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GROWTH AND NITROGEN FIXATION OF *AESCHYNOMENE* UNDER WATER STRESSED CONDITIONS

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Key words

Acetylene reduction *Aeschynomene americana* Flooding Leaf water potential Nitrogen fixation Soil moisture deficits

Summary

Studies on the tolerance of *Aeschynomene americana* L. to periods of flooding or soil moisture deficit were conducted in an attempt to elucidate nitrogen fixation as affected by soil moisture. Nitrogenase activity was not reduced significantly in pot-grown Aeschynomene plants subjected to flooding in greenhouse conditions. After 20 days of withholding water from the soil, nitrogenase activities of the drought-stressed plants were much lower than those of either the well-watered or flooded plants. Leaf water potentials were similar in flooded and control plants; however, the droughted plants had leaf water potentials that were 4 bars lower than those of the control plants. Aeschynomene plants were tolerant to long-term periods of flooding, but exhibited a reduction in nitrogenase activity and leaf water status when subjected to soil moisture deficits.

Introduction

Aeschynomene americana L. (American joint vetch) is, in frost-free areas, a short-lived perennial legume that is indigenous to tropical America. It has good potential as a forage, green manure and soil cover crop. Reports from Sri Lanka indicate that it yields well, producing a highly nutritive hay[23]. In addition, *A. americana* is capable of nitrogenase activity comparable to that of other legumes (Albrecht and Quesenberry, unpublished results) when grown under optimal environmental conditions. This species is considered a potentially valuable pasture legume for Florida, and was found to be the most productive of several forages evaluated in limited grazing trials[14].

In Florida almost 60% of the soils are subject to seasonal waterlogging[2]. Heavy seasonal rainfall on soils of poor internal drainage often causes flooding and waterlogging of pasture lands. Tolerance of wet soil is a desirable characteristic for a forage crop in many areas of Florida.

Too much or too little soil water can drastically affect growth, yield and quality of legume crops[3,5,9,10]. Water stress can also reduce nodulation, nodule growth and nitrogen fixation. Several investigators have examined the effects of water stress on nitrogen fixation in laboratory and field conditions for a wide range of

Plant and Soil 60, 309–315 (1981). 0032-079X/81/0602-0309$01.05.

legumes[6,7,8,13,15,19,21,22]. There are reports[11,16,18,20] that waterlogging depresses nitrogen fixation, largely because of oxygen deficiencies in the roots and nodules.

The objective of this study was to examine the tolerance of N_2 fixation by several accessions of *A. americana* to various soil moisture levels. These accessions of *A. americana* were subjected to periods of flooding or soil water deficits to determine stress effects on nitrogen fixation.

Materials and methods

Cuttings of 22 field-grown Aeschynomene accessions were rooted in sand and transplanted to a mixture of Wauchula sand (ultic, Haplaquod sandy, siliceous, hyperthermic), peat and perlite (2 : 1 : 1) in 15-cm black plastic pots. The soil medium had previously been treated with methyl bromide to destroy nematode and weed contaminants. Cuttings were allowed to root and become established 4 weeks in a greenhouse. Pots were then arranged in a completely random design in two flooding tanks (5 × 1 × 0.2 m). For 1 week, the water level was maintained 8 cm below the soil surface. For the second week, the water level was held 4 cm below the soil surface. The water was not stirred or disturbed except during additions in order to reduce the mixing of oxygen into the water. During the greenhouse experiment the average maximum temperature was approximately 27°C; the average minimum was 22°C, but low temperatures reached about 15°C. Relative humidities varied from 40 to 98%.

Visual observations and nitrogenase activities indicated that the flooding did not cause significant stress symptoms on any of the accessions. Nitrogenase activities were very similar to those of well-watered Aeschynomene plants observed in numerous other experiments (Albrecht and Quesenberry, unpublished data). In order to test the hypothesis that soil moisture affects nitrogenase activity, 16 plants were removed and allowed to dry for 20 days until the plants wilted; 16 plants were maintained as well-watered controls, and a corresponding group of plants remained under flooded conditions for the remainder of the experiment.

Nitrogenase activity was determined by the acetylene reduction assay. At harvest, the entire nodulated root system was excised from the plant, soil was removed by shaking, and the roots were inserted into 75-ml serum vials and sealed with rubber stoppers. A volume of air equal to the volume of acetylene to be injected was removed from the vials with a syringe and needle and the reaction was initiated by injection of acetylene to 10% (v/v). Samples of gas (0.5 ml) were removed at 30 min intervals for immediate analysis by gas chromatography. The sample vials were incubated at ambient temperatures during the C_2H_2 reduction assay. Samples were analyzed for ethylene and acetylene on a Varian[a] model 940 gas chromatograph[5]. At harvest, oxidation-reduction potentials of the soil mixture and water in the flooding tanks were measured using a portable Orion[a] meter with an Orion probe.

Before harvesting the plants for nitrogenase activity determinations, plant water status was determined by measuring leaf water and osmotic potentials with Spanner-type chamber thermocouple psychrometers[17] which had been previously calibrated with sodium chloride solutions. Chambers containing leaf material were placed in a water bath at 30°C. Readout of the thermocouple psychrometers was accomplished with a Wescor[a] HR-33T dewpoint microvoltmeter operating in the psychrometric mode. Nodule water potentials were determined by the same procedure after gently drying the nodule surface with a towel to remove surface moisture. Osmotic potentials were measured after freezing and thawing of the leaf tissue. Turgor potentials were calculated as the difference between leaf water and osmotic potentials.

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

Results and discussion

All the *A. americana* accessions survived the flooding and most produced seed during the course of the experiment. All grew slowly, with minimal lateral branching, probably due to low night temperatures in the greenhouse during the winter months. Most flooded plants showed no visible signs of water stress. Slight leaf-yellowing occurred in the early stages of flooding, and some basal leaves on some accessions turned yellow and dropped later in the experiment. This is a normal occurrence for *A. americana* and we are not certain that it is an indicator of waterlogging. Few plants showed noticeable chlorosis. None of the plants wilted, suggesting that the roots were able to absorb water under flooded conditions. When the plants were harvested, the roots had reached the bottom of the pots, in contrast to reports[4,12] of reduced root growth in other forage legumes that are not tolerant to excessive soil moisture. Soil color and odor indicated sulfate reduction in the bottoms of most pots, suggesting an anaerobic environment[1]. The pH of both the water in the flooding tank and the soil solution was 6.9. Although the redox potential was high both in the water and at the soil surface (+260 and +75 ±5 mV, respectively), it decreased rapidly with increasing soil depth (2.5 cm = −110 ± 10 mV, 5.0 cm = −125 ± 5.0 mV).

Nitrogenase activity, under the conditions of this experiment, could be detected in most waterlogged plants, even when plants were flooded for long periods. However, nitrogenase activity varied greatly among accessions, ranging from undetectable to 148.3 μmoles C_2H_4 produced · h^{-1} · g dry nodule weight^{-1}. A similar wide range of activity has been observed in this species in other experiments (unpublished observations). Nitrogenase activity measured by this method may overestimate nitrogen fixation in waterlogged plants, as the water barrier to N_2 and O_2 diffusion is removed to some extent during the

Table 1. Comparison of nitrogenase activity of flooded *Aeschynomene americana* to shoot growth rating

Measured characteristic	Visual evaluation of shoot growth			
	Superior	Good	Intermediate	Inferior
Root wt. (g)	0.3405 ± 0.0011[a]	0.2723 ± 0.0014	0.2408 ± 0.0045	0.1215 ± 0.0018
Nodule wt. (g)	0.0304 ± 0.0022	0.0069 ± 0.0025	0.0065 ± 0.0010	ND[b]
Nodule nitrogenase activity[c]	46.9 ± 1.5	116.41 ± 3.67	24.58 ± 2.67	ND[b]
Root nitrogenase activity[d]	4.18 ± 0.56	2.95 ± 0.39	0.66 ± 0.53	ND[b]

[a] Data are means of at least 4 replicate observations ± the standard error of the mean.
[b] Not determined due to insignificant nodulation.
[c] μmoles C_2H_4 formed · h^{-1} · g dry nodule weight^{-1}.
[d] μmoles C_2H_4 formed · h^{-1} · g dry root weight^{-1}.

assay procedure. The few flooded plants that showed visual sign of stress, such as poor growth or pronounced chlorosis, had few or no root nodules and reduced root biomass compared to unstressed plants.

Table 1 presents the effect of waterlogging on growth and nitrogenase activity in *A. americana*. At harvest shoot growth was given a visual rating. All plants that exhibited 'Inferior' growth inevitably lacked nodules, while plants with 'Superior' shoot development showed good nodulation. Plants rated as 'Good' and 'Intermediate' showed similar nodule biomass, but less than that of 'Superior' plants. Specific nitrogenase activity, based on nodule weight, was much higher in the plants evaluated as 'Good' than other categories. Nitrogenase activity based on root biomass, which should reflect the activity on a per plant basis, was correlated with the visual estimate of shoot quality. The exact mechanism that allows high specific nodule nitrogenase activity under these conditions has not yet been determined.

The effects of the soil water treatments on root weight, nodule weight, nodulation and nitrogenase activity are presented in Table 2. Both the flooded and droughted treatments showed increased root weights. The flooding treatment did not significantly reduce nodule

Table 2. Effect of three water treatments on root and nodule weight, percent nodulation, and nitrogenase activity in nodules of *Aeschynomene americana*

Treatment[a]	Root wt. (mg)	Nodule wt. (mg)	Percent nodulation[b]	Nitrogenase activity[c]
Dry	366.8 ± 4.9	26.4 ± 4.4	6.8 ± 0.3	9.05 ± 1.01
Flooded	227.4 ± 4.0	31.4 ± 1.5	13.0 ± 0.6	21.61 ± 1.84
Control	200.5 ± 2.8	32.9 ± 6.7	13.5 ± 0.9	18.58 ± 1.94

[a] Activity determined after 21 days of treatment. Nitrogenase activity measured by acetylene reduction. Numbers are ± the standard error of the mean.
[b] Nodule dry weight × 100/total root dry weight.
[c] μmoles $C_2H_4 \cdot h^{-1} \cdot$ g dry weight nodule^{-1}.

Table 3. Leaf water, osmotic and turgor potentials of leaves and water potentials of nodules as affected by flooding and soil water deficit

Treatment	Leaf water potential	Leaf osmotic potential	Leaf turgor potential	Nodule water potential[b]
Dry	−11.5 ± 0.9[a]	−13.6 ± 1.2	2.1 ± 0.5	−10.7
Flooded	− 5.8 ± 0.9	−12.1 ± 0.6	6.3 ± 0.8	− 2.5
Control	− 7.2 ± 0.3	−12.0 ± 0.4	4.8 ± 0.3	− 4.5

[a] Values are expressed as bars ± standard error of the mean. Potentials determined at 1300 hours after 21 days of treatment
[b] Values represent unreplicated observations.

weight, percent nodulation (nodule dry weight × 100/total root dry weight) or nitrogenase activity, whereas soil moisture deficits caused reductions in these variables. Although flooding generally represses nodulation and nodule growth[18,20], this was not evident for many of the *A. americana* accessions. The nodulation and nodule growth among accessions studied here suggest that many of the accessions have effective mechanisms for maintaining active nodules under flooded conditions.

The response of *A. americana* to drought stress was similar to that seen with other legumes[8,15,20,22]. Nodule dry weight was reduced by drought stress and nitrogenase activity was reduced about 50% in the drought-stressed plants.

Measurements of leaf and nodule water status are presented in Table 3. The data indicate that only the drought-stressed treatment resulted in significantly lower leaf water and turgor potentials. The flooded plants maintained leaf water, osmotic and turgor potentials which were similar to those of the well-watered controls. Although the samples of nodule water potentials were unreplicated, it appears that the droughted plants had much lower nodule water potentials. Leaf turgor potential appeared to be a sensitive indicator of the total root nitrogenase activity, particularly as turgor potentials fell below approximately 5 bars (Fig. 1). The components of leaf water status indicate that only the drought treatment produced a water stress. The flooded plants appeared to maintain leaf water relationships which were slightly more favorable than those of the well-watered

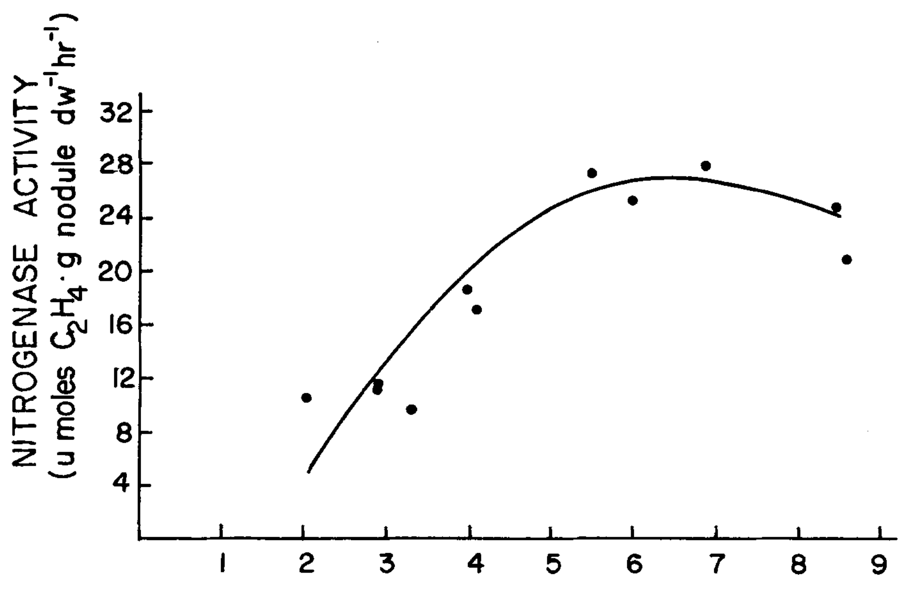

Fig. 1. Relationship of leaf turgor potential and nitrogenase activity in *A. americana*. Nitrogenase activity and leaf turgor potential measured as described in text.

controls, although the differences were small. Since turgor potential is the component of leaf water potential which is probably most closely related to physiological processes in the leaf, it appears to be an indicator of root activities such as nitrogenase activity.

Most of the *A. americana* accessions examined were able to survive long periods of flooding, and had nitrogenase activities, when assayed under similar conditions, comparable to those of non-waterlogged plants. This capacity of *A. americana* should make it useful on flooded pasture lands, and result not only in increased yields on poor soils, but also in soil fertility, forage plant persistance and lower fertilizer costs.

It has been suggested[8] for soybeans that flooded and waterlogged soil prevents gas exchange, and that this factor is responsible for poor growth and nitrogen fixation of soybeans in waterlogged conditions. Possibly *A. americana* possesses sufficient aerenchyma tissue to overcome this problem. At this time it is not possible to state which factors allow waterlogged *A. americana* to fix nitrogen. Producer observations also indicate that commercial *A. americana* is tolerant to extended periods of flooding. Accessions which show good growth and N_2 fixation under flooding in the greenhouse will be tested on seasonally waterlogged areas to measure their performance under field conditions. Experiments are planned to investigate some of these factors under waterlogged conditions.

Acknowledgements

We thank Carmen Harris, Allen Cook and Paul Nee for technical assistance, Drs. L. H. Allen, Jr., T. R. Sinclair and M. H. Gaskins for helpful discussions and Ms. Karen Woodham for typing the manuscript.

This research was supported in part by Research Agreement No. **58–7B30–9–116** with the U.S. Department of Agriculture for Tropical Agriculture Development under Section 406 of Public Law 89–808. Florida Agric. Exp. Stn. Journal Series no. **2559.**

Received 10 October 1980

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