Intra- and inter-specific competition in *Rhizobium fredii* and *Bradyrhizobium japonicum* as indigenous and introduced organisms[1]

STEPHEN F. DOWDLE[2] AND B. BEN BOHLOOL[3]
Department of Microbiology, University of Hawaii, Honolulu, HI, U.S.A. 96822
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We studied the competition between *Bradyrhizobium japonicum* and *Rhizobium fredii* isolates for nodulation of soybean *(Glycine max* L. Merrill) cultivars Williams and Ai Jiao Zao grown in three different soils in pots. Two of the soils were from People's Republic of China, one from a soybean field in Honghu with no history of *Rhizobium* inoculation, and one from a rice field in Wuhan with no history of soybean cultivation. The Honghu soil contained *B. japonicum* and *R. fredii (log* total number g^{-1} = 5.82 ± 0.58); whereas the Wuhan soil only contained *B. japonicum (log* total number g^{-1} =2.80 ± 0.52). Inoculation did not result in a significant increase in nodule number on plants in either soil. Uninoculated plants of both cultivars harbored only *R. fredii* in the Honghu soil and only *B. japonicum* in the Wuhan soil. Even when *B. japonicum* were inoculated into the Honghu *soil, R. fredii* occupied the majority of the nodules on both cultivars. In the Wuhan soil, *B. japonicum* serogroups USDA 110 and USDA 136b (= CB 1809) occupied the majority of the nodules except when an isolate of *R. fredii* from the soybean soil was added in high numbers. In a Hawaiian soil devoid of *B. japonicum* or *R. fredii, when* soybeans were inoculated with isolates of both species, most of the nodules were formed by *B. japonicum.* The *R. fredii* isolate could form up to 20% of nodules in this soil, but only on the Ai Jia Zao cultivar. In the Wuhan but not the Hawaiian soil, peat pelleting of seeds with equal numbers of two *B. japonicum* and one *R. fredii* isolates increased nodule occupancy by *B. japonicum* USDA136b serogroup significantly as compared with when the same isolates were inoculated into the soil. The results reported here highlight the critical importance of being indigenous to the competitive success of *B. japonicum* and *R. fredii* in nodulation of their soybean host.

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La competition entre les isolats de *Bradyrhizobium japonicum* et de *Rhizobium fredii pour* la nodulation du soya *(Glycine max (L.)* Merrill), a ete etudiee avec les cultivars Williams et Ai Jiao Zao croissant en pots dans trois sols differents. Deux des sols provenaient de la Republique populaire de Chine; Fun d'eux avait ete preleve d'un champ de soya de Honghu qui n'avait pas d'historicite relativement a 1'inoculation de *Rhizobium* et, 1'autre, avait ete preleve d'un champ de riz de Wuhan qui n'avait pas d'historicite concemant la *culture* de soya. Le sol de Honghu contenait du *B. japonicum et* du *R. fredii (nombre* logarithmique total g^{-1} = 5,82 ± 0,58), alors que le sol de Wuhan ne contenait que du *B. japonicum (nombre* logarithmique total g^{-1} = 2,80 ± 0,52). L'inoculation ne s'est pas traduite par une augmentation significative du nombre de nodules dans Fun et 1'autre de ces sols. Les plantes non-inocuaees des deux cultivars n'ont ete porteuses que du *R. fredii* dans le sol de Honghu et que du *B. japonicum* dans le sol de Wuhan. Lorsque du sol de Honghu fut inocule avec du *B. japonicum,* le *R. fredii* a quand meme occupe la majorite des nodules chez les deux cultivars. Dans le sol de Wuhan, les serogroupes USDA110 et USDA136b (= CB1809) de *B. japonicum* ont occupe la majorite des nodules, excepte lorsqu'un isolat de *R. fredii provenant* du champ de soya fut ajoute a forte concentration. Dans un sol hawaien depourvu de *B. japonicum* et de *R. fredii,* lorsque les cultivars de soya furent inocules avec les isolats des deux especes, les nodules furent formees en majeure partie pas le *B. japonicum.* Dans cc sol, 1'isolat de *R. fredii* a pu former jusqu'a 20% des nodules, mais seulement chez le cultivar Ai Jiao Zao. Dans le sol de Wuhan, mais non dans le sol hawaien, les graines pastillees de tourbe contenant des quantites egales en nombre de deux isolats de *B. japonicum* et d'un isolat de *R. fredii,* 1'occupation des nodules a ete augmentee de fagon significative par le serogroupe USDA 136b de *B. japonicum,* comparativement a 1'inoculation des memes isolats dans les sols. Les resultats presentes ici font ressortir 1'importance critique du caractere indigene pour le succes competitif de *B. japonicum* et de *R. fredii* dans la nodulation des soyas-h6tes.

[Traduit par la revue]

Introduction

An important objective in inoculation of legumes with rhizobia is to establish highly effective inoculum strains in the rhizosphere so they can compete successfully for nodule sites against those indigenous in the soil. With respect to soybeans in particular, inoculum strains superior in nitrogen fixation have frequently failed to compete successfully with indigenous rhizobia (Boonkerd *et* al. 1978; Ham *et* al. 1971 a; Johnson *et* al. 1965). Several studies have reported increased recovery of inoculum strains in soybean nodules by applying high cell numbers relative to the indigenous rhizobia (Bohlool and Schmidt 1973; Kapusta and Rouwenhorst 1973; Weaver and Frederick 1974). However, the numbers needed to overcome indigenous rhizobia are in many cases too excessive to be practical.

In much of the soybean-growing area in the north central United States, an area encompassing many soil types, indigeneous strains of *Bradyrhizobium japonicum* serogroup 123 dominate nodulation of soybeans (Damirgi *et* al. 1967; Ham *et* al. 1971b). Although several factors have been examined to account for the success of serogroup 123 (Ham 1980), strain characteristics that confer competitive advantage to 123 remain unknown. The competitive success of 123 was found to be unrelated to an ability to outgrow other indigenous *B. japonicum* in the host rhizosphere (Moawad *et* al. 1984). Also, when competition studies were carried out in sterile vermiculite or in soils devoid of naturalized *B. japonicum*, an isolate of 123 from a midwestern soil was found to be a poor competitor (Kosslak

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[2] Present address: Potash and Phosphate Institute, Apt. 62, no. 46 Stubbs Road, Hong Kong.

[3] Present address: NifTAL *Project,* University of Hawaii, 1000 Holomua Avenue, Paia, HI, U. S. A. 96779-9744.

and Bohlool 1985). This might have been due to factors in the midwestern soils that favored the competitive success of indigenous 123. Additionally, the 123 isolate used by Kosslak and Bohlool (1985) might have been a less competitive member of the diverse and heterogeneous serocluster 123 that has recently been characterized by Schmidt *et al.* (1986).

The adjectives "fast" and "slow" are arbitrary designation for rhizobial classification. They merely refer to the growth rate of the culture in artificial laboratory media and may not have relevance to its growth rate in the rhizosphere and its nodule occupancy on the host. Soybeans were previously thought to be nodulated only by the "slow-growing", "alkaline-producing" group of rhizobia formerly named *Rhizobium japonicum,* and now reclassified as *Bradyrhizobium japonicum*. Keyser *et al.* (1982) have reported a new group of soybean rhizobia which belong to the "fast-growing," "acid-producing" category of root-nodule bacteria. Studies have shown these rhizobia to be distinct in their microbiological and serological properties from the "typical" slow-growing types (Sadowsky *et al.* 1983, 1987; Stowers and Eaglesham 1984). A recent publication (Scholla and Elkan 1984) has proposed the designation of an entirely new species, *R. fredii,* for this group of rhizobia.

In a previous study we reported that in one soybean field in the People's Republic of China, although relatively high numbers of effective *B. japonicum* were present, the majority of nodules on soybean were formed by *R. fredii* (Dowdle and Bohlool 1985). Among these, we found *R. fredii* isolates that proved highly effective on North American cultivars Davis and Williams.

Studies designed to determine interstrain competition of fast and slow-growing rhizobia are few. Franco and Vincent (1976) studied the competition between a fast-growing isolate from *Leucaena* (ineffective on *Macroptilium atropurpureum* var. Siratro) and an effective slow-grower. They found nodulation on var. Sirato was almost entirely due to the effective slow grower unless the ratio of slow to fast growers in the inoculum was extremely favorable to the fast-growing strain. Zablotowicz and Focht (1981) compared a poorly effective fast grower and several effective slow growers isolated from cowpeas and found the fast grower produced 95% of the nodules when challenged with one slow grower, but only 6% when challenged with another slow grower. Trinick *et al.* (1983) studied effective fast- and slow-growing strains on cowpea and found at lower temperatures the fast-growing strain was a superior competitor for nodule sites, whereas at higher temperatures the slow-growing rhizobia were the better competitors. It is important to note, that the fast growers were not the host-preferred microsymbiont in terms of effectiveness in any of these studies.

McLoughlin *et al.* (1984) were the first to report on the competition pattern of *B. japonicum* and *R. fredii* in growth pouches and in two soils containing 3.5 x 105/g of slow growing *B. japonicum* belonging to serogroup 123. Their results show that the competitive ability of these organisms is different between different combinations of strains and is affected by the soil. In these studies the introduced fast-growing strains competed poorly against the indigenous slow growers. In one soil, however, two of the fast-growing strains, when applied at 10^9 cells/seed, were capable of occupying >63% of the nodules.

The present study compares the competitive ability of indigenous and introduced rhizobia for nodulation of soybeans. Competition of *R. fredii* and *B. japonicum* for nodulation of soybeans were studied in three soils: one PRE soil with an indigenous population of both species, one PRC soil with an indigenous population of only *B, japonicum,* and one from Hawaii devoid of soybean rhizobia.

Materials and methods

Soils and soybean cultivars

The chemical properties and cropping histories of the two Chinese soils used in this study have been described elsewhere (Dowdle and Bohlool 1985). The Honghu soil had been under soybean cultivation with no previous history of inoculation and had an indigenous population (6.6 x 10^5/g dry soil) of *R. fredii* and *B. japonicum.*Based on serological analysis of isolates at different dilutions (Dowdle and Bohlool 1985), populations of *R. fredii* and *B. japonicum* were estimated at approximately 10^5 and 10^4 g dry soil, respectively. The Wuhan soil had been under continuous paddy rice cultivation with no record of soybean cultivation and had an indigenous population (6.3 x 10^2/g dry soil) of only *B. japonicum*. The third soil, a Waimea very fine sandy loam (Typic Eutrandept, medial, isothermic), was collected on the island of Hawaii and had no indigenous population of soybean rhizobia. The pH of the Waimea soil determined in a 1:1 suspension in water was 6.3; and 5.8 in a 1:1 suspension in 1 M KCl. Two (*Glycine max* L. Merrill) cultivars were used in this study: cv. Ai Jiao Zao, a genetically improved, yellow-seeded cultivar released by the Oils and Root Crop Institute in Wuhan for use in Hubei province; and cv. Williams, a commercial cultivar planted in North America.

Rhizobium strains

The *R. fredii* isolate used in this study was HH003, isolated from the Honghu soil and effective on both cultivars. The *B. japonicum* isolates, WU002 and WU006, were isolated from the Wuhan soil. WU006 (serogroup USDA110) was effective on both cultivars, whereas WU002 (serogroup USDA136b) was effective on cv. Williams, but ineffective on cv. Ai Jiao Zao. The procedures used to isolate the strains were described in a previous study (Dowdle and Bohlool 1985).

Rhizobial cultures were grown and maintained in yeast extract mannitol (YEM) medium (Vincent 1970); the YEM agar slants used for the maintenance of fast-growing isolates contained 0.05% $CaC0_3$.

All of the *B. japonicum* isolates from the rice soil cross-reacted with fluorescent antibodies (FAs) against either USDA31, USDA 110, or USDA136b (same as CB1809). Isolates used in this study, WU002 and WU006, were in the USDA136b and USDAl10 serogroups, respectively (Dowdle and Bohlool 1985). The *B. japonicum* isolates from the Honghu soil could not be identified with any of the FAs against USDA serogroups; but most of the *R. fredii* isolates were reactive with one or more of the FAs against previously described isolates (Dowdle and Bohlool 1985). Isolate HH003 used in this study belonged to the PRC205 serogroup.

Soil and inoculum preparation

Since the soil had been in cold storage, the indigenous soybean rhizobia in the two Chinese soils were stimulated by planting a dense population of soybean seeds. The seedlings were removed after 10 days. All three soils were sieved (2 mm) and the number of rhizobia in the soils was determined by plant infection using a most probable number (MPN) technique (Vincent 1970). One millilitre of each dilution was inoculated onto 4-day-old *Glycine soja* seedlings growing in test tubes with Hoagland's nitrogen-free plant nutrient agar (Hoagland and Amon 1928) incubated in a Sherer model CEL4-7 controlled environment growth chamber at 27°C with a flux density of microeinsteins . m^{-2} . $s^{-1}$and a photoperiod of 16 h. The plants were examined for the presence of nodules after 4 weeks.

Cultures for inoculum preparations were grown in YEM broth until early stationary phase. The cultures were centrifuged (6000 x g) to remove excess media, and resuspended in 0.85% (w/v) saline. For each culture, a cell count was made using a Petroff-Hauser chamber and the cultures were adjusted to the same concentration with the addition of 0.85% saline. In addition, viable counts from each adjusted culture were determined by the drop plate method of Miles and Misra (Vincent 1970; Somasegaran and Hoben 1985). Mixed inoculants contained an equal number of the desired strains.

TABLE 1. Competition pattern of inoculum and indigenous *B. japonicum* and *R. fredii* strains in the Honghu soil containing both species[a]

Treatment	Inoculum size[c]	INC/IND[d]	Soybean cultivars: Ai Jiao Zao — Nodules per plant[e]	Ai Jiao Zao — % nodules identified using FAs[b] to: USDA 136b	USDA 110	PRC 205	Williams — Nodules per plant[e]	Williams — % nodules identified using FAs[b] to: USDA 136b	USDA 110	PRC 205
I. Uninoculated control			28	0	0	90	49	0	0	88
II. WU002, WU006 low	4.1	1:50	26	0	0	90	42	0	3	82
III. WU002, WU006 high	7.1	21:1	30	4	3	93	37	3	43	59
IV. WU002, WU006, HH003 low	4.2	1:40	24	0	0	91	47	0	0	95
V. WU002, WU006, HH003 high	7.2	24:1	33	3	26	70	44	5	17	75

[a]Numbers are percentages of the total numbers of nodules that reacted with the FAs used.
[b]Fluorescent antibodies (FAs) prepared against USDA 136b (CB1809), USDA 110, and PRC (USDA) 205 reacted strongly with isolates WU002, WU006, and HH003, respectively (Dowdle and Bohlool 1985). These FAs were used to type the nodule bacteroids.
[c]Values are mean log number of cells/g oven dry soil and were calculated based on viable plate count of inoculum.
[d]Ratio of inoculum (INC) to indigenous (IND) rhizobia. The log number of cells/g oven dry soil of the indigenous population was 5.82 ± 0.58 as determined by MPN.
[e]Values are the mean of three replicate plants.

Glasshouse experiment

Inocula were added to the soils at two levels and were mixed thoroughly into the soil to simulate the distribution of indigenous rhizobia. Thorough mixing of the inoculum and the soil was accomplished by first incorporating the inoculum strains into peat and then mixing the peat-rhizobia mixture into the soil to give the desired final numbers. Seeds were surface sterilized for 20 min in 4% (w/v) calcium hypochlorite, rinsed thoroughly in sterile water, and three seeds of each cultivar were sown into 16-oz plastic cups containing 350 g soil. Inoculum was also introduced into the soil with peat-pelleted seeds which were prepared using the procedures outlined by Vincent (1970). Each seed harbored approximately 1 x 10^5 cells of the desired rhizobial mixture as determined by viable count on YEM agar. Seedlings were subsequently thinned leaving one seedling of each cultivar per cup. After thinning, the top of the soil was covered with a 3-cm layer of fine gravel. Soils were maintained at 60% water-holding capacity throughout the experiment. A plastic straw (6 mm diam.) extending to the bottom of the cup facilitated watering with 1/4-strength Hoagland's nitrogen-free solution. There were three replicates for each treatment arranged in a randomized complete-block design. Plants were harvested at 4 weeks, and all nodules were collected and serotyped by immunofluorescence.

Immunofluorescence

Preparation of FAs and immunofluorescence staining of nodules are described elsewhere (Schmidt *et al.* 1968). Strain WU002 is in the same serogroup as USDA I36b (= CB 1809) and identified using FA USDA136b, whereas WU006 was identified with FA USDA110, and HH003 with FA PRC205 (Dowdle and Bohlool 1985). Smears from nodules were treated with gelatin - rhodamine isothiocyanate conjugate to suppress nonspecific staining (Bohlool and Schmidt 1968). Reflected light fluorescence microscopy was used for FA identification of nodule occupants as described previously (May and Bohlool 1983). To detect two-strain occupancy in the same nodule, transmitted light microscopy, i.e., phase contrast with an achromatic -aplanatic DIC condenser VZ, was used to visualize nonreactive cells in nodule smears.

Results

Results in Table 1 show that in the Honghu soil, with an indigenous population of *R. fredii* and *B. japonicum* (approximately 10^5 and 10^4/g dry soil, respectively), the majority of nodules on uninoculated control plants were formed by indigenous *R. fredii* that reacted with PRC 205 FA. Inoculation with low numbers of rhizobia did not alter the competition patterns significantly (Table 1, treatments 11 and IV). However, nodule occupancy on cv. Williams could be affected in favor of *B. japonicum* by including them in the inoculum in high number (Table 1, III). A comparison of treatments V with III shows an increase in WU006 occupancy on cv. Ai Jiao Zao and a decrease on cv. Williams.

In the Wuhan soil with an indigenous population of 6.3 x 10^z/g dry soil of only *B. japonicum,* the inoculum strain of *R. fredii* was unable to displace the indigenous *B. japonicum* from nodules when the ratio of inoculum : indigenous numbers was 30:1 (Table 2, II). At a higher ratio, however, a significant numbers of nodules were occupied by *R. fredii* (Table 2, 111). Inclusion of *B. japonicum* isolates in the inoculum along with the *R. fredii* reduced occupancy by the latter on both cultivars, whether applied directly into the soil (Table 2, IV) or pelleted in peat on the seed (Table 2, V).

In the uninoculated treatments in the Wuhan soil (Table 2, I), *B. japonicum* WU002 (serogroup USDA136b) formed only a few nodules on cv. Ai Jiao Zao, but the majority of them on cv. Williams. The only treatment that increased nodule occupancy by WU002 on cv. Ai Jiao Zao was when it was included in a mixture and pelleted on the seed (Table 2, V).

In the Waimea soil, devoid of soybean rhizobia, strain WU006 was the most competitive strain occupying approximately 80% of the nodules on both cultivars; strain HH003 occupied the remaining 20% of the nodules on cv. Ai Jiao Zao, while the remaining 20% on cv. Williams were occupied by WU002 (Table 3).

Discussion

China is believed to be the center of origin and diversity of soybean (Hymowitz and Newell 1981), and presumably of its microsymbiont, *Rhizobium.* Until recently, soybean cultivars used in commercial production in North America were derived from relatively a narrow genetic base (Committee on Genetic Vulnerability of Major Crops, Publications Department, U.S. National Academy of Sciences, Washington, DC). Symbiotic association of these soybean lines were also believed to be exclusively with the slow-growing group of rhizobia, now designated as *Bradyrhizobium japonicum.* However, Keyser *et al.* (1982) have described an entirely different group of rhizobia, isolated from nodules of soybeans obtained from three expeditions to China. This group is now placed in a distinct species, *R. fredii,* on the basis of biochemical and genetic evidence.

The *R. fredii* isolates of Keyser *et al.* (1982), with a few

TABLE 2. Competition pattern of inoculum and indigenous *B. japonicum* and *R. fredii* strains in the Wuhan soil containing only *B. japonicum*

Treatment	Inoculum size	INC/IND[a]	Soybean cultivars: Ai Jiao Zao — Nodules per plant	Ai Jiao Zao — % nodules identified using FAs to: USDA 136b	USDA 110	PRC 205	Williams — Nodules per plant	Williams — % nodules identified using FAs to: USDA 136b	USDA 110	PRC 205
I. Uninoculated control			25	7	59	0	28	53	37	0
II. HH003 low	4.3	30 : 1	27	2	54	10	23	41	32	3
III. HH003 high	7.3	31 000 : 1	32	0	18	86	37	21	14	41
IV. WU002, WU006, HH003 high	7.2	23 000 : 1	37	0	72	17	42	1	82	8
V. Peat-pelleted seed (WU002, WU006, HH003)[b]			34	20	30	24	32	49	38	8

[a]The log number of cells/g oven dry soil of the indigenous population was 2.80 ± 0.58 as determined by MPN.
[b]Peat on the seeds contained equal number of each strain, total of 10^5 cells/seed, as determined by plate count on YEM.

TABLE 3. Competition pattern of inoculum strains of *B. japonicum* and *R. fredii* in the Waimea soil devoid of soybean rhizobia

Treatment	Inoculum size	Soybean cultivars: Ai Jiao Zao — Nodules per plant	Ai Jiao Zao — % nodules identified using FA to: USDA 136b	USDA 110	PRC 205	Williams — Nodules per plant	Williams — % nodules identified using FA to: USDA 136b	USDA 110	PRC 205
I. Uninoculated control		0				0			
II. WU002, WU006, HH003 low	4.2	31	0	81	22	34	26	76	0
III. WU002, WU006, HH003 high	7.2	22	0	91	19	22	24	79	0
IV. Peat-pelleted seed (WU002, WU006, HH003)		27	2	89	8	29	16	89	1

NOTE: There were no significant differences ($P = 0.05$) between treatments in nodule occupancy by a serogroup.

exceptions (Hattorie and Johnson 1984; Van Rensburg *et al.* 1983; Yelton *et al.* 1983), form ineffective nodules with most North American cultivars. The same isolates, however, are highly effective with "Peking" soybean, a genetically unimproved black-seeded variety from China, and with a number of *G. soja* lines (Keyser and Cregan 1984). In a more recent collection of *R. fredii* from an uninoculated soybean field in China, Dowdle and Bohlool (1985) have found a number of isolates that were equally, or more, effective than the *B. japonicum* strain USDA I10 on two North American cultivars, Davis and Williams.

McLoughlin *et al.* (1984) have shown that the pattern of competition between fast- and slow-growing *R. japonicum* on soybeans grown in pouches an in soils containing slow-growing rhizobia varied with strains, soybean cultivar and soils. Similarily, in our study, competition between the fast- and slow-growing strains was influenced by cultivar, method of inoculation, and more dramatically by the indigenous rhizobia.

Intra- and inter-specific competition in *R. fredii* and *B. japonicum* as indigenous and introduced organisms was studied on two soybean cultivars grown in three soils. Two of the soils were from China (Dowdle and Bohlool 1985), one from a soybean field in Honghu with no history of *Rhizobium* inoculation but containing both *R. fredii* and *B. japonicum;* and another from a rice field in Wuhan with no history of soybean cultivation, and containing only *B. japonicum* population. A third soil from Hawaii was devoid of either species. Nodule occupancy data of the uninoculated control plants in the Honghu soil (Table 1) confirm our previous results (Dowdle and Bohlool 1985) that the majority of nodules on both cultivars were formed by *R. fredii* which reacted with PRC205 FA. None of the nodules reacted with FAs against *B. japonicum* strains. This was despite the fact that *B. japonicum* were present in this soil in high numbers as evidenced by their recovery at the higher dilution (10^{-2}-10^{-4}) of rhizosphere soil (Dowdle and Bohlool 1985). In the Wuhan soil (Table 2) uninoculated plants harbored nodules occupied by *B. japonicum,* the majority of which could be identified using USDA110 or USDA136b FAs.

Inoculation with *B. japonicum* and *R. fredii* did not result in significant increases in nodule numbers on either cultivar in either soil (Table 1 and 2). Nodules occupancy by the inoculum strain could be achieved in both soils, but only at high ratios of inoculum to indigenous rhizobia (Table 1, 111 and V; Table 2, 111). In the Honghu soil with an indigenous population of 6.6 x 10^5 total rhizobia/g dry soil, nodule occupancy by indigenous *R. fredii* could be reduced from 88% (Table 1, I) to 50 and 75% when high rates of *B. japonicum* or *B. japonicum* and *R. fredii,* respectively, were inoculated into the soil (Table 1,111 and IV). Likewise, in Wuhan soil with an indigenous population of 6.3 x 10^2 total *B. japonicumlg* dry soil, introduced *R. fredii* could

occupy a significant number of nodules (Table 2, III), but only when *B. japonicum* was not included in the inoculum (Table 2, IV). Pelleting of the seed with two isolates of *B.* japonicum and one of *R. fredii* favored the former (Table 2, V).

Trinick *et* al. (1983) studied competition between a fast grower and a slow grower and reported that when the total numbers in a mixed inoculum were low, the fast-growing strain was a better competitor. On the other hand, when the numbers in the inoculum were high, the slow-growing strain was the better strain and formed more nodules. Our results show that in a soil devoid of soybean rhizobia, the total number of cells in the inoculum had no influence on the competition pattern of the inoculum strains (Table 3).

Based on data from Weaver and Frederick (1974), it can be predicted that in soils in the soybean-growing region of midwestern United States, if the inoculum rhizobia are to form 50% or more of the nodules, then an inoculation rate of at least 1000 times the soil rhizobial population must be used. Our studies also illustrated that high ratios of inoculum : indigenous numbers were required to displace indigenous rhizobia from nodules. For example, in the Wuhan rice soil inoculum *R. fredii* occupied 86% of the nodules on cv. Ai Jiao Zao and 41 % of the nodules on cv. Williams, but only when its ratio in the inoculum to indigenous rhizobia 31000: 1. When HH003 was mixed into the soil in lower numbers, or mixed into the soil together with two cultured indigenous *B.* japonicum isolates, its nodule occupancy was significantly lower.

In most of the treatments, HH003 was more competitive on cv. Ai Jiao Zao than on cv. Williams (Tables 1-3). This is consistent with the observation that cv. Williams had a greater affinity for *B. japonicum*, whereas cv. Ai Jiao Zao had a greater affinity for *R. fredii,* based on the nitrogen'fixing effectiveness of the symbioses formed between the two cultivars and the various isolates from the soybean and rice soil (Dowdle and Bohlool 1985).

Competition between the slow-growing *B. japonicum* isolates was also influenced by the soybean cultivars. This was due, in large part, to the ineffective symbiotic association between cv. Ai Jiao Zao and WU002 (Dowdle and Bohlool 1985). Diatloff and Brockwell (1976) observed a similar pattern of poor competitiveness with an ineffective cultivar (Hardee) - strain CB 1809 association and high competitiveness with an effective cultivar. (Hampton) - strain CB 1809 association. Furthermore, they reported that when an ineffective strain was included in the inoculum, nodule formation by the effective strains was suppressed. This suppression effect did not occur in our study.

Although the number of rhizobia differed greatly between inoculum treatments, there was no significant effect on the number of nodules formed. Between 0 and 5% of the nodules were doubly infected by either two slow growers or by fast and slow growers. Dual occupancy of nodules by fast- and slowgrowing rhizobia has been reported before (Trinick *et* al. 1983).

The results of this study demonstrate that, at least in one soil, the *R. fredii* are highly competitive when they are native. More studies in soils from China are needed to determine the extent of the occurence of fast-growing soybean rhizobia, their competitiveness under natural conditions, and their symbiotic effectiveness on genetically diverse cultivars of soybeans.

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