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Co-selection of compatible rhizobia and vesicular-arbuscular mycorrhizal fungi for cowpea in sterilized and non-sterilized soils

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Summary. We selected two isolates of *Rhizobium* for cowpea (*Vigna unguiculata*) with sterilized soil tests and two different isolates by non-sterilized soil testing. The four rhizobia were then paired individually with either *Glomus pallidum*, *Glomus aggregatum*, or *Sclerocystis microcarpa* in separate, sterilized, or non-sterilized soil experiments. The purpose of the experiments was to determine the effect of soil sterilization on the selection of effective cowpea rhizobia, and to see whether these rhizobia differed in their effects on cowpea growth when paired with various vesicular-arbuscular mycorrhizal (VAM) fungi. Our experiments showed that the rhizobia selected in sterilized soil tests produced few growth responses in the cowpea compared to the other introduced rhizobia, irrespective of pairing with VAM fungi in sterilized or non-sterilized soil. In contrast, the two rhizobia initially selected by non-sterilized soil testing significantly improved cowpea growth in non-sterilized soil, especially when paired with *G. pallidum*. Our results suggest that it is important to select for effective rhizobia in non-sterilized soil, and that pairing these rhizobia with specific, co-selected VAM fungi can significantly improve the legume growth response.

Key words: Co-selection – Soil sterilization – Effective cowpea rhizobia – Vesicular arbuscular mycorrhizae – *Glomus pallidum* – *Glomus aggregatum* – *Sclerocystis microcarpa* – *Vigna unguiculata*

Legumes provide an important source of protein for people and livestock throughout the world. Research has demonstrated the importance of *Rhizobium* sp. and VAM fungi on the growth and yield of many legumes (Barea and Azcon-Aguilar 1983; Alexander 1984). This knowledge has had a significant impact on legume production, especially in countries where agricultural productivity is limited due to a low soil P availability (Freire 1984). P deficiency is an important limiting factor in N_2 fixation and legume production (Freire 1984; Jakobsen 1985). One of the important benefits of VAM fungi, through their synergistic effect with *Rhizobium* sp., is that they enhance the ability of the legume host to obtain P from the soil (Yost and Fox 1979; Barea and Azcon-Aguilar 1983).

Increases in legume grain production have been brought about largely by the development of high-yielding varieties and the selection of effective strains of *Rhizobium*. In many tropical countries, the indigenous rhizobia are of the promiscuous, ineffective type, which may be more aggressive in forming nodules than introduced strains. Each soil type, host variety, and climatic condition is unique for any given country, which makes the successful introduction of selected, non-native rhizobia very difficult (Halliday 1984; Williams 1984). Even with effective strains, it is not possible to predict whether they will survive, how they will function under field conditions, and how well they compete with other rhizobia for nodule occupancy (Lowendorf 1980; Alexander 1984).

As with rhizobia, criteria have been developed for the selection of effective VAM fungi for agriculture (Abbott and Robson 1982). Many of the considerations applied to *Rhizobium* sp. selection are also applicable to VAM fungi, especially their ability to compete with the indigenous soil microflora. Proper selection of effective VAM fungi for legumes is important because the tripartite association between the legume, *Rhizobium*, and VAM fungus results in a plant with a well balanced nutrient content (Barea and Azcon-Aguilar 1983). Not only can nodulated legumes benefit from the P uptake ability of VAM fungi, but some legumes do not respond to *Rhizobium* inoculation unless they are also inoculated with a mycorrhizal fungus (Azcon-Aguilar et al. 1979). Nodulated and mycorrhizal legumes generally show greater N_2 fixation, nodule biomass, plant biomass, N and P contents, and

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yield than plants colonized by the microorganisms individually (Barea and Azcon-Aguilar 1983).

Variation in the legume growth response and microbial function can be obtained by altering any biological component of the tripartite association. In soybeans (*Glycine max*), by changing the *Bradyrhizobium* isolate, mycorrhizal plants are altered in dry weight, acetylene reduction capacity, and percentage mycorrhizal infection (Pacovsky et al. 1986). Carling and Brown (1980) found that different VAM fungal species, or even different isolates of the same species, cause changes in the soybean plant yield. In cowpeas (*Vigna unguiculata*), comparisons between different plant varieties and VAM fungi have indicated that there are host variety × VAM fungus interactions (Islam and Ayanaba 1981; Ollivier et al. 1983; Miller et al. 1986). Thus, all components of the tripartite system must be considered when selecting microbial inoculants for any legume. A further complication is that dually inoculated legumes may respond differently depending on whether or not the soil was sterilized (Yost and Fox 1979; Islam and Ayanaba 1981; Habte and Aziz 1985).

Research is being conducted in Jamaica to co-select isolates of *Rhizobium* and VAM fungi for field inoculation onto cowpeas. More than 30 strains of *Rhizobium*, most of them native to Jamaica, have been characterized for their intrinsic antibiotic resistance and effectiveness on cowpeas (Ahmad et al. 1984; McLaughlin and Ahmad 1984). In addition, three VAM fungi, two *Glomus* spp., and a *Sclerocystis* sp. have been isolated from Jamaican soils and tested for their effects on the growth of cowpea. Out of 11 effective strains of *Rhizobium*, four of the most effective were selected, two each from sterilized and non-sterilized soil tests (unpublished data 1990). The purpose of the research reported here was to pair the four rhizobia, individually, with each of three VAM fungi in an effort to obtain the most effective co-selected combinations. The microbial pairings were tested in both sterilized and non-sterilized soils.

Materials and methods

The separate sterilized (autoclaved) and non-sterilized soil experiments were conducted in Jamaica. The soil for both experiments was collected on campus at the University of the West Indies, Kingston, Jamaica, from the Caribbean Agricultural Research and Development Institute's experimental farm. The black loam soil contained 2 μg g^{-1} NO_3-N, 28 μg g^{-1} P and had a pH of 6.3. For each treatment replicate, plastic bags, 25×13×10 cm, were filled with air-dried soil.

Rhizobium strains JRC14, JRC19, and JRC29 were isolated from Jamaican soils and TAL169 was obtained from NifTAL, Hawaii, USA. TAL169 and JRC19 were the most effective strains selected from sterilized soil tests while JRC14 and JRC29 were selected from non-sterilized soil testing. The three VAM fungi, *G. pallidum*, *G. aggregatum*, and *S. microcarpa*, were isolated from Jamaican soils and increased in potted plant culture with corn (*Zea mays*) in a sterilized sandy soil. The undiluted mycorrhiza inoculum produced 59.5, 54.5, and 58.3% root colonization in cowpeas from the *G. pallidum*, *G. aggregatum*, and *S. microcarpa* culture material, respectively, in a 3-week inoculum standardization test. The mycorrhizal fungus inocula consisted of spores, hyphae, root fragments, and soil from the corn pot cultures. Twenty-five grams of each of the VAM fungal inocula, containing approximately 1000 spores, were placed 2–3 cm below the seed in each VAM fungus treatment. Each bag of soil was then seeded with two surface-sterilized cowpea seeds, cv. Laura B, which were later thinned to one plant per bag. The rhizobia were grown in yeast-mannitol broth and 1.0 ml, containing 1×10^8 cells, of the appropriate inoculum was applied over each seed and covered with soil. The plants were grown for 59 days in a screenhouse with average day and night temperatures of 29 °C and 22 °C, respectively. Water was applied as needed and alternated with a dilute nutrient solution. Care was taken to prevent cross-contamination of the microorganisms.

The non-sterilized soil study consisted of a randomized factorial design of five rhizobia (JRC14, JRC19, JRC29, TAL169, and the indigenous strain) and four VAM fungi (*G. pallidum*, *G. aggregatum*, *S.* microcarpa, and the native VAM fungi). There were five replicates per treatment. The sterilized (autoclaved) soil experiment was conducted similarly, except the indigenous rhizobia and native VAM fungi were killed by autoclaving and these treatments were not inoculated. There were four replicates per treatment in the sterilized soil study.

At harvest, the shoot was severed at the soil surface, oven-dried (70 °C), and weighed. The shoot material was ground and a subsample was digested (Thomas et al. 1967) and analyzed for P (Murphy and Riley 1962) and N (Nessler test, Vanselow 1940). Nodules were removed from the roots, counted, then air-dried and weighed.

Ten nodules from each treatment were tested to confirm colonization by the introduced rhizobia, using antibiotic resistance profiles (McLaughlin and Ahmad 1984) or a fluorescent antibody assay. The roots were washed, cut into segments of 2–3 cm, and subsampled for VAM fungal colonization. The percentage mycorrhizal fungus colonization of the roots was determined using a line-intercept method (Marsh 1971) after clearing and staining to reveal the VAM fungi (Phillips and Hayman 1970). The data were analyzed by one-way analysis of variance. Significant differences between treatment means was determined by a *t*-test.

Results

Sterilized soil study

Antibiotic and fluorescent antibody assays of the nodules showed that those sampled were colonized by only the introduced rhizobia. No cross-contamination was detected and no nodulation occurred in the non-inoculated controls.

JRC14 and JRC19 produced significantly greater numbers and weights of nodules than the other rhizobia (Table 1). In plants without the rhizobial inoculant, the percentage mycorhizal colonization, shoot dry weight, and shoot N and P content were significantly ($P<0.01$) reduced compared to the *Rhizobium*-inoculated treatments. Although mycorrhizal development was significantly greater in the JRC29 versus TAL169 treatments,

Table 1. Effects of different isolates of *Rhizobium* on cowpea growth, nodulation, and mycorrhizal development in a sterilized soil

Rhizobium	Nodules (no.)	Nodule weight (mg)	Shoot weight (g)	VAM (%)	Shoot N (mg g^{-1})	Shoot P (mg g^{-1})
None	0	0	2.80b	63.9c	16.4b	1.21b
JRC14	87.6a	218.0a	4.65a	70.7ab	23.9a	2.14a
JRC19	89.1a	221.1a	4.90a	70.9ab	23.5a	2.14a
JRC29	73.9b	201.4b	4.74a	72.4a	23.3a	2.12a
TAL169	77.3b	196.8b	4.59a	69.3b	23.2a	2.09a

Numbers within columns are means of 16 observations. Values within columns not sharing the same letter are significantly different ($P\leq0.05$) based on a one-way analysis of variance and *t*-test. VAM, vesicular-arbuscular mycorrhizae

Table 2. Effects of different species of vesicular-arbuscular mycorrhizal fungi on cowpea growth, nodulation, and mycorrhizal development in a sterilized soil

Fungus	Nodules (no.)	Nodule weight (mg)	Shoot weight (g)	VAM (%)	Shoot N (mg g^{-1})	Shoot P (mg g^{-1})
None	73.1b	179.3b	3.36b	0	20.8a	1.45b
G. pallidum	87.5a	227.5a	4.73a	69.3a	23.4a	2.11a
G. aggregatum	77.9b	212.9a	4.54a	68.6a	21.6a	2.07a
S. microcarpa	90.3a	219.3a	4.71a	70.2a	22.3a	2.14a

Means of 20 observations. *G.*, *Glomus; S.*, *Sclerocystis*. For other explanations, see footnotes to Table 1

there were no differences between *Rhizobium* strains in shoot dry weight and N and P content.

No VAM fungus colonization was detected in the uninoculated controls in the sterilized soil (Table 2). The number of nodules was significantly ($P<0.001$) increased by inoculation with *S. microcarpa* and *G. pallidum* but not by *G. aggregatum*. Nodule dry weight, shoot dry weight, percentage VAM fungus colonization, and shoot P content were all significantly ($P<0.001$) increased with the VAM fungus inoculations. Shoot N was not affected.

Poor plant growth and a low nutrient content were observed in cowpeas without *Rhizobium* or VAM fungi (Table 3). JRC 14 and JRC19 increased nodule numbers more than JRC29 and TAL169 only when mycorrhizal fungi were absent, or in the presence of *G. aggregatum*, which was the least effective VAM fungus. Nodule dry weight differences occurred only in the absence of mycorrhizal fungi. The *Rhizobium* isolate had a significant effect on mycorrhiza (% VAM) development only in the presence of *G. palladium*.

VAM fungus – *Rhizobium* interactions significantly affected the cowpea shoot dry weight (Table 3). The optimum shoot weight was obtained with *G. pallidum* and JRC19 and JRC29. *G. aggregatum* increased the shoot weight when co-inoculated with JRC14 and JRC19. No shoot weight differences occurred between the four rhizobia in the presence of *S. microcarpa*.

The shoot N content was not significantly ($P>0.05$) affected by VAM fungus – *Rhizobium* interactions. The shoot P content was significantly improved in the VAM fungus + *Rhizobium* treatments compared with the single inoculation treatments (Table 3).

Non-sterilized soil study

Antibiotic resistance and fluorescent antibody assays confirmed the presence of the introduced rhizobia in each appropriate treatment. Although no cross-contamination with the introduced rhizobia was seen, we did not measure nodule occupancy by the indigenous rhizobia because no antibiotic resistance profiles had been developed for them.

Inoculation with the introduced strains of *Rhizobium* significantly increased the shoot dry weight, percentage VAM fungus colonization, and shoot N content compared with the indigenous strains(s) (Table 4). JRC14 and JRC29 significantly ($P<0.01$) improved nodule dry weight, shoot dry weight, percent VAM fungus colonization, and shoot P content over that of JRC19 and TAL169.

Table 3. Interactions among three vesicular-arbuscular mycorrhizal fungi and four isolates of *Rhizobium* in effects on cowpea growth, nodulation, and mycorrhizal development in a sterilized soil

Fungus	Rhizobium	Nodules (no.)	Nodule weight (mg)	Shoot weight (g)	VAM (%)	Shoot N (mg g^{-1})	Shoot P (mg g^{-1})
None	None	0	0	1.90c	0	14.3b	0.95c
	JRC14	84.3a	195.7a	3.42b	0	22.9a	1.65ab
	JRC19	78.0a	209.6a	3.90a	0	22.2a	1.70a
	JRC29	61.5b	167.1b	3.78a	0	22.2a	1.45b
	TAL169	68.8b	144.8c	3.81a	0	22.6a	1.50ab
G. pallidum	None	0	0	3.11c	64.6e	16.0b	1.35c
	JRC14	90.5a	230.8a	4.94b	69.5c	25.7a	2.20ab
	JRC19	95.3a	231.5a	5.50a	72.1b	25.9a	2.38ab
	JRC29	81.7a	229.0a	5.54a	74.6a	25.5a	2.60a
	TAL169	81.0a	218.9a	4.79b	67.0d	24.3a	2.15b
G. aggregatum	None	0	0	3.02c	63.3b	17.4b	1.20b
	JRC14	83.0a	217.3a	5.06a	70.7a	24.4a	2.30a
	JRC19	90.0a	219.2a	5.07a	69.4a	22.6a	2.20a
	JRC29	66.0b	199.0a	4.74b	70.5a	21.3a	2.30a
	TAL169	72.5b	216.0a	4.81b	69.0a	22.5a	2.33a
S. microcarpa	None	0	0	3.16b	63.7b	18.0b	1.33b
	JRC14	92.8a	228.2a	5.19a	71.7a	22.7a	2.43a
	JRC19	93.3a	224.2a	5.12a	71.2a	23.3a	2.30a
	JRC29	88.5a	217.3a	5.11a	72.7a	24.6a	2.25a
	TAL169	86.8a	207.4a	4.96a	71.8a	23.2a	2.40a

Means of four observations. Statistical comparisons were only made between rhizobia within each VAM fungus. For other explanations, see footnotes to Table 1

Table 4. Effects of different isolates of *Rhizobium* on cowpea growth, nodulation, and mycorrhizal development in a non-sterilized soil

Rhizobium	Nodules (no.)	Nodule weight (mg)	Shoot weight (g)	VAM (%)	Shoot N (mg g^{-1})	Shoot P (mg g^{-1})
Indigenous	101.0a	230.8ab	4.65c	56.8c	20.9b	1.61b
JRC14	106.4a	248.0a	5.49a	70.5a	23.9a	2.09a
JRC19	103.6a	226.0b	4.95b	62.8b	22.8a	1.79b
JRC29	112.2a	246.6a	5.45a	69.5a	23.5a	2.08a
TAL169	105.5a	220.9b	4.91b	61.7b	22.9a	1.78b

Means of 20 observations. For other explanations, see footnotes to Table 1

Table 5. Effects of different species of vesicular-arbuscular mycorrhizal fungi on cowpea growth, nodulation, and mycorrhizal development in a non-sterilized soil

Fungus	Nodules (no.)	Nodule weight (mg)	Shoot weight (g)	VAM (%)	Shoot N (mg g^{-1})	Shoot P (mg g^{-1})
Indigenous	101.4a	225.2b	4.71b	56.3b	21.8a	1.50c
G. pallidum	109.3a	248.3a	5.25a	67.8a	23.4a	2.05a
G. aggregatum	105.2a	230.8b	5.20a	66.8a	23.2a	1.88b
S. microcarpa	107.0a	235.7ab	5.20a	66.0a	22.7a	2.04a

Means of 25 observations. For other explanations, see footnotes to Table 1

The shoot dry weight, percent VAM fungus colonization, and shoot P content was significantly greater for the introduced than indigenous VAM fungi (Table 5). Nodule number and shoot N content were not affected by the introduced VAM fungi. The largest shoot P content was obtained by the *G. pallidum* and *S. microcarpa* treatments.

The interaction of JRC14 and JRC29 with the three introduced VAM fungi resulted in significantly ($P<0.05$) higher shoot dry weight and percent VAM fungus root colonization compared with the other rhizobia paired with the same VAM fungi (Table 6). JRC14 and JRC29 significantly improved shoot P when paired with *S. microcarpa* and nodule dry weight and shoot P when paired with *G. pallidum*.

Discussion

In the initial selection of rhizobia in sterilized soil (unpublished data 1990), JRC19 and TAL169 provided the greatest shoot dry weight, while JRC14 ranked 6th and JRC29 10th. In the non-sterilized soil selection process, JRC14, JRC29, and TAL169 gave the largest shoot dry weight and JRC19 ranked 5th. Even though TAL169 and JRC19 performed well in the initial selection process, they did not perform as well in the non-sterile soil study when paired with introduced VAM fungi. In contrast, JRC14 and JRC29, which were selected initially in non-sterilized soil, functioned optimally in the non-sterilized soil when paired with the most effective VAM fungi (*G. pallidum* and *S. microcarpa*).

Since field soils are not sterile, it has been suggested that isolates of *Rhizobium* (Boonkerd and Weaver 1982; Halliday 1984) and VAM fungi (Abbott and Robson 1982; Barea and Azcon-Aguilar 1983; Habte and Aziz 1985) be selected under non-sterilized soil conditions. Sterilization of soil alters its physical, chemical, and biological properties. Obviously, some strians of *Rhizobium* are better adapted to this changed environment than oth-

Table 6. Interactions among three vesicular-arbuscular mycorrhizal fungi, four rhizobia, and indigenous microflora on cowpea growth, nodulation, and mycorrhizal development in a non-sterilized soil

Fungus	Rhizobium	Nodules (no.)	Nodule weight (mg)	Shoot weight (g)	VAM (%)	Shoot N (mg g^{-1})	Shoot P (mg g^{-1})
Indigenous	Indigenous	99.0a	221.7a	4.27b	51.9b	19.4a	1.28b
	JRC14	102.2a	228.3a	4.81a	59.0a	23.9a	1.52a
	JRC19	101.4a	225.4a	4.75a	56.7a	22.2a	1.58a
	JRC29	102.4a	224.8a	5.04a	58.2a	20.9a	1.60a
	TAL169	102.0a	225.6a	4.68a	55.7ab	22.6a	1.54a
G. pallidum	Indigenous	105.0a	226.9b	4.73b	57.9d	22.1bc	1.74b
	JRC14	112.4a	269.4a	5.88a	76.4a	25.1ab	2.28a
	JRC19	103.2a	223.6b	5.04b	67.2c	22.5abc	1.88b
	JRC29	118.4a	291.0a	5.67a	75.1a	25.5a	2.42a
	TAL169	107.6a	226.1b	4.90b	62.6b	21.8c	1.92b
G. aggregatum	Indigenous	97.0a	222.9a	4.82b	58.1c	21.5a	1.58b
	JRC14	105.8a	251.5a	5.65a	73.8a	22.9a	2.08a
	JRC19	105.2a	226.4a	4.96b	63.8b	24.3a	1.84ab
	JRC29	113.2a	240.7a	5.57a	73.5a	24.2a	2.04a
	TAL169	104.6a	207.7a	5.00b	65.0b	23.1a	1.86a
S. microcarpa	Indigenous	103.0a	251.8a	4.77b	59.3c	20.5a	1.84b
	JRC14	105.2a	242.6a	5.63a	72.8a	23.5a	2.48a
	JRC19	104.4a	228.4a	5.04b	63.5b	22.1a	1.86b
	JRC29	114.8a	229.8a	5.52a	71.1a	23.6a	2.24a
	TAL169	107.8a	223.3a	5.04b	63.5b	23.9a	1.78b

Means of five observations. Statistical comparisons were only made between rhizobia within each VAM fungus. For other explanations, see footnotes to Table 1

ers. This artificial system, however, is not where the desired rhizobia are ultimately placed. Once in the field, the introduced rhizobia must interact with the soil biota, including VAM fungi. VAM fungi significantly improve the effectiveness of rhizobia in legumes (Carling and Brown 1980), but this effect is not always related to an increased P uptake (Ames and Bethlenfalvay 1987). The significance of our research lies not only in the mechanism of *Rhizobium* strain selection, but in the finding that the effectiveness of an individual *Rhizobium* isolate is strongly influenced by the species of VAM fungi with which it interacts. Thus, to optimize legume yields, especially in low-fertility soils, a process of co-selection for effective and compatible rhizobia and VAM fungi is recommended.

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