

Nodulation Efficiency of Legume Inoculation as Determined by Intrinsic Antibiotic Resistance†

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Patterns of intrinsic resistance and susceptibility to different levels of antibiotics were determined for strains of both fast- and slow-growing rhizobia. These patterns were stable to plant passage when they were used to identify *Rhizobium* strains in nodule suspensions or nodule isolates. The method of identification by intrinsic resistance and susceptibility patterns was reliable for identifying strains in field nodules when strains were first isolated from the nodules to provide a standard inoculum size and then typed on antibiotic-containing media. Other patterns of resistance were encountered during identification of field isolates; these patterns may have resulted from acquired resistance to certain antibiotics or from mixed infections of the nodules. The occurrence of resistance patterns identical to those of inoculant strains among native strains was directly related to the size of the soil population. High strain recovery was associated directly with high rates of inoculation.

The identification of *Rhizobium* strains that occupy nodules is mandatory in studies designed to evaluate the competitiveness and the effects of specific inoculant strains on the growth of leguminous plants. Serological methods, such as agglutination, have been used most extensively in nodule typing. Problems arise when workers attempt to identify rhizobia from inocula in nodules of plants growing in soils containing native rhizobia of the same serogroup. Agglutination may underestimate the proportion of nodules formed by the introduced strains (19). Immunodiffusion has been used in some field studies and has been shown to be more accurate than agglutination for strain identification (9). The immunofluorescence microscopy method (3) and the enzyme-linked immunosorbent assay (1) are methods for specific strain identification in legume nodules. The major limitations of these serological methods are the time and resources involved in the production of specific antisera, the lack of stable antigenic properties, and the nonspecific or shared antigens that cross-react during analysis.

Phage typing is useful if the inoculant strains are susceptible to a particular bacteriophage. This method may be highly specific or nonspecific depending on the bacterium (18), and bacteriophage may be unavailable for a particular strain.

The use of genetic markers as a means of strain identification was first demonstrated under laboratory conditions with mutants that were resistant to high levels of antibiotics (11, 21, 24, 25, 27-30) and auxotrophic mutants (15, 24). Several workers have used antibiotic resistance successfully to identify rhizobia in inoculation studies conducted in the field (4, 12, 19, 20). However, other reports have indicated that resistance to some antibiotics may be associated with reduced symbiotic effectiveness (5, 16, 24, 25, 29), decreased infectiveness (24, 33), and decreased competitiveness (5).

The use of the intrinsic antibiotic resistance of *Rhizobium* strains as a method of identification has received attention recently. Marques Pinto et al. (22) differentiated *Rhizobium meliloti* strains in nodules from *Medicago sativa* L. grown under laboratory conditions by using levels of natural resistance to kanamycin and streptomycin. Diatloff (8) used antibiotic sensitivities as supplementary criteria to characterize *Lotononis* rhizobia introduced into the field. Josey et al. (17) screened several strains of *Rhizobium leguminosarum* and *Rhizobium phaseoli* against different concentrations of eight antibiotics and found unique patterns of intrinsic resistance for all but one strain. Reliable strain identifications with reference to stock cultures could be made on the basis of these patterns when the workers examined test cultures or nodule isolates from host plants inoculated and grown under laboratory conditions. The advantages of this method of identification included its reliability in that

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resistance to low levels of antibiotics appeared to be a stable property of the *Rhizobium* strains examined, as results were reproducible over extended periods of time. In addition, this method required no cellular alterations, which may occur through mutation, selection or mutagenesis and may interfere with strain performance.

The objectives of this study were (i) to screen selected *Rhizobium* inoculant strains and field isolates for intrinsic antibiotic resistance, (ii) to identify the *Rhizobium* strains in field-collected nodules by using intrinsic antibiotic resistance and susceptibility patterns, and (iii) to determine the effect of inoculation on nodulation by applied rhizobial strains, using intrinsic antibiotic resistance and susceptibility patterns.

MATERIALS AND METHODS

Organisms. The 12 *Rhizobium* strains used in this study and their sources are shown in Table 1. Native strains were also obtained from field study sites by growing host legumes in soil samples and isolating rhizobia from nodules on the plant roots. All strains were maintained on yeast extract mannitol (YEM) agar slants (10); 4-day-old cultures (fast growers) or 8-day-old cultures (slow growers) on slants were used in the tests.

Antibiotics. Kanamycin sulfate (KAN), streptomycin sulfate (STR), tetracycline hydrochloride (TET), penicillin G (PEN), and rifampin (RIF) were obtained from Sigma Chemical Co., St. Louis, Mo., and spectinomycin (SPC) was obtained from GIBCO Laboratories, Grand Island, N.Y. Stock antibiotic solutions were prepared in 100 ml of distilled water, membrane filter sterilized (pore size, 0.45 μm ; Millipore Corp., Bedford, Mass.), transferred aseptically to sterile bottles, and stored at 4°C. Fresh stock solutions were prepared before each test. Antibiotic-supplemented media were prepared by dispensing the appropriate volume of stock antibiotic solution (to yield the de-

sired antibiotic concentration) into sterile petri dishes, followed by the addition of 20 ml of autoclaved YEM agar (cooled to 45 to 50°C).

Screening procedure. A flame-sterilized inoculating needle (20-gauge nichrome wire) was used to transfer slant growth to test plates. The amount inoculated onto each plate was about 10^5 cells for all strains. The number of rhizobia transferred was estimated by using the inoculating needle to transfer rhizobia from 4-day-old cultures (fast growers) or 8-day-old cultures (slow growers) on YEM slants to tubes of sterile peptone water and plating samples of the tube contents. The test plates contained a range of concentrations of each antibiotic on individual YEM plates. Antibiotic plates were incubated at 24°C for 4 and 8 days for fast-growing and slow-growing rhizobia, respectively. The growth on the antibiotic plates was compared with that on YEM control plates and scored as positive or negative.

Nodulation tests with *R. leguminosarum*, *R. phaseoli*, cowpea *Rhizobium*, and peanut *Rhizobium* test strains were performed on *Lens esculenta* Moench, cv. Chilean, *Phaseolus vulgaris* L. cv. Commodore, *Vigna unguiculata* (L.) Walp. cv. Mississippi Silver, and *Arachis hypogaea* L. cv. Florunner, respectively. Seeds of each host legume were surface sterilized with 5% H_2O_2 for 10 min, followed by five rinses in sterile water. Two seeds were placed in each of 10 plastic growth pouches (31) which contained sterile nitrogen-free nutrient solution. The pouches were supported in record racks. The seeds were inoculated with slant suspensions of the appropriate *Rhizobium* cultures. The plants were grown in a growth chamber that was maintained at 28°C during a 16-h light period and at 19°C during an 8-h dark period. Light was supplied by fluorescent and incandescent lamps at an intensity of 250 $\mu\text{Einsteins m}^{-2} \text{s}^{-1}$. Nodules were collected from the plants after 50 days and surface sterilized by exposure to 0.1% HgCl_2 for 1 min, followed by six rinses in sterile water. The nodules were crushed aseptically in the wells of a multipoint inoculator assembly (17). The inoculator was used to transfer the bacteria directly from individual nodules to different

TABLE 1. Sources of *Rhizobium* strains

Strain	Source
<i>R. leguminosarum</i>	
P28	F. Davidson, Research Seeds, Inc., St. Joseph, Mo.
C53	J. C. Burton, Nitragin Co., Milwaukee, Wis.
771B	Isolated from Cahaba sandy loam in Monroe Co., Miss.
<i>R. phaseoli</i>	
B84	F. Davidson
931A	Isolated from Lexington silt loam in Copiah Co., Miss.
971A	Isolated from Cahaba sandy loam in Monroe Co., Miss.
Cowpea <i>Rhizobium</i>	
CB756	J. M. Vincent, University of Sydney, Sydney, Australia, via L. Materon
CV030	Isolated from nodules on 'Magnolia Blackeye' pea plants at the Plant Science Research Center, Mississippi State, Miss.
CV041	Isolated from nodules on 'Mississippi Silver' pea plants at the Plant Science Research Center, Mississippi State, Miss.
Peanut <i>Rhizobium</i>	
CA22	J. C. Burton
C811K	Isolated from Houston clay in Monroe Co., Miss.
C870B	Isolated from Cahaba sandy loam in Monroe Co., Miss.

locations on the antibiotic plates. The crushed nodules were also streaked onto YEM agar plates, from which single colony isolates were selected, cultured on YEM slants, and then tested on antibiotic plates.

Testing procedure. Nodules were collected from field inoculation experiments with beans, peanuts, and cowpeas. Inoculated seed samples were obtained at planting for determination of inoculation rate. Samples were serially 10-fold diluted in 0.1% peptone and spread onto duplicate YEM agar plates for counting. Plants were grown in four-row plots that were replicated six times; a randomized block experimental design was used. Plant samples were dug from the outer two rows of each plot during the flowering stage of each host legume. All nodules were removed from the roots, combined, and stored at -10°C . Nodules from 1979 and 1980 field experiments were stored for 10 and 2 months, respectively, before testing. A subsample of 20 nodules was obtained from each composite sample and surface sterilized as described above. Initially, nodules were crushed and directly inoculated with the multiple inoculator onto plates containing the antibiotic concentrations determined for the particular inoculant strain. However, rhizobia from many nodules tested in this manner failed to grow even on YEM agar lacking antibiotics, and contamination occurred frequently.

Alternatively, a greater number of nodules were processed, and selection was biased for larger nodules to assure that 20 isolates were obtained per plot. The nodules were crushed and streaked onto YEM agar plates. Single-colony isolates were then selected, transferred to YEM slants, and incubated. The nodule isolates were tested on antibiotic media by using a standard stab type of inoculating needle. A grid pattern beneath the plates was followed, and this pattern allowed accommodation of 20 nodule isolates on one plate. Each test included the corresponding parent strains as controls. Antibiotic resistance patterns for nodule isolates that were identical to those determined for a strain applied in an inoculant were scored as positive (i.e., nodule occupied by the inoculant strain).

RESULTS

Table 2 shows the maximum levels of antibiotic resistance for *Rhizobium* strains. The highest levels of resistance occurred with PEN for both fast- and slow-growing species. The slow-growing strains exhibited higher levels of resistance to TET and RIF than the fast-growing strains. For example, both cowpea *Rhizobium* and peanut *Rhizobium* were resistant to TET at levels up to 35 $\mu\text{g}/\text{ml}$. Resistance to the other antibiotics varied among strains within species.

The intrinsic antibiotic resistance markers selected for differentiation of inoculant strains in nodules of field-grown plants are shown in Table 3. For *R. phaseoli*, single resistance markers that were specific for each strain were used in addition to inhibition at $<25 \mu\text{g}/\text{ml}$ because soil *R. phaseoli* strains were resistant to 25 μg of STR per ml. We found antibiotic resistance patterns that were different from the patterns of the native strains. Double markers of SPC and

TET (both at a concentration of 30 $\mu\text{g}/\text{ml}$) were used to differentiate inoculant cowpea *Rhizobium* strains from the native strains. In addition, 40 μg of RIF per ml was included in the resistance pattern for identification of strain CB756. Combinations of resistance and susceptibility to RIF, SPC, and KAN were used to identify peanut *Rhizobium* strains.

Preliminary tests with pure cultures showed that these patterns were sufficient to allow reliable identification of the inoculant strains. Crude suspensions of nodule material or isolates from nodules from plants that were inoculated with specific, single strains and grown in the growth chamber exhibited the same patterns as the stock strains grown in pure culture on YEM agar. These findings indicated that the inoculant strains could be identified directly from nodules collected from plants, thus bypassing the step of isolation from the nodules. However, when this procedure was attempted with bush bean nodules from field plots by using the multiple inoculator, rhizobia grew from only about 10% of the nodules examined, and considerable contamination was encountered. The same results occurred with cowpea and peanut nodules. The nodules from the 1979 field studies had been stored for 10 months at -10°C before testing. Apparently, the nodules had deteriorated during storage, causing a decrease in the number of recoverable rhizobia. Plate counts on selected nodules showed that most of the 1979 bush bean nodules contained no more than 1.5×10^3 rhizobia and that most of the peanut nodules contained fewer than 100 rhizobia per nodule. The detrimental effect of frozen storage on the viability of rhizobia within nodules was observed for all of the inoculant strains and isolates obtained from the uninoculated plots.

The recovery rates for *R. phaseoli* inoculant strains from bush bean nodules in the 1979 field study showed a generally low trend (Table 4). The highest rates of recovery were exhibited by strain B84 and 931A, even though the numbers of rhizobia applied per seed at planting were similar for all strains. Strain 971A may have been adversely affected by soil conditions, or it may not have been able to compete with native *R. phaseoli* for nodule sites.

Bush bean plots were located at a different site in 1980. The soil contained a native population of about 30 *R. phaseoli* per g of soil (ineffective on *P. vulgaris* cv. Commodore beans). The mean recovery of inoculant strains (Table 4) was highest for strain 971A (88%) and lowest for strain B84 (74%). The recovery rates for all strains would be expected to be closer to 100% in the presence of the low soil population. However, 18% of the nodule isolates in strain B84 plots were resistant to 25 μg of STR per ml, 10%

TABLE 2. Maximum levels of antibiotic resistance for inoculant strains and native *Rhizobium* spp. isolated from 1979 field sites

Strain	Level of resistance ($\mu\text{g/ml}$)					
	STR	SPC	KAN	TET	RIF	PEN
<i>R. phaseoli</i>						
B84	10	1	8	<0.1	5	>35
931A	5	15	10	<0.1	5	>35
971A	5	5	1	0.2	10	35
Native	25	1	10	<0.1	5	>35
<i>R. leguminosarum</i>						
P28	15	5	25	<0.1	15	>35
C53	20	5	10	<0.1	5	>35
771B	8	5	5	<0.1	10	20
Native	8	5	1	<0.1	10	35
Cowpea <i>Rhizobium</i>						
CB756	1	10	3	32	40	>45
CV030	3	10	35	32	32	>45
CV040	3	10	35	30	32	>45
Native	1	1	35	20	25	>45
Peanut <i>Rhizobium</i>						
CA22	5	5	<1	35	40	40
C811K	3	15	35	35	10	40
C870B	3	18	15	35	40	>45
Native	5	10	35	35	25	>45

TABLE 3. Antibiotic markers used in identifying inoculant strains of *Rhizobium* from nodule isolates

Strain	Intrinsic properties	
	Resistance	Susceptibility
<i>R. phaseoli</i>		
B84	STR (10) ^a	STR (25)
931A	SPC (15)	STR (25)
971A	TET (0.2)	STR (25)
Native	STR (25)	SPC (15), TET (0.2)
Cowpea <i>Rhizobium</i>		
CB756	SPC (30), TET (30), RIF (40)	
CV030	SPC (30), TET (30)	
CV041	SPC (30), TET (30)	
Native		SPC (30), TET (30), RIF (40)
Peanut <i>Rhizobium</i>		
CA22	RIF (40)	KAN (35)
C811K	KAN (35), SPC (15)	
C870B	SPC (15), RIF (40)	KAN (35)
Native	KAN (35)	SPC (15), RIF (40)

^a The numbers in parentheses are concentrations (in micrograms per milliliter).

of the nodule isolates in strain 931A plots were resistant to both 15 μg of SPC per ml and 25 μg of STR per ml, and 7.5% of the strain 971A isolates were resistant to both 0.2 μg of TET per ml and 25 μg of STR per ml.

The recovery of inoculant strains of cowpea *Rhizobium* and peanut *Rhizobium* (Table 5) illustrates that there was relatively good agreement between inoculation rate and recovery. Strain CB756 was recovered less frequently than the other strains. Even though a lower inoculation rate may have contributed to lower recovery, strain CB756 (an isolate from Australia) may

also be a poor competitor in these soils or may not be compatible with the plant cultivar used. The recovery of peanut *Rhizobium* from nodules did not differ significantly ($\alpha = 0.05$) among strains. The similar inoculation rates apparently resulted in similar recovery rates for all strains.

Table 6 shows the occurrence of the selected resistance patterns in the native populations of rhizobia. We identified no native *R. phaseoli* that expressed resistance to both SPC and TET and susceptibility to STR. However, 9% of the isolates expressed resistance to both SPC and STR, and 1.6% expressed resistance to both

TABLE 4. Proportions of 'Commodore' bush bean nodules containing inoculant strains identified by intrinsic antibiotic resistance

Inoculant strain	Inoculation rate (log rhizobia/seed)		% Recovery of inoculant strain	
	1979	1980	1979 ^a	1980 ^b
B84	8.12	8.44	55.4	74.2
931A	7.60	8.65	65.0	82.7
971A	7.70	8.18	38.8	87.6
Uninoculated	— ^c	— ^d	0.0	0.0

^a The values for the following pairs of strains were significantly different according to Duncan's multiple range test (α [level of significance] = 0.05; $S_{\bar{x}}$ [standard error of the mean] for recovery, 7.45): B84 and 971A, B84 and uninoculated, 931A and 971A, 931A and uninoculated, 971A and uninoculated.

^b The values for the following pairs of strains were significantly different according to Duncan's multiple range test (α = 0.05; $S_{\bar{x}}$ for recovery, 5.34): B84 and 931A, B84 and 971A, B84 and uninoculated, 931A and uninoculated, 971A and uninoculated.

^c There were 200 naturally occurring *R. phaseoli* cells per g of soil.

^d There were 31 naturally occurring *R. phaseoli* cells per g of soil.

TABLE 5. Proportions of 'Mississippi Silver' cowpea and 'Florunner' peanut nodules containing inoculant strains identified by intrinsic antibiotic resistance

Nodules	Inoculant strain	Inoculation rate (log rhizobia/seed)	% Recovery of inoculant strain ^a
Cowpea	CB756	7.50	67.0
	CV030	7.94	83.3
	CV041	8.27	86.2
	Uninoculated	— ^b	3.3 ^c
Peanut	CA22	8.41	77.0
	C811K	8.14	77.9
	C870B	8.32	73.7
	Uninoculated	— ^d	12.5 ^c

^a In cowpea nodules the values for the following pairs of strains were significantly different according to Duncan's multiple range test (α = 0.05; $S_{\bar{x}}$ for recovery, 3.97): CB756 and CV030, CB756 and CV041, CB756 and uninoculated, CV030 and uninoculated, CV041 and uninoculated. In peanut nodules the values for the following pairs of strains were significantly different (α = 0.05; $S_{\bar{x}}$ for recovery, 4.17): CA22 and uninoculated, C811K and uninoculated, C870B and uninoculated.

^b There were 3,480 naturally occurring cowpea *Rhizobium* cells per g of soil.

^c Percentage of soil rhizobia with antibiotic resistance patterns similar to those of any of the inoculant strains.

^d There were 1,610 naturally occurring peanut *Rhizobium* cells per g of soil.

TET and STR. A high proportion of the native peanut *Rhizobium* strains possessed the same resistance patterns as the inoculant strains. This may have been due to a highly competitive native *Rhizobium* population, which allowed more chance of similar patterns occurring in the native strains. As a rule, the higher proportions of multiple resistance patterns observed were combinations of antibiotics grouped in the aminoglycoside class. This occurrence has also been observed in *Rhizobium trifolii* isolates (13).

DISCUSSION

The antibiotic resistance patterns which we observed agree somewhat with other reports in the literature. Schwinghamer (29) examined parent strains of four fast-growing species and found that all were most resistant to PEN and most sensitive to TET. Hagedorn (13) reported a

similar pattern for *R. trifolii*. Pattison and Skinner (26) found that four fast-growing species possessed greater sensitivity to TET than slow-growing strains of *Rhizobium japonicum* and cowpea *Rhizobium*. Both slow growers and fast growers were most tolerant to PEN. Davis (7) reported that *R. phaseoli*, *R. leguminosarum*, and cowpea *Rhizobium* were sensitive to 30 μ g of TET per ml. Pankhurst (25) showed that slow-growing strains of *Lotus* rhizobia possessed higher levels of resistance to TET, RIF, and KAN than fast-growing strains. Working with three wild-type *R. japonicum* strains, Pugashetti and Wagner (27) found a high degree of sensitivity to KAN, STR, and TET and tolerance to 100 to 200 μ g of RIF per ml. Wide variability of resistance to STR in peanut *Rhizobium* has been reported (20). Cole and Elkan (6) reported that the level of resistance to 50 μ g of TET per ml

TABLE 6. Proportions of nodule isolates from uninoculated treatments resistant to the same antibiotic markers as inoculant strains (1979 field studies)

Field expt	Antibiotic marker(s)	Resistant nodule isolates (%)
Bush bean	SPC (15 µg/ml)	0.0
	TET (0.2 µg/ml)	0.0
Cowpea	SPC (30 µg/ml) + TET (30 µg/ml)	2.5
	SPC (30 µg/ml) + TET (30 µg/ml) + RIF (40 µg/ml)	0.8
Peanut	RIF (40 µg/ml)	3.3
	KAN (35 µg/ml) + SPC (15 µg/ml)	7.5
	SPC (15 µg/ml) + RIF (40 µg/ml)	1.6

among 24 strains of *R. japonicum* was quite uniform, whereas the ranges of resistance to PEN and STR were much broader. In this study we observed varying levels of resistance to STR, SPC, and KAN among strains of all species. Our results support the suggestion of Hagedorn (13) that generalizations regarding the antibiotic resistance patterns of different strains or types of *Rhizobium* are often invalid.

Inoculant size was critical in obtaining consistent growth of rhizobia from nodules on control and antibiotic-supplemented media. Josey et al. (17) showed that a sufficiently large inoculant size was required for high reproducibility and to prevent loss of viability or spontaneous mutation to antibiotic resistance. Low numbers of rhizobia detected in bean and peanut nodule suspensions were insufficient for direct transfer to plates. Single-colony isolations made from nodules were cultured so that adequate numbers (about 10^5 rhizobia) could be transferred with an inoculating needle and tested on antibiotic plates. In preliminary experiments, antibiotic typing of rhizobia could be accomplished by direct inoculation of fresh nodule suspensions. However, the rhizobial contents of these suspensions varied from 5.0×10^2 to 7.5×10^7 cells per nodule, resulting in the testing of variable numbers of rhizobia. Therefore, isolation of rhizobia from nodules was critical in obtaining a standard inoculum size for antibiotic testing and should be considered regardless of the condition of the nodule samples at the time of analysis.

Nodule occupancy by inoculant strains of rhizobia could be monitored by using patterns of intrinsic resistance and susceptibility to antibiotics. However, the single resistance markers used to identify inoculant strains of *R. phaseoli* could not be detected in a large proportion of the field-collected bean nodules in 1979 (Table 4). The conditions encountered during the 1979 study included excessive soil moisture, root disease, and possible herbicide carry-over. These adverse factors may have contributed to low nodule occupancy or alterations in the antibiotic resistance patterns of the inoculant strains

or both. In 1980, cross-resistance patterns to an antibiotic marker and to 25 µg of STR per ml were detected occasionally. This may indicate that a soil population of *R. phaseoli* possessing cross-resistant patterns escaped detection in preliminary isolation tests and became stimulated in the presence of bean plants to form nodules. However, the isolates from the few nodules obtained from uninoculated plots were resistant only to 25 µg of STR per ml and not to SPC or TET. Stimulated soil rhizobia may have acquired the resistance markers from the inoculant strains through transfer of plasmids bearing the resistance factors (2, 6, 14). Another possibility is that cells of strains 931A and 971A may have acquired spontaneous resistance to STR (14) or may have already possessed resistance and expressed it in the soil environment.

The intrinsic levels of resistance to two or more antibiotics were sufficient as markers to distinguish inoculant strains of cowpea *Rhizobium* and peanut *Rhizobium* from indigenous strains of *Rhizobium*. The use of multiple resistance markers aided in the identification of inoculant strains since some indigenous rhizobia from uninoculated plots possessed single intrinsic resistance markers identical to those of inoculant strains. Spontaneous mutants of *Rhizobium* selected for multiple resistance to high levels of antibiotics have been used in other studies for accurate identification of inoculant strains in nodules of field-grown legumes (12, 19). The intrinsic multiple resistance markers used in this study avoided the possibilities of reduced competitiveness and symbiotic effectiveness of inoculant strains that may be associated with mutant strains resistant to high levels of antibiotics (5, 24, 29). Inoculant strains were identified reliably by intrinsic antibiotic resistance or susceptibility or both and simultaneously assessed for symbiotic performance under field conditions without the possible complicating effects of genetic alterations associated with spontaneous mutation or mutagenesis.

High numbers of inoculant rhizobia applied to seeds at planting time resulted in high nodule

occupancy by the inoculant strains, as determined by intrinsic antibiotic resistance. Inoculant strains were apparently able to compete effectively with indigenous rhizobia for nodule sites. This was most pronounced with cowpea *Rhizobium* and peanut *Rhizobium*, which occupied the majority of the nodules despite the presence of $>10^3$ indigenous rhizobia per g of soil (Table 5). These results are in agreement with those of Weaver and Frederick (32) in that high inoculation rates led to high rates of recovery of inoculant strains in fields containing indigenous rhizobia.

Our results indicate that the use of intrinsic antibiotic resistance is adaptable to field studies of inoculation efficiency. Specific intrinsic antibiotic resistance patterns can be determined for inoculant strains of *Rhizobium* to differentiate them from strains residing in the soil. Also, the proportion of inoculant strains recovered from nodules of field-grown plants appears to be associated directly with the inoculation rate.

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MEMORANDUM

Date 2 Sep 88

TO: PPC/CDIE/DI, Tina Wilson/Acquisitions - 209 SA-18
FROM: S&T/AGR/RNR, Lloyd R. Frederick
SUBJECT: Reprints of Articles with Partial Support of A.I.D.
Contracts/Grants

Attached is one copy of the following article with the supporting contract/grant number:

Kremer, R.J. and H.L. Peterson, 1982.
Modulation Efficiency of Legume inoculation as
determined by intrinsic antibiotic resistance.
Appl. and Environ. Microbiol. 1982: 636-642

Contract/Grant Number: ^{DASA} AG/TAB 610-9-76
Project: 931-0610

Attachments: a/s

cc: T.Gill
D. Bathrick

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