Influence of *Bradyrhizobium japonicum* Strain and Far-Red/Red Canopy Light Ratios on Nodulation of Soybean

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ABSTRACT

Dinitrogen fixation, an important aspect of soybean [*Glycine max* (L.) Merr.] production, is affected by many environmental factors. Nodulation can be altered, for example, by changes in the ratio of far-red to red (FR/R) canopy light. The influence of canopy light spectral balance on the nodulation of 'Coker 368' soybean when inoculated with different strains of *Bradyrhizobium japonicum* was evaluated in field and growth chamber studies. Soybean seedlings were inoculated with USDA 110 (110) or Brazil 587 (587) and grown in 10-cm diameter pipes that were filled with vermiculite and inserted at 1.0-m intervals in seedling soybean rows that were oriented north–south (N/S) or east–west (E/W) on a Norfolk loamy sand (fine-loamy, siliceous, thermic Typic Kandiudult). The FR/R ratios of canopy light (relative to the ratio in incoming sunlight, which was assigned a value of 1.00), were 1.38 for N/S- and 1.26 for E/W-oriented rows. Row orientation significantly affected nodulation when 110 was the inoculum but not when 587 was the inoculum. Nodule biomass of 110-inoculated seedlings was 48% higher with E/W than with N/S row orientation. In the growth chamber study, soybean seedlings were inoculated with 110 or 587, and their shoots received high or low FR/R ratios at the end of each day. Nodulation was significantly affected by the FR/R ratio of canopy light when the inoculant was 110 but not when the inoculant was 587. Soybean seedlings that received the low FR/R ratio and were inoculated with 110 had 53% greater nodulation; the FR/R effects on nodulation were not statistically significant when the seedlings were inoculated with 587. These results show that even small changes in the FR/R ratio of canopy light can affect soybean nodulation in both field and controlled environments. They also show that this effect can be enhanced or diminished by *B. japonicum* strain.

ENVIRONMENTAL FACTORS are often the most limiting aspect of legume seed production. Drought and temperature extremes are well known and easily recognizable, but other factors are more subtle. The spectral composition of canopy light is a subtle but important environmental variable that can affect *B. japonicum*-soybean symbiosis. Kasperbauer et al. (1984) reported that exposure of shoots to a high far-red relative to red light (FR/R) ratio resulted in partitioning of more photosynthate to the shoots and less to the roots and nodules compared with plants exposed to a low FR/R ratio. The self regulation of nodulation in soybean seedlings was also found to be affected by FR and R light treatments; plants exposed to R light (a low FR/R ratio) nodulated for longer periods of time (Hunt et al., 1987b). Nodulation of soybean seedlings could also be altered by variation in the spectral composition of light reflected from different colored surfaces (Hunt et al., 1989).

Several studies have shown variation in the sensitivity of *B. japonicum* strains to environmental variable such as water deficits, temperature, and soil acidity (Keyser and Munns, 1979; Munevar and Wollum, 1981; Hunt et al., 1988). Row orientation affects the amount of reflected FR and the FR/R ratio of canopy light (Kasperbauer et al., 1984; Kasperbauer, 1987, 1988) and expression of the row orientation effect on soybean seed yield is influenced by *B. japonicum* strain (Hunt et al., 1985). Seed yield of soybean inoculated with *B. japonicum* strain USDA 110 was significantly affected by row orientation, but those inoculated with *B. japonicum* Brazil 587 were not significantly affected (Hunt et al., 1985). However, the large experimental variability of nodule numbers and masses among plants dug from the different row orientations and *B. japonicum* strain treatments precluded the determination of statistically significant differences in nodulation. This was not an unexpected result. Relatively large samples are required to obtain precision for shoot samples, and nodules are much more difficult to sample quantitatively (Hunt et al., 1987a).

The objectives of these experiments were to (i) determine the effect of row orientation on the nodulation of soybean inoculated with *B. japonicum* 110 or 587 and (ii) determine the effect of high or low end-of-day FR/R on nodulation of soybean inoculated with *B. japonicum* 110 or 587.

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MATERIALS AND METHODS

Coker 368 soybean seeds, were disinfected 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 petri dishes on filter paper saturated with a broth of the appropriate strain of *B. japonicum* that contained 10^8 cells mL^{-1}. Uniformly sized seeds (with radicles extended ≈1 cm) were transplanted ≈2.5-cm deep into sterile vermiculite in the treatment containers. Two germinating seeds were placed in each container, and the smaller seedling was removed in ≈5 d. The same procedure was used for both *B. japonicum* strains and for both field and growth chamber studies. However, the containers used in the field were larger than those used in the growth chamber (as detailed below). Plants were harvested 28 d after planting. Roots were washed with deionized water; nodules were subsequently removed and counted. Both roots and nodules were freeze-dried and weighed. Data were evaluated by analysis of variance (ANOVA) and least significant difference (LSD) by use of the Statistical Analysis Systems (SAS, 1985).

Field Study

These experiments were conducted in July 1986 and June 1987 on a Norfolk loamy sand near Florence, SC. A split-plot design with four replicates was used. Row orientation (N/S vs. E/W) was the main plot treatment, and the subplot treatment was *B. japonicum* inoculant (110 vs. 587). The main plot consisted of six 6-m rows on 0.5-m spacings with 20 plants m^{-1} of row. The subplot plants were in polyvinyl chloride (PVC) pipes (10-cm diam. by 45-cm length) which were capped on one end, sterilized by soaking overnight in

15 mL Amphyl[1] (Cole-Palmer, Chicago, IL) L^{-1} of water and rinsed with deionized water. The pipes were filled with sterile, agricultural-grade vermiculite that had been soaked overnight in one-tenth-strength N-free Hoagland's solution. The pipes were then inserted at 1-m intervals into the middle two rows of each plot and placed so that the upper 3-cm of the rim extended above the soil line (both inside and outside the pipes), to minimize entry of plot soil into the containers. Three paired-comparison containers per replicate were used.

Temperatures in the PVC pipes were monitored with a Campbell CR7 Datalogger (Campbell Scientific, Logan, UT) with copper-constantan thermocouples. Spectral composition of incoming light was measured with a Li-Cor Model LI-1800 (Li-Cor, Inc., Lincoln, NE) spectroradiometer with a remote light collector attached to a 1.5-m fiber optic probe. Measurements were made of incoming light from four compass directions (N, S, E, and W) to the canopy surface of randomized selected plants in each plot. Readings were taken ≈20 d after planting at ≈0900 h on cloudless days in both 1986 and 1987. The window of the remote light collector was pointed parallel to the ground to measure spectral distribution of incoming light that would be received by leaves near the top of the seedlings. Values presented are means of light readings from the four directions received by upper leaves of four plants in each of the two years for both N/S and E/W rows. The FR/R ratios are relative to the ratio in incoming sunlight.

Growth Chamber Study

A growth chamber study was conducted to evaluate the influence of FR/R ratio received by soybean seedling shoots on the nodulation by two strains of *B. japonicum*. A split-plot design with 12 replicates was used. The main plot treatment was FR vs. R light, and *B. japonicum* strains (110 vs. 587) were the split treatment.

The seedlings were started and inoculated as described above, and grown in 1-L plastic containers filled with moist vermiculite. The plants were grown in a growth chamber at 28 °C with 12-h daily light periods from cool-white fluorescent lamps (photosynthetic photon flux density [PPFD] = 520 $\mu mol\ m^{-2}\ s^{-1}$). Starting at emergence (Day 3), seedlings received either 5 min of FR (3.6 W m^{-2} between 700 and 780 nm), or 5 min of R (3.6 W m^{-2} between 600 and 700 nm) at the end of each day for 24 consecutive days. The R and FR units consisted of the light sources and filters previously described by Kasperbauer and Hamilton (1984). The seedlings were watered as needed with N-free Hoagland's solution during the experiment.

RESULTS AND DISCUSSION

Field Study

The PPFD and the percentages of blue light received by plants in N/S- and E/W-oriented rows were very similar (Table 1). However, the mean FR/R ratio in N/S-oriented rows was higher: 1.38 and 1.26 for the N/S- and E/W-oriented rows, respectively (relative to the ratio in incoming sunlight, which was assigned a value of 1.00). These results are similar to those obtained for soybean and bush bean (*Phaseolus vulgaris* L.) by Kasperbauer et al. (1984) and Kaul and Kasperbauer (1988). The increase in the FR/R ratio of soybean and bush bean in N/S-oriented rows was attributed to effects of heliotropic movement of leaves on direction of reflected FR, especially near the end of day (Kasperbauer, 1987, 1988). Maximum differences in the soil temperatures for row orientations were <1.0 °C. This similarity of rhizosphere temperature in the present experiment is realistic because the temperatures were recorded while plants were still so small that there was little shading of the soil surface.

Table 1. Spectral quality of soybean seedling canopy light associated with north-south (N/S) and east-west (E/W) row orientations.

Row orientation	Spectral parameters†		
	PPFD	FR/R	% blue
E/W	398	1.26	31
N/S	372	1.38	30
	NS	‡	NS

† PPFD = photosynthetic photon flux density ($\mu mol\ m^{-2}\ s^{-1}$). FR/R = ratio of far-red light (730–740 nm) to red light (640–650 nm) (see Kasperbauer, 1988, for rationale) relative to that in incoming sunlight which was assigned a value of 1.00; each value is the mean for the 4 directions × 4 plants × 2 years). % blue = percent blue light (400–500 nm) reflected relative to that in incoming sunlight. PPFD in incoming sunlight was 1290 $\mu mol\ m^{-2}\ s^{-1}$.

‡ Significantly different at the 0.07 level of probability. NS = not significant.

Table 2. Nodulation of soybean plants inoculated with *Bradyrhizobium japonicum* strains 110 and 587, and grown in rows oriented east-west (E/W) and north-south (N/S).

B. japonicum strain	Row orientation	Nodules/plant	
		No.	Mass
			mg
USDA 110	E/W	107	80
USDA 110	N/S	64	54
Brazil 587	E/W	83	80
Brazil 587	N/S	77	79
LSD (0.05)		24	22

Differences in the nodulation of plants inoculated with 110 were consistent with those expected to be produced by the different canopy light conditions. Plants in E/W-oriented rows were exposed to lower FR/R ratios; if inoculated with *B. japonicum* strain 110, they had significantly greater nodule number and nodule mass than those in N/S-oriented rows (Table 2). However, differences in nodule number and mass were not statistically significant when the inoculant was 587. Results of these field experiments were consistent with earlier work that showed differential sensitivity of *B. japonicum* strains to other environmental variations (Munevar and Wollum, 1981; Fuhrmann et al., 1986).

Growth Chamber Study

As with the row-orientation experiments, strain 110–inoculated soybean had more nodules and greater nodule mass when exposed to the lower FR/R ratio associated with end-of-day R rather than with end-of-day FR, and light treatment did not significantly affect these parameters when soybean were inoculated with 587 (Table 3). These findings are consistent with the results of the field experiments of this study and previous row-orientation studies where differences in the spectral composition of light received by soybean shoots were hypothesized to act through the phytochrome system to affect soybean seed yield more when

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

Table 3. Nodulation of soybean plants inoculated with *Bradyrhizobium japonicum* strains 110 and 587, and treated with 5 min red (R) or far-red (FR) light (low and high FR/R ratio, respectively) at the end of each day for 24 consecutive days in a controlled environment.

	End-of-day		Nodules/plant	
B. japonicum strain	Light	FR/R	No.	Mass
				mg
USDA 110	R	Low	75	154
USDA 110	FR	High	49	118
Brazil 587	R	Low	61	141
Brazil 587	FR	High	61	123
LSD (0.05)			19	30

the inoculant was 110 rather than 587 (Hunt et al., 1985).

Sensitivity of soybean nodulation to even small changes in FR/R ratios of canopy light in both field and controlled environments has been shown. The enhancement or diminishment of the FR/R ratio effects on nodulation responses by different *B. japonicum* strains suggests the importance of continued study of canopy light–phytochrome–*B. japonicum* strain interactions on nodulation and N_2 fixation by soybean.

CONCLUSIONS

1. Differences in nodulation associated with row orientation were more pronounced for soybean inoculated with *B. japonicum* strain 110 than 587.
2. Alteration of soybean nodulation by manipulation of the plant's phytochrome system with end-of-day R and FR light (low and high FR/R ratios, respectively) was more pronounced when the inoculant was *B. japonicum* strain 110 rather than 587.
3. The interaction among the *B. japonicum* symbiont, soybean plant, and light environment should be considered in the evaluation of management systems for optimal soybean production, especially on sandy soils of the southeastern Coastal Plain of the USA and similar soils elsewhere in the world.

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