Rhizobium - Host Specificities in *Phaseolus coccineus* L. and *Phaseolus vulgaris* L.[1]

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ABSTRACT

The introduction of desirable agronomic characteristics into *Phaseolus vulgaris L. is* being attempted by interspecific hybridization with *P. coccineus,* but the effects on root nodulation are unknown. Accordingly, cross-inoculation relationships between *P. vulgaris* `Red Kidney' and *P. coccineus* 'Scarlet Runner' were tested in the greenhouse with a group of 16 Rhizobium strains effective on Red Kidney and a group of 12 strains effective on Scarlet Runner. Both groups had diverse origins, including commercial sources and isolations from bean fields and other sites in California.

Only six strains, all isolates from Scarlet Runner, were effective on both hosts. Fifty percent of Scarlet Runner isolates were as effective on Red Kidney as the more effective of Red Kidney's homologous strains. Thirty percent of the California isolates tested on Red Kidney were more effective than two commercial inocula.

Strain rejection in Red Kidney was expressed in 75 % of all cases as failure to form nodules. In contrast, strain rejection by Scarlet Runner was expressed in 64 to 68 % of all cases as ineffective nodulation (nodule malfunction). If Scarlet Runner is representative of *P. coccineus* in this respect, interspecific hybridization is likely to increase rather than reduce the tendency of *P. vulgaris* to nodulate with ineffective rhizobia.

Additional index words: Rhizobium phaseoli, Phaseolus vulgaris, Phaseolus coccineus, Cross-inoculation, Nodulation effectiveness, Isolation of soil rhizobia.

MODERN cultivars of *Phaseolus coccineus* L. and *P. vulgaris* have evolved from related wild forms of the genus *Phaseolus* (6, 7, 10, 11, 17, 18). Cultivars of *P. vulgaris* exhibit a greater diversity of habit and other characteristics than do those of *P. coccineus* (6, 18), but the latter are thought to show greater vegetative vigor, seed size, disease resistance, and adaptation to cooler climates. These have been desirable characteristics for breeding programs aimed at improving *P. vulgaris* cultivars (13, 16). Graham and Halliday (8) have suggested interspecific hybridization as a means of increasing host nodulation specificity in *P. vulgaris* and improving symbiotic performance under field conditions. *Phaseolus vulgaris* has often been noted for its promiscuity (1, 8, 9, 20, 21), and for the detrimental effects of ineffective nodulation (1, 2, 12, 14, 15). *Phaseolus coccineus* may be resistant to nodulation by strains of *Rhizobium phaseoli* effective on *P.* vulgaris, but current information is limited (1, 8, 21).

Table 1. California soils sampled for rhizobial isolations.

Soil #	Location and use	Strains isolated: Red Kidney	Scarlet Runner
1	Davis, bean field	†	C1a,b
2	Davis, bean field	V2	C2a,b,c
3	Davis, university farm	V3a,b,c	C3a,b,c
4	Davis, university farm	V4a,b,c,d,e,f	†
5	Winters, bean field	V5a,b	C5a,b,c
6	Woodland, alfalfa field	†	C4a,b
7	Cache Creek river wash	†	C7a,b
8	Lodi, bean field	V8a,b	C8a,b,c
9	Stockton, bean field	no nodules	‡
10	Cal Poly campus	no nodules	C10
11	Shafter, cotton field	V11	C11a,b
12	Shafter, alfalfa field	‡	C12
13	Clovis, vineyard	no nodules	C13
14	Clovis, peach orchard	V14	no nodules
15	Chino, fallow field	‡	C15a,b
16	Gorman, high desert	V16a,b	‡
17	Davis, tomato field	no nodules	no nodules
18	Livingston, peach orchard	no nodules	no nodules

† Host variety not evaluated.
‡ No viable strains retained.

In view of these breeding interests and concern with improving N fixation in *P. vulgaris*, a study of nodulation characteristics in *P. coccineus* seems appropriate. This paper presents data on rhizobial nodulation specificities for one cultivar of each species: *P. vulgaris* `Red Kidney' and *P. coccineus* `Scarlet Runner'. Nodulation characteristics and overall effectiveness of each host were tested with commercial bean rhizobia and native strains from 16 California fields.

MATERIALS AND METHODS

Effectiveness of selected rhizobial strains was determined on ***P. coccineus*** `Scarlet Runner' and *P. vulgaris* `Red Kidney' under greenhouse conditions. Three trials were conducted in the spring and early summer of 1978 and 1979. Scarlet Runner seeds were obtained commercially from Northrup King Co. for the host isolation trial and screening Trial 1, and from Lagomarsino Seed Co. for screening Trial 2. Red Kidney seed was supplied by C. L. Tucker, Dep. of Agronomy and Range Science, Univ. of California, Davis.

Eighteen California soils were sampled (Table 1). Two pots were filled with 2 kg of each soil, air-dry and unamended. Pots were maintained at approximate field capacity by watering to weight with distilled water. Two surface-sterilized seeds of each cultivar were planted in a pot. After emergence, seedlings were thinned to one plant per pot. Plants were harvested 30 days after emergence, and crushed nodule isolates were obtained from the

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Table 2. Established *Rhizobium* strains used in cross-inoculation trials.

Strain #	Strain	Source
19	CIAT 73	*R. phaseoli* for *P. vulgaris* from
20	CIAT 135	Centro Internacional de Agricultura
21	CIAT 404	Tropical (CIAT) Cali, Colombia
22	CIAT 160	
23	CIAT 247	
24	NA 575	
25	127 K17	Commercial *P* vulgaris strains from
26	127 K44	NITRAGIN Co., Inc., Milwaukee, Wis.
27	127 K46	
28	127 K30	
29	TAL 98	Cowpea miscellany strains, from
30	TAL 209	University of Hawaii, NifTAL Project, Paia, Hawaii

largest three proximal nodules on each plant by the procedure of Vincent (19). Uniform, single-celled colonies were cultured on yeast mannitol agar. Cultures were discarded if they were not short, gram negative rods. Forty-four strains were retained. In addition, established strains for *P. vulgaris* and *Vigna* spp. were included (Table 2).

In all trials, seeds were surface sterilized by immersion in 5 % H_20_2 for 5 min, and planted at 5 cm depths at a rate of four per pot. Seedlings were thinned for uniformity to two per pot. Treatments were replicated by four pots in Trial 1 and by three pots in Trials 2 and 3. Pots were arranged in randomized complete blocks and watered to weight with distilled water throughout growth.

Plant color, budding, and flowering were noted relative to that of the uninoculated control treatments, with and without NH_4NO_3. After harvesting, plant tops were dried at 65 C and weighed. Nodule count, weight, color, and distribution were recorded. Acetylene reduction rate was measured during harvest between 1000 hours and 1400 hours. For this purpose, plant root systems were cut off below the cotyledon, placed in 970 ml canning jars fitted with serum stoppers, and incubated for 1 hour in a 10% carbide-generated acetylene atmosphere. Samples were then withdrawn for gas chromatography.

Trial 1. Test of effective common bean rhizobia on Scarlet Runner.

The growth medium was river outwash from Cache Creek, Calif., with sandy loam texture and pH 7.8. Pots with plastic liners were filled with 2 kg of dry, autoclaved soil. A basal fertilizer was mixed with the soil in each pot 1 day prior to planting, providing 3 mmole/pot of both KH_2P0_4 and K_2S0_4, 10 ppm Zn as $ZnSO_4$, and 0.1 ppm Mo as Na_2MoO_4.

Inoculum was prepared from fresh rhizobial suspensions estimated to provide 10^4 viable cells/ml, and 2 ml/seed were applied at planting. Nitrogen fertilized controls received 4 mmole NH_4NO_3 2 weeks after germination.

Soil temperature fluctuated between 20 and 28 C. A 15% moisture content was maintained throughout growth. Plants were harvested 47 days after emergence.

Trial 2. Test of Scarlet Runner isolates on Scarlet Runner.

Pots containing a mix of 2:1 horticultural perlite and fine quartz sand were watered with 400 ml of nutrient solution containing 3.75 m M $CaC1_2$, 1.25 m M MgSO,, 1.25 m M K_2S0,, 0.38 m M KH_2P0,, 0.25 m M K_2HP0,, 62.5,u M Fe-EDTA, and micronutrients. The growth medium was autoclaved for 2 to 3 hours. Nutrient solution was again added 19 days after germination.

Inoculum was prepared from fresh rhizobial suspensions estimated to provide 10^9 viable cells/ml, and seedlings each received 1.5 ml 3 to 5 days after emergence and thinning. The N fertilized controls received 5 mmole of NH_4NO_3 both 15 and 29 days after germination.

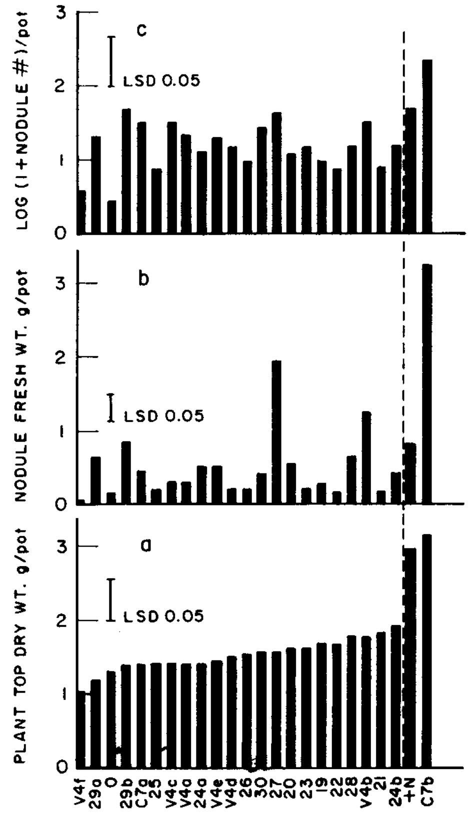

Fig. 1. Growth and nodulation of Scarlet Runner with rhizobia effective on *P. vulgaris, P. coccineus,* and Vigna spp. Strains identified by numbers according to Tables 1 and 2. Dotted line separates effective from ineffective symbioses, based on differences in plant top dry wt. (P + 0.05).

Soil temperatures were kept between 23 and 28 C by placing pots in constant temperature water baths. Forty percent moisture content was maintained in the pots. Plants were harvested from 34 to 36 days after emergence.

Trial 3. Test of Scarlet Runner and Red Kidney isolates on Red Kidney.

A combination of medium sand, 10% by volume spaghnum peat, and distilled water was autoclaved, placed in polyethylene lined pots and planted as in Trial 2. After emergence, the pot surfaces were covered with sterilized coarse perlite to reduce cross-contamination. Nutrient solution containing 1 m *M* $CaC1_2$, 1 m *M* $MgSO_4$, 1 m M $KH,_2PO_4$, 10 µ M FE-EDTA, and micronutrients was added in increments; 0.25 strength the 1st week after germination, 0.50 strength during the 2nd week, and full strength during the remaining 3 weeks. The water soluble N content of the sand was below detectable levels (< 0.016 mmole/ 100 g soil) and that of the peat was 1.06 mmole N/pot. The N fertilized controls received an additional 5 mmole NH_4NO_3 13 and 28 days after emergence.

Soil temperatures fluctuated from 24 to 31 C during the day and from 17 to 21 C during the night. Pots were maintained at $20^0/c$ moisture. Plants were harvested 34 days after emergence.

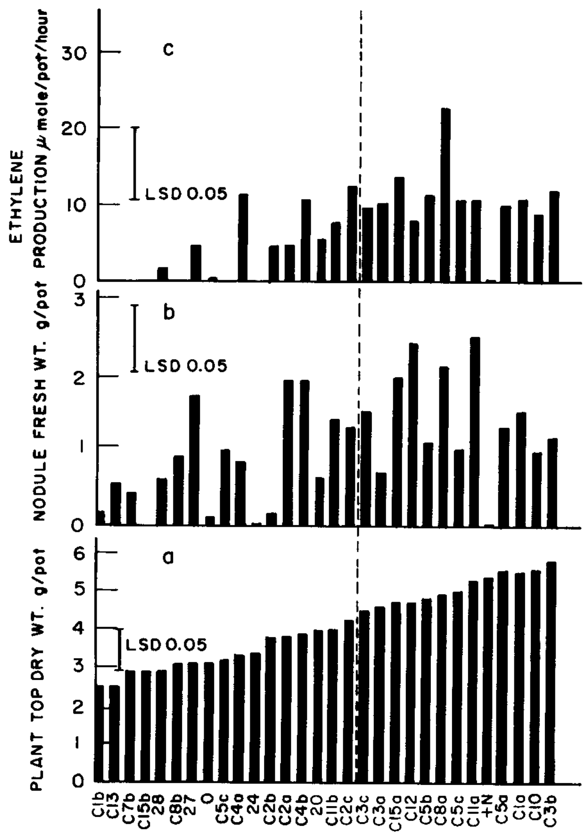

Fig. 2. Growth and nodulation of Scarlet Runner with soil isolates. Strains identified by number according to Tables 1 and 2. Dotted line separates effective from ineffective symbioses, based on differences in plant top dry weight (P + 0.05).

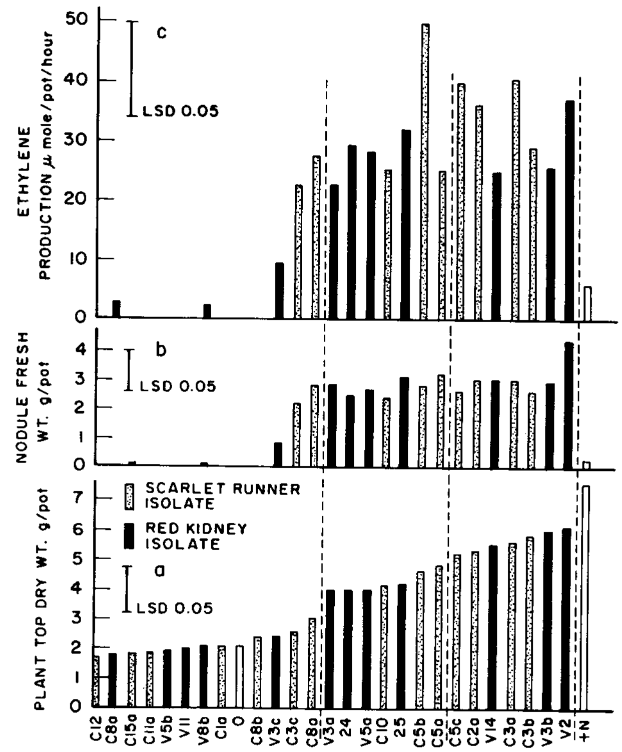

Fig. 3. Growth and nodulation of Red Kidney with soil isolates. Strains identified by number according to Tables 1 and 2. Dotted lines separate effectiveness classes based on differences in plant top dry weight (P + 0.05).

RESULTS

In all soils but no. 17 and 18, 10 to 80 nodules were produced by at least one of the host species. Nodule numbers of the two hosts correlated linearly (r^2 = 0.64). Red Kidney nodulated more heavily than Scarlet Runner on soils that were being cropped with beans at the time of sampling.

Trial 1. Test of effective common bean rhizobia on Scarlet Runner.

Strains that did not produce significantly better plant growth ($P < 0.05$) than the uninoculated control were classed ineffective; those not significantly different from the +N control were classed effective. Only one inoculum, C7b (a Scarlet Runner isolate) produced an effective symbiosis (Fig. 1a); but all produced nodules (Fig. lb and c). Two kinds of nodules were apparent: small white nodules (diameter less than 1 mm) scattered throughout the lower root system; and larger tan, pink, and white nodules (diameter larger than 1 mm) scattered in the upper half of the root system. The uninoculated controls had mainly the former type, probably from aerial sources of contamination. Only the larger nodules were counted, weighed, and included in the nodulation data.

Strain C7b gave significantly higher nodule numbers than all other treatments (Fig. 1c). Fourteen other strains also formed significantly more nodules than the control at the 0.05 probability level. These strains included 10 known to be effective on P. *vulgaris* (including two cultures of NA575), three from the cowpea miscellany (including two cultures of TAL98), and one Scarlet Runner isolate. Nine of these strains also had significantly higher nodule weights than the controls (Fig. 1b).

Ineffective nodules were often red, indicating the presence of leghemoglobin. Several symbioses produced plants which appeared moderately green from the 23rd day of growth until flowering, which began on the 28th day. These strains included CIAT73, CIAT135, NA575, 127K30, TAL98, V4a, and V4b.

Trial 2. Test of Scarlet Runner isolates on Scarlet Runner.

The growth of Scarlet Runner with its homologous soil isolates showed great variability in effectiveness (Fig. 2). Fifty percent of the strains were not significantly better (P + 0.05) than the control based on plant top dry weight. They were classed ineffective. The remaining 50% were not significantly different from the +N control and were classed effective.

Only two or three ineffective Scarlet Runner isolates had negligible nodule weight and acetylene reduction. These were strains C1b, C15b, and perhaps C2b. The remaining ineffective symbioses had substantial nodule weights and acetylene reducing activities at harvest (Fig. 2b and 2c). Some showed sporadic periods of greening, others began turning green just before harvest. Ineffec-

Table 3. Nodulation characteristics of Scarlet Runner and Red Kidney.

Host cultivar	Source of *Rhizobium*	Total # of strains tested	Insignificant or no nodulation	Ineffective nodulation	Effective symbioses
Scarlet Runner	*P. vulgaris*	16	7	9	0
	P. coccineus	24	2–3	9–10	12
Red Kidney	*P. vulgaris*	12	4	1	7
	P. coccineus	14	5	2	7

tiveness could then have been caused by delay in nodule formation or early nodule malfunction.

Four *R. phaseoli* strains repeated from trial 1 again produced variable levels of ineffective nodulation.

Trial 3. Test of Scarlet Runner and Red Kidney isolates on Red Kidney.

Nodulated plants fell into three groups, based on the L.S.D. for plant growth (P + 0.05). Symbioses in the group including the control were considered ineffective, while the other two groups were considered generally effective although variably so. Each effectiveness class contained nodule isolates from Scarlet Runner as well as those from Red Kidney (Fig. 3). Thirty-five percent of all isolates were more effective than the two commercial inocula.

Optimum growth in effective treatments was well correlated with earliness of fixation, as detected by change in plant color. All replicates of the top 10 treatments turned green 23 days after germination. Treatments Via, NA575, and V5a were not green until Day 28, and produced less plant growth than others.

Of the 12 ineffective treatments, nine were nonnodulating. Strain V3c nodulated only slightly, and plants remained yellow throughout growth. Ineffective strains V3c and C8a produced significant nodule weights and acetylene reduction rates comparable to those of the effective strains, but greening was uneven and late (after 26 days).

DISCUSSION

Cross-inoculation relationships between Scarlet Runner and Red Kidney were determined by screening 16 rhizobial strains effective on Red Kidney with Scarlet Runner as host plant, and, conversely, screening 12 strains effective on Scarlet Runner with Red Kidney as host. Both groups of strains had random and diverse origins. Plant growth differences were used to determine differences in nodulation effectiveness.

Scarlet Runner formed effective symbioses only with its own nodule isolates, and with none of the strains isolated from P. *vulgaris.* Insofar as no such specificity has been described in the literature amongst cultivars of P. *vulgaris,* it appears that species differences are important.

Scarlet Runner combined selectivity in effectiveness with high levels of ineffective nodulation (Table 3). Of all the strains found ineffective on Scarlet Runner, 64 to 68 % formed nodules resulting in variable nodule weight and activity, and variable and sporadic greening of shoots. Strain rejection by Scarlet Runner was expressed largely as nodule malfunction and not failure to form nodules.

Red Kidney showed a different pattern of specificity (Table 3). Fifty percent of the Scarlet Runner isolates were as effective on Red Kidney as Red Kidney's homologous strains. Six Scarlet Runner isolates were effective on both species. Strain rejection by Red Kidney was expressed in 75 % of all cases as failure to form nodules.

Evaluating the relative performance of each host with native strains from various California soils was less conclusive. Symbiotic performance of Scarlet Runner was not optimal, nor were sufficient numbers of Red Kidney's homologous isolates tested.

The Red Kidney isolates tested on that host came primarily from four local bean fields. Together with the Scarlet Runner isolates from the same fields, 50% were ineffective. Burton et al. (4) found that only 16% of the *R. phaseoli* in Midwestern bean fields were ineffective, and isolates from all plant nodules were evaluated. This could imply high numbers of ineffective strains in California. However, 67% of the Scarlet Runner isolates from bean fields were effective on Red Kidney, with effectiveness levels equal to or significantly greater than the effectiveness of two commercial strains. These data could imply poor selective ability in Red Kidney, rather than the absence of effective strains.

Fifty percent of Scarlet Runner's isolates were ineffective on Scarlet Runner. Effective strains, however, were isolated from California soils under a wide variety of uses at the time of sampling. Scarlet Runner nodulated as effectively as Red Kidney in soils from local bean fields, where rhizobial effectiveness would most likely have been biased in favor of Red Kidney, a dominant local cultivar. Scarlet Runner combined widespread ability to nodulate effectively with high levels of ineffective nodulation.

Although *P. vulgaris* has often been described as highly promiscuous, both Burton (3, 5) and Vincent (20) have stressed its selective ability to nodulate effectively. The cross-inoculation relationships found here support such selectivity, relative to the performance of Scarlet Runner. Interspecific hybridization could increase the relative competitiveness of ineffective rhizobia for infection of P. *aulgaris* rather than reduce it as suggested (8). But nodulation characteristics within *P. coccineus* may be variable. Both this study and that of Beckham (1) are too limited to indicate to what extent the reported characteristics reflect rhizobial and/or cultivar differences. Evaluation of cultivar differences may provide more successful germplasm for interspecific crosses.

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