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1310 Soil and Atmosphere—Water Stress in Fava Beans

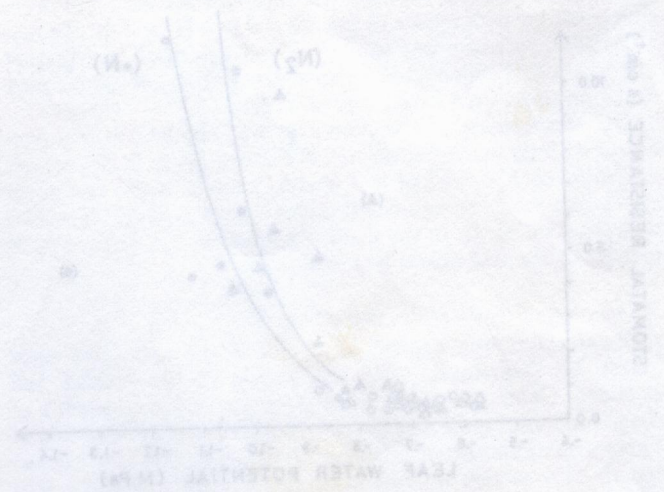


FIG. 1. Stomatal response in upper leaf lobes to diminishing leaf water potential. Each point represents a single leaf (O) +N control, (Δ) +N stressed. Curves were fitted using a program from Golden Software, Inc. Points which deviated from the fitting

increased to 2.63 and 2.54 cm² for +N and N-stressed plants, respectively.

No evidence for osmotic adjustment was found in leaves of stressed plants under these experimental conditions. On day 18, turgid leaf cells of +N and N-stressed plants had an osmotic potential of -0.65 and -0.64 MPa, respectively. In stressed +N and N-stressed plants, osmotic potentials were -0.69 and -0.66 MPa, respectively and were not significantly different ($P > 0.05$) from those of the controls.

After rewatering, the +N and N-stressed plants showed similar mid-photosynthetic potentials (P_{50}) to the controls within 20 h. At this time, the stomatal resistance of the leaves of +N-stressed plants had also decreased to control levels, while those of N-stressed plants only reached levels similar to those of the controls 3 to 4 d after rewatering (Table 1).

DISCUSSION

Fava bean control plants produced more dry matter under the +N treatment although these differences were less marked than for other legume species grown under controlled environment (Minguez and San, 1989; Ryle et al., 1983). On day 18 the shoot:root ratio of the N₂ plants was lower than the +N. This behaviour is similar to that described for *Lathyrus repens* (Ryle et al., 1981), but different to that found for indeterminate (*Cytisus* spp.) plants subjected to similar growth conditions (Minguez and San, 1989). In *G. max*, the higher production of dry matter by +N plants was almost entirely due to the larger root system of +N plants which, on the last sampling day (equivalent to day 20), was almost double that of the N₂ plants, excluding the nodules. Hence, their shoot:root ratio was significantly lower. Thus, in terms of dry matter accumulation by well-watered control plants, N₂ and G₂ may show contrasting responses due to the type of nitrogen nutrition.

[N₂] Fava bean plants decreased their shoot:root ratio in response to water stress later than +N plants during the first period of stress application (day 0 to day 18). However, as in *G. max* (Minguez and San, 1989), the plants seemed to be unable to maintain root growth in day 19 onwards, whereas by day 18 +N-stressed plants had re-allocated their dry matter allocation in favour of root production. The shoot:root ratios in which nodules have been excluded may allow the expression of dry matter allocation for its relation to root development for stressed and unstressed (Minguez and San, 1989).

+N and N₂ plants showed parallel responses in terms of leaf area, leaf transpiration and transpiration per unit leaf area since both control and stress treatments, although +N treatments generally had larger leaf areas. Leaf expansion stopped from day 13 in both stress treatments. However, in the +N plants a small increment in dry matter was maintained, producing an increase in specific leaf weight. The higher shoot weight of +N-stressed plants compared to N₂ plants on day 18 was due to the higher dry weight of stems (38.2%) petioles (8.2%)

TABLE 1. Recovery of +N and N₂ plants after 15 d of water stress as measured by mid-photosynthetic potential (P_{50}) and stomatal resistance (s_{50}) of upper leaf lobes

Days after rewatering	P_{50} (MPa)	s_{50} (s cm ⁻²)
0	0.72	0.48
1	0.71	0.47
2	0.70	0.46
3	0.69	0.45
4	0.68	0.44
5	0.67	0.43
6	0.66	0.42
7	0.65	0.41
8	0.64	0.40
9	0.63	0.39
10	0.62	0.38
11	0.61	0.37
12	0.60	0.36
13	0.59	0.35
14	0.58	0.34
15	0.57	0.33
16	0.56	0.32
17	0.55	0.31
18	0.54	0.30
19	0.53	0.29
20	0.52	0.28
21	0.51	0.27
22	0.50	0.26
23	0.49	0.25
24	0.48	0.24
25	0.47	0.23
26	0.46	0.22
27	0.45	0.21
28	0.44	0.20
29	0.43	0.19
30	0.42	0.18

The stressed plants spend only 0 hours measurements

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Response to Water Stress and Recovery of Nitrate-Fed and Nitrogen-Fixing Faba Bean

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Received 16 October 1989

ABSTRACT

Comparative studies of response mechanisms to progressive water stress were carried out in vegetative faba bean plants. These were grown under controlled environmental conditions and either received nitrate-N (+N) or were dependent on N₂-fixation (N₂). N₂-fixing plants reacted faster to water stress by increased root growth but were unable to maintain this response. +N plants showed a slower response but were able to maintain root and shoot growth throughout the treatment period. Leaf expansion was similarly affected in +N and N₂ plants but there was an increase of specific leaf weight in the former. In both +N and N₂ plants response mechanisms to water stress were aimed at postponing dehydration: they tolerated stress at high water potentials by maintaining water absorption with increased root growth and early stomatal closure. Recovery after watering the stressed plants was faster in +N plants. Stomatal resistance in leaves of N₂ plants attained similar values to those of control, non-stressed, plants after 68–82 h compared with only 20 h in +N plants.

Key words: Faba bean, water stress, nitrogen nutrition.

INTRODUCTION

In certain legume crops, nitrate-N or fixed-N nutrition leads to different patterns in plant development, e.g. soybean (*Glycine max* (L.) Merr. cvs Williams, Fiskeby), navy bean (*Phaseolus vulgaris* L. cv. Seafarer) and white clover (*Trifolium repens* L. cv. Blanca) (Cassman, Whitney, and Stockinger, 1980; Mínguez and Sau, 1989; Ryle, 1983; Ryle, Arnott, and Powell, 1981; Ryle, Arnott, Powell, and Gordon, 1983; Ryle, Powell, and Gordon, 1978). Soybean can also produce different responses to progressive water stress (Mínguez and Sau, 1989).

Stressed, nitrate-fed soybean plants had a relatively bigger root system, were able to adjust osmotically in upper leaves and showed lower threshold values of leaf water potential for stomatal closure. Nitrogen-fixing plants were more conservative in terms of water use and initiated stomatal closure at higher leaf water potentials and osmotic adjustment was delayed (Mínguez and Sau, 1989).

This paper compares the responses to progressively applied water stress of vegetative faba bean plants grown with nitrate-N (+N)- or dependent on nitrogen fixation

(N₂). The adaptation mechanisms studied, following the classification by Turner (1979, 1986), were those which allowed the plants either (a) to tolerate stress at high water potentials (maintenance of water absorption through an increase in root growth and stomatal resistance) or (b) to tolerate stress at low water potentials (maintenance of leaf turgor by osmotic adjustment). A short recovery treatment was also carried out during which the evolution of stomatal resistance was followed.

MATERIALS AND METHODS

Plant material and growth conditions

Vicia faba var. Alameda plants were grown in 3.0 dm³ pots filled with perlite in a controlled environment. Quantum irradiance at plant level was 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with an 11 h photoperiod. Temperature was maintained at 22–23 °C day/17–18 °C night and vapour pressure deficit (VPD) was 1.09 kPa day/0.3 kPa night.

The +N plants received a complete nutrient solution containing 15.7 mol m⁻³ nitrate. The N₂ plants were inoculated with *Rhizobium leguminosarum* strains VC-5 and RL 119 in peat inoculum (from the 'Rinconada' Experimental Station, Seville,

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Spain) and received nitrogen-free nutrient solution but were supplemented with nitrate to provide 1.4 mol N m^{-3} for the first 15 d. The procedure was similar to that described by Mínguez and Sau (1989) with soybean plants. The nutrient solutions were based on those used by Ryle *et al.* (1978).

The effectiveness of the symbiosis was checked in two pre-assays with the same treatments. N content (% N in dry matter estimated by Kjeldahl) of leaf blades, petioles, stems and roots was determined. No significant differences were found ($\alpha=0.05$) between treatments. In the assay presented in this paper only N content of the leaf blades was measured.

Application of water stress and recovery

Water stress was applied to plants in the vegetative stage 'phase 31' (Stupnagel, 1984), equivalent to the seventh leaf unfolded. Controls were watered every day at the beginning of the photoperiod until seepage occurred. During the first 12 d of water stress imposition, plants received water at a rate equivalent to 85% of that transpired on day 0. On the next 3 d they received the equivalent to 70% (days 13 to 15) and no water on the last 3 d (16 to 18). This followed the procedure described by Mínguez and Sau (1989).

On day 18, stressed plants were watered in excess at the end of the light period and were then watered every day until seepage for the next 4 d.

Plant determinations and water relations

Dry matter partitioning was evaluated on sampling days 0, 12 and 18 by determining the dry weights of roots, stems, petioles, and leaf blades. Leaf area was measured on each sampling day on a LI-COR model LI-3000 leaf area planimeter.

Stomatal resistance of leaves was determined on a Delta-T Mk. 3 automatic porometer. Measurements were carried out at mid-photoperiod on the last fully expanded leaves on days -1, 5, 11, 15 and 17 during water stress and on days 19, 20, 21 during recovery. On days -1, 11 and 17, measurements were taken on the same leaves chosen for mid-photoperiod leaf water potential evaluation on the next day.

Predawn (ψ_d) and mid-photoperiod (ψ_m) leaf water potentials were determined at each sampling date following the procedure of Mínguez and Sau (1989). Predawn and mid-photoperiod leaf water potentials in upper leaves were measured by the pressure chamber technique (Scholander, Hammel, Bradstreet, and Hemmingen, 1965).

Osmotic potential at full turgor (ψ_{so}) was determined in one of the leaflets from rehydrated leaves where mid-photoperiod water potential had been measured. Leaflets were frozen at -20°C , wrapped in aluminium foil. They were then thawed at a room temperature of 25°C for 1 h. Three filter paper discs were inserted between the leaflet and the aluminium foil and sap from the leaflet was expressed using a hydraulic press (Model J-14, Campbell, Utah, USA). Osmotic potential was then measured in two discs using a psychrometer (Model C52, Wescor Inc., Logan, Utah, USA) as a dew point hygrometer (Savage, Wiebe, and Cass, 1983) connected to a microvoltmeter (Model HR 33T Wescor). Equilibrium time was 7 min. If measurements differed by more than 0.05 MPa, the third disc was measured and the average value taken.

RESULTS

Leaf water potentials

Leaf water potentials at the beginning of the photoperiod, before watering the plants, (predawn ψ_d), and at mid-photoperiod (ψ_m) are shown in Table 1. Water stress

TABLE 1. *Effect of nitrogen and water stress on leaf water potentials (MPa) (predawn ψ_d and mid-photoperiod ψ_m)*

Sampling date	Day 0	Day 12	Day 18
+N Plants ψ_d			
Control	-0.32a	-0.35a	-0.37ac
Stressed	—	-0.42a	-0.54a
N ₂ Plants ψ_d			
Control	-0.30a	-0.36a	-0.34bc
Stressed	—	-0.34a	-0.49ac
+N plants ψ_m			
Control	-0.65a	-0.71a	-0.73a
Stressed	—	-0.77ab	-1.07b
N ₂ Plants ψ_m			
Control	-0.63a	-0.67a	-0.72a
Stressed	—	-0.85b	-0.95b

Values are means of five replicates. Means followed by the same letter within a column are not significantly different at 5% level of probability. These notations will be used in all subsequent tables.

lowered predawn and mid-photoperiod water potentials although these were only significantly different ($\alpha=0.05$) for ψ_m . On day 12, N₂-stressed plants had slightly lower ψ_m values than +N plants, but this trend was not maintained and by day 18 +N-stressed plants had lower ψ_m values.

Dry matter production, Shoot/root ratios

The effects of nitrogen supply and water stress on dry matter production of *V. faba* are shown in Table 2. Shoot dry matter production was highest in +N control plants but significant differences ($\alpha=0.05$) in shoot and root dry matter production between +N and N₂ control treatments only occurred on day 12. Under water stress conditions, roots of N₂ plants showed greater mass than the controls during the first 12 d, however, growth ceased between days 12 and 18. On the other hand root growth of +N-stressed plants was always less than controls, although dry matter production continued between days 12 to 18. Nodule growth was also affected by stress although there was an increase in nodule mass in the stressed plants between days 12 to 18—this increase was not statistically significant ($\alpha=0.05$).

Shoot growth was decreased by water stress for both +N and N₂ plants. However, total dry weights of N₂ plants was unaffected by 12 d stress, but was more affected than +N by 18 d stress.

N content in leaf blade dry matter was equivalent ($\alpha=0.05$) in all sampling dates and treatments: % N contents were 5.9 ± 0.2 in +N and 6.2 ± 0.2 in N₂.

When nodule dry weights were excluded, N₂ plants had a higher shoot/root ratio than +N plants on day 0 (Table 3). The shoot/root ratio of control N₂ plants did not change during the experiment, whilst that for control +N plants increased so that they had a significantly higher ratio by day 18. Under water stress conditions, shoot/root ratios of +N plants showed no response until

TABLE 2. Effect of nitrogen and water stress on shoot, root and nodule ($g\ plant^{-1}$)

Sampling time	Day 0			Day 12			Day 18		
	Shoot	Root	Nodule	Shoot	Root	Nodule	Shoot	Root	Nodule
+ N Plants									
Control	3.57a	2.04a	—	8.25a	3.96a	—	12.41a	4.78a	—
Stressed	—	—	—	6.30b	3.06b	—	8.42b	4.66a	—
N ₂ Plants									
Control	3.18a	1.43a	0.15	6.49b	3.15b	0.30a	10.50a	4.86a	0.48a
Stressed	—	—	—	5.85b	3.62ab	0.27a	6.15b	3.84a	0.32b

TABLE 3. Changes in shoot/root ratios as affected by nitrogen and water stress

Sampling day	Day 0	Day 12	Day 18
+ N Plants			
Control	1.84a	2.08a	2.64a
Stressed	—	2.08a	1.80b
N ₂ Plants			
Control	2.28b (2.03a) ^a	2.08a (1.88ab)	2.18c (1.96b)
Stressed	—	1.63b (1.50b)	1.64b (1.48c)

^aShoot/root + nodules.

day 18, whilst ratios of N₂ plants decreased on day 12 to a value which was maintained on day 18. The inclusion of nodule dry weights in the calculations of the ratios for the N₂ plants, although lowering their shoot/root values, did not alter these patterns.

Leaf area, specific leaf weight

The leaf area of N₂ control plants was smaller than that of the +N ones throughout the experiment, but differences were only significant on day 12 (Table 4). Water stress reduced the rate of increase in leaf area of both +N and N₂ plants by day 12 and stopped any increase between days 12 and 18. Similar specific leaf weights (average of 3.24 mg cm⁻²) were found in all treatments with the exception of +N stressed plants on day 18 when there was a significant increase of c. 0.6 mg cm⁻² (from 3.29 on day 12 to 3.87 mg cm⁻² on day 18).

Transpiration and transpiration efficiency

No significant differences were found between control +N and N₂ treatments in either transpiration per plant or

TABLE 4. Leaf area ($LA\ cm^2\ plant^{-1}$), transpired water per plant ($TT: g\ H_2O\ plant^{-1}\ d^{-1}$) and transpired water per unit leaf area ($T: g\ H_2O\ cm^{-2}\ d^{-1}$)

Sampling date	Day 0			Day 12			Day 18		
	LA	TT	T	LA	TT	T	LA	TT	T
+ N Plants									
Control	629.6a	182.3a	0.264a	1493a	255.1a	0.171a	2050a	349.0a	0.174a
Stressed	—	—	—	1166b	117.0b	0.101b	1158b	67.9b	0.061b
N ₂ Plants									
Control	646.9a	150.7a	0.239a	1235b	225.0a	0.185a	1699ab	324.0a	0.194a
Stressed	—	—	—	999c	103.0b	0.104b	1029b	63.5b	0.062b

per unit leaf area; the lower total transpiration of N₂ plants reflected the lower leaf areas. There was a similar decrease in both transpiration per plant and per unit area in +N and N₂-stressed plants (Table 4).

Differences in transpiration efficiency (TE: mg shoot dry matter g⁻¹ H₂O transpired; Tanner and Sinclair, 1983) were found during the 12 to 18 d period (Table 5). TE for stressed +N plants was higher than the controls, whilst that for N₂ plants was lower.

Stomatal resistance and osmotic adjustment

Changes in mid-photoperiod stomatal resistance, r_s , of upper leaves in relation to their water potentials are shown in Fig. 1. N₂ plants appeared to initiate stomatal closure at higher water potentials than +N, but these differences were not significant. On day 15 the mean value of r_s of upper leaves of +N and N₂-stressed plants was 1.53 and 4.63 s cm⁻¹, respectively. On day 17, these had

TABLE 5. Transpiration efficiencies of +N and N₂ plants subjected to water stress ($mg\ dry\ matter\ g^{-1}\ transpired\ H_2O$) in relation to shoot dry matter

Time interval	Day 0-12	Day 12-18
+ N Plants		
Control	1.8 ^a	1.2
Stressed	1.5	3.8
N ₂ Plants		
Control	1.5	2.4
Stressed	1.8	0.6

^aThese data were obtained from average dry matter production, (Table 2), and average total transpiration data, (Table 4). Only major differences are discussed.

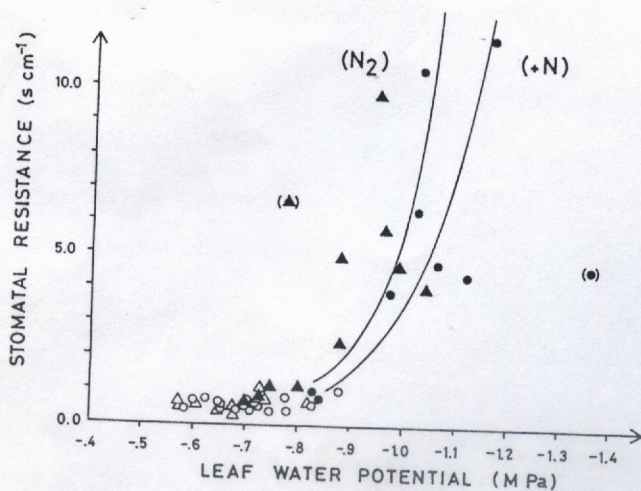


FIG. 1. Stomatal response in upper faba bean leaves to diminishing leaf water potentials. Each point represents a single leaf. (○) +N control; (●) +N stressed; (△) N_2 control and (▲) N_2 stressed. Curves were fitted using a program from Golden Software, Inc. Points within brackets were excluded from the fitting.

increased to 5.62 and 5.54 $s\ cm^{-1}$ for +N and N_2 -stressed plants, respectively.

No evidence for osmotic adjustment was found in leaves of stressed plants under these experimental conditions. On day 18, rehydrated leaves of +N and N_2 plants had an osmotic potential of -0.65 and -0.64 MPa, respectively. In stressed +N and N_2 plants, osmotic potentials were -0.69 and -0.66 MPa, respectively and were not significantly different ($\alpha = 0.05$) from those of the controls.

Recovery

After rewatering, the +N and N_2 -stressed plants showed similar mid-photoperiod potentials (ψ_m) to the controls within 20 h. At this time the stomatal resistance of the leaves of +N-stressed plants had also decreased to control levels, whilst those of N_2 -stressed plants only reached levels similar to those of the controls 3 to 4 d after rewatering (Table 6).

DISCUSSION

Vicia faba control plants produced more dry matter under the +N treatment although these differences were less marked than for other legume species grown under controlled environment (Mínguez and Sau, 1989; Ryle *et al.*, 1983). On day 18 the shoot/root ratio of the N_2 plants was lower than the +N. This behaviour is similar to that described for *Trifolium repens* (Ryle *et al.*, 1981), but different to that found for indeterminate *Glycine max* plants subjected to similar growth conditions (Mínguez and Sau, 1989). In *G. max*, the higher production of dry matter by +N plants was almost entirely due to the larger root system of +N plants which, on the last sampling day (equivalent to day 20), was almost double that of the N_2 plants, excluding the nodules. Hence, their shoot/root ratio was significantly lower. Thus, in terms of dry matter accumulation by well-watered control plants, *V. faba* and *G. max* show contrasting responses due to the type of nitrogen nutrition.

N_2 *Vicia faba* plants decreased their shoot/root ratio in response to water stress faster than +N plants during the first period of stress application (day 0 to day 12). However, as in *G. max* (Mínguez and Sau, 1989) the plants seemed to be unable to maintain root growth from day 12 onwards, whereas by day 18 +N-stressed plants had readjusted their dry matter allocation in favour of root production. The shoot/root ratios in which nodules have been excluded may allow the expression of dry matter allocation in relation to root development for increased soil exploration (Mínguez and Sau, 1989).

+N and N_2 plants showed parallel responses in terms of leaf area, total transpiration and transpiration per unit leaf area either between both control and stress treatments although +N treatments generally had larger leaf areas.

Leaf expansion stopped from day 12 in both stress treatments. However, in the +N plants a small increment in dry matter was maintained, producing an increase in specific leaf weight. The higher shoot weight of +N-stressed plants compared to N_2 plants on day 18 was due to the higher dry weight of stems (38.5%), petioles (8.5%)

TABLE 6. Recovery of +N and N_2 plants after 18 d of water stress as measured by mid-photoperiod leaf water potentials (ψ_m) (MPa) and stomatal resistance (r_s) ($s\ cm^{-1}$)

	0 h		20 h		44 h	68 h	82 h
	ψ_m	r_s	ψ_m	r_s	r_s	r_s	r_s
+N Plants							
Control	-0.73a	0.60a	-0.75a	0.69a	0.67a	0.74a	—
Stressed	-1.07b	6.68b	-0.68a	0.77a	0.78a	0.78a	—
N_2 Plants							
Control	-0.72a	0.79a	-0.68a	0.65a	0.71a	0.62a	0.67a
Stressed	-0.95b	5.77b	-0.66a	1.58b	1.87b	1.09a	0.76a

*Measurements taken 20 h after watering the stressed plants abundantly. 0 hours measurements correspond to day 18.

and leaflets (53%). This higher leaflet weight was caused by a larger leaf area (42%) and by the increase in specific leaf weight (58%). These increases were complemented by those in TE from day 12 to 18.

The +N and N₂ plants followed a similar pattern in the evolution of leaf water potential in both control and stressed conditions. The small differences in ψ_m of the N₂-stressed plants on day 12 may have been due to greater root development.

There was no evidence of osmotic adjustment in the +N or N₂-stressed plants and in both nitrogen regimes, stomatal closure occurred in response to water stress. This took place first in the N₂ plants at the threshold value *c.* -1.0 MPa as compared to *c.* -1.1 MPa for the +N plants. These differences are less clear than in *G. max* where +N-stressed plants closed stomata at significantly lower leaf water potentials than N₂-stressed plants (Minguez and Sau, 1989).

Consequently, the responses of *V. faba* to the water stress applied in these experiments are aimed at postponing dehydration. Stress is tolerated at high leaf water potentials through maintenance of water absorption by a combination of increased root growth and stomatal closure (Turner, 1979; Kramer, 1983). The differences between +N and N₂ plants are due to the increased root growth and shoot/root ratio in N₂ plants during the first part of the stress (days 0 to 12). The +N-stressed plants do not respond as fast but are able to maintain root and shoot growth during the second part of the stress (days 12 to 18).

The differences in the recovery patterns of the +N and N₂ plants may be due in part to the existence of nodules on N₂ roots. It has been proposed that, under water stress conditions, roots play a fundamental role in shoot control which could be achieved by 'signals' sent to the shoot (Passioura, 1983, 1986; Turner, 1986). The presence of high auxin and cytokinin levels in nodules is quite well known (Pate, 1958; Syono and Torrey, 1976) and several authors (Davies, Metcalfe, Lodge, and da Costa, 1986) have proposed that cytokinins and other root substances influence the physiology of the shoot, especially stomatal behaviour and leaf growth. Moreover, nodules may react more than roots to soil drying since most develop in the upper layers of the soil.

ACKNOWLEDGEMENTS

This work was financed through project No. 0254-84 from C.A.I.C.Y.T. F. Sau was sponsored by a grant from the 'Formacion de Personal Investigador' programme of the Junta de Andalucia. We wish to thank Dr F. R. Minchin for helpful discussions, Mr I. Lorite for technical

assistance and Ms Christine Mendez for the English editing.

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