Reprinted from *Crop Science*
Vol. 27, No. 5

Nodule Development in a Split-Root System in Response to Red and Far-Red Light Treatment of Soybean Shoots[1]

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ABSTRACT

A split-root (sides A and B) study with soybean [*Glycine max* (L.) Merr. ('Lee')] and USDA strain 3I1b110 *Bradyrhizobium japonicum* was conducted to assess the effect of red and far-red light treatments of shoots on the timing of *B. japonicum*-induced suppression of root nodulation. Plants were grown at 25°C in a growth-chamber with 12-h daily photosynthetic periods that ended with 5-min exposures to red or far-red light. Day 0 for inoculation was 7 days after planting at which time roots had extended into both sides of the split-root system. When side B was inoculated 2 to 4 days or longer after side A, nodulation and nodule mass on side B were significantly less than on side A. The number of nodules formed on roots of either side A or B were lower when shoots were treated with far-red than with red light. Therefore, total suppression of nodulation on side B occurred more rapidly with far-red than red light treatment. When far-red treatment was followed immediately by red treatment, plants responded similarly to the red treatment for nodule weight as well as nodule number. These data show that the timing of suppression of secondary nodulation on the roots can be affected by spectral balance of light received by the shoots, and the reversible nature of the effects of far-red light with red light strongly indicates phytochrome involvement. This interaction could have significant effects on nodulation patterns of soybean grown in different populations, row widths, and row orientations because these treatments alter the far-red/red ratio of light received in soybean canopies. It may also be important in the interpretation of greenhouse and growth-chamber studies of phenomena such as soybean nodulation and *B. japonicum* strain preference by different soybean cultivars.

***Additional index words:* *Glycine max* (L.) Merr., *Bradyrhizobium japonicum*, Nodulation suppression, Canopy light, Spectral composition, Phytochrome.**

DINITROGEN FIXATION and the *Bradyrhizobium japonicum*-soybean [*Glycine max* (L.) Merr.] symbiosis are of considerable agricultural importance, particularly in geographic areas with low N soils. Although considerable work has been directed toward improvement of *B. japonicum* strains, additions of new inoculants to field-planted soybean frequently do not increase either the nodular occupancy of the introduced strains or seed yields (4,13). These inconsistent results can be partially explained by *B. japonicum* and plant interaction with environmental variables such as drought, heat, and soil acidity (3, 6, 9, 11, 12, 14). Recently, Hunt et al. (5) showed that the response of soybean seed yield to row orientation was influenced by the strain of *B. japonicum* used for inoculation. They postulated that the far-red/red (FR/R) ratio of canopy light was involved, since they had previously shown that the FR/R ratio of canopy light was greater for north-south than east-west oriented rows. They also had shown that light environment (via phytochrome) regulated photosynthate partitioning between shoot and root as well as nodule mass and number (8). Koss-

[1] Contribution from USDA-ARS in cooperation with the S.C. Agric. Exp. Stn. Received 20 Oct. 1986.

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Published in *Crop Sci.* 27:973–976 (1987).

lak and Bohlool (10) showed that nodulation of one side of a split-root system of soybean would suppress nodulation of the other side if inoculation of the second side was delayed even a few days. They also reported that the effect was enhanced or diminished by the length of day and by photosynthetic photon flux density (PPFD) (as regulated by a shade cloth). They interpreted the response to be due to differences in photosynthate production levels. However, alteration of daylength also invokes involvement of the phytochrome system. Additionally, other workers (1) reported that under field conditions, green shade cloth increased FR/R ratio of penetrating light by about 5% and influenced plant growth. The objective of the present study was to determine whether R or FR treatment of the soybean shoot would influence the *B. japonicum*-induced suppression of subsequent root nodulation.

MATERIALS AND METHODS

A growth-chamber study was conducted using a modification of the split-root system described by Singleton (16) and Kosslak and Bohlool (10). The controlled environment was chosen because it resulted in an earlier suppression of secondary nodulation than a greenhouse environment, which was used in our preliminary experiments. A split-plot design with six replicates was used. Light (FR and R) was the main-plot treatment, and time of inoculation was the split-plot treatment.

The split-root system consisted of two adjacent polyvinyl chloride (PVC) pipes and a plastic elbow placed as an inverted "V" with one arm extending into each side (randomly designated as A and B) of the system. The PVC pipes (3.8 × 15 cm) and elbows (1.5-cm i.d., with 4-cm arms) were sterilized by soaking overnight in 15-mL Amphyl[3] L^{-1} H_2O and subsequently rinsed with deionized water. Pipes were capped on one end and filled with moist grade-3 vermiculite. A planting hole (1.0 cm) was drilled into the top of each PVC elbow. The elbows were also packed with moist vermiculite and placed with the 4-cm arms extending into the tops of two PVC pipes (sides A and B). The two pipes were taped together, and the tops were sealed with parafilm.

Soybean seeds were surface sterilized by soaking for 5 min in isopropyl alcohol (700 mL L^{-1}) and rinsed five times with deionized water. Seeds were germinated for 36 h in sterile, moist vermiculite. Seedlings with 2-cm long radicles were selected, and the tips (2–3 mm) of the radicles were removed before seedlings were planted into the hole at the top of the PVC elbow. Seedlings were watered immediately with deionized H_2O to ensure capillarity between the cut radicle and vermiculite. They were placed in a growth-chamber for preconditioning and treatment. The plants were grown at 28°C with 12-h daily light periods from 28 Sylvania VHO cool-white fluorescent lamps (PPFD = 520 μmol m^{-2} s^{-1}).

Inoculation Day 0 was 7 days after planting (DAP), at which time the roots had extended into each side of the split-root system. Seedlings were inoculated with 5 mL (1×10^8 cells mL^{-1}) of USDA 3I1b110 *Bradyrhizobium japonicum* using a hypodermic needle to place the inoculant among roots in the designated side (A or B) of the split-root system. Treatment schedules are shown in Table 1. Seedlings received the following light treatments to saturate the phytochrome system at the end of each day: (i) 5 min of FR (3.6 W m^{-2} between 700 and 780 nm); (ii) 5 min of R (3.6 W m^{-2} between 600 and 700 nm); or (iii) 5 min of FR followed immediately by 5 min of R as described by Kasperbauer and Hamilton (7). The R and FR treatments began 7 DAP and were repeated each day for 25 consecutive days. Seedlings were watered as needed with Hoagland N-free solution during the experiment.

Seedlings were harvested 32 DAP (Day 25). Stem length and dry mass of leaves and stems were measured. Roots (sides A and B) were washed with deionized water, nodules were removed and counted, and both roots and nodules were freeze-dried and weighed. Data were subjected to analysis of variance and groups of means were compared by the single degree of freedom contrast after a Box-Cox transformation $\alpha = 0.11$ to achieve homogeneity of variance (2, 17).

Table 1. Treatment codes and schedules for inoculation of split-root systems (Sides A and B) with *Bradyrhizobium japonicum*.

		Inoculation, day†	
		Side‡	
Treatment code	Single df contrast	A	B
A_0B_0		0	0
A_6B_6		6	6
	$A_{6-9}B_{6-9}$		
A_9B_9		9	9
A_0B_2		0	2
	A_0B_{2-4}		
A_0B_4		0	4
	A_0B_{4-6}		
A_0B_6		0	6
	A_0B_{6-9}		
A_0B_9		0	9
Noninoc		No inoculation	

† Inoculation Day 0 was 7 days after planting, at which time roots had extended into each side of the split-root system.
‡ A and B designate the two sides of the split-root system, and subscripts designate the inoculation time (days) after Day 0.

RESULTS AND DISCUSSION

The R and FR treatment of the shoots did not significantly affect leaf mass but did affect stem length and root mass ($P < 0.05$) as shown in previous work by Kasperbauer et al. (8). The FR-treated stems were significantly longer than R-treated stems. The grand mean for root dry mass of the FR-treated plants, 113 mg, was significantly lower ($P < 0.05$) than the grand mean of root mass for either the R or FR, R-treated plants; 142 and 148 mg, respectively. There were no significant differences in the root mass of sides A and B, and there were no significant trends in root mass on either side A or B with inoculation schedule. Thus, R and FR treatments of the shoots induced different partitioning of dry mass among plant parts rather than significantly different amounts of total dry mass accumulation per plant.

Nodule Number

The uninoculated control did not develop nodules on either side of the split-root system. When inoculation of both sides (A and B) occurred on the same day (A_0B_0, A_6B_6, or A_9B_9), nodulation always occurred on both sides, and there were no significant differences between nodule numbers of sides A and B. However,

[3] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA or the S. C. Agric. Exp. Stn. and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

Table 2. Effects of R and FR light treatment of shoots and root inoculation time on nodule number for soybean grown in a split-root system.

Inoculation schedule	Light treatment					
	R		FR		FR, R	
	Side		Side		Side	
	A	B	A	B	A	B
	no. per plant†					
A_0B_0	17 ± 4*	17 ± 3*	8 ± 2*	11 ± 1*	11 ± 4*	12 ± 6*
$A_{6-9}B_{6-9}$	27 ± 6*	22 ± 4*	21 ± 5*	19 ± 4*	24 ± 5*	18 ± 5*
A_0B_{2-4}	32 ± 5*	14 ± 3*	14 ± 3*	5 ± 2*	18 ± 4*	11 ± 3*
A_0B_{4-6}	38 ± 5*	7 ± 2*	16 ± 2*	2 ± 1	29 ± 8*	4 ± 3*
A_0B_{6-9}	38 ± 7*	2 ± 2	21 ± 4*	0 ± 0	32 ± 8*	0 ± 0
A_0B_9	39 ± 8*	0 ± 0	22 ± 5*	0 ± 0	26 ± 5*	0 ± 0
Noninoc	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

* Numbers within the same column are significantly different from the noninoculated control at $P < 0.05$ when compared by a single degree of freedom contrast. Numbers were transformed by Box-Cox transformation $\alpha = 0.11$ for homogeneity of variance before analysis.
† Values are means ± SE.

Table 3. Effects of R and FR light treatment of shoots and root inoculation time on nodule dry mass for soybean grown in a split-root system.

Inoculation schedule	Light treatment					
	R		FR		FR, R	
	Side		Side		Side	
	A	B	A	B	A	B
	mg/plant†					
A_0B_0	9 ± 2*	8 ± 1*	4 ± 1*	5 ± 1*	5 ± 2*	6 ± 3*
$A_{6-9}B_{6-9}$	8 ± 2*	7 ± 2*	6 ± 2*	6 ± 2*	9 ± 2*	6 ± 2*
A_0B_{2-4}	14 ± 2*	4 ± 1*	6 ± 2*	2 ± 1*	6 ± 0*	3 ± 2*
A_0B_{4-6}	24 ± 4*	2 ± 1*	6 ± 2*	1 ± 0	15 ± 4*	2 ± 2*
A_0B_{6-9}	29 ± 5*	1 ± 1	14 ± 5*	0 ± 0	21 ± 6*	0 ± 0
A_0B_9	26 ± 6*	0 ± 0	18 ± 4*	0 ± 0	15 ± 4*	0 ± 0
Noninoc	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

* Numbers within the same column are significantly different from the noninoculated control at $P < 0.05$ when compared by a single degree of freedom contrast. Numbers were transformed by Box-Cox transformation $\alpha = 0.11$ for homogeneity of variance before analysis.
† Values are means ± SE.

inoculation of side A suppressed nodule development from subsequent inoculation of side B (Table 2) in a manner similar to the results reported by Kosslak and Bohlool (10).

The suppression effect is easier to visualize when two inoculation times are pooled and contrasted by single degree of freedom against the uninoculated control. Therefore, data are presented in this fashion in Tables 2 to 4. The R and FR light treatments altered the timing but not the completeness of this suppression of nodulation. Nodule numbers on side B of R-treated plants were significantly greater than zero if inoculation of side B occurred 4 to 6 days or less after the inoculation of side A (Treatment A_0B_{4-6}). However, nodule numbers on side B of the FR-treated plants were significantly greater than zero only when inoculation of side B occurred 2 to 4 days or less after inoculation of side A (Treatment A_0B_{2-4}). Plants that received R immediately after FR behaved similarly to R-treated plants, which indicated phytochrome involvement. Total suppression occurred in all light treatments with a 9-day delay of inoculation to side B. Thus, we concluded that total suppression of secondary nodulation occurred sooner when light was FR and that phytochrome form in the shoots influenced timing of secondary nodule suppression on the roots.

Nodule Dry Mass

Inoculation dates and the R and FR light treatments affected nodule dry mass (Table 3) similarly to nodule number (Table 2). Dry mass of nodules on side A of inoculated plants was always significantly greater than zero. Mass of nodules on side B of R-treated plants was significantly greater than zero if inoculation of side B occurred 4 to 6 days or less after inoculation of side A. Mass of nodules on side B of the FR-treated plants was significantly greater than zero only if inoculation of side B occurred 2 to 4 days or less after side A. As with nodule number, nodule mass effects of FR-treated shoots were reversed by R. Specific nodule mass (mass/nodule) followed a similar trend to total dry mass (data not shown).

Nodule Mass to Root Mass Ratio

Inoculation timing affected this ratio much more than light treatments (Table 4), and the trends were similar to those of nodule number and mass. This indicates that the FR treatments decreased total root and nodule masses, but left the suppression mechanism relatively unaltered.

The time for total suppression of soybean root nodulation and decreased nodule mass per plant were

Table 4. Effects of R and FR light treatment of shoots and root inoculation time on ratio of nodule mass/root mass for soybean grown in a split-root system.

Inoculation schedule	Light treatment					
	R		FR		FR, R	
	Side		Side		Side	
	A	B	A	B	A	B
	ratio (× 10^3)†					
A_0B_0	125 ± 20*	174 ± 33*	93 ± 17*	92 ± 27*	75 ± 27*	154 ± 82*
$A_{6-9}B_{6-9}$	98 ± 22*	104 ± 22*	77 ± 28*	76 ± 13*	131 ± 31*	71 ± 16*
A_0B_{2-4}	218 ± 33*	58 ± 15*	101 ± 24*	55 ± 29*	123 ± 18*	51 ± 18*
A_0B_{4-6}	330 ± 43*	24 ± 8*	97 ± 23*	9 ± 6	229 ± 50*	21 ± 15*
A_0B_{6-9}	338 ± 53*	8 ± 5	207 ± 67*	0 ± 0	283 ± 58*	1 ± 0
A_0B_9	333 ± 58*	0 ± 0	327 ± 67*	0 ± 0	254 ± 61*	0 ± 0
Noninoc	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

* Numbers within the same column are significantly different from the noninoculated control at $P < 0.05$ when compared by a single degree of freedom contrast. Numbers were transformed by Box-Cox transformation $\alpha = 0.11$ for homogeneity of variance before analysis.
† Values are means ± SE × 10^3.

clearly influenced by FR treatment of the shoot, and this effect could be reversed by immediate R treatment of the shoot. Therefore, it seems evident that the timing of nodulation suppression in soybean seedlings was influenced by phytochrome manipulation in the shoot. This effect of spectral distribution of canopy light on nodulation could have important implications to soybean nodulation and performance in various cultural situations. For example, this phenomenon might be related to differences in soybean yield with different row orientations (5) that influence the FR/R ratio of canopy light, particularly near the end of day (8). Additionally, the phenomenon may be important in the interpretation of many experiments that address nodule morphogenesis and *B. japonicum* strain preference by soybean cultivars. It might also be important in the interpretation of metabolite production by certain *B. japonicum* strain-host plant combinations in particular light environments, as suggested by Neves et al. (15) in conjunction with ureide formation in greenhouse and field environments.

ACKNOWLEDGMENTS

We thank W. Sanders for technical assistance; and B. B. Bohlool, P. W. Singleton, and James Tavares, NIFTAL, Paia, Hawaii, for their discussion of the research idea and instruction in the use of the split-root technique.

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