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Nodulating Competitiveness of a Nonmotile Tn*7* Mutant of *Bradyrhizobium japonicum* in Nonsterile Soil†

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A nonmotile mutant of *Bradyrhizobium japonicum* serogroup 127 was generated by Tn*7* mutagenesis and matched with the wild type against a common competitor in studies of soybean nodulation in nonsterile soil. The Tn*7* mutant was very similar to the wild type in growth rate in culture, soybean lectin-binding ability, flagellar morphology, and nodulating capability, but it had a longer lag phase. Competing strains were distributed uniformly in soil in various ratios and at different population densities prior to planting. Mutant and wild type were equally prevalent in the seedling rhizosphere at about the time of nodule initiation, suggesting that motility conferred no advantage in rhizosphere colonization. Nodulation success of the Tn*7* mutant was lower than that of the wild type under all test conditions. Differences were greatest at low soil populations of competitors and much less pronounced at initial populations of 10^7 g^{-1}. The longer lag phase of the Tn*7* mutant may have contributed to its decreased competitiveness, especially at the higher inoculation levels. The antibiotic and motility markers were stable, and the rifampin resistance derived from the parent did not affect adversely the competitiveness of the Tn*7* mutant. We found motility to be of limited importance to the competitiveness of a strain in normal nonsterile soil, where the significance, if any, of this ability may be in migration at the immediate root surface in soils sparsely populated with rhizobial symbionts.

Attributes that account for the competitive success of certain rhizobia in the presence of related indigenous rhizobial strains are unknown. Chemotactic motility may be a factor, but evidence for that is not yet convincing. Although studies have shown that nonmotile mutants of rhizobia are quite capable of nodulating appropriate host plants (2, 11, 14), it has been suggested that random and chemotactic motility may enhance movement of rhizobia in soil and may enhance opportunities for contact with the legume root (2).

Results of competition experiments between motile and nonmotile pairs under highly artificial conditions have been interpreted as evidence that motility confers a selective advantage in interrhizobium competition (1, 8). Further evidence has been sought in autoclaved soil. Soby and Bergman (20) compared movements of motile and nonmotile pairs of *Rhizobium meliloti* in autoclaved soils of various textures and reported that efficient spreading requires active motility and chemotaxis. However, their data show that measurable movement occurred in sandy loam soil only at near-saturation moisture content and in a peat mixed with 50% coarse sand only when the soil is completely saturated. Mellor et al. (11) designed careful comparisons between a motile strain of *Rhizobium trifolii* and nonmotile mutants for their abilities to nodulate clover in an autoclaved soil described only as "yellow sand." Mixtures of equal numbers of mutant and wild-type strains applied as liquid inoculum to seedling roots resulted in a fivefold-greater success of the motile strain in forming nodules.

Further evaluation of motility as a factor in competition among rhizobia must be made in normal soil, at normal soil moisture tensions, and in the presence of normal diverse rhizosphere populations for which substances eliciting chemotactic responses are available substrates. We have attempted to provide these conditions in experiments matching motile and nonmotile competing strains uniformly distributed in a nonsterile soil planted to soybeans.

MATERIALS AND METHODS

Cultures. *Bradyrhizobium japonicum* Webster 48 (Web 48 wild type, designated Web 48 wt) was isolated from a field-grown soybean from southern Minnesota in 1969. It is a member of serocluster 123, serologically identical to strain USDA 127 (19). A spontaneous rifampin-resistant mutant, Web 48 Rif^r, served as a parent strain in plate matings to obtain a motility-minus mutant by Tn*7* mutagenesis after prolonged serial selection on soft agar failed to yield a nonmotile strain. The motility-minus mutant, designated Web 48 Tn*7*, was obtained as a Str^r exconjugant from plate matings performed in yeast extract maltose agar (15) for 48 h at a 1:4 ratio of donor *Escherichia coli* AB2463 (Rp4-Col E1::Tn*7*) to Web 48 Rif^r. Transconjugants selected on yeast extract mannitol agar, (YEM [21]) with rifampin (300 μg ml^{-1}) and streptomycin (300 μg ml^{-1}) were screened for motility in 0.025% 2,3,5-triphenyltetrazolium chloride (TTC) stabs of yeast extract maltose soft (0.25%) agar. *B. japonicum* USDA 110 was obtained originally from the U.S. Department of Agriculture, Beltsville, Md., and was used as a reference strain in competition studies.

Cultures were maintained on YEM slants and grown in broth of the same medium for soil inocula and for growth curve comparisons. This medium was also used with and without appropriate antibiotics for characterization of nodule suspensions and with TTC as a soft agar to verify the motility status of nodule occupants and to monitor possible reversion to motility.

Growth pouch nodulation assay. Nodulating features of the Web 48 strains were assessed by the most-probable-number technique in plastic growth pouches (23). Tenfold dilutions of the test strains were used to inoculate 2-day-old, preger-

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minated soybean seedlings (cv. Hodgson) at the rate of 1.0 ml seedling^{-1}. Each dilution was replicated with five pouches; each pouch contained two plants. Nodules were collected after 5 weeks of growth in a Conviron E15 plant growth chamber.

Growth curves and lectin-binding assay. Growth curves were developed in YEM broth by cell density measurements made in a Klett colorimeter with a green filter. Binding of soybean lectin on a per-cell basis was determined by means of the hemagglutination inhibition assay (21) and direct cell counts (16) during growth of the *B. japonicum* strains in YEM broth. Soybean lectin type VI (Sigma Chemical Co., St. Louis, Mo.) was used throughout, and the protocol of Robert and Schmidt (16) was followed.

Competition. Competition experiments were designed to compare the Web Tn*7* mutant with the wild type by matching each individually against a common competitor, USDA 110. The competition was carried out in nonsterile soil, necessarily free of *B. japonicum* at the outset so that the introduced rhizobia would not encounter unknown competitors and could be monitored readily by immunofluorescence (IF). Since it proved difficult to find field soils that did not nodulate soybeans, the first experiment was carried out and repeated in a Hubbard sandy loam with a low population of *B. japonicum*. This soil was 87% sand, 9% silt, 4% clay, and 2.8% organic matter content, and pH was 5.9. The soil was autoclaved for 45 min in shallow pans, leached with sterile water to remove nitrates, and dried to approximately 50% field capacity. One portion of the soil was maintained in sterile condition, amended with 2% mannitol, and used to prepare soil inocula of the competing strains. The remaining autoclaved, leached soil was mixed thoroughly with 10% *B. japonicum*-free soil from Hawaii (courtesy of B. B. Bohlool, NifTAL Paia, Hawaii) to restore microbial diversity and activity during a 2-week incubation period in preparation for use as a substrate for competition studies in pots. A third repetition which gave essentially the same results was carried out in a nonautoclaved Fayette silt loam soil (75% silt, 18% clay, 7% sand, 1.4% organic carbon) obtained in southwestern Minnesota and found to be free of *B. japonicum*.

Each strain was grown separately in the sterile soil after amendment with 2% mannitol and inoculation with 4-day-old broth cultures to a starting density of about 10^5 g of soil^{-1} Cultures were grown in this inoculant soil for 12 to 15 days and were then enumerated by IF (18). On the basis of final cell counts, which ranged from 10^8 to 10^9 g^{-1}, appropriate quantities of inoculum soil were mixed into 300-g quantities of the nonsterile "competition soil" to provide 1:1, 1:10, and 1:100 ratios of USDA 110 to Web 48 at various population densities. The amounts of inoculant soil mixed uniformly throughout the competition test soil ranged from 1 to 20 g. Water was added slowly as a spray to obtain favorable soil moisture at approximately 60% water-holding capacity. Soil inoculated with the competing strains was placed in the upper portion of a modified Leonard jar assembly comprising a tapered 1-qt (0.946-liter) plastic upper container with a tightly wound blotter paper wick extending through a hole in the base. A slightly larger bottom container, which functioned as a moisture reservoir in contact with the wick and as a support for the top unit, was sealed tightly to the top container with electrical tape.

The plant assemblies were set up immediately after inoculation with the competing strains and planted with two aseptically grown, 2-day-old soybean seedlings, cv. Hodgson. The surface of the soil was then covered with dry, sterile pebbles to a depth of about 1 cm to prevent cross-contamination. Soil pots without added competitors served as controls. Only six nodules formed in all of these controls throughout the study, and none was typed as Web 48 or USDA 110. All treatments, including controls, were replicated in three pots. Pot assemblies were placed in the Conviron growth chamber and maintained on a 16-h 23°C light, 8-h 18°C dark cycle. Plant growth was continued for 5 weeks, after which the nodules were harvested, sampled, and analyzed.

Rhizosphere soil counts. Additional replicates of the competition pots were set up in triplicate with six seeds per pot for certain treatments to allow for early rhizosphere examination at days 7 and 10. Seedling roots were carefully recovered from the soil, allowed to air dry until attached soil could be shaken free, measured for length, and placed in a small flask with a measured volume of soil extractant solution (13). Web 48 strains were counted by quantitative IF (18) and expressed as cells per centimeter of root.

Nodule analyses. Nodules were collected for both serotyping and characterization of nodule suspensions. Nodule-bearing roots were washed in tap water. Only well-formed discrete nodules were picked and pooled for all replicates within a treatment. After being blotted and air dried briefly on paper towels, 30 to 100 nodules were selected at random and processed as soon as possible for antibiotic sensitivity or motility testing or both and for serotyping. Additional nodules to be serotyped only could be kept in the refrigerator for at least a week. Nodules processed at once were cleaned by shaking for 15 min in 100 ml of water and 1 drop of Tween 80, rinsed, surface sterilized, and then crushed individually and aseptically in 2.0 ml of sterile saline. Each nodule suspension was used to prepare duplicate smears on each of two microscope slides and as inoculum for YEM, YEM-antibiotic plates, and TTC tubes as appropriate. Additional nodules as needed to total 100 per treatment for serotyping only were prepared as described above but not handled aseptically. Smears were air dried, fixed in 95% ethanol for 5 min, and stained with appropriate fluorescent antibody (FA).

FAs and fluorescence microscopy. FAs specific to Web 48 (serogroup 127) or to USDA 110 were prepared by our standard procedures (5) and used at dilutions of 1:16 in saline. FA staining procedures, procedures for microscopic enumeration of specific strains in soil, and the features of the fluorescence microscope have been described previously (5, 13, 18). For quantitative FA enumeration in soil, 50 microscopic fields were counted on each of the duplicate membrane filters.

RESULTS

Since apparent transposition of Tn*5* was lower than that of Tn*7* in preliminary trials, Tn*7* mutagenesis was attempted with several antibiotic-marked strains including the rifampin-resistant strains listed in Table 1. Transfer of Tn*7* drug resistance was well above background for strain Web 48.

Screening of Tn*7* exconjugants in TTC motility agar readily disclosed a nonmotile mutant. Repeated transfers accompanied by motility tests in TTC agar and soft-agar motility plates over a 5-year period demonstrated that the mutant was stable, with no evidence of reversion to motility. In addition to rifampin resistance from the parent strain, the nonmotile mutant carried the streptomycin, spectinomycin, and trimethoprim markers encoded by Tn*7*. Observations by electron microscopy revealed no difference in morphology among wild type, parent, and Tn*7* strains. The presence on

TABLE 1. Frequency of Tn7 transfer into several strains of *B. japonicum* with rifampin resistance to select for recipient cells and with streptomycin resistance to select for transposon Tn7 from *E. coli* AB2463

Recipient	Frequency of:	
	Spontaneous $Str^{r a}$	Tn7 transfer of Str^r $Rif^{r a,b}$
USDA 110 Rif17	$\sim 10^{-8}$	5×10^{-8}
USDA 123 Rif1	$\sim 10^{-8}$	$\sim 10^{-8}$
Web 48 Rif3	5×10^{-8}	1.5×10^{-5}

[a] Resistant to 500 μg of streptomycin ml^{-1}.
[b] Resistant to 300 μg of rifampin ml^{-1}.

the mutant of a single subpolar flagellum identical to that of the parent suggests that nonmotility was due to a defect in the motor function of the flagellum. Properties of the wild-type parent and mutant strains of Web 48 are compared in Table 2. Lack of cross-reactivity between Web 48 strains and USDA 110 to their respective FAs allowed differentiation between the two serogroups in subsequent soil experiments.

Growth of the Web 48 Tn7 mutant is compared with that of the parent and the wild type in broth culture in Fig. 1. Doubling times during log-phase growth in liquid culture were approximately the same for each of the isolates, but the mutant had a longer lag phase and a lower maximum cell density. Growth curve differences were somewhat greater in sterile soil (data not shown), where the mutant had an apparent growth rate slightly lower than that of the wild type.

The longer lag phase of Web 48 Tn7 (Fig. 1) was also evident in measurements of soybean lectin-binding polysaccharide produced as a parameter of growth in broth culture. These data, expressed as lectin bound per cell, are reported in Fig. 2. Lectin-binding-polysaccharide formation was virtually identical for the Web 48 Tn7 and Web 48 wt strains in terms of both rate of production and peak production per cell. However, both the maximum rate and the production peak of the mutant were delayed by about 24 h compared with those of Web 48 wt and strain USDA 110, which, under identical circumstances, bound lectin earlier at a greater rate and reached a 10-fold-higher peak of binding than the Web 48 strains did.

Web 48 wt and Tn7 strains along with reference strain USDA 110 were compared for their nodulation capacities in a most-probable-number plastic-growth-pouch assay (Table 3). The two Web 48 strains did not differ in ability to nodulate or in the number, size, or morphology of the nodules formed. Strain USDA 110 outperformed both Web 48 strains in the growth pouch assay of nodulation. Estimates of the proportion of infective cells in the inoculant populations based on the most-probable-number data of Table 3 indicate that the infectivity of the wild-type Web 48 and the Tn7 mutant were nearly the same (1.3 and 1.1%, respectively), while that of the reference competitor, USDA 110, was substantially higher (9.5%).

Acridine orange counts of the soil substrate used in the competition experiments confirmed a normal density of total soil bacteria at 9.2×10^8 g^{-1}. Soybean plants grown in this soil for 5 weeks in precompetition testing formed no nodules. Immediately prior to the initiation of competition, the two competing strains, either Web 48 wt and USDA 110 or Web 48 Tn7 and USDA 110, were added to the soil in the form of sterile soil-grown cultures in various ratios and were mixed thoroughly to achieve uniform distribution throughout the soil container.

TABLE 2. Characteristics of experimental strains of *B. japonicum*

Strain	FA reaction[a]		Motility on:		Selected markers[b]	Presence of single subpolar flagellum[c]
	USDA 127	USDA 110	Soft agar	TTC		
Web 48 wt	4+	—	+	+	Str^s Rif^s	+
Web 48 Rif3	4+	—	+	+	Str^s Rif^r	+
Web 48 Tn7[d]	4+	—	−	−	Str^r Rif^r	+
USDA 110	—	4+	+	+	ND[e]	ND

[a] FA dilution, 1:16. 4+, Strongly positive reaction; —, negative reaction.
[b] Streptomycin at 500 μg ml^{-1} and rifampin at 300 μg ml^{-1}.
[c] Electron microscopy courtesy of H. C. Tsien, Department of Microbiology, University of Minnesota.
[d] Tn7 insertion confirmed by ^{32}P gene probe analysis by M. J. Sadowsky, USDA Agricultural Research Center, Beltsville, Md.
[e] ND, Not determined.

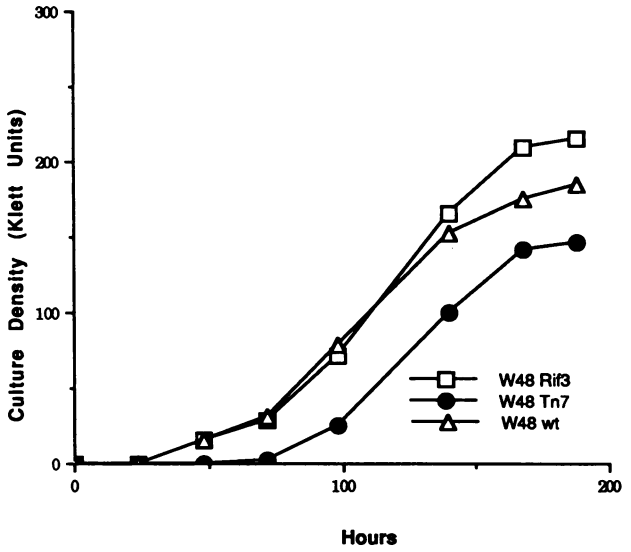

FIG. 1. Growth curve of *B. japonicum* Web 48 strains in YEM broth.

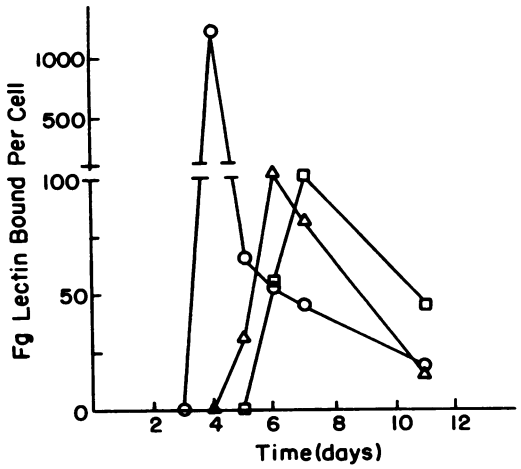

FIG. 2. Soybean lectin-binding activity of *B. japonicum* USDA 110 (○), Web 48 wt (△), and Web 48 Tn7 (□).

TABLE 3. Nodulation potential of *B. japonicum* strains for cv. Hodgson soybeans in growth pouch assay[a]

Inoculum dilution	No. of pouches with nodules (n = 5)		
	Web 48 wt	Web 48 Tn7	USDA 110
10^{-6}	4	3	5
10^{-7}	0	1	2
10^{-8}	0	0	2
10^{-9}	0	0	0

[a] Following dilution of 10^8 cells ml of broth cultures^{-1}. Two, two-day old pregerminated seedlings per pouch, five replicate pouches per dilution, 1.0 ml of inoculum seedling^{-1}.

Results of the competition experiments are summarized in Fig. 3 in terms of the composition of the nodules on test plants at early flowering. Strain USDA 110 was, as expected, clearly more competitive than either of the Web 48 strains. It dominated nodule occupancy in all pots in which equal numbers of inoculant strains competed and in which the Web 48 strain outnumbered the reference strain by a 10:1 ratio. Improved success of Web 48 competitors was brought about either by increasing the ratio of Web 48 to USDA 110 to 100:1 or by increasing the density of Web 48 in the soil to 10^7 gram^{-1}.

Nonmotile Web 48 Tn*7* was less competitive than the wild type under all experimental conditions. Whereas the wild type formed 4% of the nodules in competition with USDA 110 at a soil population ratio of 10^4:10^4 and 22% in competition with USDA 110 when the wild type had a 10-fold advantage at the 10^5:10^4 ratio, the Tn*7* mutant failed to occupy any nodules under similar conditions. The mutant performed nearly as well (25% occupancy) as the wild type (35% occupancy) when it had a 10-fold advantage at the 10^7:10^6 ratio but was substantially less competitive than the wild type at the 10^5:10^3 and 10^7:10^5 ratios.

Nodules occupied by Web 48 Tn*7* were clearly not the result of reversion to motility or of loss of antibiotic resistance by the infecting strain. Suspensions of each Web 48 nodule recovered from the Web 48 Tn*7*-USDA 110 competition pots retained the nonmotility and antibiotic resistance properties of the inoculant strain (Table 4). Also included in Table 4 are data derived from the IF enumeration of Web 48 strains in the seedling rhizospheres during the period of nodule initiation. These data show that both the wild type and the Tn*7* mutant were present in the developing rhizospheres at approximately the same density. It is particularly noteworthy that motility apparently was not a factor in the colonization of the soybean root prior to nodulation.

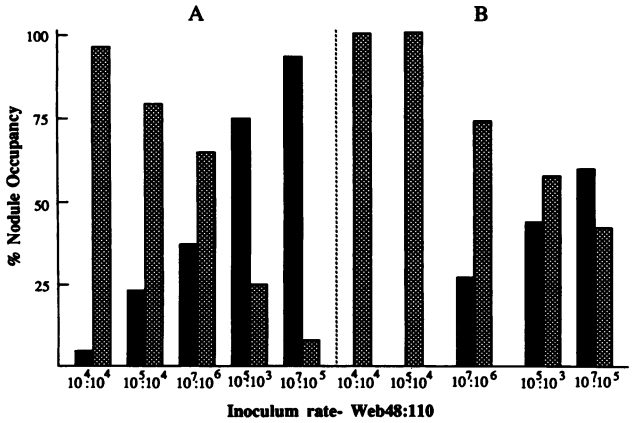

FIG. 3. Occupancy of soybean nodules by competing strains of *B. japonicum* uniformly distributed in nonsterile soil at various population densities prior to planting. Symbols: ■, Web 48 wt (A) or Web 48 Tn*7* (B); ▩, USDA 110.

The rifampin resistance associated with the Tn*7* mutant was derived from its parent. The possible impact of the Rif^r marker on competition for nodulation was assessed by comparing the performance of the Web 48 Rif^r strain with that of the wild type against the common competitor USDA 110. The Rif^r-marked strain nodulated as well as or slightly better than the wild type in competition with the reference strain in soil (Table 5). Subsequent examination of Web 48-positive nodules from several Web 48 Rif^r/USDA 110 inoculation ratios confirmed that the Rif^r marker was retained. Of 300 such nodule suspensions inoculated onto YEM agar plates with and without rifampin at 300 μg ml^{-1}, only 3 failed to grow on the medium containing rifampin.

DISCUSSION

Data from this first study to evaluate the importance of motility to rhizobia in competition for legume nodulation in nonsterile soil indicate that while motility may contribute to competitiveness, it is probably not a major factor. The approach that we used was to generate a nonmotile mutant of *B. japonicum* Web 48, to distribute it in soil to compete with a second added strain (USDA 110), and to compare the performance of the mutant with that of the wild type, competing similarly with USDA 110. By matching the wild type and the mutant separately against a common competitor rather than against each other, it was possible to capitalize on the unique capability of the IF technique to detect specific bacteria in natural soil. One antibody distinguished either of the Web 48 strains, while the common competitor, USDA 110, was detected by its serogroup-specific FA. A requirement of such an experiment is that the mutant and the wild type be very much the same except for motility. The Web 48 strains were indistinguishable serologically and were virtually identical with regard to growth rate in culture broth, lectin-binding activity, nodulation efficiency in growth pouches, and morphology (a single subpolar flagellum in each). Some differences from the wild type, however, were noted in the growth curve of the Tn*7* mutant. The mutant had a longer lag phase (Fig. 1 and 2) and a lower maximum cell density (Fig. 1) in culture. The lag phase in particular might be a factor influencing nodulation success in rhizosphere competition and must be considered along with motility as a consequence of Tn*7* mutagenesis.

Evidence that motility may confer some competitive advantage on a strain is suggested by the observation that the nodulation success of the Tn*7* mutant in competition with the reference competitor was less than that of the motile wild type under all test conditions. The most pronounced differences in competitiveness of the mutant and wild-type strains of Web 48 were at the lower population densities; that is, they were poised in the soil at either 10^4 or 10^5 g^{-1} together with the more competitive USDA 110 strain at 10^4 g^{-1}. Under these circumstances, the wild type managed to initiate some nodules (4 and 22%, respectively, at 10^4 and 10^5 g^{-1}; Fig. 3), while the mutant accounted for none. The possibility that growth delay rather than nonmotility accounted for those particular failures of Web 48 Tn*7* seems unlikely, since both mutant and wild type were equally numerous at the root surface (Table 4) at days 7 and 10, about the time when soybean nodulation is initiated (9). If,

TABLE 4. Confirmation of the stability of Web 48 strains after nodulation and of their establishment in the rhizosphere during nodule initiation

Web 48 competitor[a]	Ratio in soil	No. of nodules tested	Nodule type[b]		No. of Web 48				
			Web 48	USDA 110	Antibiotic resistance[c]	Motile	Nonmotile[d]	cm of root^{-1} on: Day 7	cm of root^{-1} on: Day 10
wt	10^6:10^4	30	21	9	0	21	0	ND	ND
Tn7	10^6:10^4	30	6	24	6	0	6	ND	ND
Tn7	10^7:10^5	28	17	11	17	0	17	ND	ND
wt	10^5:10^4	ND	ND	ND	ND	ND	ND	1.9×10^3	1.4×10^3
Tn7	10^5:10^4	ND	ND	ND	ND	ND	ND	1.8×10^3	1.0×10^3

[a] USDA 110 was added with each Web 48 strain.
[b] Typed by immunofluorescence. All nodules singly infected. ND, No data.
[c] Growth on YEM medium with streptomycin at 500 μg ml^{-1}.
[d] Assayed by TTC stab culture.

on the other hand, motility and chemotaxis contribute in any way to the inception of nodulation, it should be at the root surface, where root mucilage provides the continuous film for microbial mobility not found elsewhere in the rhizosphere (7).

Evidence that motility is not a major component of rhizobial competition is suggested by the observation that lack of motility on the part of the Tn7 mutant did not prevent its access to the root surface at low soil populations (Table 4). Conversely, motility provided no advantage for the wild type in rhizosphere colonization. These results are consistent with the numerous studies which have shown that bacteria cannot move measurable distances in soil unless the soil is at saturation or near-saturation conditions, providing interconnecting water bridges between large soil pores (3, 4, 24). Moreover, movement of bacteria in natural soil such as that used in our study is further hampered by the strong adsorptive effects of the clay and by organic colloids not found in artificial or sand systems (24). Thus in normal soil, in which larger pores are discontinuous and filled with gases, soil water occurs as discontinuous films and lenses, and soil colloids exert adsorptive effects, bacteria remain restricted to their microsites, irrespective of motility and chemotaxis. Rather than movement of rhizobia through soil to the root by either random or directed motility, the plant root encounters rhizobia as it grows through the soil. Once in contact with the rhizosphere, rhizobia begin to grow slowly and compete with other rhizosphere bacteria (13). At this stage, motility may be important to the migration and distribution of a strain in the rhizoplane, where water films can be continuous and smooth patches of root surface can occur (17).

Our data suggest that motility may be of limited importance even in the rhizoplane when soil rhizobial populations are high. At initial soil densities of 10^7 g^{-1} (Fig. 3), the nonmotile Tn7 mutant was only about one-fourth to one-third less competitive than the wild type. This relatively small difference may not have been due to motility at all but rather to the lag in growth initiation associated with the Tn7 mutant (Fig. 1 and 2). In any case, at higher population densities of rhizobia in soil, the developing legume root should encounter proportionally more rhizobia, leading to more numerous root colonization sites. With increasing initial density in the seedling rhizosphere, there should be a correspondingly lesser need for the symbiont to swim to nodulation sites.

Transposon Tn7 insertion has not been reported previously for *B. japonicum*, but has been achieved in *R. meliloti* (6) and in *Rhizobium leguminosarum* (12). In both instances the transposon insertion into a plasmid was thought to be site specific. It is possible that the Tn7 was also site specific in Web 48, with insertion in the vicinity of a locus controlling motility, since several nonmotile transconjugants were obtained.

The rifampin resistance marker derived from the Web 48 parent strain did not account for the decreased competitiveness of the Web 48 Tn7 strain in soil (Table 5). In another experiment (data not reported), the Web 48 Rif3 parent and the Web 48 wt strains were virtually identical in nodulating soybean seedlings in a most-probable-number growth pouch assay. Concern about the possible impact of the Rif^r marker was justified, since rifampin resistance was reported to be associated with significant loss of nodulating competitiveness in *R. meliloti* (10) and in *R. leguminosarum* and *B. japonicum* (22).

TABLE 5. Nodulation success of *B. japonicum* Web 48 Rif and Web 48 wt in soil in competition with *B. japonicum* USDA 110

Competitors	Ratio in soil	No. of nodules				
		Total typed[a]	Web 48	USDA 110	Mixed infection	Unidentified
Web 48 Rif[b] × USDA 110	10^5:10^5	100	32	67	1	0
Web 48 wt × USDA 110	10^5:10^5	100	15	76	1	8
Web 48 Rif × USDA 110	10^6:10^5	100	85	15	0	0
Web 48 wt × USDA 110	10^6:10^5	100	60	38	0	2
Uninoculated soil[c]		3	0	0	0	0

[a] By IF.
[b] Spontaneous mutant resistant to 300 μg of rifampin ml^{-1}.
[c] Three nodules were found in the three uninoculated control pots.

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