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Involvement of Ureides in Nitrogen Fixation Inhibition in Soybean[1]

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The sensitivity of N_2 fixation to drought stress in soybean (*Glycine max* Merr.) has been shown to be associated with high ureide accumulation in the shoots, which has led to the hypothesis that N_2 fixation during drought is decreased by a feedback mechanism. The ureide feedback hypothesis was tested directly by measuring the effect of 10 mM ureide applied by stem infusion or in the nutrient solution of hydroponically grown plants on acetylene reduction activity (ARA). An almost complete inhibition of ARA was observed within 4 to 7 d after treatment, accompanied by an increase in ureide concentration in the shoot but not in the nodules. The inhibition of ARA resulting from ureide treatments was dependent on the concentration of applied ureide. Urea also inhibited ARA but asparagine resulted in the greatest inhibition of nodule activity. Because ureides did not accumulate in the nodule upon ureide treatment, it was concluded that they were not directly inhibitory to the nodules but that their influence mediated through a derivative compound, with asparagine being a potential candidate. Ureide treatment resulted in a continual decrease in nodule permeability to O_2 simultaneous with the inhibition of nitrogenase activity during a 5-d treatment period, although it was not clear whether the latter phenomenon was a consequence or a cause of the decrease in the nodule permeability to O_2.

The physiological basis of N_2 fixation inhibition by water deficits in legume nodules is not clearly understood. A potential physiological basis for this water-deficit sensitivity may be that drought stress decreases the P_o (Weisz et al., 1985), as has been shown with other stresses such as temperature, salinity, or nitrate (Hunt and Layzell, 1993; Serraj et al., 1994; Denison and Harter, 1995). The role of O_2 limitation in the response of nitrogenase activity to drought stress has been discussed extensively (Diaz del Castillo and Layzell, 1995; Purcell and Sinclair, 1995; Serraj and Sinclair, 1996b; Serraj et al., 1999). However, the mechanisms by which drought affects P_o have not been elucidated. It is not clear whether drought stress has a direct effect on P_o, or whether the decrease in P_o is a consequence of a decrease in nodule activity.

An alternative explanation for the decrease in nitrogenase activity under drought could be a feedback mechanism involving the accumulation of N compounds. Pate et al. (1969) proposed that lower rates of water movement out of the nodule during drought stress may restrict export of products of N_2 fixation, and the accumulation of these products would inhibit nitrogenase activity. Others have suggested that N_2 fixation in legumes might be regulated by a feedback mechanism involving N metabolism and the pool of reduced N in the plant (Silsbury et al., 1986; Parsons et al., 1993; Hartwig et al., 1994). Oti-Boateng and Silsbury (1993) reported an inhibition of nitrogenase activity in fava bean after plant uptake of Asn or Gln.

Soybean (*Glycine max* Merr.) usually exports more than 80% of the N compounds out of the nodules in the form of the ureides Aln and Alac. They are transported in the xylem to the shoots, where they are catabolized (Winkler et al., 1987). High accumulation of petiole ureides has been measured during the inhibition of N_2 fixation by drought in both controlled (de Silva et al., 1996; Serraj and Sinclair, 1996a) and field (Purcell et al., 1998) environments. Furthermore, in a comparison of grain legume species, Sinclair and Serraj (1995) reported that those species exporting ureides from the nodules had N_2 fixation that was drought sensitive. Those species that exported little or no ureide had N_2 fixation that was relatively drought tolerant.

An important possibility is that the accumulation of ureides in soybean nodules under soil-water deficits might trigger a feedback mechanism that results in decreased N_2 fixation activity (Sinclair and Serraj, 1995; Serraj et al., 1999). This paper reports a series of experiments to investigate the hypothesis of a ureide feedback inhibition of N_2 fixation in soybean. First, ureide levels were measured in plant tissue (nodules, roots, and shoots) upon the imposition of water deficits to confirm that ureide levels increased in the nodules themselves, and not just in the shoot. Second, the influence of ureides on nodule activity was examined by introducing ureides, along with other compounds, into soybean plants. These experiments were designed to

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Abbreviations: Alac, allantoic acid; Aln, allantoin; ARA, acetylene reduction activity; P_o, nodule permeability to O_2; pO_2, partial pressure of O_2.

examine the time course of the response and to determine the concentration response. Third, data were collected to determine if P_o and the response of N_2 fixation to pO_2 were also sensitive to introduced ureides.

MATERIALS AND METHODS

Accumulation of Ureides in Soybean Tissues under Drought (Experiment 1)

Soybean (*Glycine max* Merr. cv Biloxi) plants were inoculated with a commercial preparation of *Bradyrhizobium japonicum* (Nitragin[3], Milwaukee, WI) and grown in pots (2.5 L) filled with a loamy sand-soil mixture in a greenhouse (Serraj and Sinclair, 1996a). One plant was allowed to develop in each of 15 pots under well-watered conditions for 4 to 6 weeks. When the plants had produced four or five fully expanded leaves, all pots were watered to saturation. Seven pots were maintained as controls in well-watered conditions, whereas eight drought-treatment pots were allowed to dehydrate during the next 12 d as a result of plant transpiration. The mean nodule dry weight of these plants was 0.55 g, reflecting a good level of nodulation.

Four drought-treatment plants were harvested on d 7 and 10 of the dry-down experiment. Leaf water potential was measured at midday on the uppermost, fully expanded leaf using a pressure chamber. Ureides were extracted from freshly harvested nodule, root, and leaf-blade tissues. Samples of about 0.8 g fresh weight were extracted with 1.0 mL of 0.2 M NaOH in a boiling water bath for 30 min. Ureide concentration in the extracts was determined colorimetrically according to the method of Young and Conway (1942) and is expressed on a fresh weight basis.

Techniques of Ureide Application (Experiment 2)

To measure the effects of ureides on nodule activity in soybean, we compared two different techniques of ureide application: stem infusion and ureide addition to hydroponic solutions. For stem infusion, the plants were grown as described above for 4 weeks, and then prepared for infusion by inserting three 26-gauge hypodermic needles into the stem of each plant at the three top internodes. The needles were connected with plastic tubing (Fisher Scientific) to 10-mL syringes filled with the infusion solution, which was continuously injected into the stem using an infusion apparatus (Sage Instruments, Boston, MA) at a constant flow rate of 0.5 mL h^{-1}. This stem-infusion procedure was similar to the technique described previously by Grabau et al. (1986), in which metabolites were found to be distributed throughout the soybean shoots. Two solutions were infused: 10 mM ureides (5 mM Alac plus 5 mM Aln) and deionized water (control). The infusion solution was renewed every day during the 7-d infusion period by filling the syringes with the appropriate solution. The effects of stem infusion on N_2 fixation were measured by daily assays of ARA using the procedure described below.

For the hydroponic method, the plants were germinated, inoculated with commercial inoculant (Nitragin), and grown in 1-L Erlenmeyer flasks in a greenhouse on a nutrient solution containing the following concentrations of macro- and microelements: $CaCl_2$ (3.3 mM), $MgSO_4$ (2.05 mM), K_2SO_4 (1.25 mM), KH_2PO_4 (0.25 mM), H_3BO_3 (4 μM), $MnSO_4$ (6.6 μM), $ZnSO_4$ (1.55 μM), $CuSO_4$ (1.55 μM), $NaMoO_4$ (0.12 μM), and FeEDTA (40 μM). The nutrient solution was changed twice weekly, the pH of the solution was maintained close to 7.0 by adding 0.2 g L^{-1} $CaCO_3$, and air was continuously bubbled through the solution at a flow rate of 2 L min^{-1} (Serraj and Sinclair, 1996b). The volume of nutrient solution was initially sufficient to immerse the roots, and was then adjusted with root growth down to 20% of the flask volume, so that the nodules developed above the nutrient solution. The nodule dry weight per plant when subjected to treatment was 0.25 to 0.50 g. The intact, nodulated root systems of 4-week-old plants were exposed to ureide treatment by replacing the nutrient solution in the flask with a nutrient solution containing 10 mM ureides (5 mM Alac plus 5 mM Aln). ARA was measured daily using the procedure described below.

Effect of Different N Compounds and Ureide Concentrations (Experiments 3, 4, and 5)

Because the stem-infusion technique was more tedious than the hydroponic method, all of the following experiments were done with plants grown hydroponically in a greenhouse. The intact, nodulated root systems of 4-week-old plants were exposed to different treatments by replacing the nutrient solution in the flasks with a new solution containing various N compounds. In experiment 3, the effects on nodule activity of 10 mM urea, Aln, Alac (K salt), and Asn were compared with nodule activity in control plants and with 10 mM KCl and malate treatments. Experiment 4 examined the effect of Aln and Alac concentrations (5, 10, and 20 mM) on nodule activity. Finally, the effects of 2.5 and 5.0 mM Alac, and the recovery of ARA after ureide removal, were investigated in experiment 5. In the latter experiment, plants were harvested and oven dried for 2 d, and the shoots and nodules were individually ground. Ureide content was determined on each plant part using the colorimetric method of Young and Conway (1942) and is expressed on a dry weight basis.

Nodule Oximetry Measurements (Experiments 6 and 7)

There are several possible changes in the nodule in response to the ureide treatment. To investigate whether a potential N feedback mechanism could be linked to some changes in P_o, plants were grown hydroponically and exposed to solutions containing ureide or Asn. P_o was measured by oximetry (Denison and Layzell, 1991). This method is based on computer-monitored measurement of the in vivo changes in leghemoglobin oxygenation (measured spectrophotometrically) of individual nodules after

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply approval or the exclusion of other products that may also be suitable.

exposure of nodules to gas mixtures containing either 20 or 100 kPa O_2.

For experiment 6, the plants were grown in Erlenmeyer flasks for 3 weeks in a greenhouse as described previously. Approximately 4 d before beginning the oximetry measurements, the plants were transferred to 2-L clear pouches (Ziploc, Dow Chemical, Indianapolis, IN) with 200 mL of nutrient solution and moved to the laboratory under artificial light (600 μmol m^{-2} s^{-1} for 20 h). Permeability was initially measured on three or four nodules (2–4 mm external diameter) per plant, three plants per treatment, using the oximeter. After the initial permeability measurement, each nodule was tagged with a droplet of methylene blue. The nutrient solution was then replaced with one of four treatment solutions: control, ureide (Alac), malate, or nitrate. The added compounds were all at a concentration of 2.5 mM. Four days after the plants were exposed to the various treatment solutions, P_o was again measured with oximetry for each tagged nodule. After measurement, tagged nodules were individually harvested and fresh weight was determined to estimate the diameter of the nodule, assuming a spherical nodule and a bulk density of 1.

For experiment 7, 4-week-old plants were exposed to ureides by replacing the nutrient solution in the Erlenmeyer flasks with nutrient solution only, nutrient solution containing 10 mM Alac, or nutrient solution containing 10 mM Asn. Nodule ARA was measured daily, as described below, during a 5-d period. Permeability was also measured daily on three or four nodules (2–3 mm external diameter) per plant, three plants per treatment. Nodules used for the permeability measurements were harvested immediately after the oximeter measurement for determination of nodule diameter and fresh weight.

Acetylene Reduction Assays and Response to pO_2

For all experiments described above except experiment 6, ARA was assayed on undisturbed, intact plants sealed in the Erlenmeyer flasks (Serraj and Sinclair, 1996b). An open-flow system with a steady flow of 1 L min^{-1} was used to subject the nodules to a gas mixture of 21 kPa O_2, 69 kPa N_2, and 10 kPa acetylene, which was generated with mass-flow meters (MKS Instruments, Andover, MA). Ten minutes were allowed for steady-state conditions to be achieved. Outflow ethylene concentration was determined with a gas chromatograph equipped with a flame-ionization detector. Previous tests (Serraj and Sinclair, 1996b) showed that this assay system using greenhouse-grown plants resulted in no acetylene inhibition. After gas samples were obtained for the ARA measurements, the nodulated roots were continuously flushed with acetylene-free atmospheric gas. The values of ARA were expressed on a per plant basis, and then commonly normalized against either control plants or initial rates.

The response of nodule ARA to O_2 enrichment was measured on the plants from experiment 7 treated or not with 10 mM Alac for 5 d. The purpose of this experiment was to determine if ARA in Alac-treated plants could be recovered to levels achieved by nontreated plants when subjected to elevated pO_2 to overcome the potential limitation of nodule permeability changes. ARA response to pO_2 was measured by varying the O_2 and N_2 partial pressures (Serraj and Sinclair, 1996b). The mass flow meters were used to vary the composition of the gas mixture and to maintain a 0.5 L min^{-1} constant flow during the assay. To prevent inhibition of nitrogenase activity by a sudden O_2 increase, the increase in pO_2 was imposed gradually at a rate of 0.6 kPa min^{-1} rather than by a step change. ARA was measured at 10, 20, 30, 40, 55, and 70 kPa O_2 for every pO_2. After reaching the desired pO_2, 10 kPa acetylene was introduced for only 10 min and then the outflow ethylene concentration was measured.

RESULTS

Accumulation of Ureides under Drought

Drought stress resulted in a significant increase in ureide concentration in soybean tissues. Under well-watered conditions and high leaf water potentials, ureide concentration

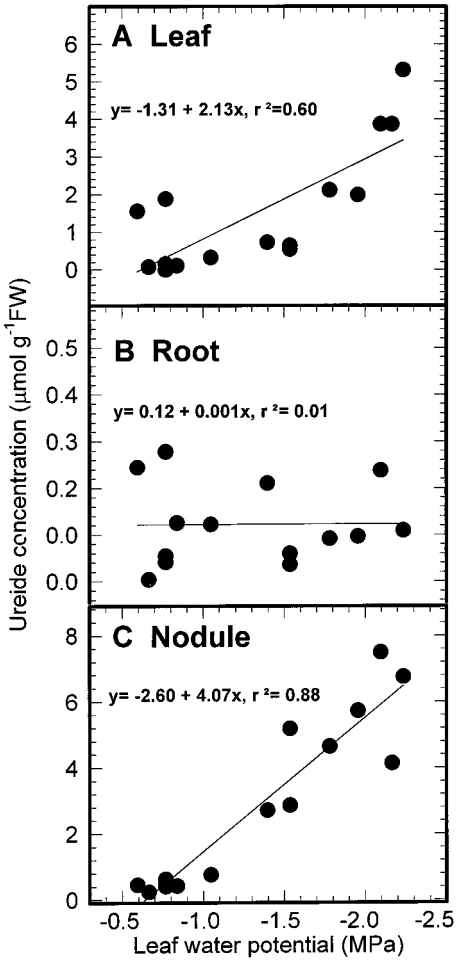

Figure 1. Leaf-blade (A), root (B), and nodule (C) ureide concentrations of soybean plants as a function of leaf water potential in a soil dehydration experiment (experiment 1). Each data point is for individual plants harvested from the control and after 7 and 10 d of stress. FW, Fresh weight.

was very low in nodule, root, and leaf-blade tissues (Fig. 1). During soil dehydration leaf water potential decreased substantially, indicating a fairly severe level of drought stress, which was associated with an accumulation of ureides in the leaf blades and nodules. Ureide concentration in the leaf blades and nodules exceeded 4 μmol g^{-1} fresh weight when the plants were severely stressed (Fig. 1). The concentration of ureides in the roots was extremely low and remained unchanged during the drought-stress treatment.

Stem Infusion and Ureide Application in Hydroponic Solution

Two techniques of plant exposure to ureides were compared: stem infusion and addition of 10 mM ureide to the hydroponic solution. Both treatments resulted in a dramatic decrease of ARA (Fig. 2). ARA was unaffected during the first 3 d after the initiation of the stem-infusion treatment and started to decrease almost linearly after this time (Fig. 2A). On d 7, ARA of treated plants was less than 15% of that for plants infused with deionized water. Ureide application in the root nutrient solution, however, resulted in a faster inhibition of ARA compared with the stem-infusion treatment. Exposure of nodulated soybean roots to hydroponic solution with 10 mM ureide resulted in a decline of ARA within 48 h after treatment (Fig. 2B). After 4 d of treatment, ARA was almost nonexistent for ureide-treated plants, whereas ARA was still increasing in the control plants.

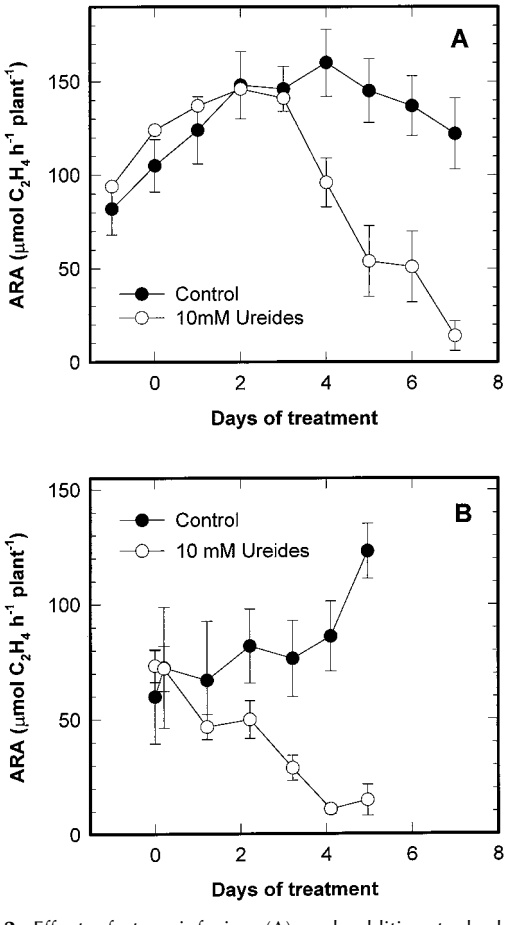

Figure 2. Effect of stem infusion (A) and addition to hydroponic solution of 10 mM ureides (5 mM Aln plus 5 mM Alac) (B) on ARA (experiment 2). Values are means ± SE of four replicates.

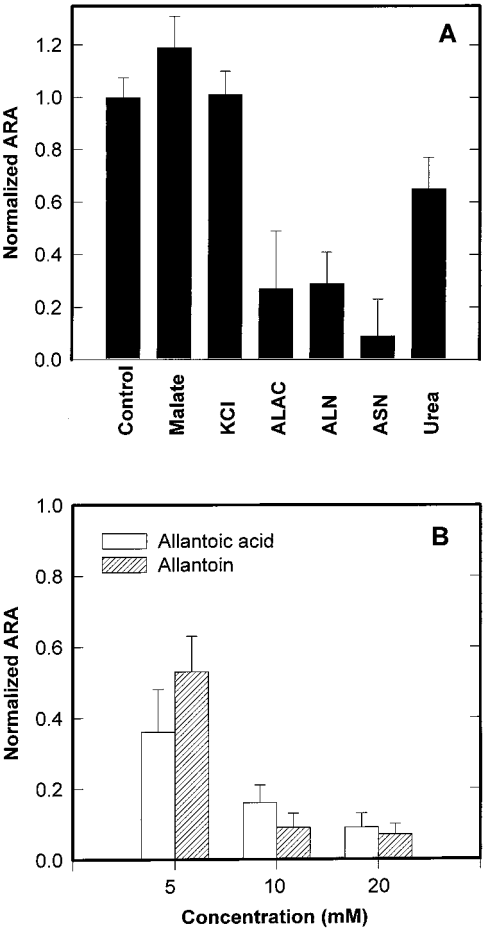

Figure 3. Effect of 10 mM of various compounds 3 d after treatment (A, experiment 3) and concentrations of Aln and Alac 4 d after treatment (B, experiment 4) on ARA of 4-week-old cv Biloxi soybean plants grown hydroponically. Values were normalized against daily values of ARA for control plants. Values are means ± SE of four replicates.

Although both stem infusion and ureide addition to hydroponic solution resulted in an inhibition of nodule ARA, the first technique was more tedious and less reproducible. Therefore, only the hydroponic solution method was used in all subsequent experiments.

Effects of Different N Compounds on Nodule Activity

To examine further the effects of ureides on nitrogenase activity, we compared the effects of 10 mM of various N compounds (Alac, Aln, Asn, and urea) and other compounds (KCl and malate) on ARA after 3 d of treatment. All N compounds showed an inhibitory effect on ARA, whereas nonnitrogenous compounds showed no signifi-

cant effect on ARA (Fig. 3A). Among the N compounds, urea prompted the smallest decrease in ARA, i.e. only 25%. By contrast, there was a 90% inhibition of ARA by Asn. Ureides induced a 70% inhibition of ARA compared with the control, with no difference detected between Aln and Alac.

The effects of Aln and Alac on nodule ARA were compared further at different concentrations. Both ureide compounds resulted in large declines in ARA (Fig. 3B), with no significant difference between the effects of Aln and Alac at any concentration. The ARA decline was less in the 5 mM treatment (an average 56% decrease after 96 h) than in the 10 and 20 mM treatments (an average 88% decrease after 96 h).

Recovery of Nodule ARA from Ureide Inhibition

To investigate the reversibility of ureide effects, ARA recovery was studied after treatment with 2.5 mM (Fig. 4A) and 5 mM (Fig. 4B) Alac. The 2.5 mM ureide treatment prompted only a 40% decrease of ARA, which was significant only after 4 d of treatment and stabilized to this value on the 5th and 6th d (Fig. 4A). After 6 d, one-half of the plants were changed to a Alac-free nutrient solution, whereas the other plants were changed to a new 2.5 mM Alac nutrient solution. Thereafter, ARA decreased another 30% in the Alac-treated plants, whereas there was an ARA recovery in the other plants, reaching 80% of the control. The 5 mM treatment induced a 60% decrease in ARA within 3 d after treatment (Fig. 4B). Switching the plants to an Alac-free nutrient solution resulted in an ARA recovery to only 53% of the control.

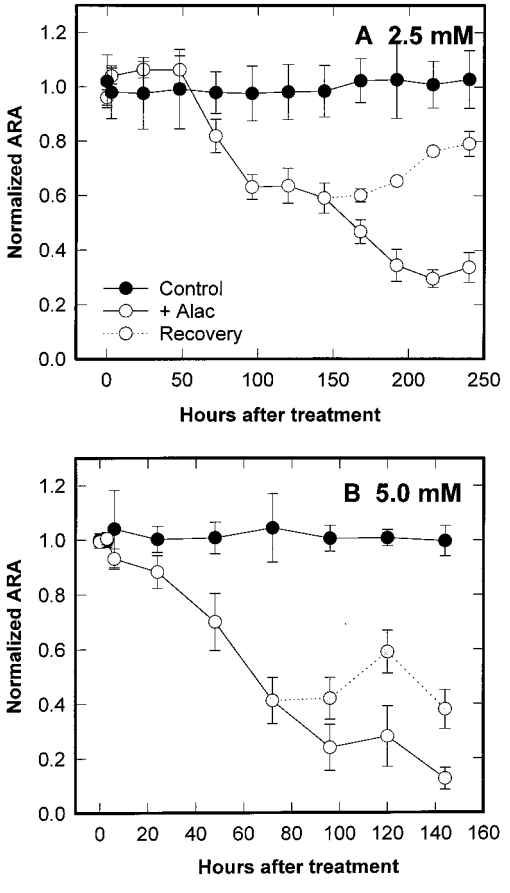

Figure 4. Effect of 2.5 mM (A) and 5.0 mM (B) Alac on ARA of 4-week-old cv Biloxi soybean plants grown hydroponically (experiment 5). ARA recovery was measured after Alac removal from the nutrient solution. Data were double normalized for daily values of ARA compared with the control plants and compared with the mean value for 0 and 3 h. Values are means ± SE of four replicates.

Ureides were measured in shoots and nodules at the end of this experiment. Both Alac concentration treatments resulted in ureide accumulation in shoots; there was a 5-fold increase with 5 mM Alac and a 4-fold increase with 2.5 mM Alac (Table I). Plants recovered from the 5.0 mM Alac treatment showed a 3-fold increase in shoot ureide compared with the controls, whereas the 2.5 mM treatment showed a decreased shoot ureide concentration compared with the control. By contrast, nodules did not show any treatment difference for ureide concentration regardless of Alac concentration (Table I).

Effects of Ureide on P_o

The response of P_o to 2.5 mM Alac treatment was compared with the response to nitrate and malate treatments at the same concentration. The treatments with the N compounds resulted in a change in P_o. After 4 d of treatment both nitrate and ureide applications resulted in a significant 30% decrease in P_o, whereas P_o after treatment with malate was statistically equivalent to the control (Table II).

Nodule ARA and P_o were measured simultaneously during a 5-d period after the addition of either 10 mM Asn or 10 mM Alac to the nutrient solution. After 2 d there was a significant decrease in both ARA (Fig. 5A) and P_o (Fig. 5B) in response to the Asn treatment. The measured variables seemed to be nearly stable after 3 d, with ARA at 0.3 to 0.4 of the control and with P_o at 0.5 to 0.6 of the control, respectively. The response of ARA and P_o to the ureide treatment was somewhat different from the response to the Asn treatment. There was not a statistically significant decrease in either ARA or P_o until d 3 in response to the ureide treatment. The severity of the decrease in the Alac treatment was also not as great as in the Asn treatment, with ARA at about 0.6 and P_o at about 0.7 of the control. V_{max}, as calculated from the oximeter data, also decreased in the same pattern as ARA and P_o in response to the Asn and Alac treatments (data not shown).

To investigate the extent of O_2 limitation in nodules in response to ureide treatment, we examined the ARA response to changes in external pO_2 at 5 d after ureide treatment (Fig. 6). An increase in pO_2 induced a stimulation of nodule ARA in the control plants, whereas increasing pO_2 did not result in a statistically significant ARA recovery in ARA for the ureide-treated plants.

DISCUSSION

Previous research demonstrated that ureides accumulated in soybean petioles in response to developing water

Table I. *Ureide concentration in shoots and nodules of 4-week-old soybean plants after treatment with 2.5 or 5 mM Alac, or after treatment with Alac followed by recovery after ureide removal, compared with control plants (experiment 5)*

Plants correspond to data reported in Figure 5 harvested after the last ARA measurement. Data are means ± SE of four replicates.

Treatment	2.5 mM Alac		5.0 mM Alac	
	Shoot	Nodule	Shoot	Nodule
	μmol ureide g^{-1} dry wt			
Control	5.3 ± 0.6	2.7 ± 0.1	5.7 ± 1.3	1.9 ± 0.6
Ureide	21.8 ± 3.6	2.1 ± 0.4	29.4 ± 7.5	1.6 ± 0.7
Recovery	3.1 ± 0.2	3.0 ± 0.1	16.5 ± 4.8	0.9 ± 0.7

deficits (de Silva et al., 1996; Serraj and Sinclair, 1996a; Purcell et al., 1998). This study of the ureide concentrations in plants exposed to drought confirmed not only that leaf ureide levels increased but also that there was a large increase in nodule ureide concentration (Fig. 1). This result is consistent with the increases in nodule ureide levels observed by Purcell and Sinclair (1995) after exposure to PEG and by Walsh et al. (1989) in response to the imposition of stresses other than water deficits on soybean plants.

The observed increase in nodule ureide concentration is consistent with the hypothesis that ureides will accumulate in the nodule as a result of the action of any factor that decreases phloem flow to the nodules (Walsh et al., 1989; Streeter and Salminen, 1993; Serraj et al., 1999). This hypothesis results from the apparent dependence of xylem water flow from the nodule on the inflow of water in the phloem. Therefore, factors that decrease phloem flow to the nodules decrease the export rate from the nodules, and consequently, N_2 fixation products such as ureides will accumulate. Certainly, the development of soil-water deficits is likely to be an important factor contributing to decreased phloem flow to the nodules, which then results in the inhibition of nitrogenase activity (Serraj et al., 1999).

Oti-Boateng and Silsbury (1993) found an inhibition of nitrogenase activity after nodule exposure to Asn or Gln. Neo and Layzell (1997) increased phloem N_2 levels by exposing soybean plants to an atmosphere enriched in ammonia, which resulted in decreased N_2-fixation activity. Whereas the concentration of most nitrogenous compounds increased, Gln had the greatest increase. Neo and Layzell (1997) suggested that Gln was the most likely candidate for the feedback signal compound. However, nitrate inhibition studies with soybean implicated changes in Asn concentration in the regulation of N_2-fixation activity (Mizukoshi et al., 1995; Bacanamwo and Harper, 1997).

Table II. P_o after 4 d of exposure to 2.5 mM Alac, nitrate, or malate (experiment 6)

Data are means ± SE of 8 to 12 nodules.

Treatment	P_o	Ratio with Control
	μm s^{-1}	
Control	5.17 ± 0.39	1.00
Alac	3.53 ± 0.24	0.68
Nitrate	3.65 ± 0.49	0.70
Malate	5.71 ± 0.40	1.10

The main objective of this research was to determine if the accumulation of ureides in soybean plants with developing water deficits is involved in a feedback inhibition of N_2-fixation activity. Both stem infusion with ureides and exposure of hydroponically grown plants to ureides resulted in decreases in ARA (Fig. 2). The inhibitory effect on ARA was dependent on the concentration of the ureide treatment (Fig. 3B). However, other N compounds, including Asn and urea, also had inhibitory effects on nodule activity (Fig. 3A).

However, the results of our experiments indicated that ureides may not be the direct signal compounds. Whereas ureide application resulted in large increases of ureides in the shoot, ureide levels did not increase in the nodules (Table I). Furthermore, exposure of the plants to Asn resulted in a greater (Fig. 3A) and faster (Fig. 5A) decrease in

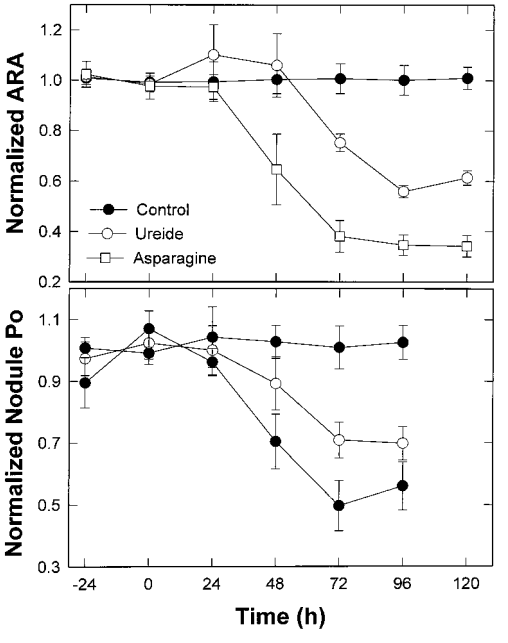

Figure 5. Effect of 10 mM Alac and 10 mM Asn on ARA (top) and P_o (bottom) in 4-week-old cv Biloxi soybean plants grown hydroponically (experiment 7). P_o was measured on three or four nodules on each plant with the nodule oximetry method (Denison and Layzell, 1991). Data were double normalized for daily values compared with the control plants and compared with the mean value for −24 and 0 h. Values are means ± SE of three plants.

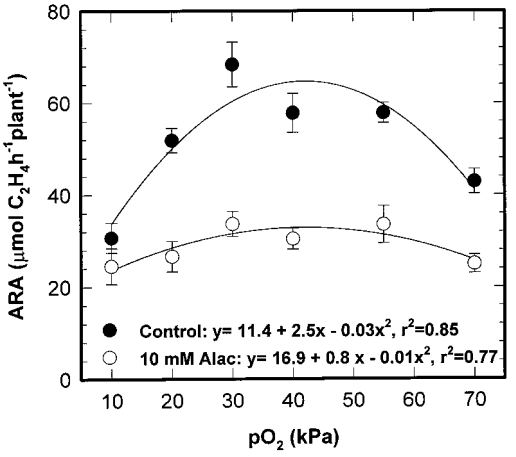

Figure 6. Effect of external pO_2 on ARA of soybean nodules exposed or not to 10 mM Alac during 5 d in experiment 7. Each value is the mean ± SE of three replicates.

ARA than was observed with Alac. Therefore, it seems more likely that a catabolic product of ureide resulting from the high concentrations of ureides in the shoot (Table I) might be more closely associated with the triggering mechanism for the observed feedback response. Such a conclusion is consistent with the suggestion of Mizukoshi et al. (1995) and Bacanamwo and Harper (1997) that Asn might be closely linked to a feedback inhibition of nitrogenase activity.

The possibility of a shoot-derived signal on nitrogenase activity has also been indicated in other experiments. Research using both grafting and split-root experiments indicated feedback regulation of N_2 fixation that may not originate in the roots but in the shoots (Silsbury et al., 1986; Rafin and Roumet, 1994). We found in grafting experiments with soybean (Serraj and Sinclair, 1996a) that the drought tolerance of N_2 fixation was associated with both the root and the shoot. Reciprocal grafts were done between the drought-tolerant cv Jackson and the drought-sensitive cv Biloxi. Those plants that had cv Jackson rootstock were as drought tolerant as cv Jackson itself. Those plants with cv Jackson as the scion and cv Biloxi as the rootstock also showed a high level of drought tolerance, although it was not as great as that of cv Jackson. Therefore, the shoots of cv Jackson contained a trait, which we now speculate may be associated with ureide catabolism, that contributed to drought tolerance for N_2 fixation. In experiment 5 the recovery from 2.5 mM Alac treatment (Fig. 4A) showed a decrease in shoot ureide, and the poor recovery from the 5.0 mM Alac treatment (Fig. 4B) was associated with the maintenance of a high concentration of ureide in the shoot (Table I).

A hypothesis to explain the feedback response involving ureides might be based on the regulation of P_o (Parsons et al., 1993; Streeter, 1993; Purcell and Sinclair, 1994; Denison and Harter, 1995). Our results showed a decrease in P_o that paralleled ARA inhibition associated with the Asn and ureide treatments (Fig. 5), indicating that the inhibition of nitrogenase activity could be driven by a pO_2 limitation within the nodules. It was not possible to determine from our data whether the decrease in P_o was directly responsible for decreasing nodule activity, or whether a decrease in nodule respiration led to an increase in nodule pO_2, which then triggered a decrease in P_o.

If decreased P_o was the main consequence of the ureide treatment, then increased pO_2 would help to overcome this inhibitory effect on nodule activity. However, increasing pO_2 5 d after imposition of the ureide treatment failed to induce a recovery of nodule activity (Fig. 6). This indicates that mechanisms other than O_2 diffusion may be involved in the long-term response to the ureide inhibition. The lack of response to pO_2 after exposure of the roots to ureides for 5 d is similar to the results of Serraj and Sinclair (1996b) on plants that had low activity after being subjected to prolonged osmotic stress. Consistent with their conclusion, O_2 limitations seem to be less important in limiting nodule activity in the case of severe stages of water-deficit stress or exposure to ureide. Recent evidence (Gordon et al., 1997) indicates that a limitation on carbon metabolism in the nodule may become important under prolonged drought stress.

In summary, ureides were shown to accumulate in nodules as a result of a water-deficit treatment and to be potentially important in a feedback inhibition of N_2-fixation activity. The results of these experiments indicated that ureide accumulation may trigger the accumulation of an intermediate compound, with Asn being a potential candidate. The importance of ureides being involved in the high sensitivity of ureide-transporting legumes to water deficits may result from decreased phloem flow after water deficit and a resulting increase in ureide concentrations in the plant. Accumulating ureides and Asn may feed back into the nodules to inhibit nitrogenase activity.

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