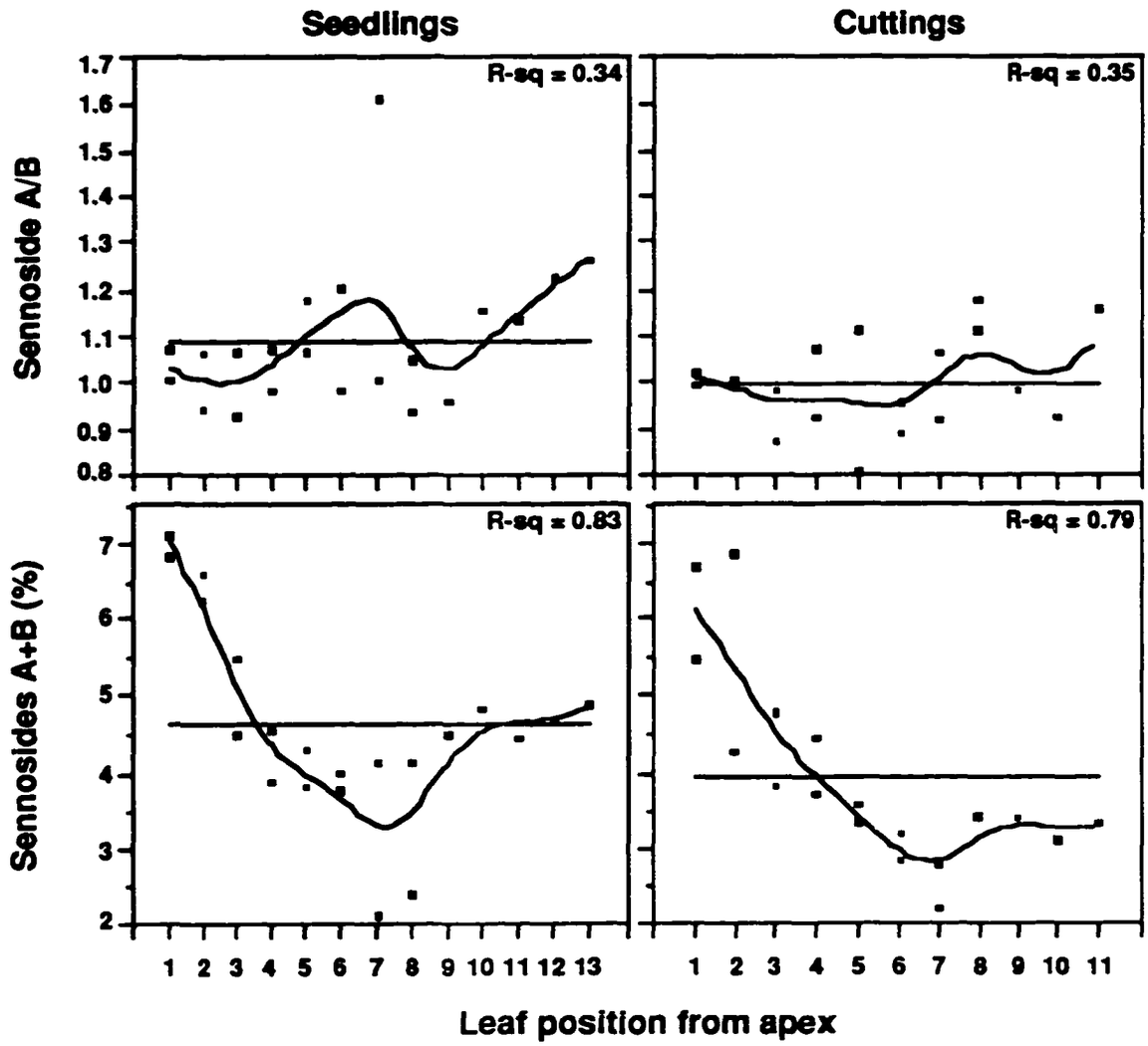


**Figure 4. 21** Sennoside A:B ratio and A+B content in the leaflets of leaves at varying degrees of maturity. All the leaves from one branch each in two seedling and two cutting plants were stripped and individually analyzed. Scatter plots are superimposed with λ 1 splines. Straight line through the scatter indicates the mean.

**Leaf elemental analyses in response to drought, nitrogen and deflowering**

As shown in the Table 4. 1, droughted plants accumulated 15% more total N, and 13%, 50% and 25% less K, Ca and Mg, respectively, in the foliage than the watered plants. No effect of foliar nitrogen application on leaf macronutrient analyses was detected (Table 4. 2). Furthermore, drought or foliar nitrogen application showed no effect on the content of any micronutrient in the leaves (data not presented).

Deflowered plants possessed 15% and 20% more P and K, respectively, compared to flowering plants, as presented in the Table 4. 3. However, the flowering plants had 9% greater Ca than the deflowered plants. Nitrogen, Mg and S concentrations were unaffected by deflowering. The only leaf micronutrient influenced by deflowering was Zn, a 19% increase in the deflowered plants (Table 4. 4). There was no effect of the nitrogen levels used (0, 40 and 80 kg $ha^{-1}$) on any macro- or micronutrient except for the 15% increase of Mg in the plants applied with 40 kg $ha^{-1}$ of nitrogen compared to the other two nitrogen levels (data not presented).

**Table 4. 1** Macronutrient analysis of leaflets of droughted and watered plants pooled across nitrogen levels. Mean with SE.

| Water status | Element (%) | | | | | |
|---|---|---|---|---|---|---|
| | N | P | K | Ca | Mg | S |
| **Watered** | 3.37 (0.24) | 0.36 (0.01) | 2.26** (0.03) | 1.64** (0.07) | 0.59* (0.02) | 0.28 (0.00) |
| **Droughted** | 3.87* (0.13) | 0.41 (0.02) | 2.00 (0.06) | 1.09 (0.12) | 0.47 (0.03) | 0.28 (0.01) |
| **ANOVA** | | | | | | |
| **N** | **6** | **6** | **6** | **6** | **6** | **6** |
| **F** | **6.30** | **5.30** | **14.78** | **14.47** | **10.28** | **0.00** |
| **P** | **< 0.05** | **0.06** | **< 0.01** | **< 0.01** | **< 0.05** | **1** |
| **R-sq** | **0.25** | **0.32** | **0.56** | **0.60** | **0.52** | **0.00** |

*, ** element concentration significantly greater than the other water status at P<0.05 and 0.01, respectively.

**Table 4. 2** Macronutrient analysis of leaflets. Totals for plants with and without foliar nitrogen application pooled across water status. Mean with SE.

| N application | Element (%) | | | | | |
|---|---|---|---|---|---|---|
| | N | P | K | Ca | Mg | S |
| **Foliar N** | 3.75 (0.25) | 0.41 (0.02) | 2.13 (0.07) | 1.28 (0.19) | 0.51 (0.04) | 0.28 (0.01) |
| **No foliar N** | 3.49 (0.17) | 0.36 (0.01) | 2.12 (0.08) | 1.45 (0.09) | 0.54 (0.03) | 0.28 (0.01) |
| **ANOVA** | | | | | | |
| **N** | **6** | **6** | **6** | **6** | **6** | **6** |
| **F** | **0.84** | **2.45** | **0.01** | **0.45** | **0.25** | **0.10** |
| **P** | **0.39** | **0.17** | **0.92** | **0.52** | **0.63** | **0.76** |
| **R-sq** | **0.06** | **0.22** | **0.00** | **0.06** | **0.04** | **0.01** |

**Table 4. 3** Macronutrient analysis of leaflets. Totals for flowered and deflowered plants pooled across nitrogen levels. Mean with SE.

| Flowering | Element (%) | | | | | |
|---|---|---|---|---|---|---|
| | N | P | K | Ca | Mg | S |
| **Flowered** | 4.54 (0.02) | 0.27 (0.01) | 2.05 (0.04) | 2.80* (0.05) | 0.28 (0.01) | 0.31 (0.00) |
| **Deflowered** | 4.58 (0.03) | 0.31** (0.01) | 2.46** (0.10) | 2.55 (0.07) | 0.30 (0.01) | 0.31 (0.00) |
| **ANOVA** | | | | | | |
| **N** | **6** | **6** | **6** | **6** | **6** | **6** |
| **F** | **1.25** | **10.20** | **12.44** | **9.14** | **2.19** | **0.06** |
| **P** | **0.29** | **< 0.01** | **< 0.01** | **< 0.05** | **0.16** | **0.80** |
| **R-sq** | **0.11** | **0.43** | **0.55** | **0.48** | **0.22** | **0.01** |

*, ** value significantly greater than the other in the same column at P<0.05 and 0.01, respectively.

**Table 4. 4** Selected micronutrient and Al concentrations of leaflets of flowered and deflowered plants, pooled across nitrogen levels. Mean with SE.

| Flowering | Element (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | **B** | **Zn** | **Mn** | **Fe** | **Cu** | **Al** |
| **Flowered** | 41.66 (0.91) | 30.00 (0.73) | 26.16 (0.47) | 200.83 (18.21) | 8.50 (0.22) | 76.66 (7.89) |
| **Deflowered** | 38.50 (2.59) | 35.66** (1.11) | 27.00 (0.68) | 187.50 (12.17) | 9.83 (0.60) | 113.83 (36.82) |
| **ANOVA** | | | | | | |
| **N** | **6** | **6** | **6** | **6** | **6** | **6** |
| **F** | **1.33** | **18.06** | **1.00** | **0.37** | **4.32** | **0.97** |
| **P** | **0.27** | **< 0.01** | **0.34** | **0.55** | **0.06** | **0.35** |
| **R-sq** | **0.11** | **0.64** | **0.09** | **0.03** | **0.30** | **0.09** |

** value significantly greater than the other in a column at P<0.01.

## DISCUSSION

### Effects of drought and foliar nitrogen application on leaf sennoside concentration and elemental composition

A short term drought without defoliation increases the sennoside concentration in the dried leaves whereby would render a yield advantage in a single crop. A severe, long term drought does not influence the sennoside concentration in the dried leaves. It only reduces the sennoside A and B yields per plant due to drought-induced reduction in leaf biomass. However, in both severe and short term droughts when leaf xylem water potential drops below -1.5 MPa leaf photosynthesis decreases dramatically (Chapters one and two). Thus, sennoside precursors may mediate the early physiological stress manifested by low $P_{net}$ under low plant water potential, for instance, as osmolytes, osmoprotectants or carbon reserves. However, after the plants morphologically adapt to the drought through means such as defoliation and reduced leaflet size the amounts of sennoside precursors appear to decline.

Foliar nitrogen application increases the leaf yield, whereby the sennoside yield per plant, when plants were not water-stressed. Leaves from the plants without foliar nitrogen application, however, contained higher sennoside A+B concentration than the plants with nitrogen application. Pareek *et al.* (1983) reported a 31% increase in estimated sennoside yield (kg $ha^{-1}$) mainly due to increased leaf biomass in response to 50 kg $ha^{-1}$ of nitrogen, compared to the control in a sandy loam soil. Sennoside yield per plant in severely droughted plants is unaffected by foliar nitrogen application. Again, similarity in leaf biomass of droughted and nitrogen-applied plants versus droughted plants without nitrogen application (Chapter one) explains this. Lower leaf elemental contents

(K, Ca, Mg) in the water-stressed plants clearly indicate the dependence of plant nutrient status on the water status (Chapin, 1991), and both nutrient and water stresses jointly would have contributed to the lower leaf yield. Greater leaf nitrogen content in droughted plants indicates the efficient reallocation of nitrogen from the lower shedding leaves to the remaining young leaves near the apex. However, the contents of K, Ca and Mg the elements relatively less mobile in the plant were lower in the top foliage of droughted plants compared to the watered plants. The lack of soil solution for the nutrients to flow toward the roots, as well, causes this (Chapin *et al.* 1987; Marshner, 1995). However, $P_{net}$ was markedly lower in both droughted and watered plants when they were deprived of foliar nitrogen application (Chapters one). This suggests the possible association of biosynthesis of sennoside precursors with the reduced photosynthesis induced by nitrogen stress, as well. Biosynthesis of various secondary metabolites induced by physiological stress is well documented (Skriver and Mundy, 1990; McCue and Hanson, 1990; Bohnert *et al.*, 1995).

In the experiment that tested the combined effects of drought and foliar nitrogen application (Chapter one), the plants were maintained deflowered until they were placed outside the glasshouse, and drought and nitrogen treatments were stopped for the following 12 weeks. These plants in mature pods maintained leaf sennoside concentrations comparable to when they were kept deflowered, irrespective of the water and nitrogen status they had been subjected to. This probably rules out the possibility of a simple translocation-based reduction of leaf sennosides (Lohar *et al.*, 1979) during pod development phase in the previously deflowered plants. Thus, if the leaves are the source of sennosides in pods (Atzorn *et al*, 1981; Lemli, 1985) then the leaves in the previously deflowered plants have the capacity to continuously produce sennosides (or precursors), to

replenish their previous sennoside content following the export into the pods. Furthermore, this would allude to the effect of different environmental conditions that are associated with the different experiments on the leaf sennoside concentration.

***Crop type and sennoside concentration***

Seedlings, cuttings and ratoon possessed high, moderate and low rates of $P_{net}$, respectively (Chapter two). Sennoside content was highest in ratoon followed by seedlings and cuttings. In ratoon, the smaller photosynthetic source supports a biomass of non-photosynthetic tissues comparable to the biomass of non-photosynthetic tissues of seedlings. However, with the older root stock, ratoon may not be as efficient as seedlings in resource acquisition and transport that are essential for increasing $P_{net}$ (Chapter two). Higher sennoside contents in ratoon leaves could indicate the possible functional relationship of sennoside precursors to these plant physiological and nutritional states (stress) that result in low $P_{net}$. Cuttings carry the smallest photosynthetic source and the smallest total biomass as well, thus do not experience such intrinsic plant stress as the ratoon. Sennoside yields per plant in the crop types follow the similar pattern as the total leaf biomass (seedlings > ratoon > cuttings) rather than the leaf sennoside concentration in each crop.

***Effect of deflowering on sennoside concentration***

In an overview of the patterns of leaf $P_{net}$ and sennoside concentrations of the flowered and deflowered plants (Figures, 3. 3 and 4. 13), $P_{net}$ in deflowered plants sharply dropped from two months to two and one-half months of age with sennoside contents increasing. During the same time, although the $P_{net}$ of flowered plants decreased as well, sennoside concentrations were unchanged.

From two and one-half to three months, $P_{net}$ remained the same in either deflowered or flowered plants with sennoside concentrations continuing to rise in deflowered and to be unchanged in the flowered plants. However, from three to three and one-half months with $P_{net}$ declining, sennoside concentrations dramatically increased in both flowered and deflowered plants. Lohar *et al.* (1979) reported that the leaf sennoside concentration decreased with the onset of flowering and fruiting. In contrast, Pareek *et al.* (1983) observed that removal of flowers and flower buds between 45 and 60 days after sowing slightly decreased the leaf sennoside concentration. In my deflowering experiment, however, flower buds were removed from the time of their emergence, approximately from five weeks after seeding, to the end of the experiment. These patterns of fluctuations in $P_{net}$ and leaf sennoside concentration testify that the relationship between sennoside producing secondary metabolism and the concurrent primary photosynthetic metabolism is affected by the flowering physiology.

The mean weight of the leaflets plus pods of a flowered plant was 23% less than the mean weight of leaflets of a deflowered plant. Similarly, flowered plants produced 20% less leaf sennoside concentration than the deflowered plants. Atzorn *et al.* (1981) showed that the sennoside concentrations of organs other than leaves and pods are negligible. Although flowers are rich in sennosides, they were absent by four months of age when the biomass was assessed. Thus, a positive correlation exists between the leaf sennoside concentration and the total capacity of the sennoside depository (leaves plus pods). This again suggests that there could be physiological/phytochemical mechanisms leading to the reduction of sennoside precursors in the pod bearing plants other than their simple translocation from leaves to pods.

However, the late flower drop and early pod drop caused by cold ambient air temperature in the early fall when the plants were three to four months old resulted in pod yields lower than the potential yield (Figure 3. 8). This loss of sennoside reservoirs may be another reason for the decreased sennoside levels in the flowered plants. Furthermore, the increase in sennoside A+B concentration with the start of flower and pod drop, which represents a limiting sink, alludes to the existence of source (leaves) and sink (pods) relationship for sennoside precursors. However, deflowered plants showed a greater increase of sennoside A+B concentration than flowered plants at three to four months than in the earlier days. This suggests that the greater leaf sennoside yields in both flowered and deflowered plants toward three and half months of age could be an age- or weather-related response, as well. Thus, further investigations must be undertaken to ascertain whether the sennosides in pods are solely leaf-derived, and whether the increase in leaf sennoside contents after three months is due to environmental changes or age. In such an experiment it is essential that even weather prevails from vegetative through late reproductive stages, and that biomass and sennosides of pods and leaves are quantified simultaneously at close time intervals.

Deflowering, unlike nitrogen or long term drought, increases both sennoside concentration and sennoside yield per plant. Moreover, deflowering clearly promoted the growth of all vegetative organs (Chapter three), and permitted higher foliar nutrient concentrations than the plants with flowers. Thus, deflowering cannot be considered a stress at the whole plant level. Although increased leaf P and K levels in the deflowered plants would have contributed to the greater biomass of vegetative organs compared to the flowered plants, these elements were already adequate in the flowered plants to maintain a higher $P_{net}$

than the deflowered plants. Adequate supplies of soil macro- and micronutrients are evident from the leaf elemental contents, and from the fact that nitrogen application showed no effect on the elemental analyses.

***Sennoside A:B ratio***

Sennoside A:B ratio varies with both external environmental and plant physiological conditions. For instance, in the experiments with plants grown in the glasshouse, the mean sennoside A:B ratio always remained more than one. In the deflowering experiment on the garden-plot, however, plants maintained sennoside A:B ratio of more than one only up to three months. Then it dropped sharply to less than 0.45 due to both an increase in sennoside B and a decrease in sennoside A concentration (Figure 3. 8). Although the drop in sennoside A concentration does not exactly match the increase in sennoside B concentration, this suggests the possible inter-convertibility of the two compounds or other intermediary metabolites of the sennoside synthetic pathway. This is not an age-related response since the plants of the same age (three and half months or older) grown in the glasshouse continued to show sennoside A:B of more than one. This probably could be attributed to the cold weather that set in after the plants were three months old and prevailed toward four months of age. Furthermore, deflowering does not influence the sennoside A:B ratio.

Crop type, as well, bears a clear impact on sennoside A:B ratio. Ratoon showed a sennoside A:B of more than 1.5, a statistically significant increase compared to seedlings and cuttings. This is due to relatively greater increase in the concentration of sennoside A than B in ratoon compared to other crop types. Again, the physiological and nutritional stress and associated low photosynthetic metabolic rates could be considered important factors in sennoside A:B ratio

similar to increased A+B concentration in ratoon. This is further exemplified by the increased sennoside A+B concentration and A:B ratio by the drought-induced low plant water potential, and differential diurnal rhythms of sennoside A and B in flowered versus deflowered plants (Figures 4. 5, 4. 11 and 4. 19, and Chapters one and two).

***Diurnal fluctuations of sennosides***

Diurnal fluctuations of sennoside A and B were observed in both flowering and deflowered plants, but they followed different patterns. The sennoside A:B ratio and A+B concentration, increased toward evening in deflowered plants but slightly decreased in flowering plants. In the deflowered potted plants, $P_{net}$ gradually declined in the course of the day from 8:00 hr to 17:30 hr (Chapter two). Thus, the sennoside A+B concentration of dried leaves in the deflowered plants again follows an inverse relationship with $P_{net}$. The different diurnal pattern of sennoside A+B accumulation in the flowering plants demands further investigation; especially because of the high variability with the low R-sq of the cubic splines for both sennoside A+B concentration and A:B ratio.

***Sennoside metabolism and leaf maturity***

It is established that the sennosides are absent in the fresh plant material of senna (Atzorn *et al.* 1981) or of other sennoside sources (Labadie, 1970). Atzorn *et al.* (1981) reported that the amount of postharvest water loss from leaves is strongly correlated to the formation of sennosides, and that either gradual air-drying at room temperature or rapid drying at 60°C allows formation of sennosides. Furthermore, they argued that sennosides may not form solely from 8-glucosidorheinanthrone, the supposed monomeric precursor, due to the presence of this metabolite in both fresh and dry leaves in equal concentrations.

The varying sennoside A:B ratio under different environmental conditions in my experiments support this idea and furthermore, hints at the possibility of different precursor metabolites forming in the fresh leaves in response to environmental triggers. Furthermore, it suggests that the precursors of sennoside A and B would play different functional roles especially according to the changes in external environment.

The biosynthesis of anthraquinones is known to be possible via the acetyl malonate or the shikimate pathways (Harborne and Turner, 1984). However, acetyl malonate pathway yields anthraquinones with substituents on A and C rings while the shikimate pathway produces anthraquinones without such substituents. Thus, acetyl malonate pathway can be considered the predominant biosynthetic pathway of sennoside precursors (Vickery and Vickery, 1981). Molecular and phytochemical investigations to identify the intermediate metabolites and the translational products that govern specific steps of this pathway have not been conducted yet. However, they are necessary for a comprehensive understanding of the possible inter-convertibility of anthraquinone metabolites, including sennoside A and B, in response to the environmental regimes.

Srivastava and Luthra (1993) reported that the reduced photosynthesis caused by Fe deficiency in *Mentha piperita* was associated with lower yields of essential oils that were biosynthesized via mevalonic acid pathway. They proposed the dependence of the pathway on primary metabolites for energy as one possible reason for this. In this respect, the higher sennoside concentration in the leaves with lower primary photosynthetic metabolic rates in our studies could be due to the differences in the causes of the suppression of primary metabolism, and in the biosynthetic pathway of the sennoside precursors itself. Furthermore,

sennosoide secondary metabolism may derive energy from older photosynthate pools not necessarily from the current photosynthesis. My data suggest a functional role of sennoside precursors under the low photosynthetic primary metabolic rates (e.g. moisture and nitrogen stress, and ratoon versus seedling crop).

Higher leaf sennoside content in the younger leaves of the top canopy as opposed to the mature leaves of the lower canopy in well-watered seedlings and cuttings suggests a possible defence-related function of anthraquinones. Srivastava *et al.* (1981) and Gupta *et al.* (1977) also have observed higher sennoside yields in younger leaves than the mature leaves. However, it could be assumed that the rate of $P_{net}$ in expanded leaves would gradually decrease from top to bottom of the canopy due to tissue aging and increased mutual shading. If the sennoside precursors in the young leaves are synthesized *in situ* and not transported from old leaves, then this exemplifies the capacity of senna to biosynthesize higher levels of anthraquinones even in the leaves with high levels of $P_{net}$ in the absence of nutritional or environmental stresses. This is further supported by the higher sennoside contents in seedlings than cuttings where $P_{net}$ in seedlings is consistently higher than in cuttings.

Furthermore, the presence of greater sennoside concentrations in the dried younger leaves than in the mature leaves largely explains the greater sennoside concentrations in the deflowered plants as opposed to the flowering plants. Removal of flower buds maintains the plants in the vegetative physiological phase which causes continuous production of leaves. Thus, although the leaves at the same nodal positions are stripped from both deflowered and flowered plants for

sennoside quantifications it is more likely that the leaves from the deflowered plants are younger than the leaves from the flowered plants.

In summary the following treatments have been demonstrated to have a positive effect on sennoside yield:

**1. deflowering 2. harvesting of young leaves 3. correct choice of crop 4. short term drought 5. foliar nitrogen spray 6. correct harvesting time of the day**

These practices show a great promise from agricultural and industrial viewpoints. Therefore, they must be considered subjects of intensive field research. Studies on deflowering should involve evaluations of young leaf picking and deflowering performed together as a test component technology since both these practices are costly and time consuming in a farming context. In general, cuttings may not be a promising choice of a crop due to poorer sennoside yield per plant and weaker root system (especially for dry agroecosystems, Chapter two) compared to other two crop types. Cropping pattern studies must be undertaken site-specifically to realize the advantage of the ratoon crop mainly owing to its higher leaf sennoside contents, and relatively low cost that would be involved in this extra return. Imposition of short term droughts, timed leaf picking during day and foliar nitrogen spray could be more conveniently field-investigated compared to the other above-mentioned crop cultural practices.

# CHAPTER 5

## EFFECTS OF DROUGHT AND FOLIAR NITROGEN APPLICATION ON LEAF ULTRASTRUCTURE

### SUMMARY

Electron microscopic investigations were undertaken to assess the effects of drought and foliar nitrogen application on leaf surface and mesophyll ultrastructure. A network of crystalline epicuticular wax was observed on both adaxial and abaxial surfaces. Stomatal density was greater on the adaxial surface than the abaxial surface. In severe drought, the reduction in number of stomata per leaflet was not as great as the reduction in leaflet size. Adaxial stomatal density was significantly increased by drought. Number of stomata per plant was 74% reduced by the drought largely due to defoliation. Foliar nitrogen supplement decreased the stomatal density on the adaxial surface but increased it on the abaxial surface. Stomatal size was increased by foliar nitrogen application but decreased by drought. Trichome density was 10 times greater on the abaxial surface than the adaxial surface. Drought and nitrogen stress increased the trichome density on both adaxial and abaxial surfaces. Chloroplasts were smaller in the droughted plants than the watered plants. A dense collection of thick walled cells with a corresponding pattern of epicuticular wax was found in the bundle sheath extension of the mid vein and in the leaflet margins. Drought and nitrogen stress caused no injury to the photosynthetic apparatus or other cell organelles under the conditions of the experiment.

### INTRODUCTION

The results presented in the previous chapters exemplify the morphological responses and physiological mechanisms that confer the drought tolerance in

senna. This was evident, for instance, by reduction in total leaf area and paraheliotropic leaf orientation, and the recovery of photosynthesis and sennoside synthesis upon restoration of water regime, respectively. Furthermore, effects of drought and foliar N application on gas exchange characteristics appeared to be related to stomatal activity and the sennoside synthesizing secondary metabolism. However, whether these responses are also accompanied by leaf morphological responses, particularly at the surface and mesophyll cellular ultrastructural level, remains to be investigated.

Leaf characteristics influence the survival of plants in harsh environments (Upadhyaya and Furness, 1994), and potential use of agrochemical sprays on crops (Bitterlich and Upadhyaya, 1989; Kutík and Bergmannová, 1991; Ricotta and Masiunas, 1992). Leaf ultrastructural changes in response to drought stress have been reported in different species (Ristic and Cass, 1992; Upadhyaya and Furness, 1994; Bondada *et al.*, 1996). However, investigations on leaf ultrastructural responses of senna to environmental constraints such as drought and nitrogen stress have not been investigated. Thus, the main objective of this chapter is to present the quantitative assessments of the leaf surface ultrastructural responses to drought and nitrogen stresses. These findings will also help strengthen the understanding of the results of the foliar nitrogen spray (Chapter one). Furthermore, transmission electron microscopic investigations were undertaken to detect whether leaf mesophyll tissues show signs of injury under the specific conditions of the experiment.

## MATERIALS AND METHODS

### Plant material and preparation of specimens

The plants that were subjected to drought and foliar nitrogen treatments (Chapter 1) were used for investigations on leaf ultrastructure. Three leaflets were stripped from the second fully expanded leaf from the apex in each plant on the tenth day of the fifth drought cycle (Chapter 1). Approximately five mm long pieces were excised cross-wise from these leaflets (each piece with full width of the leaflet) from each plant using razor blades, and immediately fixed in 3% gluteraldehyde in 0.1 M Na-K phosphate buffer (pH 7.2) for one hour at room temperature. The fixed leaflet pieces were then post-fixed for one and half hour in 1% $OsO_4$ in Na-K phosphate buffer. Upon post-fixing, the tissues were rinsed in Na-K phosphate buffer and dehydrated in graded ethanol (50%, 70%, 95%, 100%, 100%, 100%), and propylene oxide (three changes). For mesophyll cellular investigations, the dehydrated pieces were embeded in Spurr's medium (Spurr, 1969) and polymerized at 65°C for 72 hr. Each block was trimmed exposing the embeded tissue which was then sectioned using a Reichert Ultracut-S ultramicrotome with a diamond knife. Sections of 60-90 nm were mounted on 300-meshed copper grids and stained with uranyl acetate-methanol (Stampak and Ward, 1964) followed by lead citrate (Reynolds, 1963). Sections were examined in a Hitachi H-7000 electron microscope at 75 kilovolts.

For leaf surface ultrastructural investigations, the leaflet pieces dehydrated as above were critical point dried in $CO_2$ using a Tousimis Samdri-790. They were then coated with gold (100 Å) in a Hummer II sputter coater. Central zones of each side of the mid vein on either adaxial or abaxial surface of ten leaflet pieces

per treatment were examined using a Hitachi S-2700 scanning electron microscope. Number of stomata and trichomes were counted on the computer-aquisitioned image of leaf fields of 258,829 and 6,472,048 $\mu m^2$ at the magnification of 350 and 70, respectively. Length and breadth of the stomatal aperture and of stoma (to the outer boundary of guard cells) were measured on the image at the magnification of 1000.

## RESULTS

### *Leaf surface ultrastructure*

Figures 5. 1 through 5. 8 are scanning electron microscopic views of leaf and trichome surfaces, and of leaf cross sections of the plants that underwent different treatment combinations. The results of quantitative assessments of stomata trichomes are summarized in Figures 5. 9 through 5. 11, and Tables 5. 1 and 5. 2.

#### Qualitative characteristics

Dense, interconnected crystalline epicuticular wax deposits were observed on both adaxial and abaxial surfaces (Figures 5. 1, 5. 2 and 5. 7). Leaf margins and the large mid veins were covered with ridges of epicuticualr wax that were deposited more thickly than the other areas of the leaf blade. These ridges corresponded to bundles of thick walled cells lying parallel along the longitudinal axis of the leaflet (Figure 5. 5). The leaf epidermal cells were individually well-recognizable both on the adaxial and abaxial surfaces in all the plants (Figure 5. 1 and 5. 3). Epicuticular wax deposition on the adaxial surface of the droughted plants that were deprived of nitrogen supplement was not as continuous as of the other plants (Figure 5. 4). A large bundle sheath extension was associated with the mid vein, and the palisade mesophyll spread isobilaterally (Figure 5. 8).

Trichomes were unicellular and mostly uniseriate while glandular (globular) trichomes were rare (Figure 5. 3). The uniseriate trichomes were 56-160 µm long, and 9-19 µm wide. They were bent near the base, and oriented parallel to the leaf surface to a height of approximately 25 µm, and their tips were directed toward the distal end of the leaflet (Figure 5. 8). Similar orientation of the trichomes was observed on the fresh, intact leaflets (both young expanding and mature) of the plants, when observed through a dissecting microscope. Shapes of micropapillate sculpturing on the uniseriate trichomes were round, oval or ridge-like in shape (Figure 5. 6).

**Stomatal density**

Stomatal density was 26% higher on the adaxial surface than the abaxial surface when the data were pooled across nitrogen and drought treatments (Figures 5. 1 to 5. 6, F=26.44, P < 0.001, R-sq=0.14). Statistical power of this test was ca 0.99 with LSN of 26. Stomatal density increased by 15% on the adaxial surface in response to drought (F=6.43, P<0.05, R-sq=0.07; statistical power=0.70, LSN=51) although, only 7% increase when pooled across the two surfaces, was not significant.[1] Plants that were not applied with foliar nitrogen had 33% higher stomatal density on the adaxial surface than the plants that were applied with foliar nitrogen (F=38.12, P<0.001, R-sq=0.30; statistical power=0.99, LSN=12). In contrast, on the abaxial surface, the stomatal density was 27% higher in the plants with foliar nitrogen application than the plants without it (F=13.67, P<0.001, R-sq=0.14; statistical power=0.94, LSN=26). Droughted plants had 23% greater stomatal density on the adaxial surface compared to the watered plants, when foliar nitrogen spray was not applied (F=23.83, P<0.001, R-sq=0.38; statistical power=0.99, LSN=10), but the

[1] F=1.94, P=0.16, R-sq=0.01

difference was nonsignificant in the plants that were applied with foliar nitrogen.[1] However, in the plants that were deprived of foliar nitrogen application, stomatal density was 19% higher on the abaxial surface when watered compared to droughted (F=8.05, P<0.01, R-sq=0.17).

As presented in the Table 5. 1, the number of stomata per leaflet was 33% reduced by the drought (F=142.33, P<0.001, R-sq=0.63). Statistical power of this test was nearly one with LSN of six. Number of stomata per plant was 74% reduced by drought (F=111.03, P<0.001, R-sq=0.58). This test, as well, was associated with a statistical power of nearly one and LSN of six. In the watered plants, foliar nitrogen application increased stomatal number per plant by 1.4 times (F=204.00, P<0.001, R-sq=0.80) with no significant effect on stomatal number per leaflet. In the droughted plants, nitrogen application showed no significant effect on stomatal count per leaflet[2] but reduced the stomatal number per plant (F=10.46, P<0.01, R-sq=0.22).

[1] F=0.45, P=0.50, R-sq=0.01
[2] F=1.54, P<0.22, R-sq=0.04

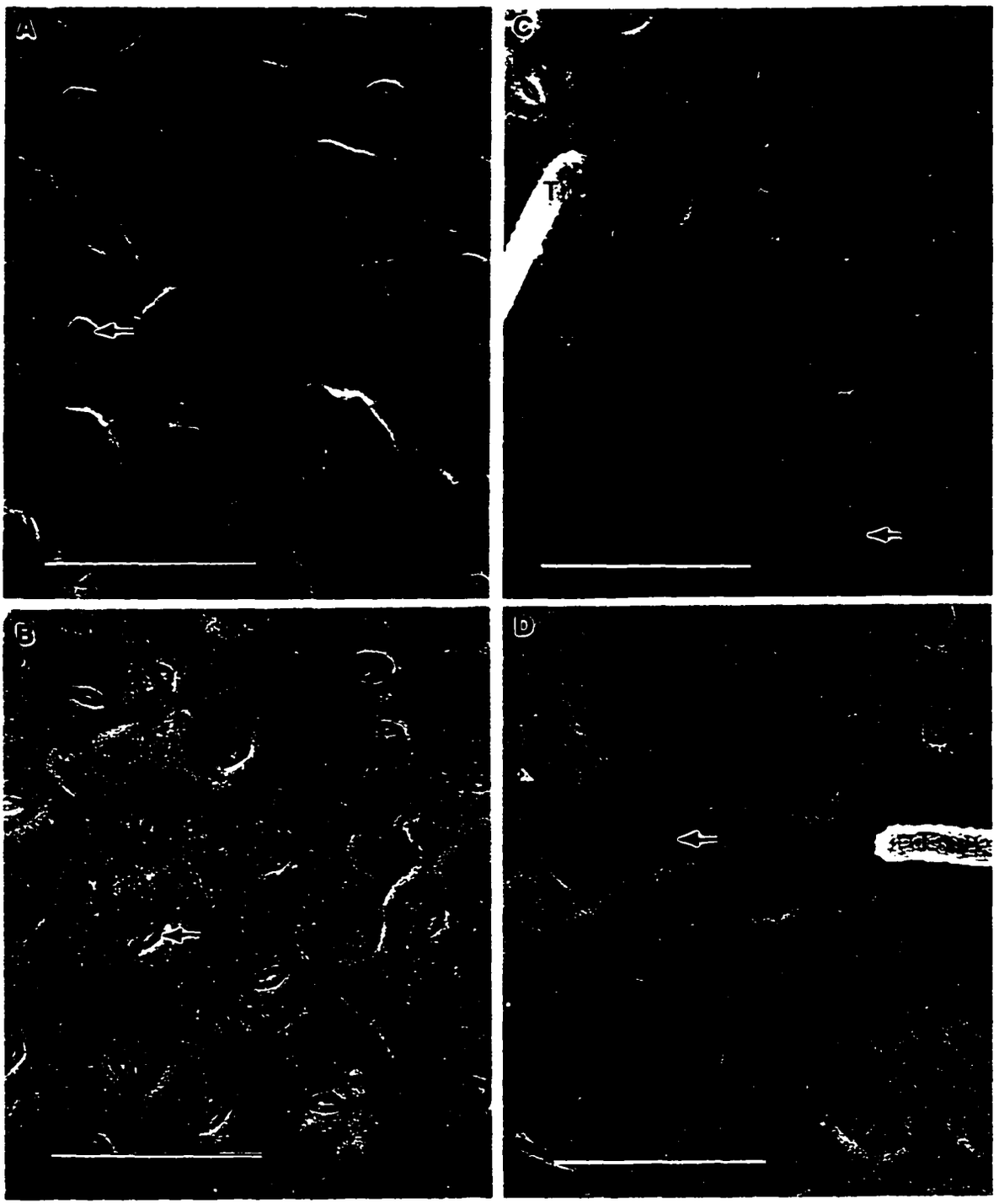


**Figure 5. 1** Characteristics of the adaxial leaf surface as influenced by drought and foliar nitrogen spray. A, watered and no foliar nitrogen; B, watered and foliar nitrogen applied; C, droughted and no foliar nitrogen; D, droughted and foliar nitrogen applied. T, trichome; Arrow head, stoma. Scale bar = 85.7 μm.

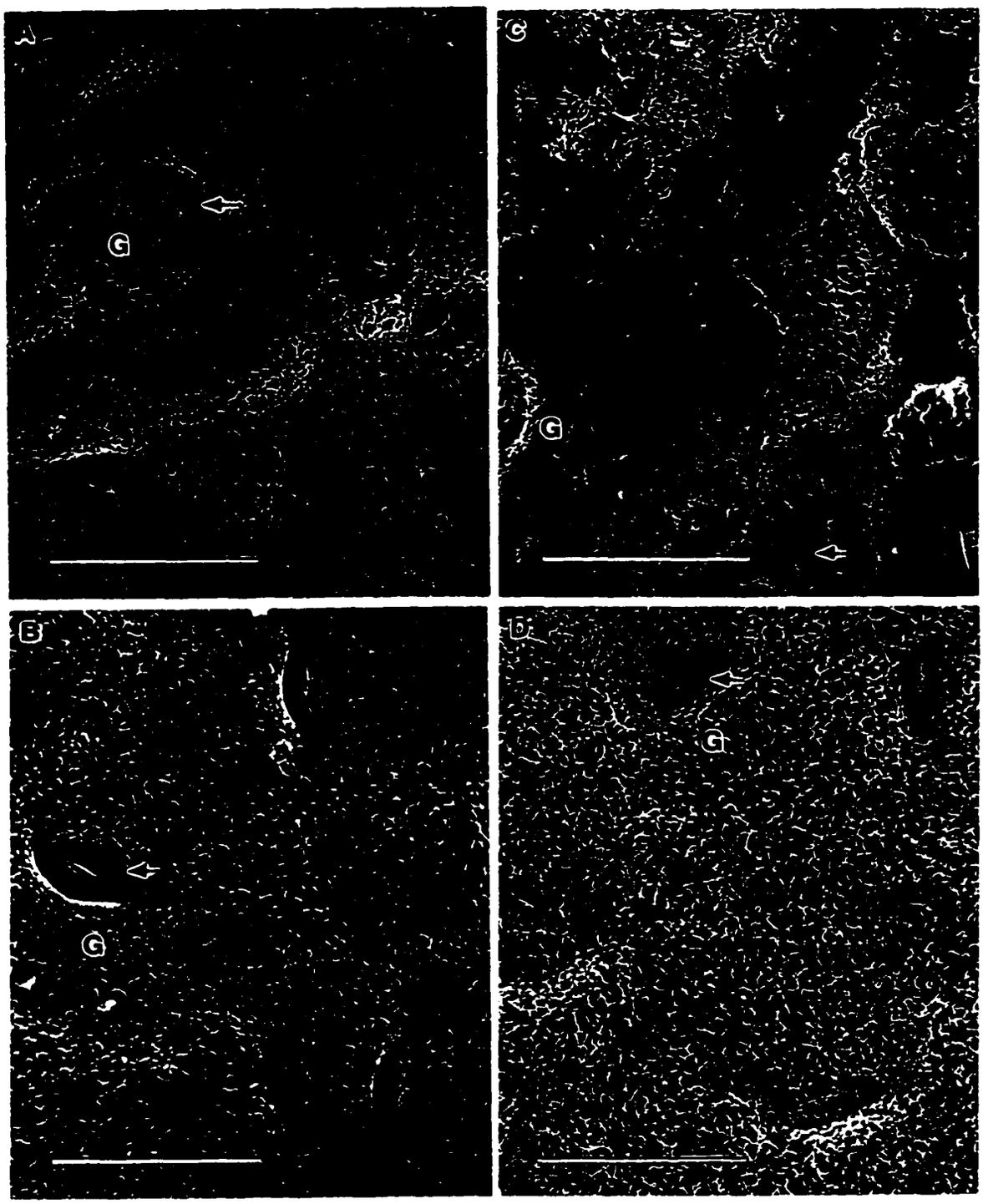


**Figure 5. 2** A close-up view of the adaxial leaf surface, showing crystalline epicuticular wax. A, watered and no foliar nitrogen; B, watered and foliar nitrogen applied; C, droughted and no foliar nitrogen; D, droughted and foliar nitrogen applied. G, guard cell; Arrow head, stoma. Scale bar = 30 μm.

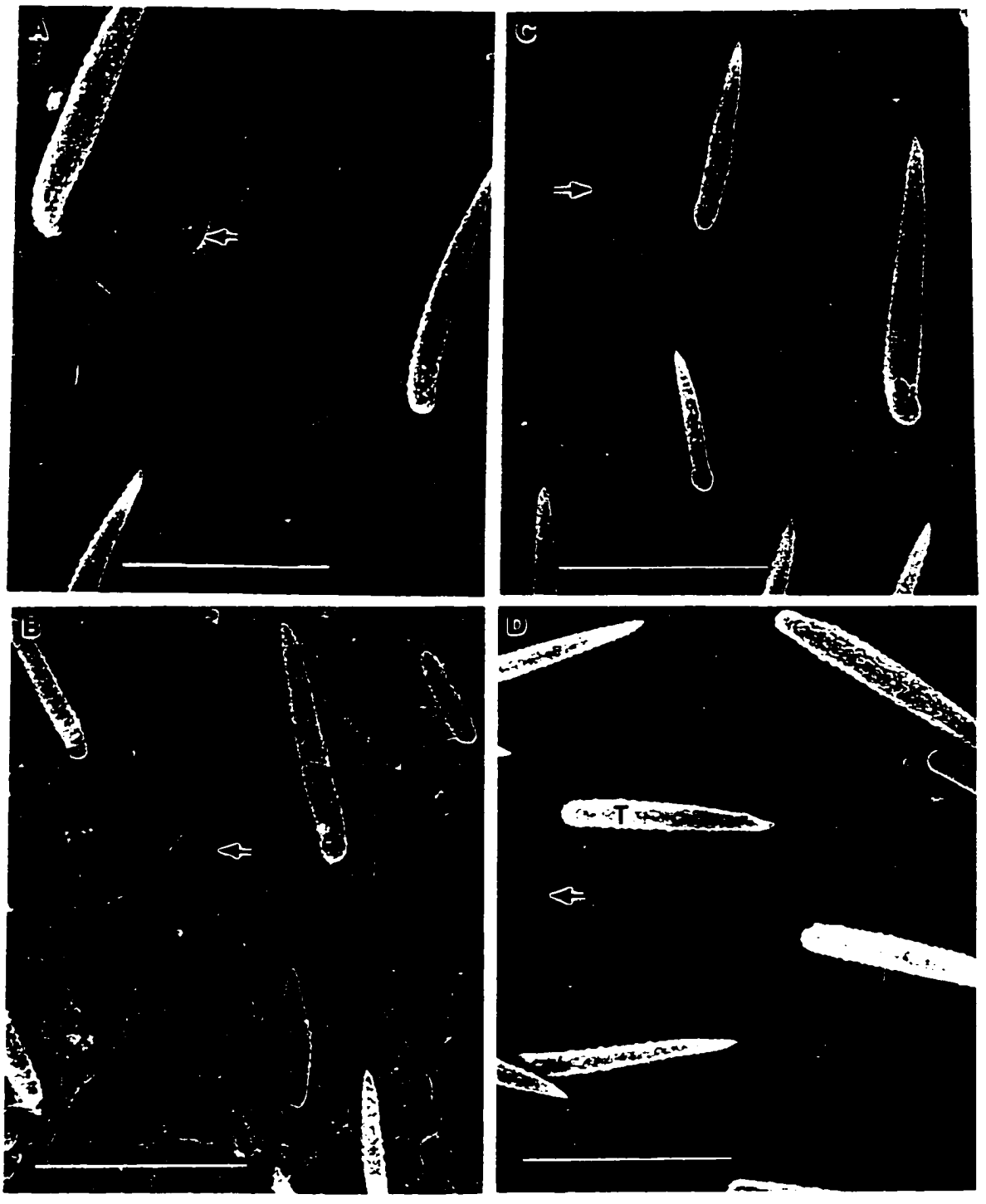


**Figure 5. 3** Ultrastructure of leaf abaxial surface. A, watered and no foliar nitrogen; B, watered and foliar nitrogen applied; C, droughted and no foliar nitrogen; D, droughted and foliar nitrogen applied. T, trichome; Arrow head, stoma. Scale bar = 85.7 μm.

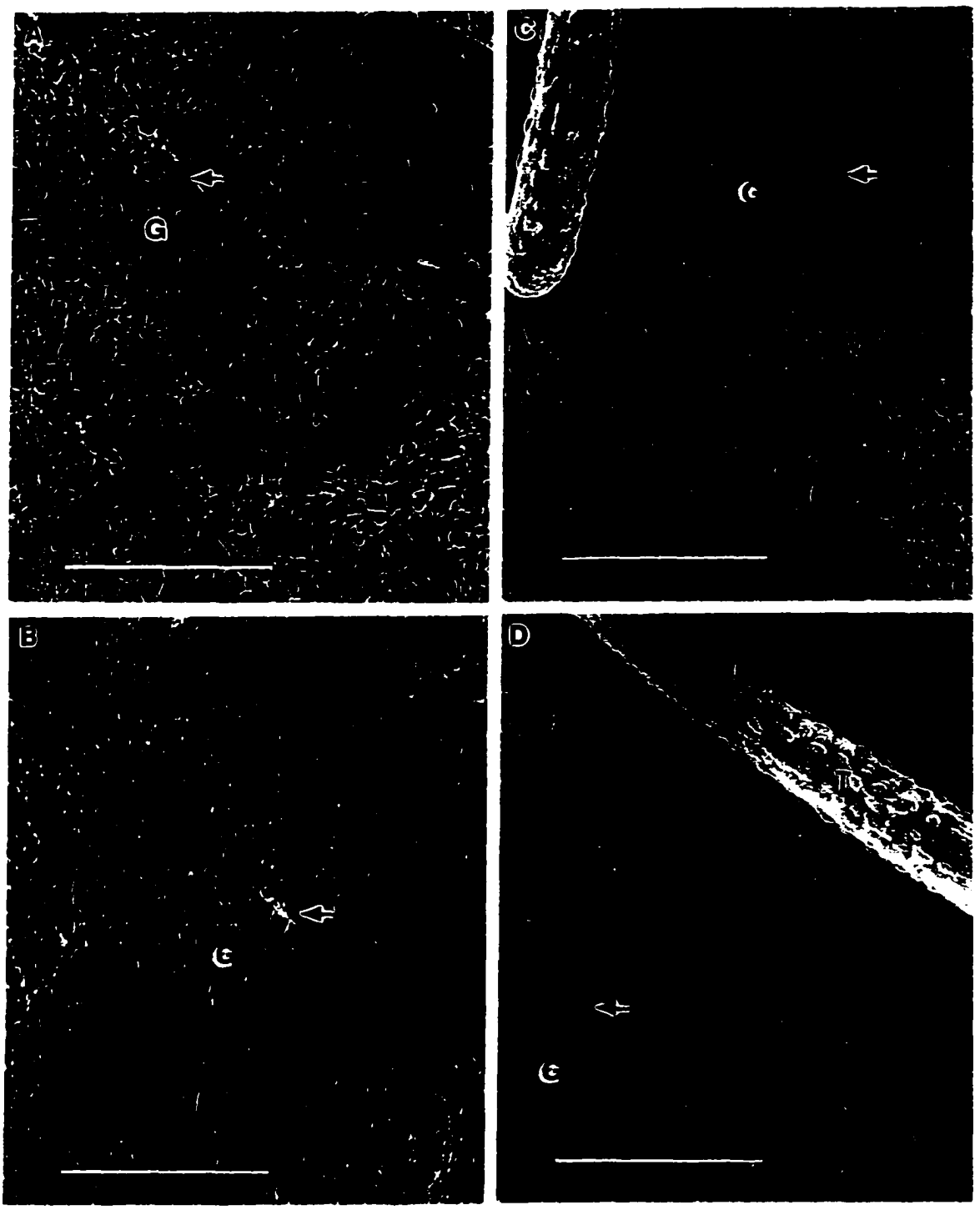


**Figure 5. 4** A close-up view of leaf abaxial surface showing crystalline epicuticular wax. A, watered and no foliar nitrogen; B, watered and foliar nitrogen applied; C, droughted and no foliar nitrogen; D, droughted and foliar nitrogen applied. G, guard cell; T, trichome; Arrow head, stoma. Scale bar = 30 μm.

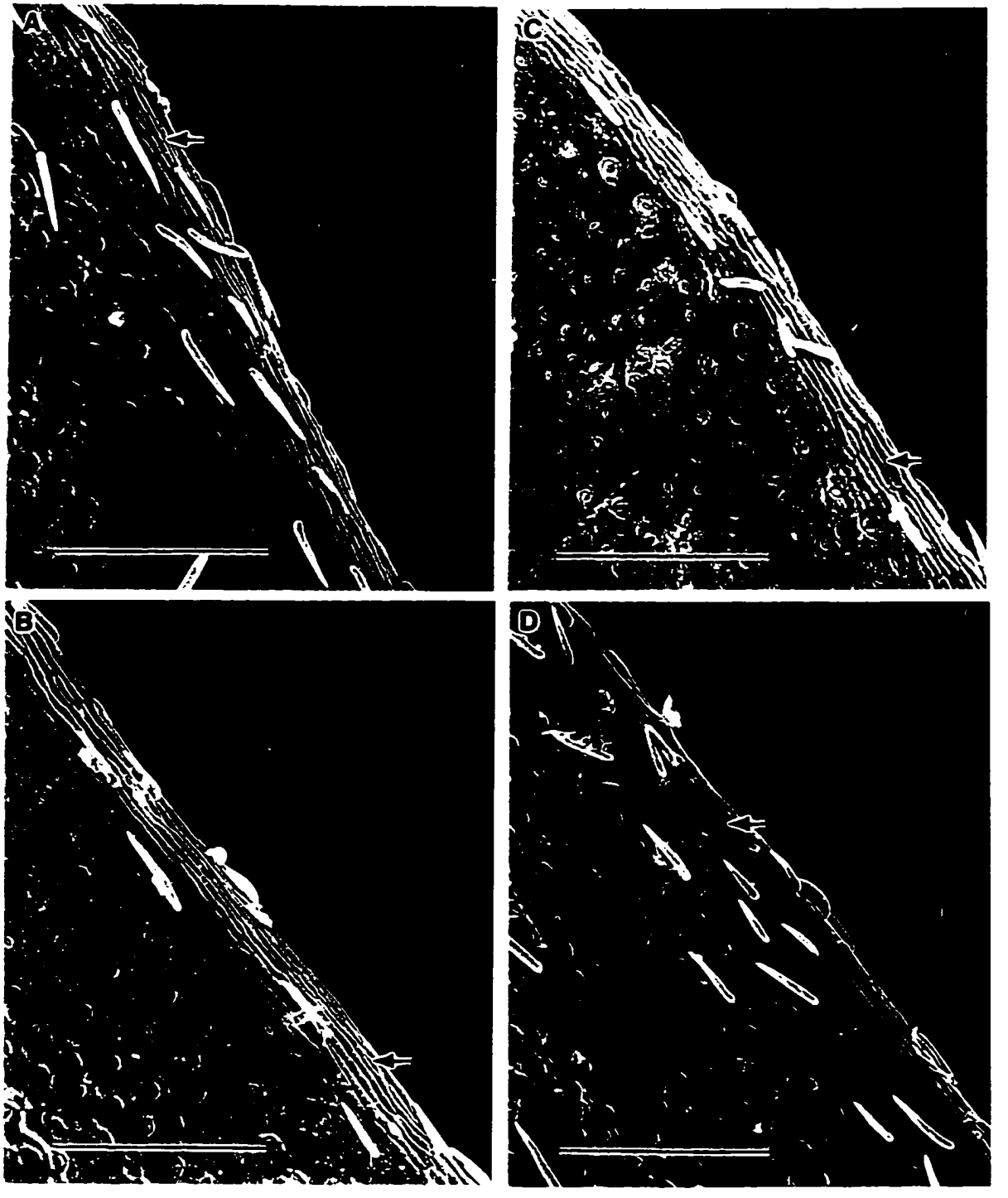


**Figure 5. 5** Adaxial leaf edge of the plants showing patterns of heavy wax deposit along the leaf margin (arrow head). A, watered and no foliar nitrogen; B, watered and foliar nitrogen applied; C, droughted and no foliar nitrogen; D, droughted and foliar nitrogen applied. Scale bar = 300 μm.

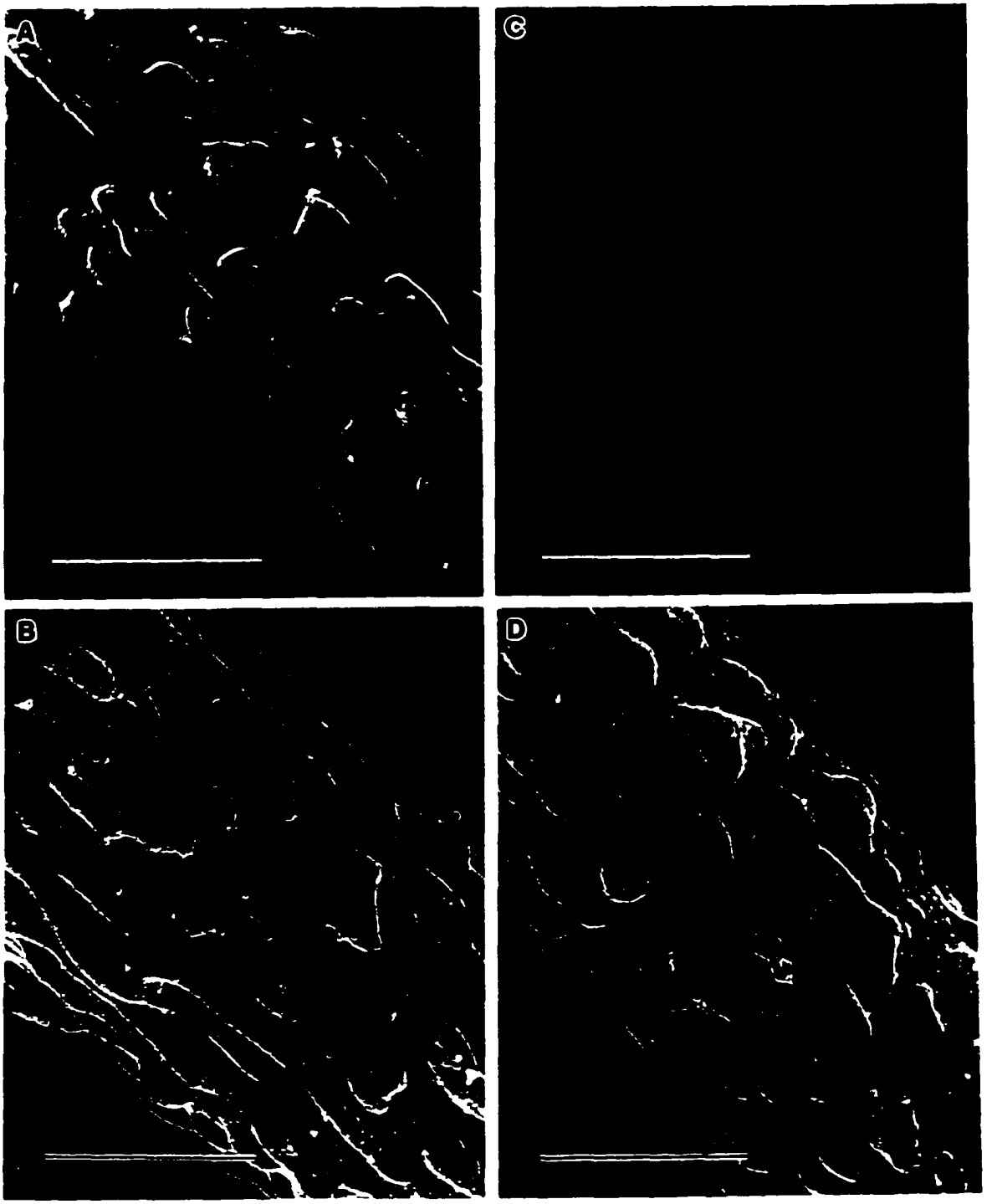


**Figure 5. 6** Close-up view of trichomes showing micropapillate sculpturing. A, watered and no foliar nitrogen; B, watered and foliar nitrogen applied; C, droughted and no foliar nitrogen; D, droughted and foliar nitrogen applied. Scale bar = 7.5 μm.

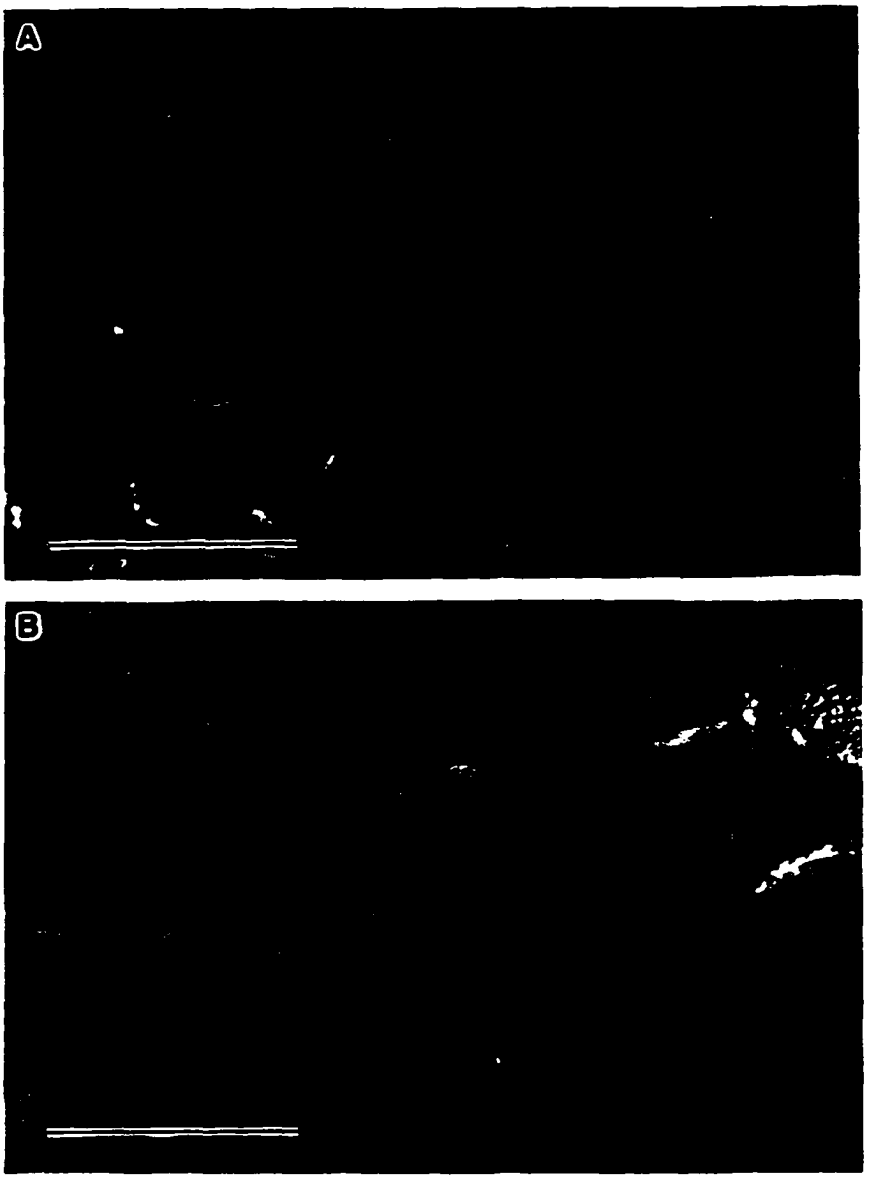


**Figure 5. 7** Close-up views showing epicuticular crystalline wax (A, adaxial leaf surface of a watered plant without foliar nitrogen spray, scale bar = 3. 33 μm), and orientation of trichomes (B, abaxial surface of a droughted plant without foliar nitrogen spray, scale bar = 30 μm).

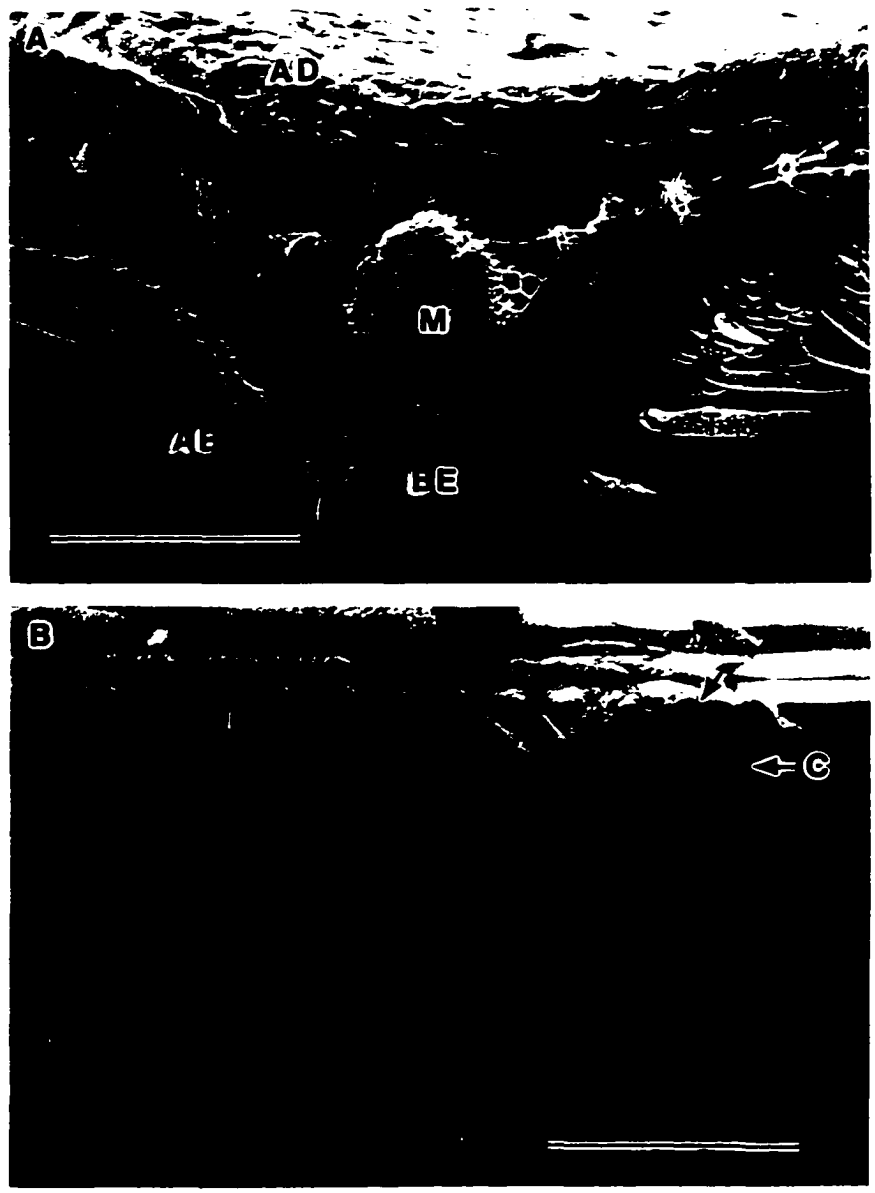


**Figure 5. 8** Leaflet cross sections of droughted plants without foliar nitrogen spray. A, section via the mid vein, AD, adaxial; AB, abaxial; P, palisade mesophyll; M, mid vein; BE, bundle sheath extension. Scale bar = 150 μm. B, leaf edge showing heavy epicuticular wax deposit (arrow head) and the corresponding colenchymal cells (C). Scale bar = 50 μm.

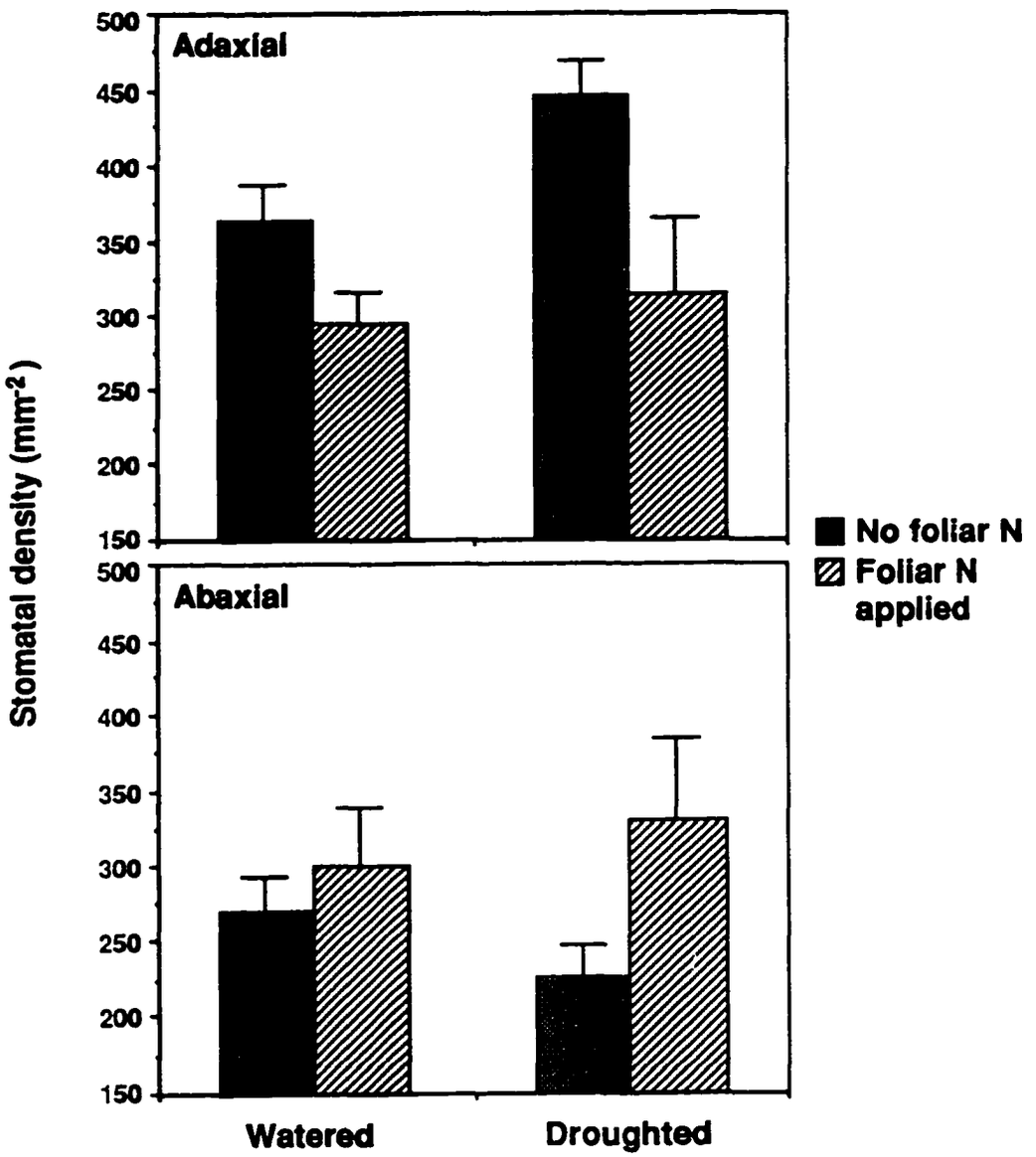


**Figure 5. 9** Effects of drought and foliar nitrogen application on stomatal density. N=20 fields on leaflets. Error bar represents 95% CI of the mean.

**Table 5. 1** Effect of drought and foliar nitrogen application on the number of stomata per leaflet and per plant. N is the number of fields on leaflets.

| Treatment | N | Stomata/Leaflet ($x10^4$) | Stomata/Plant ($x10^6$) |
|---|---|---|---|
| **Droughted** | | | |
| **No foliar N** | 20 | 7.31 | 13.14* |
| **Foliar N applied** | 20 | 7.77 | 11.29 |
| **Total** | 40 | 7.54 | 12.22 |
| **Watered** | | | |
| **No foliar N** | 20 | 11.08 | 30.03 |
| **Foliar N applied** | 20 | 11.73 | 72.25** |
| **Total** | 40 | 11.40** | 51.11** |

* Significantly greater than the corresponding total in column, or greater than nitrogen status within a water regime (** P<0.001, * P<0.01).

**Trichome density**

Abaxial surface had 10 times more trichomes than the adaxial surface (Figure 5. 2; F=448, P<0.001, R-sq=0.74). The statistical power of this model was nearly one with LSN of only five. The highest trichome densities, 7.1 $mm^{-2}$ on the adaxial and 67.8 $mm^{-2}$ on the abaxial surfaces, were observed in the droughted plants without foliar nitrogen spray. Trichome density doubled on the adaxial surface (F=58.08, P<0.001, R-sq=0.38), and increased 59% on the abaxial surface (F=100.23, P<0.001, R-sq=0.37), in response to drought. Each of these tests was associated with a statistical power of nearly one with LSN of 10. Plants that were deprived of foliar nitrogen spray had 45% and 44% greater trichome density on the adaxial and abaxial surfaces, respectively, compared to the plants that received the foliar nitrogen (adaxial surface, F=16.01, P<0.001, R-sq=0.10; abaxial surface, F=62.47, P<0.001, R-sq=0.23). The statistical power for these tests was ca 0.85 and ca 0.99 with LSNs of 36 and 16, respectively. Deprivation of nitrogen caused 64% and 16% increase in the trichome density on the abaxial surface in the droughted and watered plants, respectively (interaction between nitrogen and drought, F=28.23, P<0.001, R-sq=0.10).

**Aperture size**

Stomata of adaxial and abaxial surfaces had statistically similar aperture lengths and breadths.[1] Drought decreased the aperture length by 20% only in the plants that were deprived of foliar nitrogen. No significant effect of drought was detected on the aperture breadth in either nitrogen-treated or -deprived plants.[2] However, in the droughted plants, foliar nitrogen spray increased the aperture length by 19%

[1] For aperture length, F=0.52, P=0.46, R-sq=0.00: for aperture breadth, F=0.06, P=0.80, R-sq=0.00
[2] F=0.1, P=0.75, R-sq=0.00

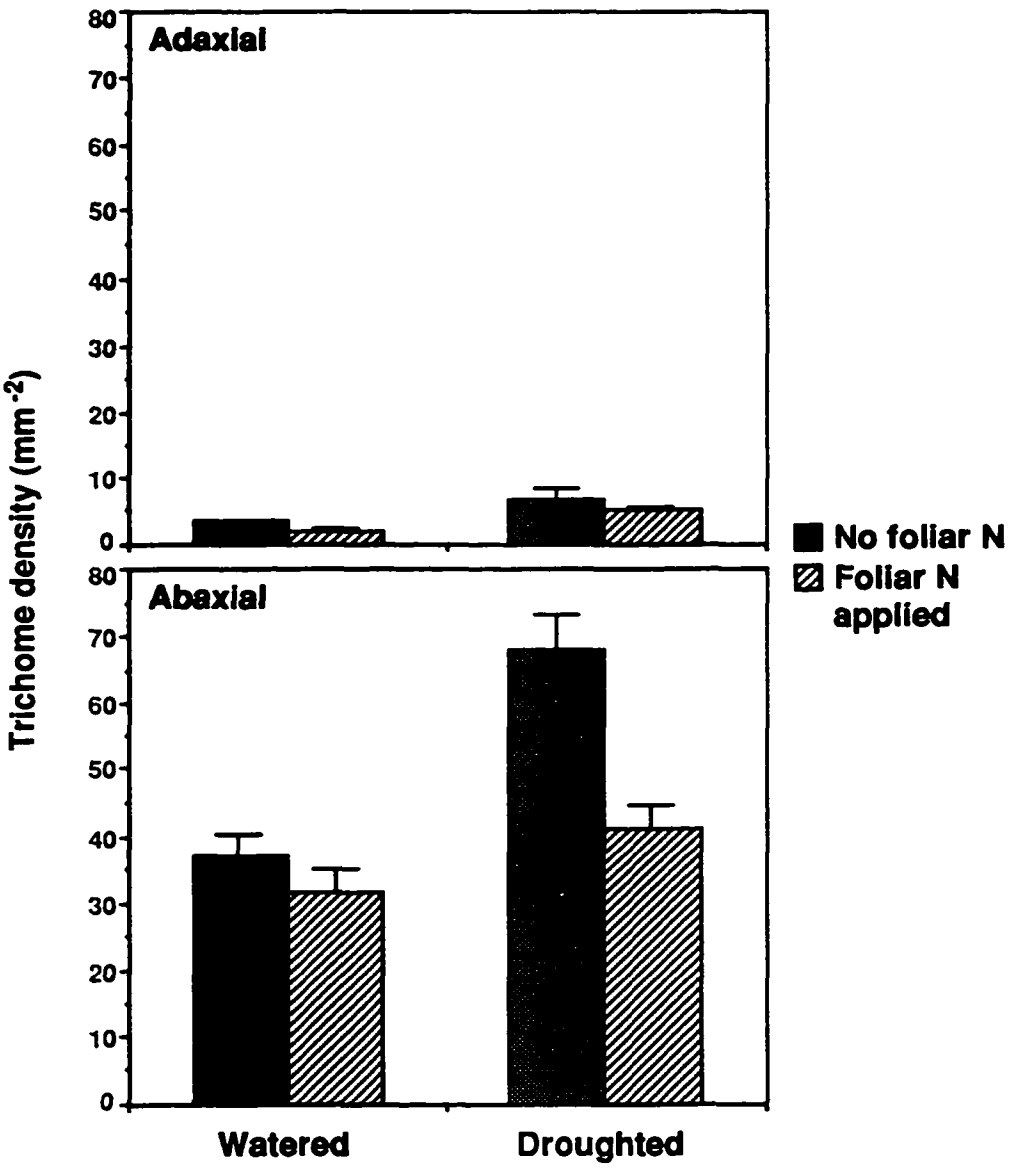


**Figure 5. 10** Trichome density as influenced by drought and foliar nitrogen spray. N=20 leaf fields. Error bar represents 95% CI of the mean.

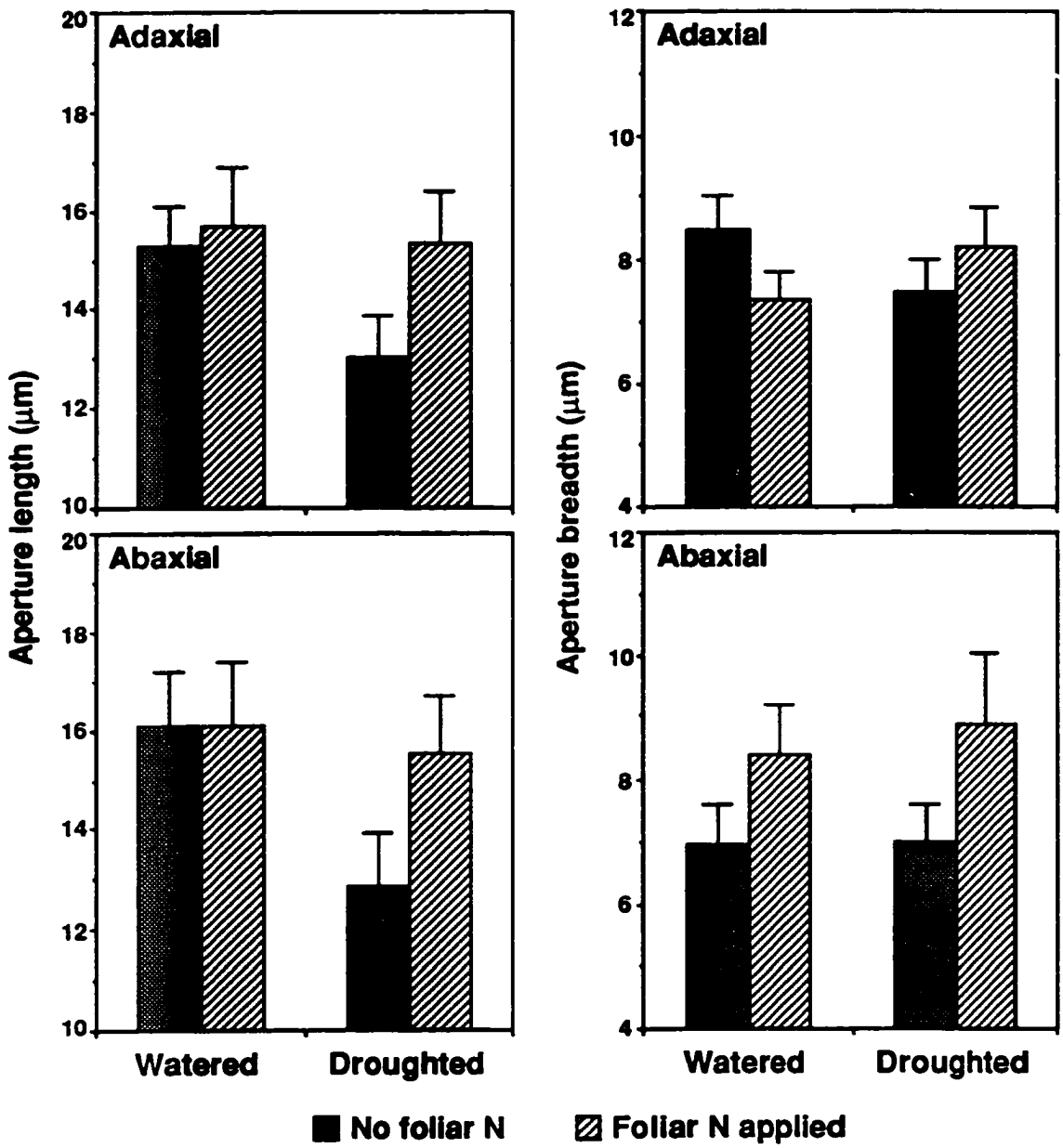


**Figure 5. 11** The influence of drought and foliar nitrogen application on the length and breadth of the stomatal aperture. N=20 leaf fields. Error bar represents 95% CI of the mean.

and aperture breadth by 18% (aperture length, F=23.82, P<0.001, R-sq=0.23; aperture breadth, F=10.82, P<0.01, R-sq=0.12). The value of length multiplied by breadth was 20% greater in the plants that were applied with foliar nitrogen versus without nitrogen when data were pooled across water status (F=12.09, P<0.001, R-sq=0.07). Statistical power of this test was ca 0.93 with LSN of 54. However, this value was only 9% greater in the watered plants compared to droughted plants when the data were pooled across nitrogen treatments, a nonsignificant difference.[1]

**Stomatal size**

Measures of the length and the breadth of the stomata (guard cells) are summarized in the Table 5. 1. The stomatal length and breadth of droughted plants were generally lower than the watered plants. However, only the 12% decrease in the stomatal length on the abaxial surface in droughted plants compared to watered plants was statistically significant (F=5.66, P< 0.05, R-sq=0.06). Furthermore, measures of the stomatal size were generally greater in the plants applied with foliar nitrogen, except for the lengths in the watered plants. However, only the 14% increases in the stomatal breadth on abaxial surface in each water status were statistically significant (for droughted plants, F=4.90, P<0.05, R-sq=0.11; for watered plants, F=4.3, P<0.05, R-sq= 0.10). Although, the stomatal length was 9% greater on the adaxial surface than the abaxial surface (pooled across drought and nitrogen treatments, F=7.61, P<0.01, R-sq=0.04), there was no significant difference in the stomatal breadth.[2]

The value of the stomatal length multiplied by breadth was 22% less in the

[1] F=2.77, P=0.09, R-sq=0.07
[2] F=1.60, P=0.20, R-sq=0.00

**Table 5. 2** Influence of drought and foliar nitrogen application on stomatal size. Mean with SE.

| Experimental | N | Length (μm) | | Breadth (μm) | |
|---|---|---|---|---|---|
| | | Adaxial | Abaxial | Adaxial | Abaxial |
| **Droughted** | | | | | |
| **No foliar N** | 20 | 28.9(0.6) | 25.8(1.3) | 30.1(0.7) | 31.7(0.9) |
| **Foliar N applied** | 20 | 32.9(1.1) | 27.8(1.9) | 34.7(0.9) | 36.2(1.7)* |
| **Total** | 40 | 30.9(0.7) | 26.8(1.1) | 32.4(0.7) | 33.9(1.0) |
| **Watered** | | | | | |
| **No foliar N** | 20 | 32.4(1.2) | 33.9(1.2) | 33.1(1.5) | 32.1(1.3) |
| **Foliar N applied** | 20 | 31.8(1.2) | 27.3(1.7) | 33.9(0.9) | 36.5(1.6)* |
| **Total** | 40 | 32.1(0.8) | 30.6(1.1)* | 33.5(0.8) | 34.3(1.1) |

* Significantly higher than the other total (in length), or other nitrogen treatment in a given drought treatment (in breadth), in the column ($P<0.05$).

droughted plants, only when they were deprived of foliar nitrogen supplement ($F=15.87$, $P<0.001$, R-sq=0.17), and was 9% less when pooled across nitrogen treatments ($F=4.31$, $P<0.05$, R-sq=0.27), compared to the watered plants. Effect of nitrogen application on this value (8% less in nitrogen deprived plants) was nonsignificant at the sampling size of the experiment.[1] The whole model test of this value was associated with a power of ca 0.93 with LSN of 54.

### *Leaf cellular and chloroplast ultrastructure*

Figures, 5. 12 to 5. 15 present the transmission electron microscopic views of the mesophyll parenchyma and vascular tissues, and chloroplasts of the plants under different treatment combinations.

No signs of cellular or organelle injury by drought or nitrogen stress were detectable in any tissue that was observed. In all the treatments, cell walls of the palisade mesophyll were contiguously lined internally with the chloroplasts. Chloroplasts in both palisade and spongy mesophyll were thinner and devoid of fully-formed starch inclusions in the droughted plants compared to watered plants (Figure 5. 12 and 5. 13). In a close-up view of chloroplasts, the thylakoid membranes were more clearly seen in the plants that were sprayed with foliar nitrogen compared to nitrogen-stressed plants, without regard to the plant water status. Chloroplasts of the droughted plants appeared to have more plastoglobuli than the watered plants especially when nitrogen-stressed (Figure 5. 14).

Minor veins were compactly surrounded by bundle sheath cells (Figure 5. 15). Some vascular parenchymal cells in the minor veins had chloroplasts that were

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[1] $F=3.14$, $P=0.07$, R-sq=0.19

more developed and clearly visible in the watered plants compared to the droughted plants. Companion cells were larger than the sieve tube elements, in the phloem of all the plants.

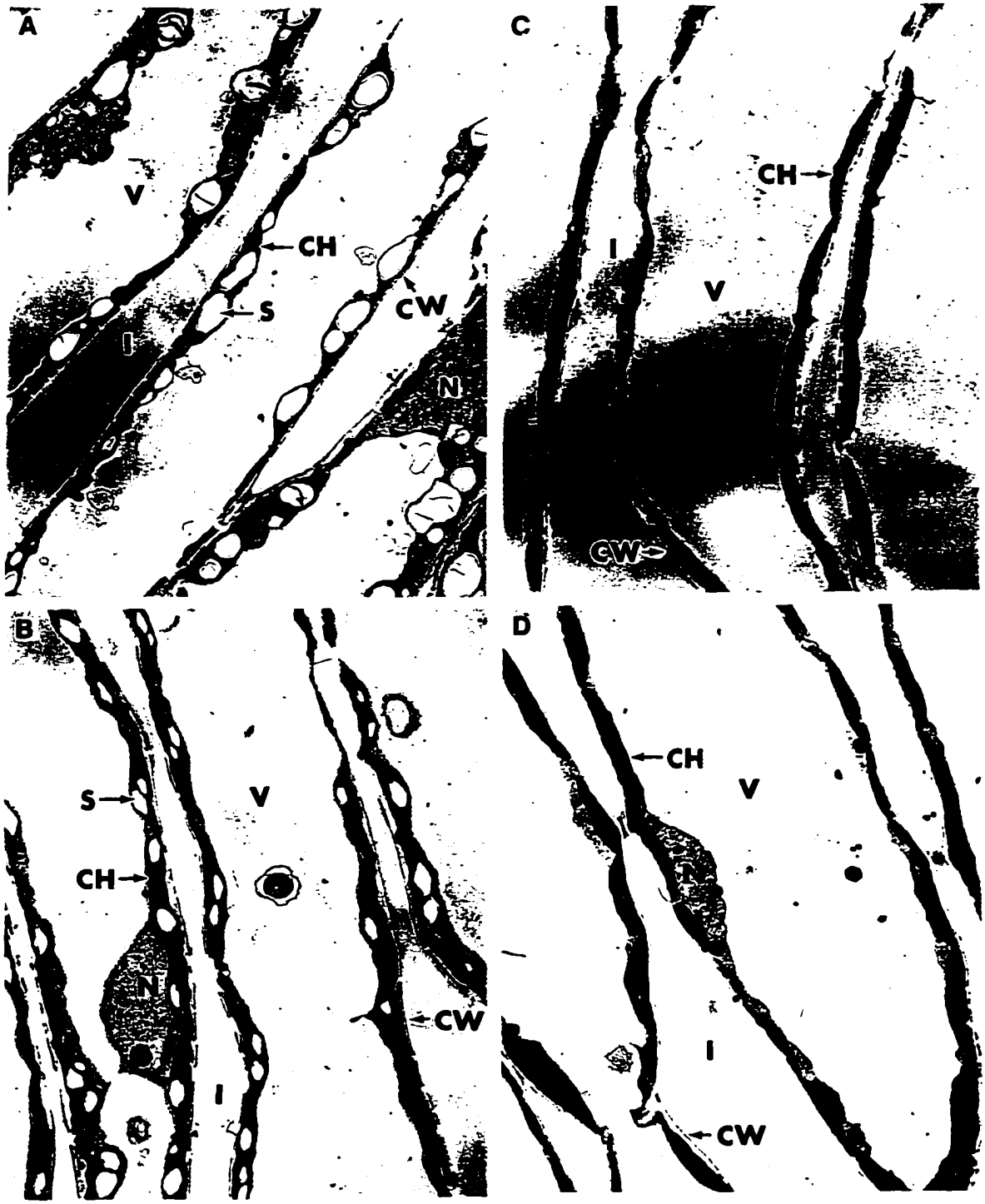



**Figure 5. 12** Ultrastructure of palisade mesophyll (2100X). A, watered and no foliar nitrogen; B, watered and foliar nitrogen applied; C, droughted and no foliar nitrogen; D, droughted and foliar nitrogen applied. CH, chloroplast; CW, cell wall; I, intercellular space; N, nucleus; S, starch grain; V, vacuole.

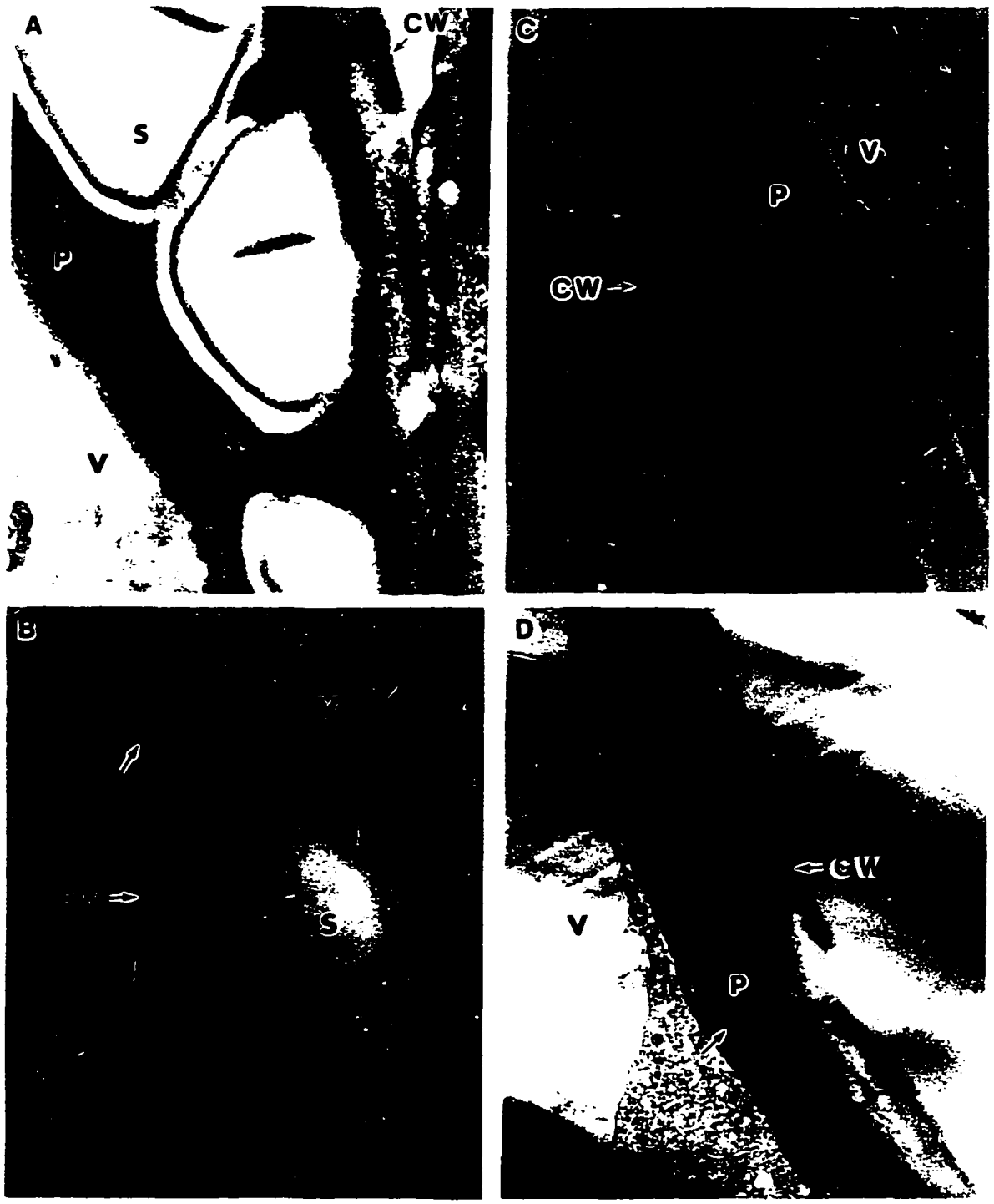



**Figure 5. 13** Close-up view of palisade chloroplasts (19400X). A, watered and no foliar nitrogen; B, watered and foliar nitrogen applied; C, droughted and no foliar nitrogen; D, droughted and foliar nitrogen applied. CW, cell wall; P, plastoglobule; S, starch grain; V, vacuole; Arrow head, thylakoid.

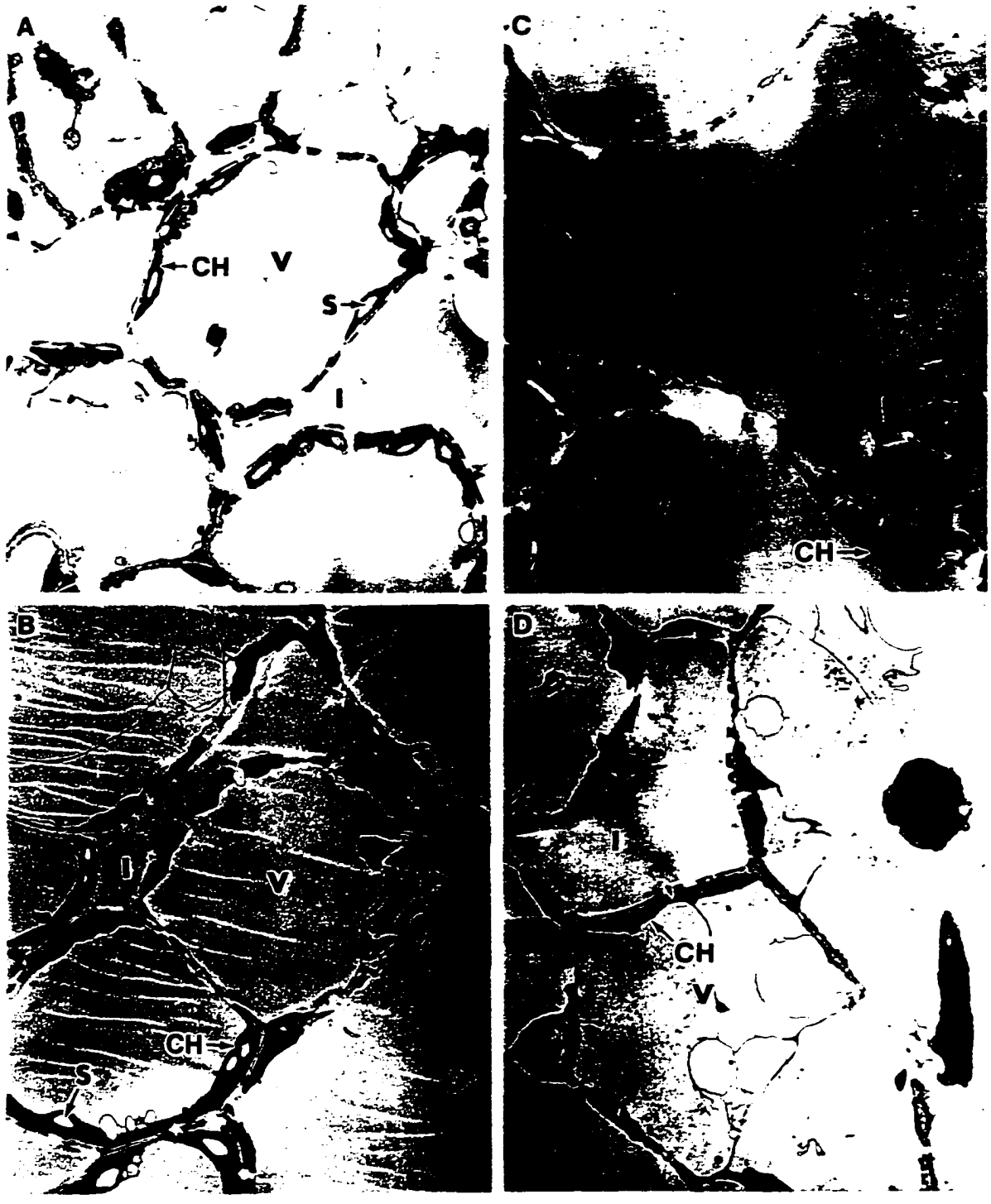



**Figure 5. 14** Ultrastructure of spongy mesophyll (2100X). A, watered and no foliar nitrogen; B, watered and foliar nitrogen applied; C, droughted and no foliar nitrogen; D, droughted and foliar nitrogen applied. CH, chloroplast; I, intercellular space; S, starch grain; V, vacuole.

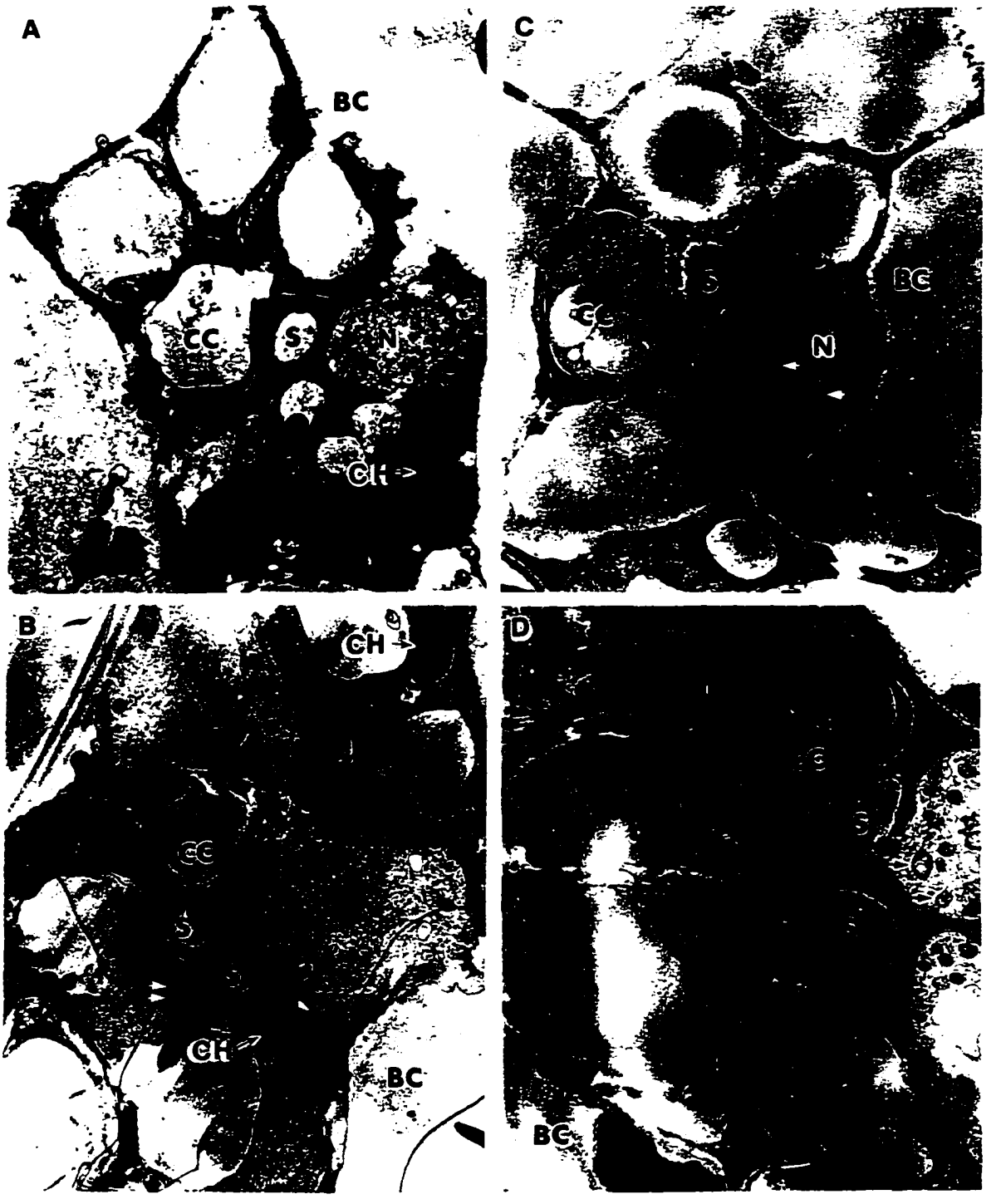



**Figure 5. 15** Cross sectional view of minor veins (4400X). A, watered and no foliar nitrogen; B, watered and foliar nitrogen applied; C, droughted and no foliar nitrogen; D, droughted and foliar nitrogen applied. BC, bundle sheath cell; CC, companion cell; CH, chloroplast; N, nucleus; S, sieve tube element; Arrow head, mitochondrion.

## DISCUSSION

Senna leaves are amphistomatic with more stomata on the adaxial surface than the abaxial surface. Furthermore, the stomatal density is relatively high, considering a herbaceous plant. Appearance of stomata on the adaxial surface and acquisition of high stomatal densities are typical xeromorphic characteristics (Bolhàr-Nordenkampf and Draxler, 1993). Interestingly, the adaxial stomatal density shows a greater sensitivity to plant water status compared to the abaxial surface, and clearly increases in drought. However, when the plants were drought- or nitrogen-stressed stomata tended to be smaller.

The degree of drought-induced reduction in number of stomata per leaflet (33%) was proportionately less than the reduction in leaflet size (38%). Thus, the stomatal density was greater (15% on adaxial, 7% adaxial and abaxial pooled) in the droughted plants compared to watered plants. However, the number of stomata per plant was 76% reduced by the drought. This trend closely correlated with the effect of drought on total leaf area per plant which was more a function of leaflet number than the leaflet size (Chapter one). This exemplifies the significance of drought-deciduousness in senna for controlling the total number of stomata per plant, and the adaptive advantage in the failure of nitrogen application to increase leaf area per plant in the severely droughted plants even when nitrogen was supplied through foliage. The smaller leaflet size in the drought would have resulted from the reduced cell size as observed in the stomatal size.

Chapter 2 presented data that show active stomatal closure as diurnal irradiation increases. However, stomatal closure under high irradiance may lead to high leaf temperatures (Gates, 1980) and photoinhibition (Powles, 1984;

Bolhàr-Nordenkampf and Draxler, 1993) causing significant metabolic damage (Berry and Björkman, 1980). Thus, plants that are adapted to high irradiation must carry mechanisms that reduce absorptivity of irradiance and maintain optimum leaf temperatures. In senna, the paraheliotropism that reorients the leaflets parallel to the sun's rays (Chapter two), drought-induced reduction of total leaf area (Chapter one) that greatly control the stomatal count per plant, epicuticular wax deposits on both adaxial and abaxial surfaces, and increased stomatal frequency with decreased stomatal size, would ensure this heat balance. Presence of lower stomatal and higher trichome densities on the abaxial surface than adaxial surface, therefore, could be regarded as adaptive responses that would effectively reduce transpiration and absorptivity as leaves reorient exposing the abaxial surface under irradiated, water-stressed conditions. The contribution of similar attributes to reduced transpiration and leaf absorptivity is well documented in other species (Ehleringer and Mooney, 1978; Mulroy, 1979; Forseth and Ehleringer, 1983). Furthermore, the prostrate orientation of the trichomes in senna would imply their probable importance in reducing absorptivity by increasing reflectivity, besides lowering the direct transpiration by increasing the boundary layer resistance.

Trichome density is clearly increased by drought and nitrogen stress on both adaxial and abaxial surfaces. This would mean an advantageous adaptation in the context of tropical dryland farming where crops are submitted to both these constraints simultaneously. Development of xeromorphic structures under moisture and nitrogen deficiency is well known (Bolhàr-Nordenkampf and Draxler, 1993). However, Upadhyaya and Furness (1994) reported that uniseriate trichome density decreased with increasing moisture stress in *Centaurea diffusa*, and with increasing light intensity in *Cynoglossum officinale*. Therefore, the

contribution of leaf trichomes to the stress-adaptive capacity should be evaluated in the context of the overall physiological and morphological responses to the given environmental stress in the given species.

The isobilateral leaf anatomy, possession of a layer(s) of palisade parenchyma on the abaxial epidermis as well, is a xeromorphic character (Bolhàr-Nordenkampf and Draxler, 1993). This would reduce transpiration greatly due to the restriction of the inter-connected network of free surfaces of the cells in spongy mesophyll to a small central strip in the leaf, away from the stomatal connection to the dry atmosphere. Furthermore, senna leaves avoid injurious effects of wilting even when the photosynthesis is undetectably low at around -2 MPa of leaf xylem water potential (Chapter 1). This could result from the substantially large central bundle sheath extension, high frequency of minor veins across the leaflet, and the densely organized thick walled cells coupled with heavy epicuticular wax deposits along the leaflet edges. These thick walled cells stained purple with Toludine Blue O, in fresh, free hand sections of leaflets from well watered plants, suggesting that they represent collenchyma (O'Brien *et al.* 1964).

Prominence of palisade mesophyll over the spongy mesophyll in senna increases the internal free surface area of the photosynthetic tissue. Internal free surface area provides the diffusion pathways for $CO_2$, $O_2$ and water (Bolhàr-Nordenkampf and Draxler, 1993). This leaf anatomy, with efficient stomatal activity and other xeromorphic characteristics discussed above would also be important in the maintenance of a minimum safe rates of photosynthetic primary metabolism and heat balance during severe drought, and in the rapid resumption of $P_{net}$ when the water supply is restored. The data presented in Chapters, one and two exemplify this strategy which is further reflected by the absence of any signs of injury to the

photosynthetic apparatus or other cell organelles under the conditions of this experiment. For instance, chloroplasts acclimated to the drought stress by becoming thin, and by losing the capacity to develop elaborate thylakoid systems and high chlorophyll contents compared to watered plants (Chapter one), but maintained membrane integrity. Fetene *et al.* (1990) reported similar responses in the chloroplasts of ***Bromelia humulis***, a CAM plant, under high light intensities. However, Ristic and Cass (1992) observed that chloroplast rupture is diagnostic of severe drought in maize. Higher $P_{net}$ in plants that were sprayed with foliar nitrogen, compared to nitrogen-stressed plants, in a given plant water status, could be attributed to the well-developed thylakoid system, as well, besides higher chlorophyll content.

The movement of chloroplasts to anticlinal walls constructs a "light pipe" which reduces absorptivity and avoids photoinhibition in the palisade mesophyll (Bolhàr-Nordenkampf and Draxler, 1993). This would also increase the availability of light to the spongy and adaxial palisade mesophylls which also helps explain the high $P_{net}$ in senna. Furthermore, the lower palisade mesophyll layer may be important in using reflected light from light-colored (e.g. sandy) soils in sunny tropical agroecosystems, as well.

## CONCLUSIONS AND FUTURE DIRECTIONS

The information generated through the experiments of this research contributes toward increasing the productivity of senna in several ways. First, the findings indicate the potential use of common environmental stresses of the tropical agroecosystems such as drought and limited availability of nitrogen to produce a sennoside yield advantage. Secondly, it was possible to identify other component technologies that show great promise for increasing sennoside yields in the dried leaves namely, deflowering, foliar nitrogen spray, harvesting of young leaves, choice of the crop type and the harvesting time of day. Thirdly, the data help understand the physiological and developmental mechanisms that confer stress tolerance in senna, particularly drought resistance. Fourthly, the relationship between the rate of net photosynthesis and the leaf sennoside concentration under specific treatment and environmental variables was revealed.

Site-specific field research should develop methods to use short term drought and nitrogen stress for increasing sennoside yield $ha^{-1}$. However, the test component technologies must be designed essentially in the context of the positive correlation between the total leaf biomass and the sennoside yield per plant. Furthermore, when the long term drought in the field is expected to cause significant reduction in leaf biomass, plant population density would be an important factorial treatment in these investigations.

Foliar nitrogen spray, deflowering, harvesting of young leaves and ratooning should each be tested in combination with other specific factors that interact with these individual component technologies. For instance, foliar nitrogen spray can benefit only if the biomass production capacity of the crop is not hindered by

other environmental stresses (e.g. light and moisture constraints). Deflowering and harvesting of young leaves which are both high labour- and time-intensive crop cultural practices, must be assessed together, and against the cost of their adoption. Furthermore, loss of pods and seeds, a form of harvest and planting materials, respectively, should be considered in comparison to the yield advantages of deflowering. Given the high promise of deflowering for increasing sennoside yield $ha^{-1}$, research to produce and test the use of a non-flowering cultivar of senna would be interesting, as well. The gains of ratooning should be evaluated against the returns of alternative use of the farm space in the next cropping season. The differences in the patterns of diurnal fluctuation of sennoside concentrations between the flowering and deflowered plants suggests the need for studying harvesting time of the day under the specific conditions of the given cropping system.

A multitude of physiological and developmental responses collectively confer the high degree of drought resistance in senna. Tight stomatal regulation of gas exchange, highly elastic leaf chlorophyll content and net photosynthetic rate, reduction of transpirational surface area that greatly minimizes the stomatal count per plant, shunting of resources from the lower shedding leaves to the marginal top leaf mass, very high proportional allocation of root biomass into a deep tap root, and the characteristics that would mean efficient heat balance such as paraheliotropic leaflet movement and leaf surface with specific patterns of incidence of stomata, trichomes and wax deposits are important attributes in this regard.

The treatments that suppress net photosynthesis tend to increase the leaf sennoside concentration. Thus, investigations on the effect of the use of

photosynthesis-suppressants such as antitranspirants and abscisic acid on leaf sennoside content, at the optimum leaf biomass, would be interesting. In my research however, with the exception of deflowering, the treatments that suppressed net photosynthesis (e.g. drought, nitrogen stress, ratoon vs. seedlings) reduced the leaf biomass, whereby the sennoside yield per plant, the most immediate component of the sennoside yield $ha^{-1}$. Thus, judicious crop management that would permit the correct degree of stress-induced suppression of photosynthetic primary metabolism without significantly decreasing the leaf biomass is important to sustain high sennoside yields.

**LITERATURE CITED**

## Chapter 1

Al-Dakan, A. A., M. Al-Tuffail and M. A. Hannan. 1995. *Cassia senna* inhibits mutagenic activities of benzo[a]-pyrene, aflatoxin $B_1$, shamma and methyl methanesulfonate. *Pharmacology and toxicology* 77:288-292.

Atzorn, R., E. W. Weiler and M. H. Zenk. 1981. Formation and distribution of sennosides in *Cassia angustifolia*, as determined by a sensitive and specific radioimmunoassay. *Planta Medica* 41:1-14.

Bernath, J. 1986. Production ecology of secondary plant products. *In* Herbs, spices, and medicinal plants: Recent advances in botany, horticulture, and pharmacology I. Oryx Press. Arizona. 185-190.

Cameron, J. C. 1992. Constipation related to narcotic therapy, a protocol for nurses and patients. *Cancer Nurs.* 15:372-377.

Chapin, F. S., A. J. Bloom, C. B. Field and R. H. Waring. 1987. Plant responses to multiple environmental factors. *Bio Science* 37:49-57.

Cohen, J. 1977. Statistical power analysis for the behavioral sciences. Academic Press, NY.

Department of Agriculture Sri Lanka (DASL). 1990. Climatological data. Land Use Division. Peradeniya.

Farnsworth, N. R. 1973. *In* Phytochemistry Vol. III. (Ed. Miller, L. P.) Van Nonrand Reinhold Comp., NY.

Feldman, D. S., Jr. and J. Gagnon. 1986. StatView. Abacus, Inc..

Folkard, C. 1995. Encyclopedia of herbs and their uses. Herb Society of America. Dorling Kindersley Publishing Inc. 352.

Geiger, D. R. and J. C. Servaites. 1991. Carbon allocation and response to stress. *In* Response of plants to multiple stresses (Eds. Mooney, H. A., W. E. Winner and E. J. Pell) Academic Press Inc. 103-127.

Gupta, R. 1980. Medicinal and aromatic plants. *In* Handbook of agriculture. Ed. Jaiswal, P. L. Indian Council of Agricultural Research. New Delhi. 1196-97.

Gupta, R., S. K. Pareek, V. K. Srivastava, S. Singh and M. L. Maheshwari. 1982. Recent advances in improvement of senna. *In* Proc. National Seminar on Med. and Arom. Plants (Eds. Muthukrishanan, C. R., S. Muthuswami and J. B. M. Abdul Khader).

Henry, L. T. and C. D. Raper. 1991. Soluble carbohydrate allocation to roots, photosynthetic rate of leaves, and nitrate assimilation as affected by nitrogen stress and irradiance. *Bot. Gaz.* 152:23-33.

Husain, A., J. R. Sharma., H. S. Puri and B. R. Tayagi. 1984. Genetic resources of important medicinal and aromatic plants plants in South Asia. IBPGR. Rome. 6-24.

Irwin, H. S. and R. C. Barneby. 1982. The American Cassiinae. A synoptical revision of Leguminosae tribe Cassieae subtribe Cassiinae in the new world. Memoirs of the New York Botanical Garden 35:481-482.

Kalyanasundaram, N. K., D. R. Amin, A. I. Patel and K. C. Dalal. 1980. Effect of nitrogen and phosphorus on yield and quality of senna leaflets. *Ind. J. Pharm. Sci.* 43:100-102.

Lemli, J. 1986. The chemistry of senna. *Fitoterapiya* 57:33-40.

Marquard, R. D. and J. L. Tipton. 1987. Relationship between extractable chlorophyll and an in situ method to estimate leaf greenness. *HortScience* 22:1327.

Pahor, M., A. Mugelli, J. M. Guralnik, A. Manto, L. Carosella, A. Sgadari and P. U. Carbonin. 1995. Age and laxative use in hospitalized patients. *Aging Milano* 7:128-135.

Pareek, S. K., V. K. Srivastava, M. L. Maheshwari, S. Mandal and R. Gupta. 1983. Investigation in agronomic parameters of senna (*Cassia angustifolia* Vahl) as grown in north western India. *Int. Nat. J. Trop. Agric.* 1:139-144.

Perry, L. M. and J. Matzger. 1980. Medicinal plants of East and South-east Asia MIT Press. Cambridge.

Rascio, A., M. C. Cedola, M. Topani, Z. Flagella and Z. Wittmer. 1990. Leaf morphology and water status changes in *Triticum durum* under water stress. *Physiol. Plant.* 78:462-467.

Robert, W. P., O. Bjorkman, M. M. Caldwell, J. E. Keeley, R. K. Monson and B. R. Strain. 1987. Carbon gain by plants in natural environments. *Bio Science* 37:21-25.

Smith, W. K. and P. S. Nobel. 1978. Influence of irradiation, soil water potential and leaf temperature on leaf morphology of a dessert broadleaf, *Encelia jarmosa* Gray (Compositae). *Am. J. Bot.* 65:429-432.

Weerasuriya, S. M. and D. T. Kincaid, 1998. Photosynthesis of wild *Ricinus communis* in contrasting biomes of Sri Lanka. *Bull. Torrey Bot. Club* In review.

Wintermans, J. F. G. M. and A. De Mots. 1965. Spectrophotometric characteristics of chlorophylls and their pheophytins in ethanol. *Biochim. Biophys. Acta* 109:448-453.

Witkowski, E. T. F. and B. B. Lamont. 1991. Leaf specific mass confounds leaf density and thickness. *Oecologia* 88:486-493.

Ziegenhagen, D. J., E. Zehnter, W. Tacke, T. Gheorghiu and W. Kruis. 1992. Senna vs. bisacodyl in addition to Golytely lavage for colonoscopy preparation - a prospective randomized test. *Z-Gastroenterol* 30:17-19.

de Soyza, A. G., A. C. Franco, R. A. Virginia, J. F. Reynolds and W. G. Whitford. 1996. Effects of plant size on photosynthesis and water relations in the desert shrub *Prosopis glandulosa* (Fabaceae). *Am. J. Bot.* 83:99-105.

Ehleringer, J. and I. Forseth. 1980. Solar tracking by plants. *Science* 210:1094-1098.

Ehleringer, J. and O. Björkman. 1978. Pubescence and leaf spectral characteristics in a desert shrub *Encelia farinosa*. *Oecologia* (Berl.) 36:151-162.

Fay, P. A. and A. K. Knapp. 1993. Photosynthetic and stomatal responses of *Avena sativa* (Poaceae) to a variable light environment. *Am. J. Bot.* 80:1369-1373.

Fitter, A. H. and R. K. M. Hay. 1987. Environmental physiology of plants. Academic Press, NY.

Forseth, I. N. and J. R. Ehleringer. 1983. Ecophysiology of two solar tracking desert winter annuals. IV. Effect of leaf orientation on calculated daily carbon gain and water use efficiency. *Oecologia* 58:10-18.

Freckman, D. W. and R. A. Virginia. 1989. Plant-feeding nematodes in deep-rooting desert ecosystems. *Ecology* 70:1665-1678.

Kao, W, -Y. and I. N. Forseth. 1991. The effects of nitrogen, light and water availability on tropic leaf movements in soybean (*Glycine max*). *Plant, Cell and Environment* 15:703-710.

Koch, M. S. and P. S. Rawlik. 1993. Transpiration and stomatal conductance of two wetland macrophytes (*Cladium jamaicense* and *Typha domingensis*) in the subtropical everglades. *Am. J. Bot.* 80:1146-1154.

Marquard, R. D. and J. L. Tipton. 1987. Relationship between extractable chlorophyll and an *in situ* method to estimate leaf greenness. *HortScience* 22:1327.

Martin, E. S., M. E. Donkin and R. A. Stevens. 1983. Stomata. pp. 10-14. Edward Arnold Ltd. London.

Mooney, H. A. 1980. Photosynthetic capacity of populations of *Heliotropium curassavicum* L. originating from different thermal regimes. *Oecologia* (Berl.) 45:372-376.

Smith, M., Y. Wu and O. Green. 1993. Effect of light and water-stress on photosynthesis and biomass production in *Boltonia decurrens* (Asteraceae) a threatened species. *Am. J. Bot.* 80:859-864.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry, 2nd Ed. W. H. Freeman and Company, New York.

Wong, S. C., I. R. Cowan and G. D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* 282:424-426.

## Chapter 3

Bolhàr-Nordenkampf, H. R. and G. Draxler. 1993. Functional leaf anatomy. *In* Photosynthesis and production in a changing environment: a field and laboratory manual, Eds. Hall, D. O., J. M. O. Scurlock, H. R. Bolhàr-Nordenkampf, R. C. Leegood and S. P. Long. Chapman and Hall Publishing Co. London. 91-111.

Bunce, J. A. 1988. Differential responses of photosynthesis to water stress in three soybean cultivars. *Plant Physiol. Biochem.* 26:415-420.

Ellsworth, D. S. and P. B. Reich. 1996. Photosynthesis and leaf nitrogen in five amazonian tree species during early secondary succession. *Ecology* 77:581-594.

Fay, P. A. and A. K. Knapp. 1993. Photosynthetic and stomatal responses of *Avena sativa* (Poaceae) to a variable light environment. *Am. J. Bot.* 80:1369-1373.

Field, C. B. 1983. Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control of the program. *Oecologia* 56:341-347.

Field, C. B. and H. A. Mooney. 1986. The photosynthesis nitrogen relationship in wild plants. *In* Givnish TJ. Ed. *On the economy of plant form and function.* Cambridge Univ. Press. 25-55.

Foyer, C. H. 1987. The basis of source-sink interaction in leaves. *Plant Physiol. Biochem.* 25:649-657.

Foyer, C. H. 1988. Feedback inhibition of photosynthesis through source-sink regulation in leaves. *Plant Physiol. Biochem.* 26:483-492.

Henry, L. T. and C. D. Raper, Jr. 1985. Cyclic variations in nitrogen uptake rate in soybean plants. *Plant Physiol.* 78:320-322.

Hicks, D. R. and J. W. Pendleton. 1969. Effect of floral bud removal on performance of soybeans. *Crop Sci.* 9:435-437.

Ismande, J. and B. Touraine. 1994. N demand and the regulation of nitrate uptake. Update on mineral nutrition. *Plant Physiol.* 105:3-7.

Layne, D. R. and J. A. Flore. 1995. End-product inhibition of photosynthesis in *Prunus cerasus* L. in response to whole-plant source-sink manipulation. *J. Am. Soc. Hort. Sci.* 120:583-599.

Marquard, R. D. and J. L. Tipton. 1987. Relationship between extractable chlorophyll and an in situ method to estimate leaf greenness. *Hortscience* 22:1327.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd Ed. Academic Press. 250-253.

Micallef, B. J., K. A. Haskins, P. J. Vanderveer, K. S. Roh, C. K. Shewmaker and T. D. Sharkey. 1995. Altered photosynthesis, flowering, and fruiting in transgenic tomato plants that have an increased capacity for sucrose synthesis. *Planta* 196:327-334.

Olsthoorn, A. F. M., W. G. Keltjens, B. van Baren and M. C. G. Hopman. 1991. Influence of ammonium on fine root development and rhizosphere pH of Douglas-fir seedlings in sand. *Plant Soil* 133:75-81.

Radin, J. W. and J. S. Boyer. 1982. Control of leaf expansion by nitrogen nutrition in sunflower plants: role of hydraulic conductivity and turgor. *Plant Physiol.* 69:771-775.

Raper, C. D. and L. T. Henry, 1991. Soluble carbohydrate allocation to roots, photosynthetic rate of leaves, and nitrate assimilation as affected by nitrogen stress and irradiance. *Bot. Gaz.* 152:23-33.

Raper, C. D., Jr, D. L. Osmond, M. Wann and W. W. Weeks. 1978. Interdependence of root and shoot activities in determining nitrogen uptake rate of roots. *Bot. Gaz.* 139:289-284.

Schierenbeck, K. A. and J. D. Marshall. 1993. Seasonal and diurnal patterns of photosynthetic gas exchange for *Lonicera sempervirens* and *L. japonica* (Caprifoliaceae). *Am. J. Bot.* 80:1292-1299.

Stitt, M. and A. Krapp. 1995. An evaluation of direct and indirect mechanisms for the "sink-regulation" of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady-state transcript levels after cold-girdling source leaves. *Planta* 195:313-323.

von Caemmerer, S. and G. D. Farquhar. 1984. Effects of partial defoliation, changes of irradiance during growth, short-term water stress and growth at enhanced $p(CO_2)$ on the photosynthetic capacity of leaves of *Phaseolus vulgaris* L. *Planta* 160:320-329.

Wittenbach, V. A. 1983. Effect of pod removal on leaf photosynthesis and soluble protein composition of field grown soybean. *Plant Physiol.* 73:121-124.

Yadava, U. L. 1986. A rapid and nondestructive method to determine chlorophyll in intact leaves. *Hortscience* 21:1449-1450.

**Chapter 4**

Al-Dakan, A. A., M. Al-Tuffail and M. A. Hannan. 1995. Cassia senna inhibits mutagenic activities of benzo[a]-pyrene, aflatoxin $B_1$, shamma and methyl methanesulfonate. *Pharmacology and toxicology* 77:288-292.

Atzorn, R., E. W. Weiler and M. H. Zenk. 1981. Formation and distribution of sennosides in *Cassia angustifolia*, as determined by a sensitive and specific radioimmunoassay. *Planta Medica* 41:1-14.

Auterhoff, H. and F. C. Scherff. 1960. Die dianthrone der pharmazeutisch interessierenden hydroxyanthrachinone. *Arch. Pharmaz.* 293:918-925.

Bohnert, H. J., D. E. Nelson and R. G. Jensen. 1995. Adaptations to environmental stress. *Plant Cell* 7:1099-1111.

Chapin, F. S. 1991. Effects of multiple environmental stresses on nutrient availability and use. *In* Response of plants to multiple stresses. Ed. Mooney, H. A., W. A. Winner, E. J. Pell. Academic Press. 67-88.

Chapin, F. S., A. J. Bloom, C. B. Field and R. H. Waring. 1987. Plant responses to multiple environmental factors. *Bio Science* 37:49-57.

Gupta, R. 1984. Senna cultivation in India. *Indian Horticulture.* Jan-March 1984. 19-21.

Gupta, R., V. K. Srivastava and M. L. Maheshwari. 1977. *Indian J. Pharmacy* 39:109.

Harborne, J. B. and B. L. Turner. 1984. Plant chemotaxonomy. Academic Press. 166-167.

Labadie, R. P. 1970. The anthracene derivatives in *Rhamnus frangula* L. 1 The aglycones. *Pharm. Weekblad.* 105:189-195.

Lemli, J. 1985. The chemistry of senna. *Fitoterapia* 57:33-40.

Lohar, D. R., R. K. Bhatia, S. P. Garg and D. D. Chawan. 1979. Seasonal variation in the content of sennoside in senna leaves. *Pharmaceutisch Weekblad Scientific Edition.* 1:206-208.

McCue, K. F. and A. D. Hanson. 1990. Drought and salt tolerance:Towards understanding and application. *Trends Biotechnol.* 8:8:358-362.

Pareek, S. K., V. K. Srivastava, M. L. Maheshwari, S. Mandal and R. Gupta. 1983. Investigation in agronomic parameters of senna (*Cassia angustifolia* Vahl) as grown in north-western India. *Internat. J. Trop. Agric.* 1:139-144.

Sharma, H. K., K. S. Daiya and D. D. Chawan. 1980. *Pharmaceutisch Weekblad Scientific Edition* 2:573-575.

Skriver, K. and J. Mundy. 1990. Gene expression response to abscisic acid and osmotic stress. *Plant Cell* 2:503-512.

Srivastava, N. K. and R. Luthra. 1993. The relation between primary and secondary metabolism in peppermint under Fe-stress. *J. Essent. Oil Res.* 5:525-534.

Srivastava, V. K., M. L. Maheshwari, S. Sing and R. Gupta. 1981. Dynamics and localization of anthracene compounds during growth of senna plants, *Cassia angustifolia* Vahl. *Herba Hungarica* 20:1-2.

Stuppner, H. and S. Sturm. 1996. LC-MS and CZE of dianthrones from *Cassia angustifolia* and *acutifolia*. *Chromatographia* 42:697-703.

Suman, N., P. Sewark and R. Safar. 1990. Techniques for isolation of sennoside A and B from the leaflets of *Cassia angustifolia* Vahl. *Indian Drugs* 27:427-428.

Vickery, M. L. and B. Vickery. 1981. Secondary Plant metabolites. Macmillan Press Ltd. 88-105.

## Chapter 5

Berry, J. and O. Björkman. 1980. Photosynthetic response and adaptation to temperature in higher plants. *Ann. Rev. Plant Physiol.* 31:491-543.

Bitterlich, I. and M. K. Upadhyaya. 1989. Leaf surface ultrastructure and susceptibility to ammonium nitrate injury. *Can. J. Bot.* 68:1911-1915.

Bolhàr-Nordenkampf, H. R. and G. Draxler. 1993. Functional leaf anatomy. *In* Photosynthesis and production in a changing environment: a field and laboratory manual, Eds. Hall, D. O., J. M. O. Scurlock, H. R. Bolhàr-Nordenkampf, R. C. Leegood and S. P. Long. Chapman and Hall Publishing Co. London. 91-111.

Bondada, B. R., D. M. Oosterhuis, J. B. Murphy and K. S. Kim. 1996. Effect of water stress on the epicuticular wax composition and ultrastructure of cotton (*Gossypium hirsutum* L.) leaf, bract, and boll. *Environ. Exp. Bot.* 36:61-69.

Ehleringer, J. R. and H. A. Mooney. 1978. Leaf hairs: effects on physiological activity and adaptive value to a desert shrub. *Oecologia* 37:183-200.

Fetene, M., H. S. J. Lee and U. Lüttge. 1990. Photosynthetic aclimation in a terrestrial CAM bromeliad, *Bromelia humilis* Jacq. *New Phytol.* 114:399-406.

Forseth, I. N. and J. R. Ehleringer. 1983. Ecophysiology of two solar tracking desert annuals. IV. Effects of leaf orientation on calculated daily carbon gain and water use efficiency. *Oecologia* 58:10-18.

Gates, D. M. 1980. Biophysical Ecology. Springer Verlag Publishing Co., Berlin.

Kutík, J. and E. Bergmannová. 1991. Quantitative comparison of chloroplast ultrastructure in mesophyll cells of Triazine resistant and Triazine sensitive *Amaranthus retroflexus* L. plants. *Biologia Plantarum* (Praha) 33:510-512.

Mulroy, T. W. 1979. Spectral properties of highly glaucous and non-glaucous leaves of a succulent rosette-plant. *Oecologia* 38:349-357.

O'Brien, T. P., N. Feder and M. E. McCully. 1964. Polychromatic staining of plant cell walls by Toludine Blue O. *Protoplasma* 54:367-373.

Powels, S. B. 1984. Photoinhibition of photosynthesis induced by visible light. *Ann. Rev. Plant Physiol.* 35:15-44.

Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* 17:208-212.

Ricotta, J. A. and J. B. Masiunas. 1992. Relationship of leaf surface characteristics to Acifluorfen tolerance in Tomato (*Lycopersicon esculentum*) and related species. *Weed Science* 40:402-407.

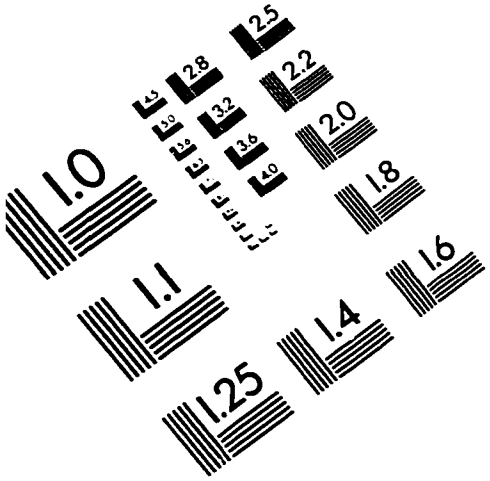
Ristic, Z. and D. D. Cass. 1992. Chloroplast ultrastructure after water and high-temperature stress in two lines of maize that differ in endogenous levels of abscisic acid. *Int. J. Plant Sci.* 153:186-196.

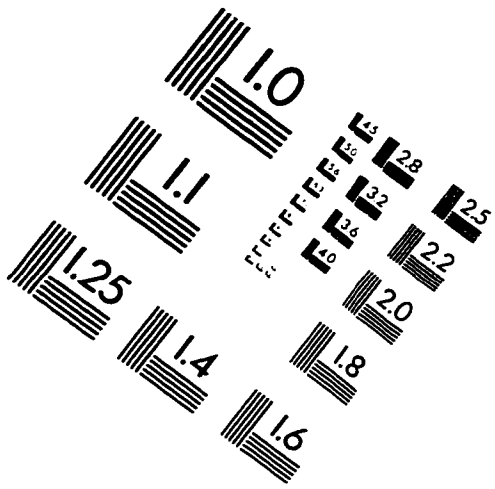
Spurr, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrast. Res.* 26:311.

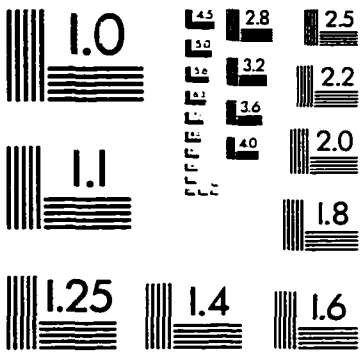
Stampak, J. G. and R. T. Ward. 1964. An improved method for electron microscopy. *J. Cell Biol.* 22:697-701.

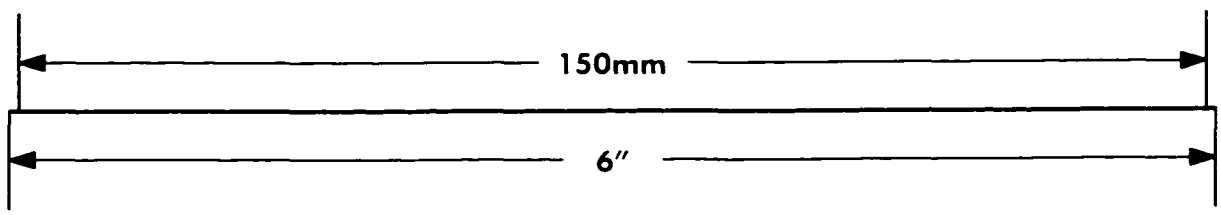
Upadhyaya, M. K. and N. H. Furness. 1994. Influence of light intensity and water stress on leaf surface characteristics of *Cynoglossum officinale*, *Centaurea* spp., and *Tragopogon* spp. *Can. J. Bot.* 72:1379-1386.

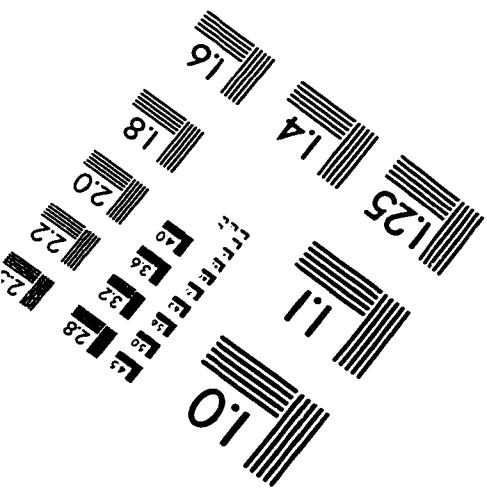
# IMAGE EVALUATION TEST TARGET (QA-3)

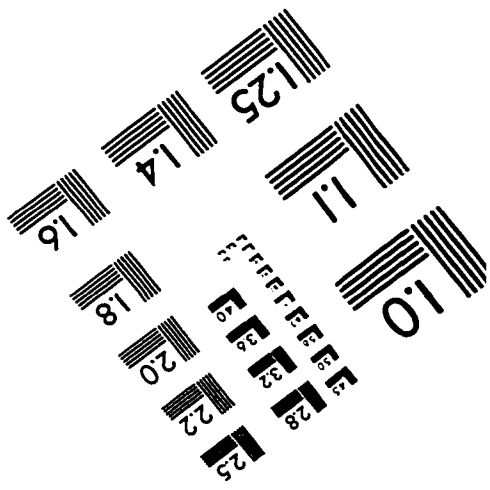
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