APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1982, p. 98–106 Vol. 44, No. 1
0099-2240/82/070098-09$02.00/0

Competitive Abilities of *Rhizobium meliloti* Strains Considered to Have Potential as Inoculants

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Received 3 September 1981/Accepted 5 March 1982

Twenty four strains of *Rhizobium meliloti* considered to have potential for inoculant production were grouped in pairs and tested for their ability to compete for nodulation on *Medicago sativa*, *Medicago truncatula*, and *Medicago littoralis*. At the outset, each pair of strains, which consisted of a wild type and a selected streptomycin-resistant mutant of another strain, was tested in an autoclaved soil. Six strain pairs, each consisting of a good and a poor competitor, reacted consistently when tested in each of five other autoclaved soils; eight pairs consisting of strains with comparable competitive abilities varied in their reactions in some of the soils, or even in the same soil when retested. An effect of soil pH on competitive ability was observed with some of these strains. Not all of the strains identified as good competitors on one or more of the *Medicago* spp. in the autoclaved soils were able to nodulate these plants satisfactorily in a field soil containing an established population of *R. meliloti*. Strain RF24, which seemed to be the best competitor on each of the three *Medicago* spp., grouped among the less effective strains on two of the legumes. Two strains of *R. meliloti* frequently used for inoculant production differed markedly with regard to competitive ability; this places some doubt on the relevancy of singling out competitive ability for special attention when selecting a strain for inoculant production.

Properties such as nitrogen-fixing ability, ability to survive and multiply in the soil, and competitiveness in nodule formation (2, 5–7, 10, 13) have been singled out as essential for a strain of *Rhizobium* to be suitable for inoculant production. It therefore seems reasonable to assume that a *Rhizobium* strain which has performed consistently well in inoculants under diverse soil conditions in the presence of competing *Rhizobium* populations possesses these properties.

One of our functions is to provide suitable strains of *Rhizobium* to commercial inoculant manufacturers in South Africa. The task of selecting the best strain for a particular legume is frequently complicated by the availability of an appreciable number of strains which are indistinguishable with regard to their nodulating and nitrogen-fixing abilities, as indicated by field trials in various parts of the country. Although the consistently good performance of these strains in the field should be sufficient proof that they possess those properties considered essential for an inoculant strain, our field trials suffer from a major shortcoming: they are mostly conducted on sites relatively free of rhizobia capable of nodulating the legume concerned. Information on the ability of these strains to compete for nodulation with an established *Rhizobium* population (6, 15) was therefore lacking.

To bridge this gap and to aid in the selection of the most suitable strain(s), we decided to specifically screen strains which have passed our field tests for their ability to compete for nodulation. A technique was decided on in which the competitive ability of a selected mutant resistant to a single antibiotic (3) was compared with that of the wild type of another strain in soil free of other rhizobia. Agar as a test medium was rejected for two reasons. First, relatively few nodules were formed in agar in preliminary experiments, which tended to give unreliable results. Second, the probability was high that double infections in agar would result in nodules containing both strains (4).

Three *Medicago* spp. important to South African agriculture and 24 strains of *R. meliloti* obtained over a period of 2 decades from various countries, or isolated locally, were selected for this study. Most of these strains were similar in their ability to nodulate various *Medicago* spp. effectively under a range of field and climatic conditions.

The performance in these competition experiments of two well-proven inoculant strains was expected to give a fair indication of the relevancy of our approach to single out competitive ability as a final criterion for the selection of a strain for inoculant production.

TABLE 1. Available information on strains of *R. meliloti* used

Strain no.	Synonym(s)	Obtained from:	Country of origin	Yr obtained
RF1	3 DOa20	U.S.A.		1955
RF4			R.S.A.[a]	1959
RF5			R.S.A.	1966
RF6			R.S.A.	1959
RF7			R.S.A.	1959
RF8			R.S.A.	1959
RF9			R.S.A.	1959
RF10			R.S.A.	1960
RF11			R.S.A.	1966
RF12	CB119	Australia	Australia	1962
RF14[b]	U45	Australia	Uruguay	1968
RF14B[b]	U45; 735	Zimbabwe	Uruguay	1968
RF15	WA39	Australia		1962
RF18	396; PDD Luc	Zimbabwe	New Zealand	1968
RF19	423; K8	Zimbabwe	Holland	1968
RF20	497; 3DOa27	Zimbabwe	Turkey	1968
RF22			R.S.A.	1964
RF23			R.S.A.	1963
RF24			R.S.A.	1963
RF27	A145	Holland		1969
RF29			R.S.A.	1967
RF31			R.S.A.	1967
RF32			R.S.A.	1967
RF33			R.S.A.	1967

[a] R.S.A., Republic of South Africa.
[b] Strains RF14 and RF14B were serologically identical.

MATERIALS AND METHODS

***Rhizobium* strains and preparation of inocula.** The *R. meliloti* strains used were obtained from the South African Rhizobium Collection. The origin of each strain is shown in Table 1. The inoculum of each strain used in the various experiments was obtained by suspending in sterile distilled water the growth of a 6-day-old culture on yeast extract-mannitol (YM) agar (1). The cells were washed by repeated centrifugation and resuspension in sterile water before the suspension was standardized at a desired concentration by means of a Petroff-Hausser counting chamber.

Streptomycin-resistant (Str^+) mutants of each strain were obtained by streaking a culture on YM agar containing 125 μg of streptomycin per ml. Growth from selected colonies was restreaked onto YM agar plates containing 250 or 500 μg of streptomycin per ml to confirm resistance. At least five Str^+ mutants of each strain were compared with the wild-type strains on *M. sativa* for their ability to fix nitrogen and compete for nodulation. Mutant and wild-type strains were maintained on YM agar slants at 4°C.

Nitrogen-fixing ability of strains. The nitrogen-fixing ability of each strain was determined by inoculating seedlings of *M. sativa* cultivar Karoo, *M. truncatula* cultivar Cyprus, and *M. littoralis* cultivar Harbinger, respectively, in washed quartz sand in Leonard jars (16). The plants were supplied with nitrogen-free Hoagland nutrient solution (9) and maintained in a greenhouse the temperature of which was between 8 and 24°C. After 8 weeks, plants were harvested, and the dry mass of the tops was determined.

Competitive ability: greenhouse experiments. The ability of each of the 24 strains to compete for nodulation was determined in a sandy loam of unknown composition in open clay pots containing 2 kg of soil. Before use, the soil was treated with steam at 120°C for 5 h to kill any rhizobia present. The soil, as well as

TABLE 2. Soil types used in competition experiments

Soil type[a]	Description	Chemical analysis[b] (ppm)					Soil pH[c]
		P	K	Ca	Mg	Al	
Avalon	Yellow-brown clay	1	260	519	347	48	5.6
Katspruit	Black-greyish peat	3	313	626	324	32	5.8
Doveton	Red clay loam	12	110	1,258	267	3	5.4
Cartreff	Grey sand	13	109	273	33	36	5.5
Southwold	Yellow sandy loam	17	169	692	99	99	4.9

[a] As classified by MacVicar et al. (11).
[b] Analysis by soil testing laboratory, Department of Agricultural Technical Services.
[c] pH was measured in a 1:1 soil-water suspension.

five other soils used in subsequent greenhouse experiments (Table 2), received superphosphate equivalent to 500 kg hectare^{-1}. Lime was added to obtain a pH (soil-to-water ratio, 1:1) of ca. 6.5 to 7.0. Each pot had a layer of gravel at the bottom to prevent spillage of soil through the drainage hole. Pots were watered to approximately field capacity when necessary, using unsterilized tap water.

The *Rhizobium* strains were studied in pairs for competitive ability on *M. sativa*, *M. truncatula*, and *M. littoralis*. Because it was impossible to test pairs of strains in all combinations, strains were grouped in pairs in a subjective fashion based on previous experience of the capabilities of many of the strains. It was attempted in the first round to combine strains expected to be good competitors with ones suspected of being less competitive, for example, on *M. sativa*, a strain highly compatible (effective) with this host and one considered more compatible with either *M. truncatula* or *M. littoralis*. After the first series of tests, the better strain of each combination was retested against the other strain(s) until it was eliminated (see Fig. 1–3). Each pair of strains was tested twice, i.e., wild-type A against Str$^+$ mutant B and Str$^+$ mutant A against wild-type B.

Surface-sterilized seeds of a *Medicago* sp. were sown into a pot before the soil was inoculated with equal numbers of cells of the two *Rhizobium* strains of a pair. Suspensions containing ca. 3×10^9 cells of each of the strains were applied evenly to the soil surface before being carefully washed into the soil with 250 ml of sterile water. Each treatment was replicated three times. The pots were maintained in a greenhouse with temperatures ranging from 8 to 24°C. After germination, the seedlings in a pot were thinned and allowed to develop for 8 to 9 weeks. The root system of a plant from each pot was harvested intact by carefully removing all adhering soil under running tap water. The entire root system was surface sterilized for 3 min with 0.1% $HgCl_2$ containing a wetting agent, before being thoroughly washed with sterile water. A total of 32 nodules from each plant selected from the crown downward were removed and squashed separately by

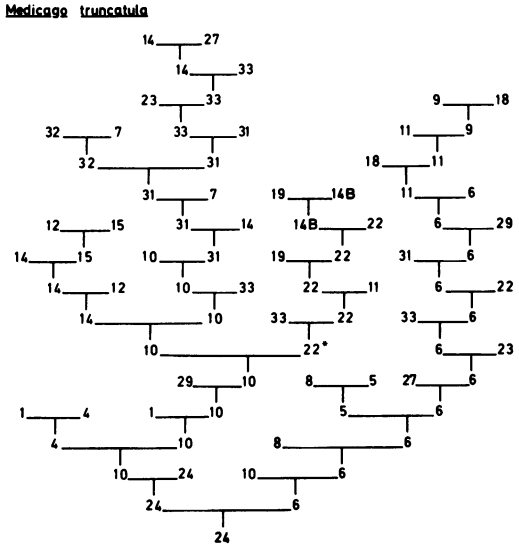

FIG. 2. Combinations in which RF strains of *R. meliloti* were paired in competition experiments on *M. truncatula*. *, Strain RF22 not significantly better than RF33.

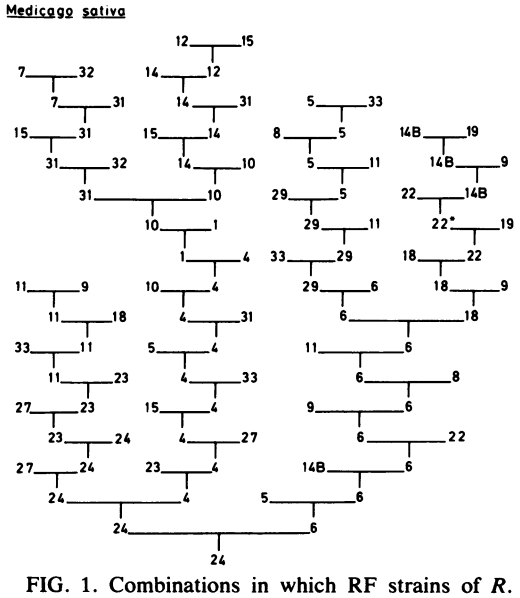

FIG. 1. Combinations in which RF strains of *R. meliloti* were paired in competition experiments on *M. sativa*. *, Strain RF22 not significantly better than RF14B.

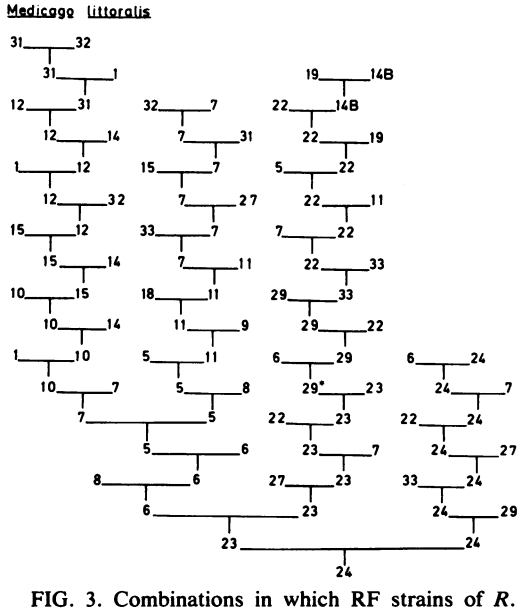

FIG. 3. Combinations in which RF strains of *R. meliloti* were paired in competition experiments on *M. littoralis*. *, Strain RF29 not significantly better than RF6.

means of sterile forceps onto plates of YM agar and YM agar containing 250 μg of streptomycin per ml. It was possible to test eight nodules on a single plate divided into segments. Agar plates were incubated at 27°C for 4 days before being examined. The incidence of each strain of a pair in nodules was examined by the Pearson χ^2 test (14) to determine whether strain ratios found in nodules of replicate plants differed significantly from an expected 1:1 ratio.

Field experiments. The ability of some strains to compete in soil containing an established *Rhizobium* population was determined in a field experiment in an area where *Medicago* spp. were frequently cultivated. The numbers of rhizobia present in the soil were calculated by means of the plant dilution method (16), using *M. sativa* as the test plant. Soil pH was measured in a 1:1 soil-water suspension.

The soil in three blocks, one each for *M. sativa*, *M. truncatula*, and *M. littoralis*, was fertilized with appropriate amounts of lime and superphosphate before seedbeds were prepared. Blocks were separated by 7 m of undisturbed soil. Each block consisted of five rows (replicates) 1 m apart. A row was divided into eight plots (treatments) of 1 m each; plots were separated from one another by 1-m distances within the row. Peat inoculant prepared for each of the strains tested was applied to seed with a 1.0% methylcellulose sticker before it was sown in appropriate plots randomized within each of the rows of a block. An uninoculated control plot was included in each row. The numbers of rhizobia in the various peat inoculants were determined by means of plate counts immediately before inoculation of the seed. After 9 weeks, three plants were removed at random from each of the five replicates of a treatment. Isolations were made from all of the nodules on YM agar with and without streptomycin.

After 1 year, remaining plants of the perennial *M. sativa* were still growing in the original rows; the two annual *Medicago* spp. had reseeded themselves in and around the rows in which they had been established the previous year. To determine how well the inoculant *Rhizobium* strains had survived in the soil, uninoculated seeds of each of the *Medicago* spp. were sown in rows as close as possible to those of the year before. After 9 weeks, 10 plants from a row next to each of the five replicate rows of the previous year's treatments were removed. A total of 160 nodules were taken from the 10 plants of each replicate and squashed onto YM agar with and without streptomycin.

RESULTS

Various Str^+ mutants were compared with their respective wild-type strains with regard to nitrogen fixing and competitive abilities on each of the three *Medicago* spp. One mutant of each strain, with properties similar to that of the wild type, was selected for further study.

A pair of strains consisting of the wild type of one strain and the selected Str^+ mutant of another was tested for the ability to compete for nodulation on *M. sativa*, *M. truncatula*, and *M. littoralis*. No discrepancy was found in the results obtained with any pair of strains when the combination of wild-type A and Str^+ mutant B was reversed to wild-type B and Str^+ mutant A. The experimental procedure and the results obtained in an autoclaved sandy loam in the greenhouse are shown in Fig. 1–3. The nitrogen-fixing ability of each strain is shown in Table 3.

The best competitors for nodule sites were as follows: on *M. sativa*, RF24, RF6, and RF4; on *M. truncatula*, RF24, RF10, RF6, and RF22; on *M. littoralis*, RF24, RF23, RF22, RF29, and RF6 (Fig. 1–3). RF24 was the best competitor on each of the three *Medicago* spp.

The best competitors were not all among the most effective strains (Table 3). Although none of the strains on *M. sativa* differed significantly in effectiveness, the best competitor, strain RF24, was at the lower end of the scale (Table 3). With *M. truncatula*, three of the best competitors, RF6, RF10, and RF22, were highly effective, with strain RF24 among the least effective. With *M. littoralis*, three of the four best competitors were among the most effective strains; strain RF29 was only partially effective (Table 3).

In a subsequent experiment, strains of *R. meliloti* which performed best in competition experiments in the sandy loam on *M. sativa* and *M. truncatula* (Fig. 1 and 2) were retested in this soil as well as in five other autoclaved soils (Table 1). A poor competitor, strain RF14, was also included. The results are shown in Tables 4 and 5.

When strains with good competitive abilities were combined, the test medium (soil type) seemed to have determined their relative success, for example, RF4 with RF6 (Table 4), RF24 with RF10, RF22 with RF6, and RF10 with RF22 (Table 5). However, with some of these strain combinations, the results obtained in the sandy loam (Tables 4 and 5) were also the reverse of those obtained in the same soil in the initial screening experiment (Fig. 1 and 2).

Soil-strain interaction was further indicated by the fact that RF10 was superior to RF6 when paired in each of the six soil types (Table 5) but was inferior to RF22 in the Katspruit soil; RF22 in turn lost to RF6 in the same soil (Table 5). Once again, it should be noted that RF10 had lost to RF6 in the initial screening experiment in the sandy loam (Fig. 1).

The effect of soil pH on the competitive ability of some of the strains of *R. meliloti* was determined in an unsterilized sandy loam with pH adjusted with $CaCO_3$ (Table 6). A significant change in the relative proportions of cells of strains of each of the pairs RF4 with RF6 and RF10 with RF24 occurred with a decrease in soil pH.

Finally, some of the most promising strains of *R. meliloti* were used in a field experiment to

TABLE 3. Dry mass[a] of plants of each of three *Medicago* species nodulated by strains of *R. meliloti*

Determination for	*M. sativa* (g)	Determination for strain no.	*M. truncatula* (g)	Determination for strain no.	*M. littoralis* (g)
RF4[b]	2.50	RF6[b]	2.98	RF10	2.57
RF27	2.47	RF31	2.96	RF19	2.55
RF15	2.34	RF19	2.85	RF12	2.45
RF32	2.30	RF10[b]	2.79	RF23[b]	2.43
RF10[b]	2.23	RF12	2.74	RF9	2.35
RF23	2.04	RF7	2.69	RF27	2.30
RF5	2.04	RF22[b]	2.65	RF6[b]	2.30
RF33	2.03	RF27	2.58	RF18	2.29
RF19	2.02	RF9	2.49	RF31	2.28
RF6[b]	2.01	RF18	2.47	RF33	2.26
RF11	2.00	RF32	2.38	RF22[b]	2.23
RF12	1.99	RF23	2.30	RF15	2.19
RF9	1.88	RF33	2.15	RF24[b]	2.15
RF31	1.88	RF1	1.90	RF11	2.13
RF18	1.87	RF20	1.90	RF32	2.10
RF24[b]	1.79	RF11	1.88	RF8	2.05
RF1	1.76	RF14B	1.77	RF1	2.03
RF29	1.73	RF5	1.75	RF4	1.90
RF7	1.70	RF15	1.70	RF20	1.89
RF14B	1.69	RF29	1.66	RF7	1.89
RF22	1.68	RF14	1.59	RF5	1.70
RF23	1.65	RF4	1.58	RF14B	1.66
RF14	1.64	RF8	1.58	RF29[b]	1.64
RF8	1.55	RF24[b]	1.57	RF14	1.60
Uninoculated	0.07		0.07		0.11
LSD[c]					
0.01	0.86		1.01		0.94
0.05	0.77		0.90		0.87
CV[d]	20.9%		25.3%		22.2%

[a] In grams per plant dry mass. Each value is the average of five replicates.
[b] Strains considered to be among the best competitors on the respective *Medicago* spp. (see Fig. 1–3).
[c] LSD, Least significant difference.
[d] CV, Coefficient of variation.

inoculate seeds of *M. sativa*, *M. truncatula*, and *M. littoralis* before the seeds were sown into soil containing a naturalized population of *R. meliloti*. Although it was attempted to have peat inoculants available that would contain at least 10^9 cells of the various strains g^{-1}, plate counts at the time of inoculation showed that inoculants of each of two strains, RF14 and RF24, fell short of this goal (Table 7). Strains RF6 and RF10 were good on each of the three *Medicago* spp.;

TABLE 4. Ability of strains of *R. meliloti* to compete for nodulation on *M. sativa* in each of six autoclaved soils[a]

Combination of strains tested	Strain which formed most nodules in following soil:					
	Sandy loam	Avalon	Katspruit	Doveton	Cartreff	Southwold
RF12 × RF6[b]	RF6	RF6	RF6	RF6	RF6	RF6
RF14 × RF4[b]	RF4	RF4	RF4	RF4	RF4	RF4
RF14 × RF24[b]	RF24	RF24	RF24	RF24	RF24	RF24
RF6 × RF24[c]	RF24[d]	RF24	RF24	RF24	RF24	RF24
RF6 × RF4[c]	RF4[d,e]	RF4[e]	RF6	RF6	RF6	RF6

[a] See Table 2 for soils used.
[b] Combination of a poor and a good competitor (Fig. 2).
[c] Combination of two good competitors (Fig. 2).
[d] The difference in the numbers of nodules formed by the strains of a pair was not significant.
[e] When first tested in the sandy loam (Fig. 2), strain RF4 lost to strain RF6.

TABLE 5. Ability of strains of *R. meliloti* to compete for nodulation on *M. truncatula* in each of six autoclaved soils[a]

Combination of strains tested	Strains which formed most nodules in following soil:					
	Sandy loam	Avalon	Katspruit	Doveton	Cartreff	Southwold
RF14 × RF24[b]	RF24	RF24	RF24	RF24	RF24	RF24
RF14 × RF6[b]	RF6	RF6	RF6	RF6	RF6	RF6
RF14 × RF10[b]	RF10	RF10	RF10	RF10	RF10	RF10
RF14 × RF22[b]	RF22	RF22	RF22	RF22	RF22	RF22
RF6 × RF10[c]	RF10[d]	RF10	RF10	RF10	RF10	RF10
RF6 × RF22[c]	RF22[d,e]	RF22	RF6	RF22	RF6	RF6
RF6 × RF24[c]	RF24	RF24	RF24	RF24	RF24	RF24
RF10 × RF24[c]	RF24	RF10	RF24	RF10	RF10	RF10
RF10 × RF22[c]	RF22[d]	RF10	RF22	RF22	RF10	RF10
RF22 × RF24[c]	RF24	RF24	RF24	RF24	RF24	RF24

[a] See Table 2 for soils used.
[b] Combination of a poor and a good competitor (Fig. 2).
[c] Combination of two good competitors (Fig. 2).
[d] When first tested in the sandy loam (Fig. 1), strain RF22 lost to strains RF6 and RF10, respectively, strain RF10 lost to RF6.
[e] The difference in the numbers of nodules formed by the strains of a pair was not significant.

strain RF4 was among the best on *M. sativa* and *M. littoralis*. Considering the relatively low number of cells of RF24 in the inoculant, it did fairly well on each of the three plant species and completely outperformed strains RF14 and RF22 (Table 7). Note that strains RF6 and RF10, which were inferior to RF22 on *M. truncatula* in three of six autoclaved soils (Table 5), completely outperformed RF22 in the field experiment (Table 7).

TABLE 6. Occurrence in *M. sativa* nodules of *R. meliloti* strains applied as mixed inocula to seed in an unsterilized sandy loam[a] with pH adjusted with $CaCO_3$

Strains in inoculum (Wild type × Str^+ mutant)	Number of nodules formed in soil with pH:					
	7.2		6.8		6.0	
	Wild type	Str^+ mutant	Wild type	Str^+ mutant	Wild type	Str^+ mutant
RF24 × RF6	74	93[b]	28	138	60	108
RF6 × RF24	109	59	86	82[b]	91	70[b]
RF4 × RF24	123	45	115	53	119	49
RF24 × RF4	67	101	47	116	43	120
RF4 × RF10	131	37	130	38	121	47
RF10 × RF4	43	125	58	110	48	120
RF4 × RF6	63	105	69	99	74	94[b]
RF6 × RF4	98	70	93	75[b]	96	72[b]
RF6 × RF10	97	71	92	76[b]	105	63
RF10 × RF6	44	134	48	120	49	119
RF10 × RF24	81	87[b]	76	92[b]	101	67
RF24 × RF10	88	80[b]	106	61	66	102
RF4 × RF4[c]	81	87[b]	86	82[b]	78	90[b]
RF6 × RF6[c]	88	80[b]	72	97[b]	84	84[b]
RF24 × RF24[c]	84	84[b]	79	89[b]	88	80[b]

[a] Soil free of other rhizobia which nodulate *M. sativa*.
[b] Ratio does not differ significantly from 1:1.
[c] Controls.

TABLE 7. Ability of strains of *R. meliloti* to form nodules on each of three *Medicago* spp. in soil containing naturalized rhizobia[a]

Strain of *R. meliloti*[b]	Cells g of inoculant^{-1} ($\times 10^9$)	Occurrence of inoculum strains[c] in nodules[d] of					
		M. sativa		*M. truncatula*		*M. littoralis*	
		No.	%	No.	%	No.	%
RF4	3.4	21.2	71.6	9.0	31.6	19.2	64.4
RF6	8.1	25.0	91.9	22.8	75.0	18.4	59.7
RF10	5.8	22.2	84.1	21.0	70.0	15.0	52.1
RF22	6.1	7.4	26.3	4.8	17.5	6.4	22.4
RF14	0.8	4.0	13.1	2.2	8.5	8.4	30.2
RF23	1.6	19.4	62.5	5.0	19.7	17.0	59.8
RF24	0.7	13.2	51.6	9.4	32.6	13.6	54.4
Uninoculated		0	0	0	0	0	0

[a] Average number of naturalized rhizobia which nodulated *M. sativa* was 7.4×10^3 g of soil^{-1}.
[b] Streptomycin-resistant mutants.
[c] Average number of nodules formed by the inoculum strain on a plant; five replicates were used.
[d] Total numbers of nodules analyzed were: *M. sativa*, 1,288; *M. truncatula*, 1,242; *M. littoralis*, 1,240.

Uninoculated seeds of each of the three *Medicago* spp. were sown into the same field plots 1 year later to act as trap plants for surviving inoculant strains. Whereas most strains survived in low numbers, RF6 was outstanding, forming between 26 and 28% of the nodules on plants of each of the three plant species (Table 8). Strains RF4 and RF10, which were comparable to strain RF6 in forming nodules when introduced into the soil the previous year (Table 7), apparently failed to establish any better than the other strains used.

DISCUSSION

The relative abilities of strains of *Rhizobium* to compete for nodulation are determined, or strongly influenced, by factors such as strains involved, the host plant, the abiotic and biotic environments, and interactions among these factors (5, 8, 10, 17). Competitive ability is a complex phenomenon not easily related to specific properties or conditions, as was apparent from this investigation. Results obtained in a simplified environment must therefore be interpreted with caution.

Although the technique of studying strains in pairs for competitive ability was laborious, it provided additional and relevant information on the strains studied. The concern that mutants resistant to a single antibiotic might give misleading results because of back mutation to wild type proved unfounded, as results obtained with a stain combination of wild-type A and Str$^+$ mutant B were without exception consistent with those obtained with the reverse combination of wild-type B and Str$^+$ mutant A.

The extent to which results of competition experiments in a single test medium (in this case, an autoclaved soil) could be relied on, or extrapolated, was indicated when some of the strain pairs were retested in each of six autoclaved soils. A pair of strains which consisted of a good and a weak competitor (Fig. 1 and 2) reacted consistently in each of the six soils used (Tables 4 and 5). Only when strains with apparently

TABLE 8. Occurrence of inoculant strains of *R. meliloti* in nodules of uninoculated *Medicago* spp. sown into soil 1 year after inoculated seed of the same species had been sown

Inoculant strain	Inoculant strains present in nodules of uninoculated					
	M. sativa		*M. truncatula*		*M. littoralis*	
	No.	%	No.	%	No.	%
RF4	4	5.0	2	2.5	7	8.8
RF6	22	27.5	21	26.3	22	27.5
RF10	3	3.8	7	8.8	6	7.5
RF22	2	2.5	1	1.3	5	6.3
RF14	0	0	0	0	2	2.5
RF23	3	3.8	5	6.3	3	3.8
RF24	5	6.3	1	1.3	3	3.8
Uninoculated	0	0	0	0	0	0

comparable competitive abilities were paired, such as RF4 with RF6 on *M. sativa* (Table 4), or RF6 with RF22, RF10 with RF24, and RF10 with RF22 on *M. truncatula*, did some variation in reactions occur in different soil types. This variation, linked to the inconsistent reactions obtained when some of these strain pairs were retested in the same soil (Tables 4 and 5), is considered evidence of similar competitive abilities of strains under the conditions of the experiment rather than a marked effect of soil type. However, a significant change in the relative proportions of cells of strains of at least two pairs, RF4 with RF6 and RF10 with RF24, occurred with a change in soil pH (Table 6).

The screening procedure in the greenhouse thus provided a good indication of the relative potential of the strains of *R. meliloti* to compete for nodulation. In deciding to what extent these results pertained to our concept of a good inoculant strain, the performance of a strain in the competition experiments had to be compared with that in the field trial. Taking into account that strains RF14, RF23, and RF24 were present in relatively small numbers in inoculants used in the field experiment, good competitors identified in the greenhouse, such as RF4, RF6, and RF10 on *M. sativa*, RF6 and RF10 on *M. truncatula*, and RF23 and RF24 on *M. littoralis*, were also good nodulators of the same legume(s) in the field. Also consistent with the poor competitive ability of strain RF14 in the greenhouse on each of the three *Medicago* spp. (Fig. 1–3) was its inferior performance in the field. Strain RF22 was an exception; it was considered a good competitor on *M. truncatula* and *M. littoralis* (Fig. 1 and 3) but failed on all of the legumes in the field.

Competitive ability is but one of several qualities of a good inoculant strain (2, 15, 17). This raises the question of the extent to which this property justifies special emphasis, as does effectiveness, when selecting a strain for inoculant production. Relevant were the different results obtained with strains RF10 and RF14 (U45), which have been used in inoculants for many years. Whereas strain RF10 could be grouped among the good competitors on the *Medicago* spp. tested, strain RF14 was among the least competitive in this study.

The behavior of strain RF14 was unexpected and hardly reconcilable with its reputation as an inoculant strain in more than one country (R. J. Roughley, personal communication). It seems unlikely that a strain unable to compete for nodulation under the favorable conditions of the experiment would be able to do so in the field in the presence of an established population of *R. meliloti*. Possible incompatibility between RF14 and the respective cultivars of the three legume species used seems unlikely as an explanation of its poor performance, as it has been used successfully in inoculants for these legumes (unpublished data). Also, the possibility that the culture of strain RF14 in our possession is a mutant less competitive than the original strain, U45, has to be considered. In this regard, we refer to the fact that the two cultures of strain U45, RF14 and RF14B, originally obtained from different sources (Table 1), were remarkably similar with regard to their nitrogen fixing and competitive abilities; strain RF14 is still being used extensively in inoculants in South Africa with apparent success.

This study supports the view that the ability of a *Rhizobium* strain to compete for nodulation is unrelated to other single features of the *Rhizobium*-host symbiosis (7); for example, the best competitors were not necessarily among the most effective strains (12). Secondly, competitiveness was also unrelated to the ability of a strain to survive in soil. Although strain RF6 was outstanding in this respect, other strains which apparently lacked surviving ability were equally competitive or effective (Table 8).

The investigation narrowed down the number of strains that could seriously be considered as candidates for inoculant production. A few additional field trials with strains RF4, RF6, and RF10 in soils containing established *Rhizobium* populations would have provided a more definite answer. Although strain RF10 has already proven itself in inoculants, inoculant manufacturers find its gummy nature undesirable (unpublished data). Strain RF6 is considered the most promising at this stage and will probably replace RF14 in inoculants in South Africa. It was also among the most effective in earlier studies, when 16 strains of *R. meliloti* were tested on each of 25 lines and cultivars of *M. truncatula* (13).

ACKNOWLEDGMENTS

We thank Martie Kriel, Susan Dutrieux, and Elsabè Marx for technical assistance.

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