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Nodulation of *Glycine max* by Six *Bradyrhizobium japonicum* Strains with Different Competitive Abilities†

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The root nodule locations of six *Bradyrhizobium japonicum* strains were examined to determine if there were any differences which might explain their varying competitiveness for nodule occupancy on *Glycine max*. When five strains were added to soybeans in plastic growth pouches in equal proportions with a reference strain (U.S. Department of Agriculture, strain 110), North Carolina strain 1028 and strain 110 were the most competitive for nodule occupancy, followed by U.S. Department of Agriculture strains 122, 76, and 31 and Brazil strain 587. Among all strains, nodule double occupancy was 17% at a high inoculum level (10^7 CFU pouch^{-1}) and 2% at a low inoculum level (10^4 CFU pouch^{-1}). The less competitive strains increased their nodule representation by an increase in the doubly occupied nodules at the high inoculum level. Among all strains, the number of taproot and lateral root nodules was inversely related at both the high and low inoculum levels ($r = -0.62$ and -0.69, respectively; $P = 0.0001$). This inverse relationship appeared to be a result of the plant host control of bacterial infection. Among each of the six strains, greater than 95% of the taproot nodules formed at the high inoculum density were located on 25% of the taproot length, the nodules centering on the position of the root tip at the time of inoculation. No differences among the six strains were observed in nodule initiation rates as measured by taproot nodule position. Taproot nodules were formed in the symbiosis before lateral root nodules. One of the poorly competitive strains (strain 76) occupied three times as many taproot nodules as lateral root nodules when competing with strain 110 (nodules were harvested from 4-week-old plants). Among these six wild-type strains of *B. japonicum*, competitive ability evidently is not related to nodule initiation rates.

Legume nodulation by *Rhizobium* and *Bradyrhizobium* species is a complex process requiring the coordinated expression of both bacterial and plant genes. Flavonoids in plant root exudate induce the expression of the common nodulation (*nod*) genes in both *Rhizobium* (13) and *Bradyrhizobium* (8; A. J. Nieuwkoop, Z. Banfalvi, M. G. Schell, and G. Stacey, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, K46, p. 210) species. The expression of the *Rhizobium meliloti* common *nod* genes in turn stimulates the earliest detectable plant response of root hair curling and cortical cell division (13).

Bhuvaneswari et al. (1) reported that nodulation is restricted to a zone above the apex of the soybean root corresponding to the zone of no root hairs and that the susceptibility of this zone to infection is a transient property. Newly emerged soybean root hairs are more susceptible to root hair curling than are young root hairs, and mature root hairs are the least prone to curl (17). Turgeon and Bauer (17) proposed that the propensity for root hair curling is the physiological cause for the restricted zone of infection.

Pierce and Bauer (14) showed in soybeans that all infections resulting from a second inoculation (15 h after the first) were aborted and proposed that there was a rapid host-mediated response which controls nodulation. Suppression by the plant has been reported to occur at a stage after meristem formation but before nodule emergence (2) and has been termed autoregulation (12). Delves et al. (3), using grafts between supernodulating mutants and wild-type soybeans, found evidence that plant regulation of nodule formation is controlled by the shoot. Similar plant control over infection has been reported in clover (16), and in soybeans the regulation was shown to be dependent on the plant cultivar and rhizobial strain (7).

If root infectibility is a transient property because of the rapid host suppression of nodulation, then the rate at which rhizobial strains initate infections may influence interstrain competition for nodule occupancy. A strain which is slow to initiate infections is possibly less competitive than a strain which initiates nodules more rapidly, since later nodule formation would be suppressed by the plant. This hypothesis was tested by examining the positional patterns of nodulation of six wild-type *Bradyrhizobium* strains with different competitive abilities.

MATERIALS AND METHODS

Growth of bacteria and plants. *Bradyrhizobium japonicum* 110, 122, 76, and 31 were obtained from D. Weber (U.S. Department of Agriculture, Beltsville, Md.). Strain 1028 was isolated from a North Carolina soil (11), and strain 587 was isolated from a Brazil soil (obtained from the culture collection of C. Vidor). All strains were grown and maintained on a yeast extract-mannitol medium (18). In all experiments, a 0.1-ml sample of an 8-day-old starter culture of stationary-phase cells was placed in 10 ml of yeast extract-mannitol medium, and the cells were grown for 3 days to the early log phase of growth on an orbital shaker (200 RPM). The cells were harvested by centrifugation (8 min at 12,000 × *g*), washed once with 0.85% NaCl, resuspended, and diluted to the appropriate density in sterile distilled water after being counted in a Petroff-Hausser chamber. Viable bacterial cells were verified by plate counts on yeast extract-mannitol agar.

Seeds (diameter, 5.5 to 6.4 mm) of soybeans (*Glycine max* (L.) Merr. cv. Centennial) were surface sterilized by two sequential exposures (10 min each) to a 4% $CaOCl_2$ solution supplemented with 1 drop of Tween 20 100 ml^{-1}, followed by repeated rinses with sterile distilled water. Seeds were

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germinated for 2 days in sterile, moistened vermiculite and then transferred to sterile Dispo (Northrup King Co.) growth pouches. Seedling roots were inoculated 1 day after transfer to the growth pouches. The plants were grown under fluorescent lights (60 microeinsteins m^{-2} s^{-1} in a 16-h day-night cycle) and watered on alternate days with distilled water or a nitrogen-free plant nutrient solution (10) modified by reducing $CaSO_4$ from 7 to 2 mM and replacing KH_2PO_4 plus K_2HPO_4 with 1 mM KH_2PO_4 to avoid salt precipitation when autoclaving. The nutrient solution acidity was adjusted to pH 6.2 with 1 N KOH.

Nodulation response to inoculum density. The effect of the bacterial inoculum density on nodule formation was examined. Cells of strains 110 and 587 were diluted and separately added to plant roots in growth pouches (two seedlings per pouch) to produce inoculum densities ranging from 10^8 to 10^3 CFU $pouch^{-1}$. The nodule numbers and locations (taproot or lateral root) were measured 4 weeks after inoculation.

Competition assays. The competitiveness for nodule occupancy of five of the strains was compared with that of one of the strains, strain 110. Washed cells of the five competing strains (122, 76, 31, 1028, and 587) were combined in a 1:1 ratio with reference strain 110 in water. This suspension was diluted to a density of 10^4 or 10^7 CFU ml^{-1}, and 0.5 ml was added to each root of 3-day-old soybean seedlings in growth pouches (two seedlings per pouch; 1.0 ml of inoculum $pouch^{-1}$). Nodules were harvested 4 weeks later, and the occupying strain was identified by an enzyme-linked immunosorbent assay technique (4). Additionally, the location on the taproot was measured for each nodule identified. Between 48 and 80 nodules from 12 plants were serotyped for each treatment.

Nodule initiation rate assays. The method of Bhuvaneswari et al. (1) was used to compare the nodule initiation rate of the six strains of bradyrhizobia. This method relates the position of nodules on the taproot to the time at which they were initiated. Plants were inoculated in plastic growth pouches as described above, except that the six strains were added separately at two inoculum levels, 10^7 and 10^4 CFU ml^{-1}, and plants were grown for 2 weeks instead of 4. The positions of the root tip and the zone of no root hairs were marked on the plastic pouch overlaying the root at the time of inoculation. In one experiment, the inoculation of strain 110 was delayed 3.5 and 7 h after the position of the root tip was marked at time 0. Two weeks after inoculation, the location and number of the nodules on the taproot were measured relative to the position of the root tip at the time of inoculation. Most nodules were readily visible, and a dissecting microscope was used to distinguish the smaller nodules. During the 2 weeks of growth, nodules developed on the lateral roots but were not counted in these assays.

RESULTS

Nodulation and competition among strains of *B. japonicum*. Both total and taproot nodule numbers increased significantly on soybeans grown in plastic growth pouches when plants were inoculated with increasing densities of either strain 110 or strain 587 (Table 1). Strain 587 maintained higher numbers of nodules at the two lowest inoculum levels, athough the difference was only significant between taproot nodules at an inoculum of 10^3 CFU (Student-Newman-Keuls test, $P = 0.05$). In a separate experiment with two inoculum levels (10^4 and 10^7 CFU $pouch^{-1}$), six strains of *B. japonicum* formed significantly more taproot and total nodules at the higher inoculum level than at the lower one (see Fig. 2).

TABLE 1. Effect of inoculum density on numbers of nodules formed by *B. japonicum* 110 and 587[a]

Inoculum density (log CFU $pouch^{-1}$)	No. of nodules per plant with the following strain:			
	110		587	
	Taproot	Total	Taproot	Total
8.3	10.4a	14.3a	10.9a	13.8a
7.3	8.8ab	15.4a	10.8a	11.6a
6.3	7.4b	9.4b	10.7a	12.8a
5.3	6.4b	9.3b	5.0b	7.2b
4.3	1.9c	5.2c	3.9b	7.6b
3.3	0.6c	5.2c	1.5c	6.2b
LSD	2.4	2.9	2.4	2.9

[a] Values are the means for 12 plants. Within each column, values followed by the same letter are not significantly different from each other (LSD, $P = 0.05$).

The inoculation rate affected competition for nodule occupancy among five strains in competition with strain 110, although the differences observed at the two levels were due primarily to increases in the percentage of nodule double occupancy at the high inoculum level (Table 2). The increased frequency of double occupancy was consistently at the expense of single occupancy of the more competitive reference strain 110. The single occupancy of the other strains either remained unaffected (strains 1028, 122, and 76) or was increased significantly (strains 31 and 587) at the high inoculum level as compared with the low one (chi-square analysis, $P < 0.05$). Generally, strains 110 and 1028 were the most competitive, followed by strains 122, 76, 587, and 31.

A significant inverse relationship was observed between the number of taproot and lateral root nodules formed by the six strains (inoculated separately) at an inoculum of 10^7 CFU

TABLE 2. Competitive relationships among six strains of *B. japonicum* at two inoculum levels[a]

Strain	% Nodule occupancy at the following inoculum level:			
	10^4 CFU $plant^{-1}$		10^7 CFU $plant^{-1}$	
	Single	Double	Single	Double
110	58a	4	49a	17
1028	38a		34a	
110	85a	0	60a	20
122	15b		20b	
110	83a	1	69a	14
76	16b		17b	
110	90a	5	63c	23
587	5b		14d[b]	
110	96a	1	74c	12
31	3b		14d[b]	

[a] Strain 110 was used as a reference strain. Values represent the nodule occupancy of each strain as measured by the percentage of single and double antiserum reactions. Five strains were paired in a 1:1 ratio with reference strain 110. Forty-eight to 80 nodules were harvested from 12 plants per strain combination. For each pair, values followed by the same letter are not significantly different from each other (chi-square analysis, $P < 0.5$).

[b] The increase in competitiveness of strains 587 and 31 at the high inoculum level as compared with the low one was significant (chi-square analysis, $P < 0.05$). Differences between the two levels were not significant for strains 76, 1028, and 122.

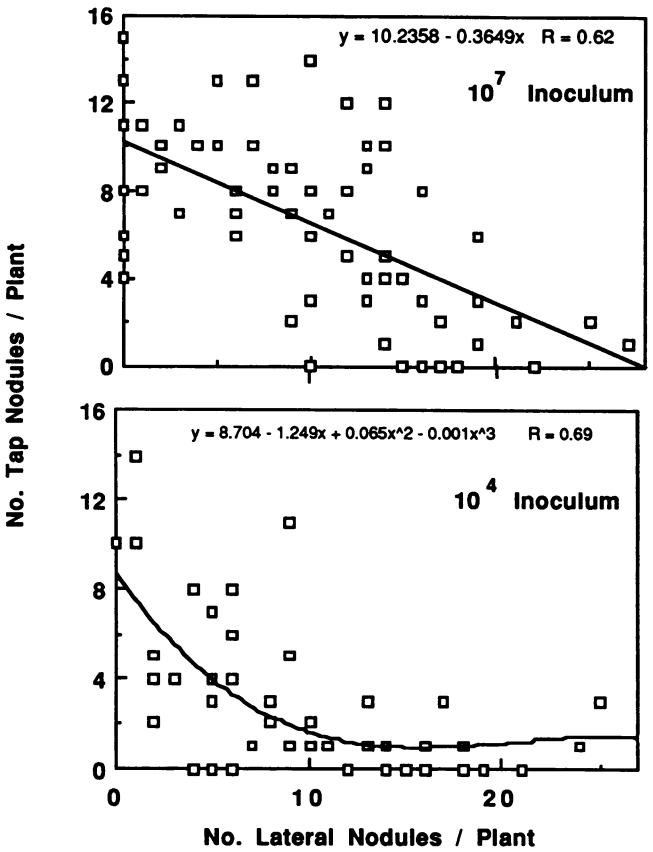

FIG. 1. Relationship between numbers of taproot and lateral root nodules formed per plant at inocula of 10^4 and 10^7 CFU for six strains of *B. japonicum* (each strain was inoculated separately). Nodules were harvested from 68 and 54 plants for the high and low inoculum densities, respectively. Some values were observed more than once, so symbols may represent more than one value. Pearson correlation coefficients were significant at $P < 0.01$.

(Fig. 1; $r = -0.62$; $P < 0.01$). Although the proportions of taproot and lateral root nodules changed, there was a fairly constant total number (± standard error) of nodules per plant (16.4 ± 0.6) formed at the high inoculum density. At the low inoculum density, the number of taproot and lateral root nodules was also inversely related (Fig. 1; $r = -0.69$; $P < 0.01$).

Nodule initiation rate. An experiment was designed to measure whether a delay in inoculation could be measured by a change in nodule location on the taproot (Table 3). Taproot nodules appeared on the plant root system before lateral nodules did and became visible at 5 to 6 days after inoculation. When inoculation was delayed as little as 3.5 h after the root tip location was marked at time zero, the percentage of nodules above the root tip position at the time of inoculation decreased from 30 to 11%, and the uppermost taproot nodule was 8.9 mm below the root tip position at the time of inoculation (Table 3).

Figure 2 shows the taproot nodule positions of the six strains relative to the root tip mark made at the time of inoculation on soybeans. Taproots averaged 220 mm in length 2 weeks after inoculation, and each of the six strains formed over 95% of their taproot nodules within a 55-mm-long segment of the taproot. This restricted area of nodulation corresponded approximately to the zone of no root hairs at the time of inoculation (after this zone underwent growth and cell elongation).

TABLE 3. Effect of delayed inoculation after marking of the root tip at time zero[a]

Inoculation time (h)	Avg. position of upper-most nodule (mm from root tip)	Avg. no. of nodules above root tip mark	% of total nodules above root tip mark
0	7.4a	3.4a	30a
3.5	−1.5b	0.6b	11b
7.0	−4.1b	0.2b	4b

[a] Nodule position and number were measured relative to the position of the root tip at the time of inoculation. Strain 110 was used as the inoculum. Negative values indicate a position below the root tip mark. Within each column, values followed by the same letter are not significantly different from each other (LSD, $P = 0.05$).

Analysis of the data shown in Fig. 2 revealed that at the high inoculum level, the number of taproot nodules per plant and the percentage of nodules above the root tip mark did not differ significantly among the six strains (least significant difference [LSD], $P = 0.05$). The only parameter which differed among the strains at the high inoculum level was the position of the uppermost nodule. The average position of the uppermost nodule formed by strain 31 was significantly higher on the taproot than the average position for the other strains (14.7 mm above the root tip mark versus 9.8 mm, respectively; the difference was significant by the LSD statistic [$P = 0.05$]). A similar nodule distribution was observed at the low inoculum level as compared with the high one, although the position of the uppermost nodule did not differ significantly among the strains (Fig. 2).

The location on the soybean root system of nodule occupants was measured from the competition experiment reported in Table 2. The poorly competitive strains occupied a higher percentage of taproot nodules than lateral nodules at the high inoculum level, although the difference was only significant with strain 76 (Table 4). At the low inoculum level, there were fewer taproot nodules, but the occupancy of strain 76 was likewise skewed in favor of taproot nodule occupancy (39%) as compared with lateral root occupancy (9%) (data not shown). In a separate experiment with log- and stationary-growth-phase cultures of strain 76 competing against strain 110, strain 76 again occupied significantly more taproot nodules than lateral root nodules. Strain 76 occupied 33 and 73% of taproot nodules and only 0 and 7% of lateral root nodules (log- and stationary-growth-phase cultures, respectively [unpublished data]).

DISCUSSION

Strains 110 and 1028 were the most competitive strains for nodule occupancy in these experiments. The rankings of strain competitiveness under these growth chamber-plastic growth pouch conditions were consistent with those in greenhouse studies with plants grown in vermiculite: 110 = 1028 >> 587 = 31 (J. Fuhrmann, Ph.D. thesis, North Carolina State University, Raleigh, 1985; C. Vidor, unpublished data). Strains 122 and 76 were not tested in the greenhouse. Although repeatedly observed under both growth chamber and greenhouse conditions, the competitive relationships reported here do not necessarily represent relationships observed under environmentally complex field conditions. For example, one of the least competitive strains in the greenhouse (strain 31) is one of the most dominant in southeastern United States soils (S. M. Mpofu and A. G. Wollum II, unpublished data).

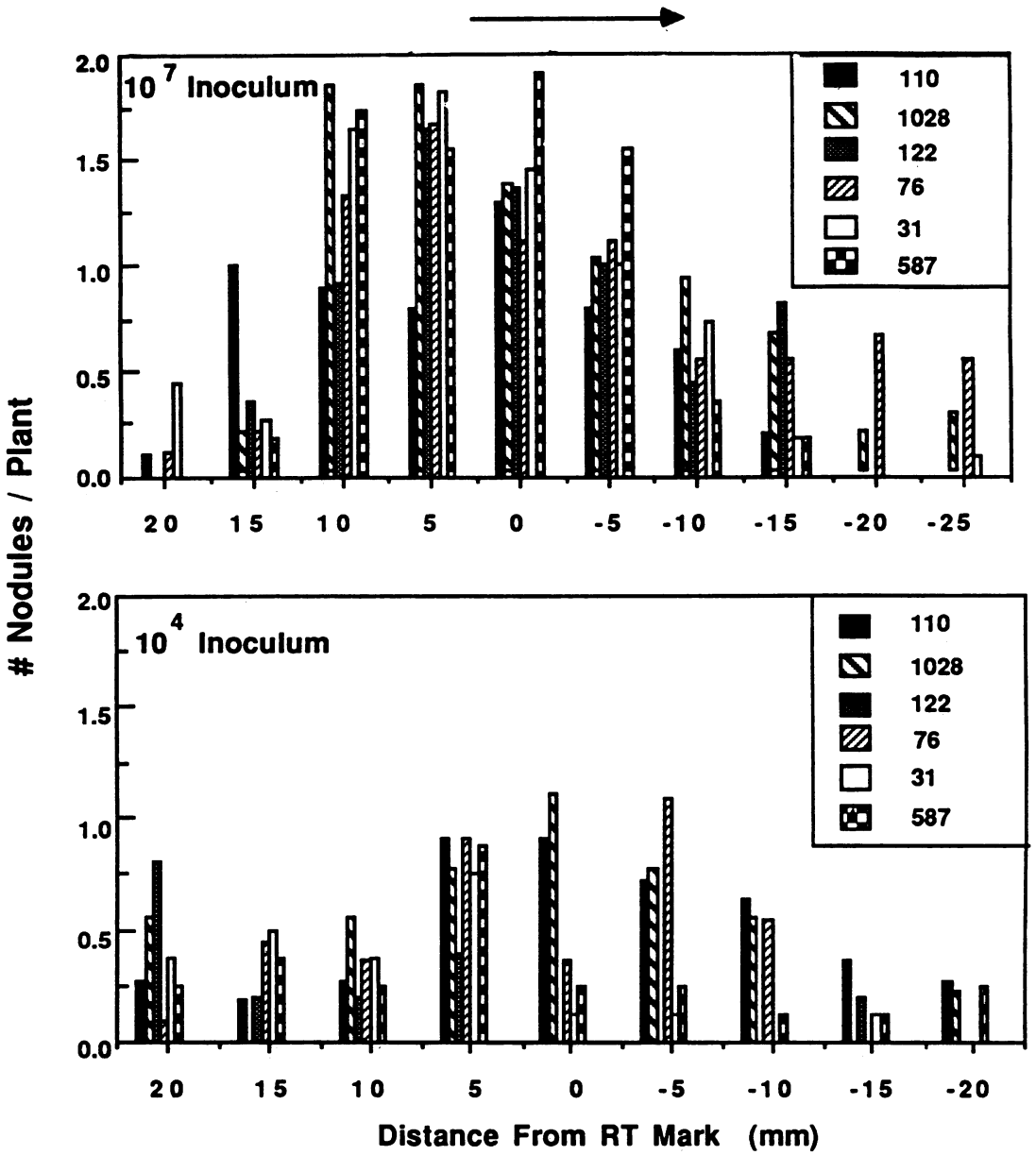

FIG. 2. Taproot nodulation patterns for six strains of *B. japonicum* at two inoculum densities as measured by the distance from the root tip (RT) mark made at the time of inoculation. At the high and low inoculum densities, there were averages of 7.3 and 3.3 taproot nodules per plant, respectively (the difference was significant by the LSD statistic [$P < 0.05$]). Negative values indicate a position below the RT mark, and the arrow shows the direction of root growth.

The overall competitive relationships did not change with varying inoculum densities, although nodule double occupancy increased significantly at the higher inoculum level. Increased nodule double occupancy at high inoculum levels was first reported by Lindemann et al. (9). The less competitive strains increased their nodule representation at the high inoculum level through their presence in the doubly-occupied nodules. Poorly competitive strains may gain access to nodules via infection threads initiated by more competitive strains. Supportive evidence for one strain using the infection initiated by another is the observation that a *Rhizobium fredii* strain (U.S. Department of Agriculture strain 257) incapable of nodulating soybeans cohabited nodules on cultivar McCall when inoculated with a *nod*$^+$ strain (U.S. Department of Agriculture strain 191) (7). Cohabitation of a *nod* strain with a *nod*$^+$ strain has also been observed in clover (16).

The inverse relationship between taproot and lateral root nodules may be a result of plant control over nodule formation to allow sufficient nodulation. For example, if only a few taproot nodules are formed, the plant would not inhibit later nodulation on the lateral roots; if sufficient taproot nodules are formed, lateral infections would be suppressed. The constant total number of nodules per plant provides evidence for this plant control. It is possible that a certain threshold level of developing infections induces the release of a compound which, by its presence in the root or root exudate, inhibits further nodule formation. Rolfe and Gress-

TABLE 4. Percent nodule occupancy and nodule location (taproot or lateral root) for five *B. japonicum* strains competing against strain 110 (10^7 CFU plant^{-1})[a]

Strain	% of total nodule occupancy (no. of nodules) on:	
	Taproots	Lateral roots
76[b]	36 (22)	12 (49)
122	28 (36)	17 (12)
31	23 (13)	14 (56)
587	23 (22)	15 (39)
1028	35 (59)	34 (13)

[a] Values are from single reactions only. Data are from the competition experiment reported in Table 2.

[b] Strain 76 taproot occupancy and lateral root occupancy were significantly different from the expected equivalency between the two (chi-square analysis, $P < 0.05$). Taproot occupancy and lateral root occupancy for the other strains were not significantly different.

hoff (15) have suggested that it is the plant flavonoid inducer/inhibitor ratio which may determine the fate of infections.

At the lower inoculum levels, the limiting number of bradyrhizobia became an important factor controlling nodulation. It is probable that all inoculated bacteria did not come into contact with the seedling root because of the inoculum diffusing throughout the paper wick of the growth pouch. However, if only 10% of the inoculated cells were available for nodulation, there still would have been approximately a 40-fold excess of cells as compared with the number of nodules formed, since only five to six nodules formed at the lowest inoculum level. A similar nodulation response to inoculation level has been observed by Weaver and Frederick (19) in rhizobium-free soil. We have hypothesized that not all cells in a given population of soybean rhizobia are capable of nodulation because of their inability to attach to soybean roots. However, the results suggest that the proportion of cells capable of root attachment is a dynamic property of a cell population (G. B. Smith and A. G. Wollum II, submitted for publication).

The timing of nodule initiation was directly related to nodule position on the taproot. With a 3.5-h delay of inoculation, taproot nodules were formed significantly lower on the root. Bhuvaneswari et al. (1) also found that root cells which had been susceptible to infection at the time of inoculation were no longer infectible within a matter of a few hours and that nodule position could be used to measure the time of nodule initiation.

Hahn and Hennecke (5) have reported a correlation between a delay in nodulation and a reduction in nodulation competitiveness of *B. japonicum* deletion mutants. Similarly, using nodulation-defective mutants, Sargent et al. (16) proposed that competitive ability might be correlated with the nodule initiation rate of *R. leguminosarum* biovar trifolii. The present study was designed to examine this hypothesis directly, and no such correlation was observed among six wild-type strains of *B. japonicum*. The taproot nodulation patterns of the six strains were very similar, with greater than 95% of the taproot nodules being formed within a restricted zone on the taproot, centering on the position of the root tip at the time of inoculation. On the basis of the evidence that taproot nodule position is related to timing of nodule initiation, the least competitive strains initiated nodules at the same rate as the more competitive strains.

Additionally, if a strain was a poor competitor because it needed time in the plant rhizosphere to become capable of nodulation, then one would expect the poor competitor to occupy more lateral root nodules formed later in the symbiosis than taproot nodules. For example, a *B. japonicum* mutant (HS111) which requires root exudate preincubation before it can nodulate at the rate of the parent strain has been isolated (6). However, in the study reported here, there was no significant difference in taproot and lateral root nodulation among three of the four poorly competitive strains, and one strain (strain 76) actually occupied a significantly higher percentage of taproot nodules than lateral root nodules when competing against the more competitive strain 110.

The nodulation data for the six strains of *B. japonicum* used in this study do not support the hypothesis that poor competitiveness for nodule occupancy is a result of delayed nodulation. These six strains had widely different competitive abilities but nodulated soybean taproots in very similar patterns, and one of the poor competitors (strain 76) occupied more taproot nodules formed earlier than lateral nodules formed later. In agreement with these results, Zdor and Pueppke (20) recently reported that a strain of serogroup 123 infected soybeans earlier than did strain U.S. Department of Agriculture strain 138 but that the latter was more competitive.

Much useful information and interesting hypotheses have been generated from experiments with nodulation-defective mutants (5, 6, 16). However, the results of the present study and that of Zdor and Pueppke (20) demonstrate the importance of investigating the behavior and physiology of wild-type strains to test and validate conclusions drawn from mutant strains.

LITERATURE CITED

1. **Bhuvaneswari, T. V., B. G. Turgeon, and W. D. Bauer.** 1980. Early events in the infection of soybean by *Rhizobium japonicum*. Plant Physiol. **66**:1027–1031.
2. **Calvert, H. E., M. K. Pence, M. Pierce, N. S. Malik, and W. D. Bauer.** 1984. Anatomical analysis of the development and distribution of *Rhizobium* infections in soybean roots. Can. J. Bot. **62**:2375–2384.
3. **Delves, A. C., A. Mathes, D. A. Day, A. S. Carter, B. J. Carroll, and P. M. Gresshoff.** 1986. Regulation of the soybean-*Rhizobium* nodule symbiosis by shoot and root factors. Plant Physiol. **82**:588–590.
4. **Fuhrmann, J., and A. G. Wollum II.** 1985. Simplified enzyme-linked immunosorbent assay for routine identification of *Rhizobium japonicum* antigens. Appl. Environ. Microbiol. **49**:1010–1013.
5. **Hahn, M., and H. Hennecke.** 1988. Cloning and mapping of a novel nodulation region from *Bradyrhizobium japonicum* by genetic complementation of a deletion mutant. Appl. Environ. Microbiol. **54**:55–61.
6. **Halverson, L. J., and G. Stacey.** 1985. Host recognition in the *Rhizobium*-soybean symbiosis. Plant Physiol. **77**:621–625.
7. **Heron, D. S., and S. G. Pueppke.** 1987. Regulation of nodulation in the soybean-*Rhizobium* symbiosis. Strain and cultivar variability. Plant Physiol. **84**:1391–1396.
8. **Kosslak, R. M., R. Bookland, J. Barkei, H. E. Paaren, and E. R. Appelbaum.** 1987. Induction of *Bradyrhizobium japonicum* common nod genes by isoflavones isolated from *Glycine max*. Proc. Natl. Acad. Sci. USA **84**:7428–7432.
9. **Lindemann, W. C., E. L. Schmidt, and G. E. Ham.** 1974. Evidence for double infections within soybean nodules. Soil Sci. **118**:274–279.
10. **McClure, P. R., and D. W. Israel.** 1979. Transport of nitrogen in the xylem of soybean plants. Plant Physiol. **64**:411–416.
11. **Munevar, F., and A. G. Wollum II.** 1981. Growth of *Rhizobium japonicum* strains at temperatures above 27°C. Appl. Environ. Microbiol. **41**:272–276.
12. **Nutman, P. S.** 1952. Studies on the physiology of nodule formation. III. Experiments on the excision of root-tips and nodules. Ann. Bot. **16**:81–102.
13. **Peters, N. K., J. W. Frost, and S. R. Long.** 1986. A plant flavone,

luteolin, induces expression of *Rhizobium meliloti* nodulation genes. Science **233**:977–980.
14. **Pierce, M., and W. D. Bauer.** 1983. A rapid regulatory response governing nodulation in soybean. Plant Physiol. **73**:286–290.
15. **Rolfe, B. G., and P. M. Gresshoff.** 1988. Genetic analysis of legume nodule initiation. Annu. Rev. Plant Physiol. **39**:297–319.
16. **Sargent, L., S. Z. Huang, B. G. Rolfe, and M. A. Djordjevic.** 1987. Split-root assays using *Trifolium subterraneum* show that *Rhizobium* infection induces a systemic response that can inhibit nodulation of another invasive *Rhizobium* strain. Appl. Environ. Microbiol. **53**:1611–1619.
17. **Turgeon, B. G., and W. D. Bauer.** 1985. Ultrastructure of infection-thread development during the infection of soybean by *Rhizobium japonicum*. Planta **163**:328–349.
18. **Vincent, J. M.** 1970. A manual for the practical study of the root-nodule bacteria. Blackwell Scientific Publications, Ltd., Oxford.
19. **Weaver, R. W., and L. R. Frederick.** 1972. Effect of inoculum size on nodulation of *Glycine max* (L.) Merrill, variety Ford. Agron. J. **64**:597–599.
20. **Zdor, R. E., and S. G. Pueppke.** 1988. Early infection and competition for nodulation of soybean by *Bradyrhizobium japonicum* 123 and 138. Appl. Environ. Microbiol. **54**:1996–2002.