

Growth and Specific Nodule Activity of Soybean during Application and Recovery of a Leaf Moisture Stress¹

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ABSTRACT

Soybean plants growing at day/night temperatures of 30/18, 26/18, and 22/18 C were subjected to a single drying and recovery cycle during an 18- to 19-day period in the early to midpod development stage. Leaf total electrochemical water potential was reduced to about -24 bars during the 4-day drying cycle at all temperatures, but recovered to control levels upon rewatering. The changes in dry matter accumulation in whole plants and plant parts, specific activity of nodules as measured by acetylene reduction, and levels of adenosine phosphates in nodules were measured periodically during stress and recovery.

Vegetative and reproductive growth were about equally suppressed by the leaf moisture stress. Both rate of appearance and number of pods were reduced. However, a similar average weight per pod for both stressed and control plants at the conclusion of the recovery period suggests that individual pod development is not irreversibly affected by a single stress cycle and that yield potential is restricted by a decrease in number of pods or seed. Dry matter accumulation in plants and pods was unaffected by temperature.

Specific nodule activity and energy charge of nodules declined concurrently with leaf moisture potential. Recovery of specific nodule activity following rewatering lagged behind recovery of leaf moisture potential, but energy charge of nodules recovered as rapidly as leaf moisture potential upon rewatering. Thus, the delayed recovery of specific nodule activity does not appear to be related to recovery of energy charge of the nodules.

Low moisture potentials in soybean leaves inhibit photosynthesis and nitrogen fixation activity of the nodules as measured by acetylene reduction (10, 11). Net photosynthesis and acetylene reduction, both measured *in situ*, dropped simultaneously after water was withheld. Upon rewatering, leaf moisture potential returned to its initial level within hours, but neither acetylene reduction nor net photosynthesis had fully recovered 3 days later. Experiments in which external CO₂ was withheld (without water stress) indicated that although the decline in acetylene reduction could be related to a simultaneous decrease in photosynthate availability, the recovery of acetylene reduction lagged behind that of net photosynthesis following resupply of external CO₂ (11). It seems likely that the recovery of acetylene reduction following

water stress depends on processes other than recovery of photosynthetic capacity of leaves.

Roots apparently have a lower priority for photosynthates than leaves (14, 15). Hence, a decline in root activity (including acetylene reduction) might be concomitant with a stress-induced decline in photosynthetic activity, but subsequent recovery of root activities might lag behind recovery of photosynthesis. Acetylene reduction requires a supply of photosynthate to roots for production of ATP by nodule respiration (2-4). Although additional photosynthate supply would be required as carbon chains to support nitrogen fixation, restoration of adenylate energy charge of nodules would be an indication of resurgence of photosynthate availability to nodules.

A single cycle of moisture stress and recovery can reduce final seed yield of soybeans. Sionit and Kramer (13) subjected plants of the determinate cultivars "Ransom" and "Bragg" at several stages of reproductive development to leaf moisture potentials of about -23 bars before rewatering. A stress applied during pod formation or pod filling caused greater yield reduction than a stress applied during flower induction or flowering. The yield reduction by a stress during pod filling was attributed to a decrease in seed size whereas the reduction by a stress during pod formation was related primarily to a reduction in either pod number (for "Ransom") or seed number per pod (for "Bragg").

The experiments of Huang *et al.* (10, 11) were confined to the vegetative growth phase and did not evaluate growth during recovery from moisture stress. The experiment of Sionit and Kramer (13) employed noninoculated plants supplied with a complete nutrient solution and evaluated effects of applied stresses on growth and yield only at pod maturity. Therefore, one objective of this study was to evaluate components of growth, yield, and potential for acetylene reduction by nodules of soybeans during application and recovery of a leaf moisture stress. Also, both the studies on soybeans by Huang *et al.* (10, 11) and Sionit and Kramer (13) were at single temperature regimes (day/night temperatures of 29/24 and 28/17 C, respectively). Because prior work (unpublished) with corn indicated that the effect of moisture stress on growth and metabolism is influenced by temperature, another objective of this study was to evaluate the effects of temperature on the plant responses to applied moisture stress.

MATERIALS AND METHODS

Plant Growth Conditions. Seeds of a determinate soybean (*Glycine max* [L.] Merrill cv. Hill) were germinated at 25 C for 2 days in moist Vermiculite. Three germinated seeds with radicles 1.5 to 3 cm long were planted in 20.3-cm diameter, 4-liter plastic pots filled with a 2:1 mixture (v/v) of a peat moss-Vermiculite material and gravel. The plants were grown at day/night temperatures of 30/18, 26/18, and 22/18 C (± 0.25 C) in controlled-environment rooms of the phytotron unit of Southeastern Plant Environment Laboratories at North Carolina State University (5). During a 9-

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h day period a combination of cool-white fluorescent and incandescent lamps provided a PPF⁴ of 67.0 to 73.5 nE cm⁻²s⁻¹ between 400 and 700 nm and PR of 10 to 13 w m⁻² between 700 and 850 nm. PPF⁴ and PR were measured 95 cm from the lamps with a cosine-corrected Lambda⁵ quantum/radiometer/photometer and sensors. Day and night transitions of light and temperature were abrupt and coincident. RH was greater than 70% at all temperatures. Atmospheric CO₂ concentrations were monitored by IR gas analysis and maintained at 350 to 400 μl/l by injection of commercial grade gas. Beginning at planting, the photoperiod was kept effectively long to prevent floral induction by interrupting the dark period from 2300 to 0200 with the incandescent lamps. These lamps provided PR of 9 to 12 w m⁻² and PPF⁴ of 6.9 to 7.0 nE cm⁻²s⁻¹. Plants were watered in the mornings with a nutrient solution and in the afternoon with deionized H₂O to the drip point. All elements in the nutrient solution except nitrogen and chlorine were as reported for the phytotron (5). Substitution of MgCl₂ and CaCl₂ for Mg(NO₃)₂ and Ca(NO₃)₂ resulted in a nitrogen-free solution with a chlorine concentration of 132 μg/ml. Nitrogen was added as NH₄NO₃ to provide nitrogen concentrations of 10 μg/ml from planting to the V2 growth stage, 5 μg/ml from V2 to V3 stage, and 0 μg/ml from V3 to final sampling at the R6 stage (Table I). Upon emergence at 5 days after planting and again 5 days later, a commercial preparation of *Rhizobium japonicum* suspended in deionized H₂O (20 g dry inoculum/l) was applied at a rate of 25 ml per plant. The plants were thinned to two per pot at all temperatures at 10 days after planting and to one per pot at the V2 stage. Inductive short day photoperiods were begun at the V3 stage by discontinuing the 3-h interruption during the dark period.

Application and Measurement of Leaf Moisture Stress. Half of the plants at each temperature were subjected to a moisture stress by withholding water and nutrient solution at the beginning pod (R3) stage. Water and nutrient application were continued for the other half. The total water potentials of leaf discs from two plants of each moisture treatment were measured with double junction isopiestic thermocouple psychrometers (16). Leaf water potential measurements were made on uppermost fully expanded leaf tissue immediately following the morning watering. When the leaf moisture potential reached -22 to -24 bars, the stressed plants were rewatered with nutrient solution and then continued on the same schedule for water and nutrient solution application as the control plants. For the remainder of the sampling period, leaf moisture potentials of plants were measured six or seven times at irregular intervals for 14 days (at 30/18 and 26/18 C) or 15 days (at 22/18 C) after water was resupplied to the stressed plants. At the final sampling, plants were at the full seed (R6) stage.

Measurement of Specific Nodule Activity. Nitrogen fixation activity of intact nodules on excised root systems was assayed by the acetylene reduction technique (9). Two plants from each treatment were selected randomly for the assay on each sampling date. The intact root systems were excised and gently separated from the substrate. The excised root system, together with any substrate still attached to root tissue, was placed in a 2-liter, wide-mouth glass jar. The jar was capped with a screw-type lid fitted with a septum to permit injection and withdrawal of gas samples. Acetylene was injected within 3 min after excision of roots. After incubation for 1 h at a temperature of 23 to 25 C, gas samples were collected in triplicate from each root system and stored in 1.0-ml tuberculin syringes. The rate of ethylene formation was linear throughout the incubation period for both control and stressed plants at each temperature. Ethylene production was

Table I. Effect of Day/Night Temperatures on Timing of Developmental Stages of Soybeans

Day/Night Temperature	Developmental Stages ^a							
	VC	V1	V2	V3	V5	R1	R3	R6
C	days after planting							
30/18	5	9	13	17	23	40	48	66
26/18	5	10	14	18	24	41	49	67
22/18	5	13	17	21	31	48	54	73

^a Criteria of Fehr and Caviness (7) were used in determining developmental stages.

determined by injection of a 0.5-ml aliquot of the gas sample into a Hewlett-Packard 5831A gas chromatograph using a hydrogen flame ionization detector and a 2-m stainless steel column containing 80- to 100-mesh Porapak R (Waters Associates, Inc., Milford, Mass.). Specific nodule activity was calculated from the mean of the triplicate gas samples and dry weight of nodules for each root system.

Measurement of Adenylates. Adenylate levels in nodule tissue were determined for two plants from each treatment on each sampling date using a modification of the procedure described by Gruenhagen and Moreland (8) and Moreland *et al.* (12). Between 0.25 and 0.35 g fresh nodules were gathered randomly (with respect to size and position on the root system) from each plant, weighed and immediately placed in an extraction medium of 3 ml of 7% (v/v) HClO₄ at 0 C. Duplicate samples of the extraction medium containing nodules were homogenized for 30 s at 0 C using a Brinkmann model PT-10 Polytron operating at maximum speed. The homogenates were adjusted to pH 7.6 with KOH in the presence of 2.0 ml of 0.2 M Hepes buffer and centrifuged at 10,000g for 10 min. Aliquots of the extracts were incubated with pyruvate kinase and myokinase to enzymically convert ADP and AMP to ATP. The aliquots were analyzed for ATP using the luciferin-luciferase system (Sigma FLE-50) with an Aminco Chem-Glow Photometer. Internal ATP standards were used to calculate a quenching factor to correct for the interferences of HClO₄ and other materials present in the extract. ADP and AMP concentrations were calculated by difference after enzymic conversion. The adenylate pool of the nodules was used to calculate energy charge according to the concept of Atkinson (1).

Measurement of Plant Dry Weights. As plants were sampled for moisture potential, specific nodule activity, and adenylates, fresh weights were taken of all tissues to be used for analyses and of remaining tissues. The remaining tissues of each separate plant part (leaves, stem and petioles, roots, nodules, and pods) were dried at 65 C and weighed. The dry weights were corrected for tissue used in analyses on the basis of dry weight to fresh weight ratios.

Statistical Procedures. The emphasis of this study was the difference in response between control and stressed plants rather than in absolute response. In the case of leaf water potential, specific nodule activity, and nodule adenylate charge, it was assumed that variations in measured values for control plants during the experimental period were random or procedure-related. To assist in presentation of the results, an adjusted parameter value for control plants throughout the experimental period was calculated as the mean of measured values at all samplings within a temperature. Then, adjusted parameter values for stressed plants at individual sampling dates were calculated as the differences between measured values for control and stressed plants at each sampling date subtracted from the adjusted value for control plants according to the relationship

$$A_s = A_c - (M_c - (M_s)) \quad (1)$$

where A_s and A_c are adjusted parameter values, and M_s and M_c are measured parameter values for stressed and control plants, respectively. Dry matter accumulation in whole plants, pods,

⁴ Abbreviations: PPF⁴: photosynthetic photon flux density; PR: photomorphogenic radiation.

⁵ The use of trade names in this publication neither implies endorsement by the North Carolina Agricultural Research Service of the products named nor criticism of similar ones not mentioned.

nodules, and roots and number of pods were expected to change over the sampling period. These data were fitted to regression models by a forward selection procedure (6). Beginning with a linear model, successive nonlinear elements of the independent variable, days after water withheld, were added to the model until no significant (0.05) deviation for the variance ratio of the partial sum of squares was obtained. The equation for the preceding regression model was then accepted as an adequate description of the data.

RESULTS

Leaf Moisture Potential. The average leaf moisture potential for control plants was significantly higher at the 22/18 C temperature than at the warmer temperatures (Fig. 1). The leaf moisture potentials dropped rapidly after water was withheld and reached the predetermined minimum value of about -24 bars on the 4th day at all temperatures. Upon rewatering, initial recovery was rapid with potentials reaching about -13 bars within the 1st day after rewatering. Thereafter, differences in potentials between control and stressed plants were not significant. The lack of an effect of temperature on rate of expression of recovery from a leaf moisture stress can be attributed to selection of cultural and environmental conditions during growth. By establishing the temperature treatments at seeding and initiating short day inductive photoperiods at a common morphological growth stage after

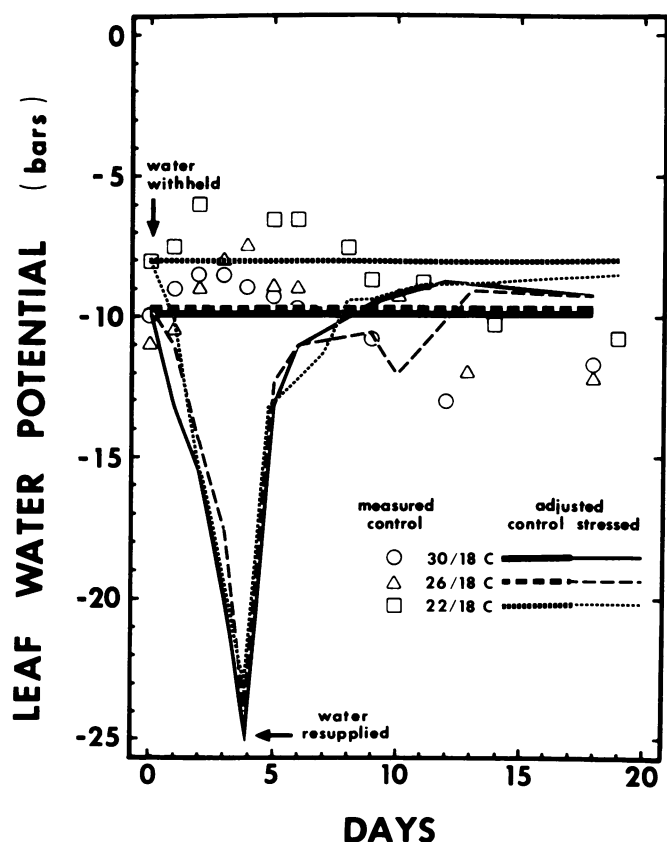


FIG. 1. Response and recovery in leaf moisture potential of soybeans to applied moisture stress at three day/night temperatures. Plots for adjusted control are within temperature means of measured potentials over all sampling times. Adjusted potentials for control plants at 22/18 C are statistically different at the 0.05 level probability from that of either 26/18 or 30/18 C. Adjusted plots for stressed plants are derived by equation 1 and reflect the difference between measured potentials for stressed and control plants. The *t* statistics for differences between control and stressed plants are significant at the 0.15 level of probability between 2 and 5 days for all temperatures.

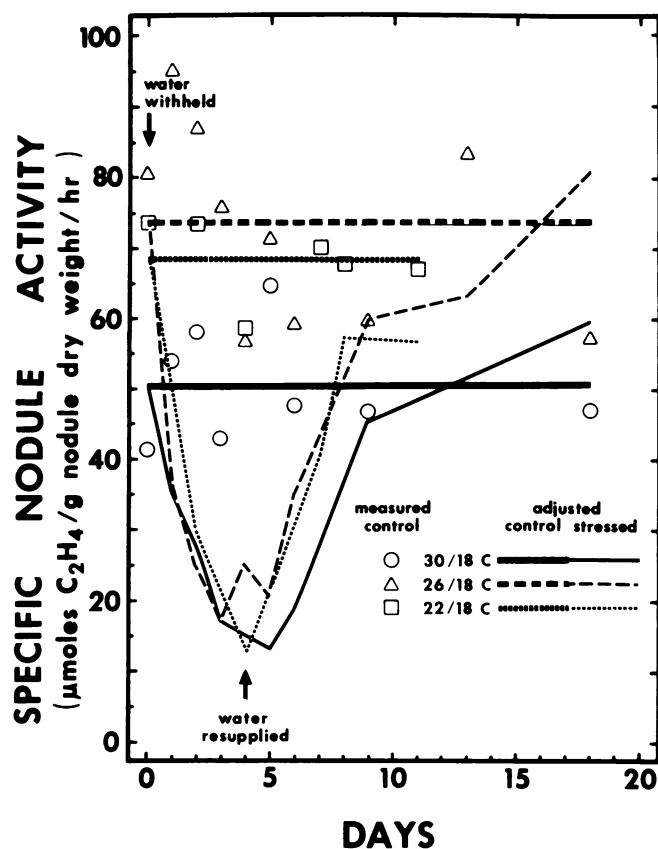


FIG. 2. Response and recovery in specific nodule activity of soybeans to applied moisture stress at three day/night temperatures. Plots for adjusted control are within temperature means of measured activities over all sampling times. Adjusted activities for control plants at 22/18 C are statistically different at the 0.01 level of probability from that of either 26/18 or 30/18 C. Adjusted plots for stressed plants are derived by equation 1 and reflect the difference between measured activities for stressed and control plants. The *t* statistics for differences between control and stressed plants are significant at the 0.15 level of probability between 2 and 7 days for all temperatures.

steady-state growth had begun rather than at a common chronological age, the effect of temperature on initial growth was counteracted and plants at the three temperatures were approximately the same size (30–35 g total dry weight per plant) when water stress was applied at the beginning pod stage. Because vapor pressure deficits at the three temperature treatments were similar (approximately 5, 6, and 8 mm Hg at 22, 26, and 30 C day temperatures and 4 mm Hg at the 18 C night temperature), transpirational demands of the plants with similar leaf mass (12–14 g leaf dry weight per plant) were not greatly different.

Specific Nodule Activities. The average specific nodule activity of control plants at 30/18 C was significantly lower than those at the cooler air temperatures (Fig. 2). (Substrate temperatures were not measured but are presumed to vary relative to air temperatures.) However, the specific nodule activities had decreased to a similar level for all temperatures at the end of the 4-day stress period. Recovery of specific nodule activity lagged behind the recovery of leaf moisture potential (*cf.* Fig. 1) and remained significantly lower than control levels until 4 to 5 days after rewatering.

Adenylates. The energy charge of nodules responded rapidly to both the imposition and relief of a leaf moisture stress (Fig. 3). The decrease in energy charge at the end of the 4-day stress period was greater in stressed plants at 30/18 C (30%) than at 26/18 and 22/18 C (19%). Recovery of energy charge at all temperatures nearly paralleled the recovery of leaf moisture potential (Fig. 1)

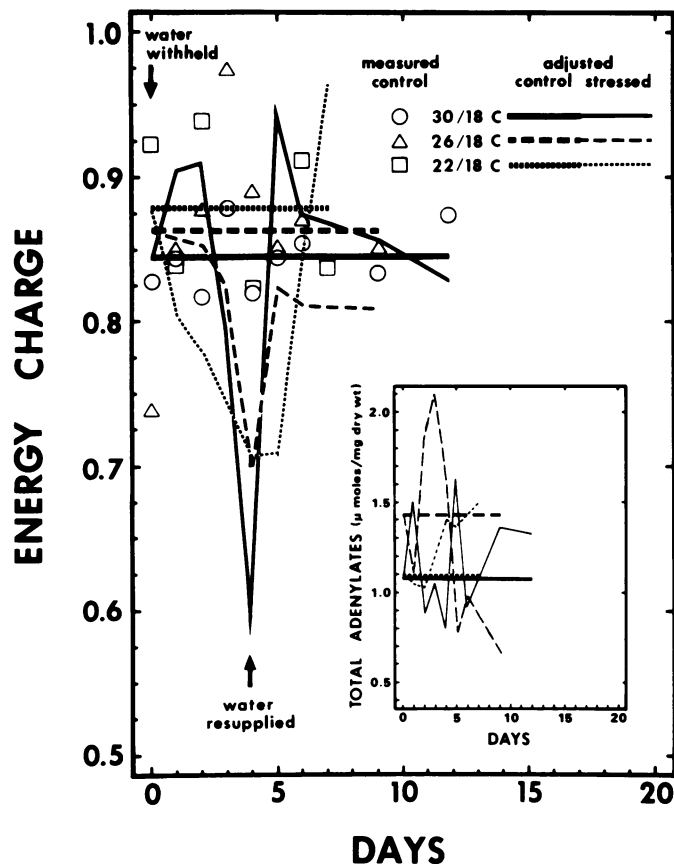


FIG. 3. Response and recovery in nodule adenylate charge of soybeans to applied moisture stress at three day/night temperatures. Plots for adjusted control are within temperature means of measured charges over all sampling times. Adjusted plots for stressed plants are derived by equation 1 and reflect the differences between measured charges for stressed and control plants. The t statistics for differences between control and stressed plants are significant at the 0.15 level of probability between 3 and 4 days for all temperatures.

and did not undergo the lag in recovery found for specific nodule activity (Fig. 2). Thus, the lag in recovery of specific nodule activity was not associated with the energy charge of the nodules. The total adenylate concentration in nodules (Fig. 3) demonstrated no discernible pattern of response to the imposed leaf moisture stress, but in control plants was significantly greater at the 0.05 level of probability at 26/18 C than at 30/18 and 22/18 C. The lack of correlation between total adenylate levels in nodules and leaf moisture potential indicates that neither is directly involved in the delayed recovery of specific nodule activity.

Growth and Yield. Application of a single cycle of water stress and recovery reduced the dry matter accumulation both for whole plants and pods during the 18- to 19-day interval between beginning pod stage and full seed stage (Fig. 4). Temperature had no significant effect at the 0.10 level of probability on either initial or final dry weight of plants or pods. The reduction in dry weight of whole plants attributed to the stress (about 10 g per plant) was greater at the conclusion of sampling than that for pods alone (about 5 g per plant). The residual effects during early pod development of a moisture stress applied at the beginning pod stage were about equally divided between vegetative and reproductive growth. The linear response of dry matter accumulation by whole plants and the quadratic response by pods indicate that the reduction in rate of vegetative growth was an immediate effect of leaf moisture stress and the reduction in reproductive growth was a delayed effect. This is supported by the rapid resumption in rate of dry matter accumulation by whole stressed plants at nearly that of control plants when only the poststress period is considered (Fig. 4). It also is consistent with the effects of the stress on changes in number and size of pods (Fig. 5). Pods continued to appear for about 2 weeks after appearance of the first pods. The rate of appearance of pods, as well as the number of pods remaining at the full seed stage, was suppressed by the application of a leaf moisture stress at the beginning pod stage (Fig. 5). The early rate of pod development, as measured by average weight per pod, also was suppressed by the moisture stress (Fig. 5). However, the average pod weight at the full seed stage was similar for stressed and control plants. These results for "Hill," which agree with data taken by Sionit and Kramer (13) at pod maturity for "Ransom" and "Bragg," suggest that the development of individual pods is

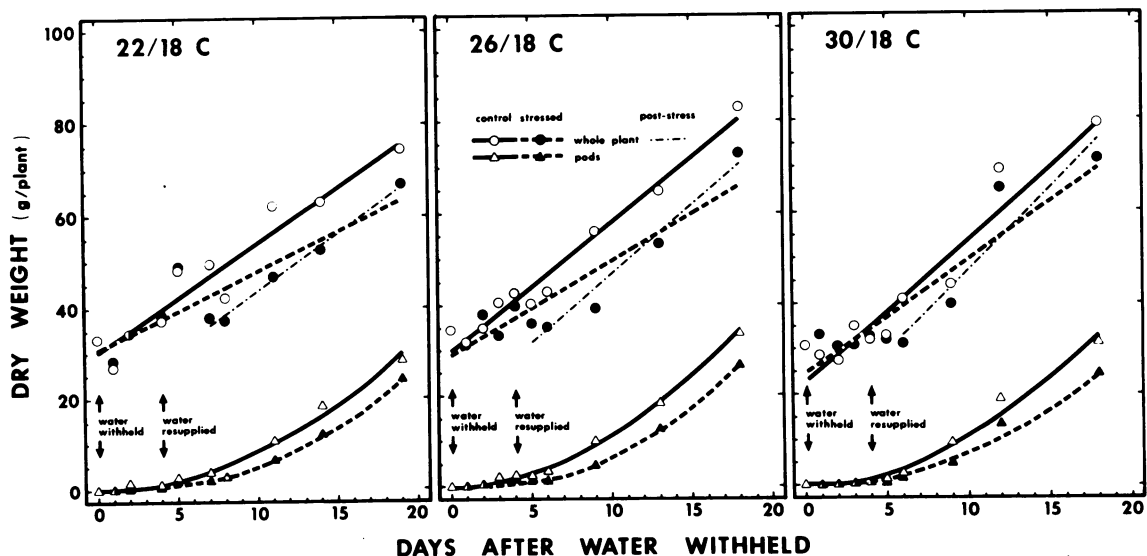


FIG. 4. Effect of a moisture stress on dry matter accumulation in whole plants and pods at three day/night temperatures. In the linear regression equation, $y = a + bx$ for whole plant dry weights (y) at 22/18, 26/18, and 30/18 C, for control plants $a = 30.0, 30.2,$ and $23.5,$ and $b = 2.4, 2.8,$ and $3.1;$ for stressed plants $a = 30.5, 29.0,$ and $25.1,$ and $b = 1.8, 2.0,$ and $2.5;$ for poststress plants $a = 18.8, 13.2,$ and $12.4,$ and $b = 2.5, 3.2,$ and $3.5.$ In the quadratic regression equation $y = a + bx + cx^2$ for pod dry weights (y) at 22/18, 26/18, and 30/18 C, for control plants $a = -0.02, -0.07,$ and $-1.11,$ $b = 0.03, 0.15,$ and $0.39,$ and $c = 0.081, 0.096,$ and $0.082;$ for stressed plants $a = 0.45, 0.64,$ and $0.33,$ $b = -0.36, -0.43,$ and $-0.01,$ and $c = 0.085, 0.103,$ and $0.079.$

not irreversibly affected by a moisture stress applied during early pod formation and that the yield potential of the plant is reduced by a decrease in the number of pods.

The data for dry weight of roots (Fig. 6) are variable and the regression equations nonsignificant, possibly because the difficulty in separating peat moss used in the substrate from root tissue prevented quantitative recovery of roots. Relative to effects of the applied moisture stress on dry weight of total plants, the moisture stress had little effect on dry weight of roots because root growth apparently ceased before application of the moisture stress (note the apparent lack of growth of roots for control plants). The dry weight of nodules (for which retrieval was less impeded by the peat moss) continued to increase during the sampling period, but the amount of increase was restrained by the moisture stress (Fig.

6). The rate of nodule growth was slower for control and stressed plants at 22/18 C than at 26/18 and 30/18 C.

DISCUSSION

This experiment utilized a single cycle of an abrupt moisture stress in pot culture. The results should not be interpreted as characteristic of plant response to the more gradual onset of moisture stress which normally occurs in a field situation. The results of this experiment do connect the separate reports of Huang *et al.* (10, 11) and Sionit and Kramer (13) which also used single cycles of moisture stress in pot culture. A single cycle of application and recovery of a leaf moisture stress during pod formation of nodulated soybeans resulted in a temporary depression of specific

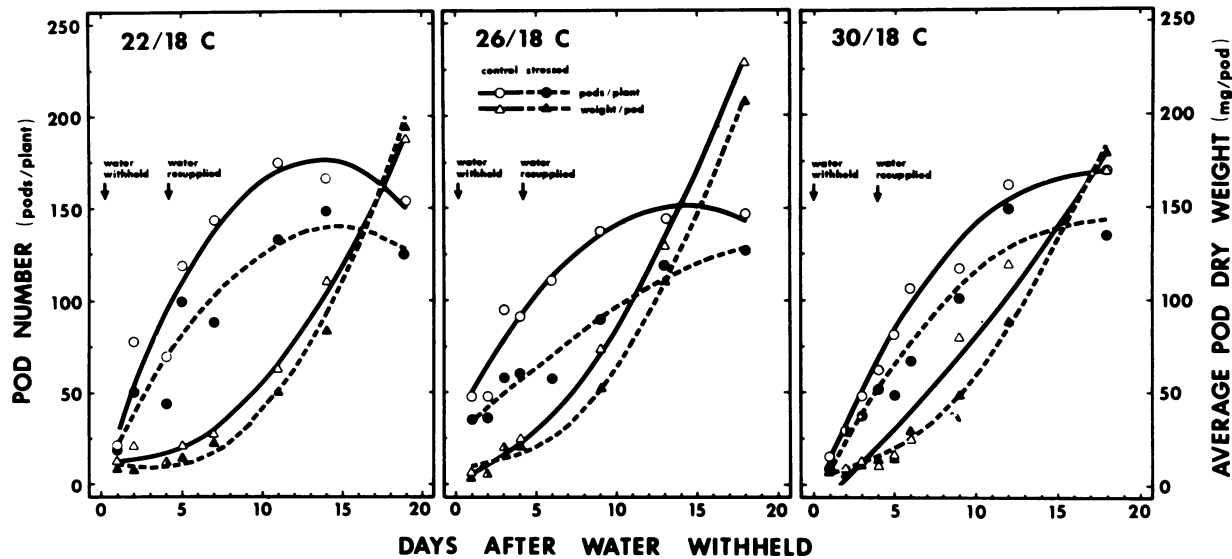


FIG. 5. Effect of a moisture stress on number and size of pods at three day/night temperatures. In the quadratic regression equation $y = a + bx + cx^2$ for pod number (y) at 22/18, 26/18, and 30/18 C, for control plants $a = 7.85, 32.45,$ and $-8.48, b = 24.88, 16.71,$ and $20.74,$ and $c = -0.915, -0.588,$ and -0.601 ; for stressed plants $a = 3.58, 24.73,$ and $-12.28, b = 18.36, 8.40,$ and $17.59,$ and $c = -0.616, -0.143,$ and -0.502 . In the quadratic regression equation $y = a + bx + cx^2$ for dry weight per pod (y) at 22/18, 26/18, and 30/18 C, for control plants $a = 14.22, 3.14,$ and $-12.45, b = -1.72, 2.25,$ and $7.52,$ and $c = 0.573, 0.575,$ and 0.165 ; for stressed plants $a = 17.12, 10.53,$ and $3.87, b = -5.29, -1.63,$ and $0.53,$ and $c = 0.758, 0.701,$ and 0.517 .

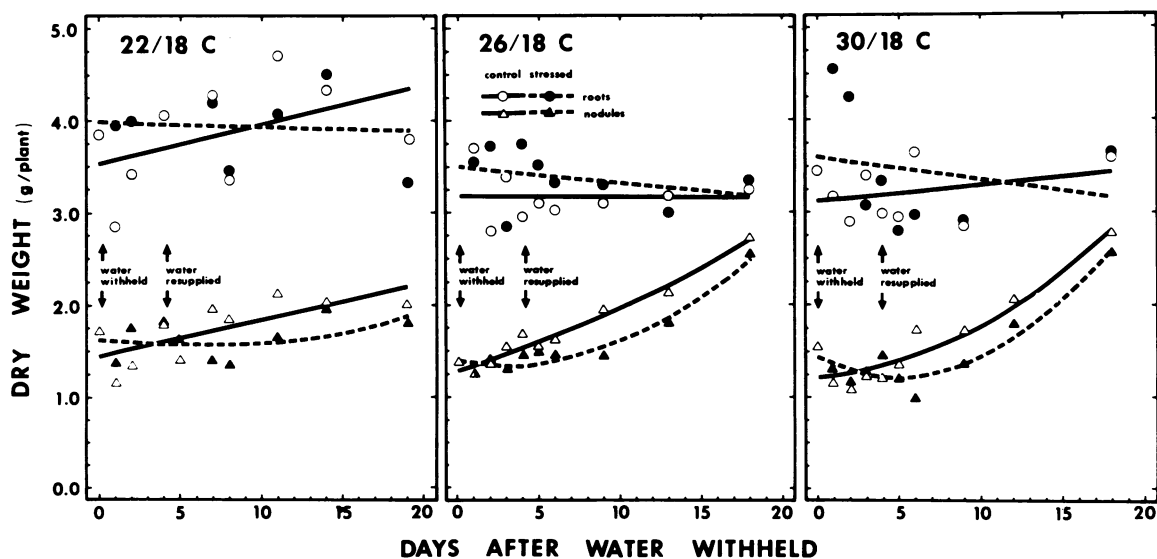


FIG. 6. Effect of a moisture stress on dry matter accumulation in roots and nodules at three day/night temperatures. In the linear regression equation $y = a + bx$ for root dry weights (y) at 22/18, 26/18, and 30/18 C, for control plants $a = 3.54, 3.18,$ and $3.13,$ and $b = 0.04, -0.01,$ and 0.02 ; for stressed plants $a = 3.98, 3.50,$ and $3.58,$ and $b = -0.01, -0.02,$ and -0.03 . In the quadratic regression equation $y = a + bx + cx^2$ for nodule dry weights (y) at 22/18, 26/18, and 30/18 C, for control plants $a = 1.45, 1.31,$ and $1.24, b = 0.04, 0.05,$ and $0.01,$ and $c = 0.001, 0.002,$ and 0.004 ; for stressed plants $a = 1.64, 1.39,$ and $1.42, b = -0.02, -0.03,$ and $-0.08,$ and $c = 0.002, 0.005,$ and 0.008 .

nodule activity as measured by acetylene reduction (Fig. 2) and in a reduction of the number of pods per plant (Fig. 5). The leaf moisture potential recovered rapidly upon rewatering (Fig. 1), but recovery of specific nodule activity was delayed several days beyond recovery of leaf moisture potential (Fig. 2). In a separate study in a controlled-temperature greenhouse at 26/22 C, the net CO₂ exchange rate for leaves of stressed plants at the same growth stage did not return to control levels until several days after recovery of leaf moisture potential (unpublished data). During and immediately after the stress period, there was an interruption of growth of pods. Since the average weight of pods for stressed plants was equal to that for control plants (Fig. 5), the difference in weight of pods per plant resulted from effects on number of pods.

Nitrogenase activity in nodules is closely related to the energy state of the nodules (3). In this experiment both the decline and revival in energy charge of nodules (Fig. 3) were associated with the moisture status of the leaves (Fig. 1); but, only the decline in specific nodule (nitrogenase) activity (Fig. 2) was associated with the moisture status of leaves. As was concluded for recovery of photosynthesis (11), recovery of specific nodule activity following a severe water stress was dependent upon more than restoration of energy charge. The mechanism responsible for the delay in recovery of specific nodule activity is not evident from the results of this experiment.

It is evident from this experiment that while temperature affected the specific nodule activity of control plants, time of recovery of specific nodule activity to control levels following relief of a leaf moisture stress was relatively unaffected. It also is evident that the effects of temperature on specific nodule activity were not reflected in either vegetative or reproductive growth of plants. Specific nodule activity was significantly less for control plants grown at 30/18 C than at either 26/18 or 22/18 C, but total and pod dry weights were not altered significantly by these temperatures. Either temperature affected the relationship between potential nitrogenase activity as measured by acetylene reduction and the actual rate of dinitrogen fixation or altered the ratio between

dry matter and nitrogen accumulation during the experimental period. In view of the greater than 2-fold increase in dry matter accumulation during the experimental period, the latter explanation seems unlikely.

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