Cotton responses to simulated insect damage: light-use efficiency, canopy architecture and leaf nitrogen content as affected by loss of reproductive organs

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Running title: light-use efficiency in damaged cotton

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INTRODUCTION

Herbivory induces a wide range of morphological and physiological plant responses including changes in photosynthetic activity (Karban & Myers 1989; Trumble, Kolodny-Hirsh & Ting 1993; Rosenthal & Kotanen 1994). Responses of leaf and plant photosynthesis to arthropod herbivory have been mostly investigated in defoliated plants but also in plants damaged by mesophyll feeders, gall formers, phloem feeders, stem borers and root feeders (Welter 1989). Photosynthetic responses to selective damage affecting reproductive structures have been largely neglected.

In cotton crops, key pests (Lepidoptera, Hemiptera) feed preferentially on flowerbuds and young fruits (Hearn & Fitt 1992). Fruiting structures usually shed after damage and manual fruit removal induces many of the plant responses observed after insect attack (Brook et al. 1992; Sadras 1995). The effect of this type of damage on photosynthesis is unknown but it has been speculated that photosynthetic efficiency may increase in response to fruit loss (Sadras 1995). The rationale underlying this hypothesis is that loss of reproductive organs that delays fruit set could lead to: (a) an increase in root growth and nitrogen uptake; (b) an increase in leaf growth and nitrogen reduction, and (c) a delay in (or reduced rate of) leaf nitrogen depletion associated with fruit growth. Since leaf (Evans 1989) and canopy photosynthesis (Sinclair & Horie 1989) are closely linked to leaf nitrogen content, higher levels of leaf nitrogen resulting from more assimilation and less depletion could increase the photosynthetic capacity of defruited crops in relation to undamaged controls. More main-stem nodes and longer internodes typical of defruited cotton (e.g. Dale 1959) may generate changes in canopy structure and light distribution that could also affect crop photosynthetic efficiency (Niklas 1988, Takenaka 1994).

Most measurements of photosynthesis in studies of plant responses to herbivory have been conducted on single leaves and the problems of scaling up to canopies or natural stands are complex (Trumble *et al.* 1993). Light-use efficiency (ε = g dry matter per MJ of photosynthetically active radiation intercepted by the canopy) has been successfully used to quantify photosynthesis of crops (Monteith 1994) and natural plant communities (Runyon *et al.* 1994). To test the hypothesis that cotton photosynthetic efficiency increases after reproductive damage, light-use efficiency of crops in which flowerbuds and young fruits have been

Experiment 2

Crops of cv. Siokra V-15 (okra-leaf type) were sown on October 12, 1994. Plants grew under conditions of high availability of resources similar to those in exp. 1, i.e. low density (5 m^{-2}) and high fertility (120 kg N ha^{-1}). Two treatments were compared: undisturbed controls (C) and damaged (D) crops in which all flowerbuds and young fruits were removed at 85 DAS. This was a less severe treatment than the one applied in exp. 1 in which all reproductive organs were removed during a three-week period. Treatments were laid-out in a randomised block design with four replicates and standard ANOVA was used for the analysis of response variables. Plot dimensions were similar to those in exp. 1.

In both experiments, every time that D plants were defruited, control plants were handled likewise to reproduce thigmomorphogenetic effects associated with the damage treatment (Braam 1993).

Measurements

Plant samples (shoot + tap root) were taken from random sections in the central row on each plot nine times between 56 and 161 DAS in exp. 1 and six times between 93 and 169 DAS in exp. 2 (no. plants per sample ≥ 4). Plant parts were separated and weighed after drying to constant weight. Green leaf area of two whole plants was measured with a leaf area meter to estimate specific leaf weight, and this variable used to estimate plant leaf area of the sample from leaf biomass values. Milled leaf laminae were analysed for nitrogen using a near-infrared protein analyser calibrated as in Rochester et al. (1993).

The fraction of photosynthetically active radiation (PAR, 0.4-0.7 μm) intercepted by the canopy at noon (Qn) was obtained from measurements with a ceptometer (Decagon, Delta-T Devices Ltd, Cambridge) at the top (PAR_t) and bottom of the canopy (PAR_b), i.e. $Qn = 1-(PAR_b/PAR_t)$. Measurements were taken at approximately weekly intervals.

In exp. 1, lengths of mainstem internodes were measured in three mature plants of CS7S using the procedure of Lang (1973). In exp. 2, fruiting-branch angles (with respect to the vertical) and fruit fresh weight were measured in control plants at 107 DAS.

coefficients were also calculated for crops of cv. DP5690 and a near-isogenic line protected by *Bacillus thuringiensis* insecticidal proteins (Llewellyn *et al.* 1994). Leaf area and light transmission were measured at 177 DAS using the same procedures explained before and k was calculated as ln (1-Q)/LAI. Since these crops were exposed to severe pressure by *Helicoverpa spp* after 120 DAS (C. Mares pers. comm.), differences in k can be attributed to damage caused by naturally occurring larvae.

RESULTS AND DISCUSSION

Seasonal growth, PAR interception, and light-use efficiency

In Exp. 1, growing conditions and damage treatments significantly affected dry matter production and the amount of PAR intercepted by the crops (both $P<0.0001$; Table 1). No effect of cultivar was found on dry matter production ($P>0.75$) or PAR interception ($P>0.28$). No interactions between cultivar and other variables were found either.

Control crops under high availability of resources produced 1.7 times more biomass and intercepted 1.5 more PAR than crops grown under low availability of resources. Thus, differences in seasonal growth between these crops were associated with differences in both light interception and ε (Table 1).

Under favourable growing conditions, defruited crops produced 1.6 times more dry matter and intercepted only 10% more light than control crops. Under low availability of resources, damage did not change either dry matter production or light interception. Overall, these data show that removal of fruiting structures increased seasonal ε in crops grown under high but not under low availability of resources.

Exp. 2 confirmed that selective damage affecting reproductive structures may increase light-use efficiency of crops grown under favourable conditions: with the same amount of PAR intercepted, damaged crops produced almost 20% more dry matter than undamaged controls ($P = 0.09$; Table 2). The increase in ε due to damage in Exp. 2 (ca. 20%; Table 2) was less than the increase observed in Exp. 1 (27%; Table 1) probably because damage was less severe in the second experiment (see Methods).

canopy photosynthesis reported in the literature (Peng & Krieg 1991; Wullschleger & Oosterhuis 1990a) support that, in the present study, changes in light-use efficiency calculated on a dry-matter basis can be considered to reflect true changes in canopy photosynthetic capacity.

Leaf nitrogen

Leaf nitrogen concentration (LNC) declined with plant age in all treatments ($P<0.0001$, Fig. 2). Under favourable growing conditions, the rate of decline in LNC of damaged crops was slower (cv CS7S) or similar (cv Siokra S324) than that in undisturbed controls. Under low availability of resources, LNC after ca. 90 DAS declined faster in damaged crops than in undamaged controls (growing conditions x damage treatment interaction was significant at $P<0.0001$).

Since leaves are a major source of nitrogen for growing fruits, it was expected that flowerbud removal and delay of fruit growth could delay leaf nitrogen depletion and hence contribute to the maintenance of higher LNC in damaged crops in relation to controls (Sadras 1995). This premise, however, does not consider variations in LNC with ontogeny that are the result of leaf dry matter being accumulated faster than nitrogen (e.g. Crowther 1941). Thus, changes in LNC during the season result not only from a "depletion" effect but also from a "dilution" effect that is specially important when vegetative growth is dominant. To separate these effects, LNC was plotted against the total amount of nitrogen in the foliage (Fig. 3). For crops grown under high availability of resources, a clear pattern emerged that had two phases. First, LNC declined with time despite net gains in total leaf nitrogen. The end of this phase, in which LNC changes could be attributed to the dilution effect, coincided with the initiation of active fruit growth (cf. Fig. 1 for the definition of fruit growth periods). In the second phase, both LNC and total nitrogen declined, indicating that the changes in LNC were mostly associated with depletion of leaf nitrogen. Fig. 3 shows that a major effect of damage was to extend the duration of the dilution phase by delaying fruit growth. The relative magnitude of the dilution and depletion effects underlies the patterns of change in LNC shown in Fig. 2. For CS7S, depletion of nitrogen from leaves in control crops after 80 DAS changed LNC faster than the dilution of nitrogen in damaged crops which continued vegetative growth for a longer period, leading to the expected greater LNC in damaged crops. In contrast, both dilution and depletion affected similarly the availability of resources decreased k, probably reflecting the effect of leaf size on light penetration in the canopy as found by Wullschleger & Oosterhuis (1990a) in nitrogen-stressed cotton.

Damaged crops also had a lower coefficient of extinction in Exp. 2 (Table 2). Again, the magnitude of this difference was less than that observed in Exp. 1 (Fig. 5) in which crops were more severely damaged. Crops damaged by *Helicoverpa spp.* also had lower extinction coefficients than crops of transgenic cotton protected by insecticidal proteins of *Bacillus thuringiensis* (Table 3).

Since simulated fruit removal (Fig. 5, Table 2) and natural damage by *Helicoverpa spp.* larvae (Table 3) consistently decreased k, it is of interest to analyse both the causes underlying this effect and its consequences on light-use efficiency. Two changes in plant structure have been found that may underly the lower k of damaged crops. First, top internodes were longer in damaged plants than in controls, as illustrated in Fig. 6. Differences in height between normal (damaged) and transgenic (control) crops are a reflection of changes in internode length and/or number (Table 3). Changes in internode length, internode number and plant height are consistent responses of cotton to loss of flowerbuds (e.g. Dale 1959; Sadras 1995) that may affect light penetration in the canopy (Niklas 1988, Takenaka 1994). Second, changes in k may be associated with the weight of fruits that bends fruiting branches generating a more horizontal leaf distribution (Marani & Ephrath 1985) and hence reducing light penetration in control canopies relative to defruited ones. Measurements taken in Exp. 2 showed a substantial effect of fruit load on fruiting-branch angle: it varied from ca. 60° for branches with no fruit to about 90° for branches with heavy fruit load. Fruit weight accounted for 39% of the variation in branch angle ($P<0.0001$). The correlation was improved when fruit weight per unit branch length was used as independent variable (Fig. 7).

In order to analyse whether the observed changes in k could account for part of the variation in light-use efficiency of crops grown under high availability of resources, we followed Charles-Edwards, Doley & Rimmington (1986) who proposed that a lineal relationship holds between

Although the underlying mechanisms need to be further explored, we can conclude that the observed increase in light-use efficiency was not simply the result of delayed canopy senescence and slower depletion of leaf nitrogen in comparison with undamaged controls. Rather, changes in plant and canopy structure and hence changes in distribution of light were important factors affecting canopy photosynthesis. More and longer internodes and branches which were more vertical likely contributed to better profiles of light distribution and enhanced light-use efficiency of damaged canopies. Three-dimensional plant models (Room et al 1993) could be used for more detailed analyses of the effects of damage on canopy structure. Further work is also necessary to determine whether changes in nitrogen partitioning at the plant and leaf levels may have influenced canopy photosynthesis. At the plant level, changes in the profile of leaf nitrogen distribution following changes in the pattern of light distribution need to be explored (Werger & Hirose 1991; Sadras, Hall & Connor 1993; Hikosaka, Terashima & Katoh 1994). At the leaf level, changes in partitioning between thylakoid proteins, Rubisco and storage proteins (Evans 1989, Staswick 1994) in response to flowerbud removal cannot be discarded.

Irrespective of the mechanisms involved, changes in canopy architecture and photosynthetic capacity should be considered when analysing compensatory growth after damage by pests that induce abscission of reproductive organs in cotton.

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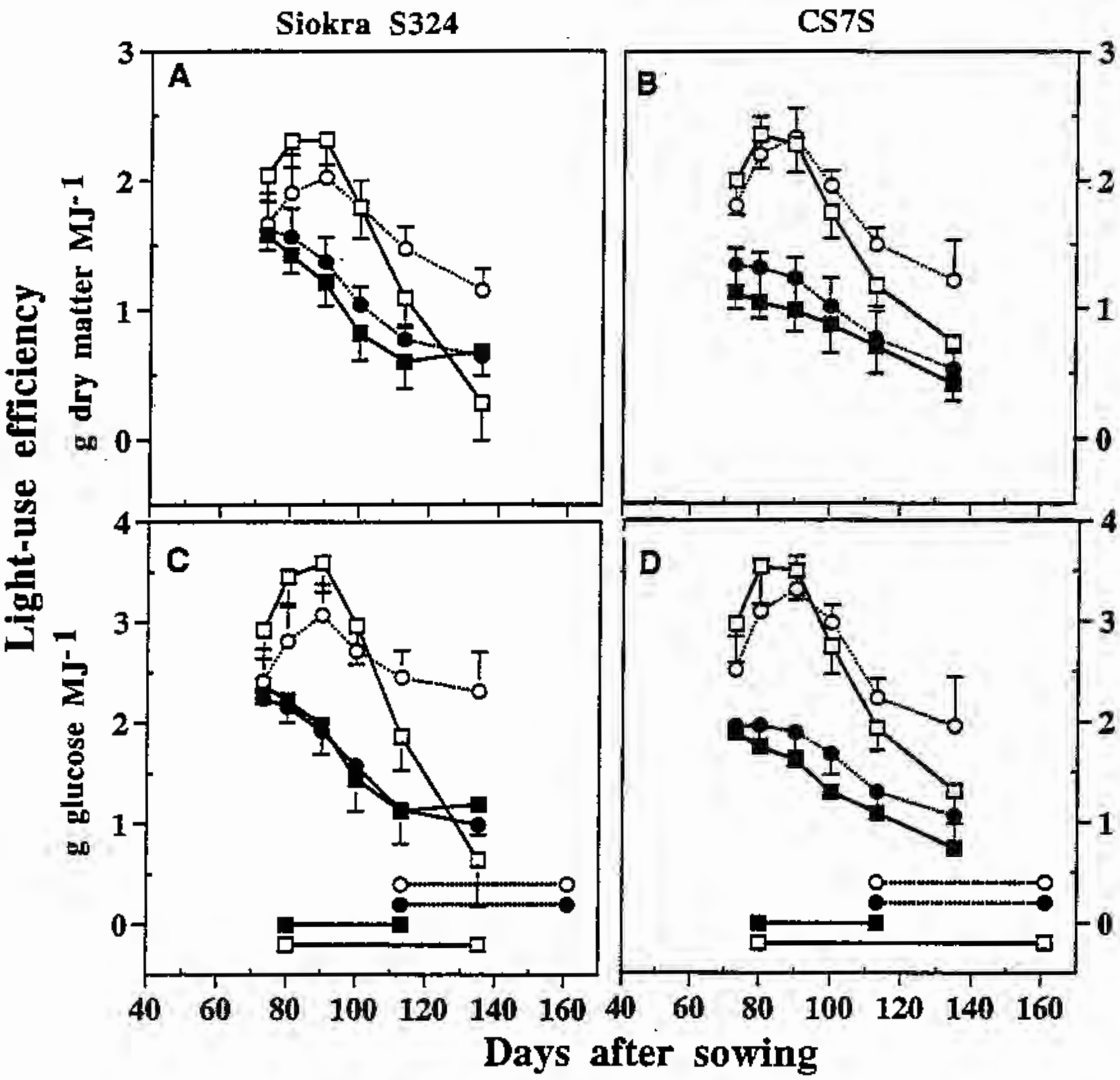
Table 1. Dry matter production, PAR interception and light-use efficiency of damaged and undisturbed cotton crops grown under contrasting availability of resources (period from 56 to 161 days after sowing, Exp. 1). Values are averages (± s.e.) across cultivars which behaved similarly (see text). Seasonal light-use efficiency is the slope of the regression between dry matter and cumulative PAR interception ($n = 64$, $r^2 \geq 0.92$, $P<0.0001$).

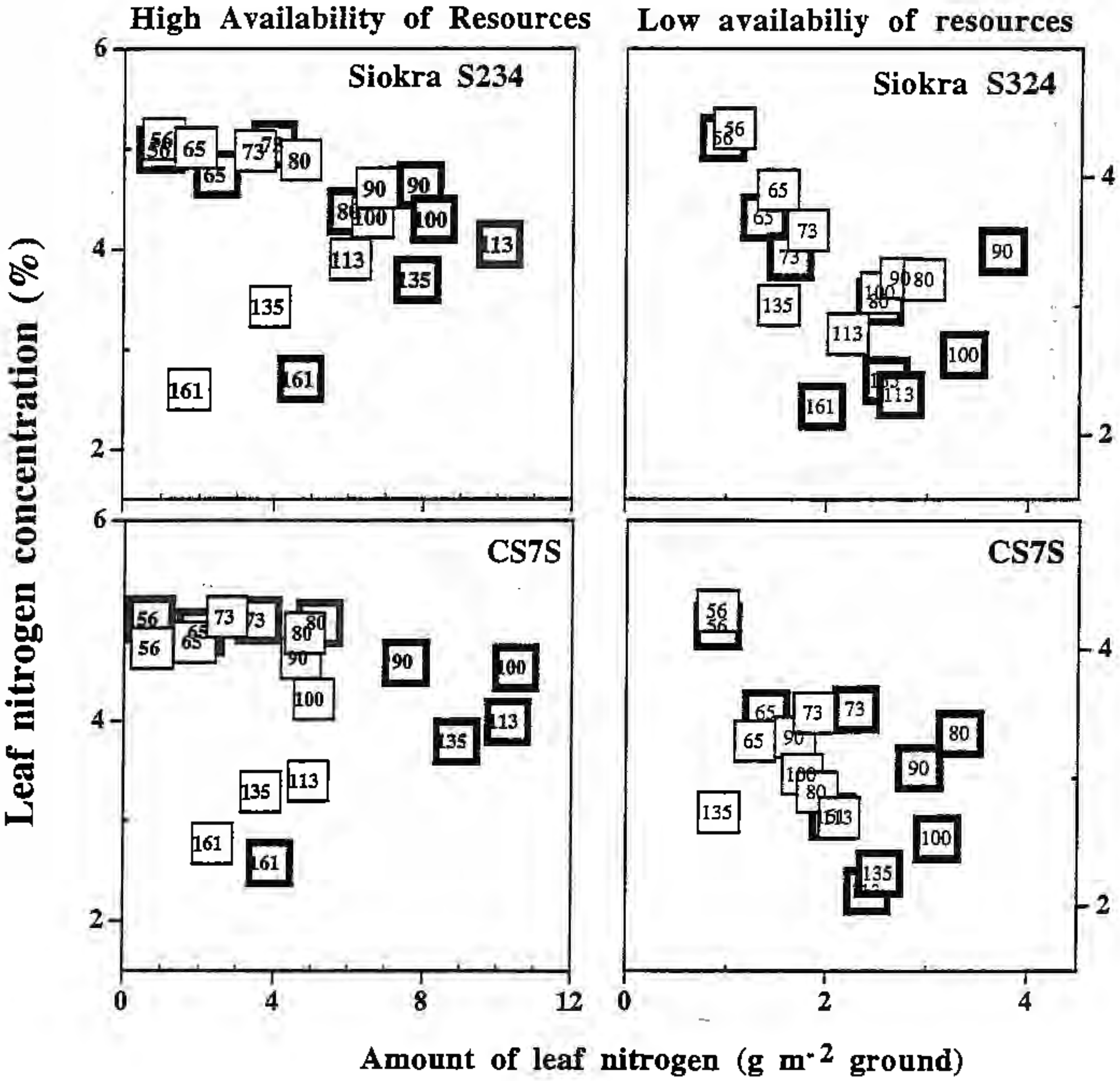
Treatment		Response variable		
damage	availability of resources	dry matter ($g\ m^{-2}$)	PAR interception ($MJ\ m^{-2}$)	light-use efficiency ($g\ MJ^{-1}$)
control	high	745 ± 63	722 ± 27	1.33 ± 0.049
damaged	high	1208 ± 88	798 ± 21	1.69 ± 0.037
control	low	444 ± 27	491 ± 53	1.19 ± 0.041
damaged	low	490 ± 33	491 ± 25	1.18 ± 0.036

Table 3. Comparison of transgenic and normal cotton crops (cv. DP5690) at 177 DAS. Values are averages ± s.e. (n=3).

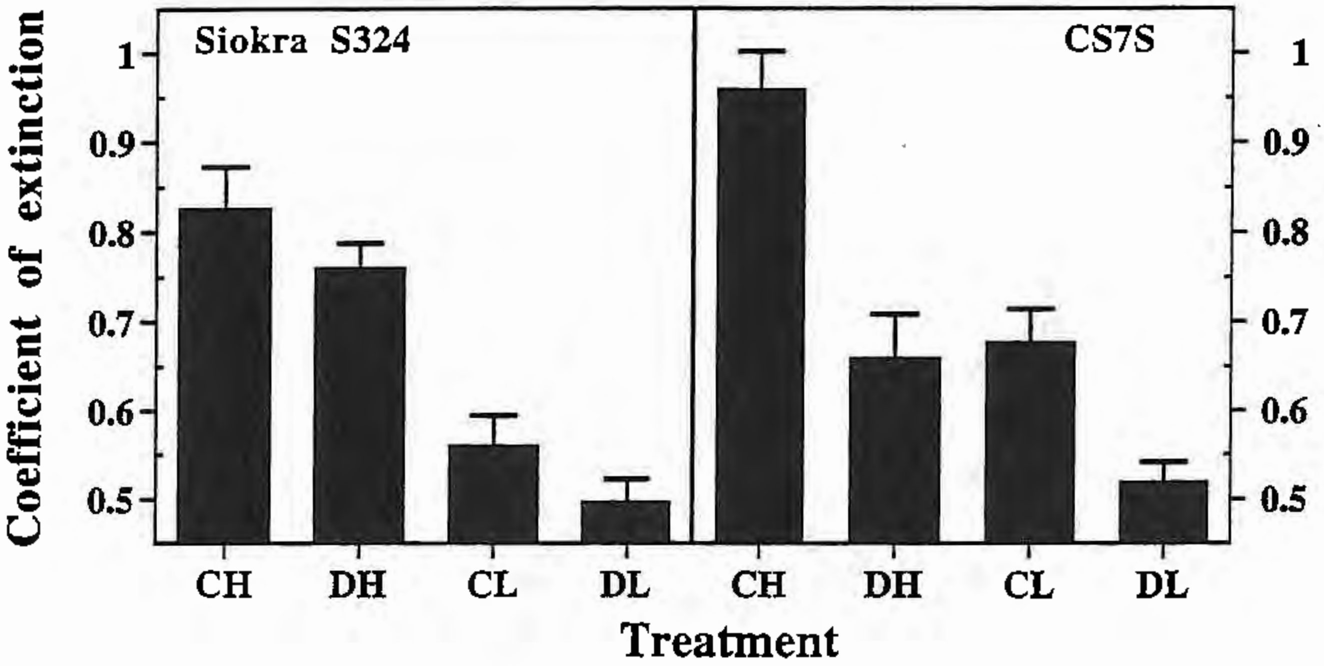
Variable	Transgenic (protected)	Normal (damaged)
Damage indicators:		
Number of fruit (plant^{-1})	202 ± 33	109 ± 8
Proportion of damaged fruit (%)	10 ± 7	71 ± 4
Canopy characteristics:		
Height (m)	0.98 ± 0.09	1.19 ± 0.01
Coefficient of extinction	0.70 ± 0.03	0.86 ± 0.09


Siokra S324
CS7S
Light-use efficiency
g dry matter MJ-1
g glucose MJ-1
A
B
C
D
3
2
1
0
4
3
2
1
0
40
60
80
100
120
140
160
Days after sowing

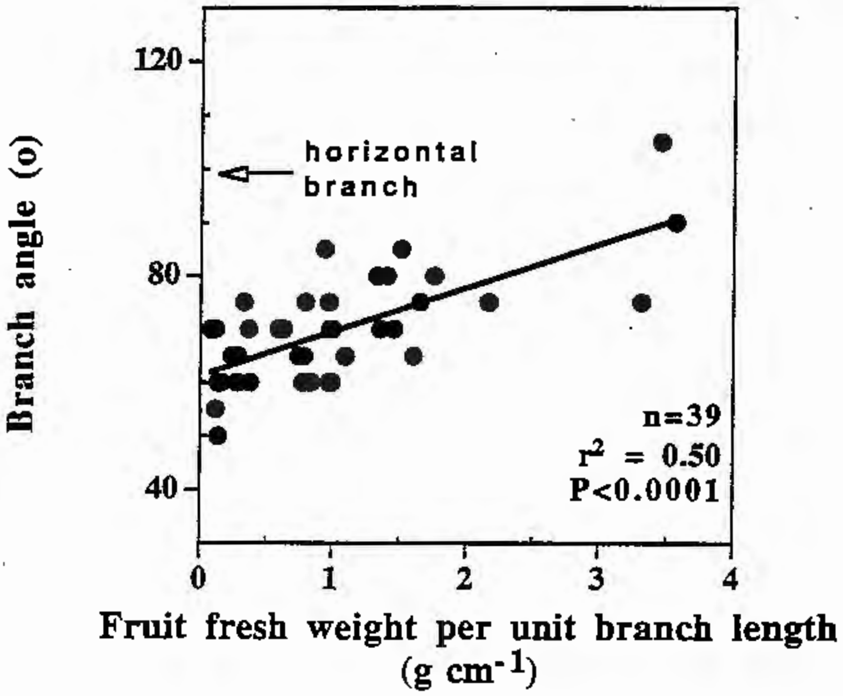
Sadras, FIG. 1


High Availability of Resources
Low availabiliy of resources
Siokra S234
Siokra S324
CS7S
CS7S
Leaf nitrogen concentration (%)
Amount of leaf nitrogen (g m-2 ground)
6
4
2
0
4
8
12
0
2
4
56
65
73
80
90
100
113
135
161

Sadras, FIG 3


Siokra S324
CS7S
Coefficient of extinction
1
0.9
0.8
0.7
0.6
0.5
CH
DH
CL
DL
CH
DH
CL
DL
Treatment

Padras, Fig 5


120
80
40
Branch angle (o)
horizontal branch
n=39
$r^2 = 0.50$
$P<0.0001$
0
1
2
3
4
Fruit fresh weight per unit branch length
(g cm^{-1})

Sadras, FIG. 7