

A Comparative Study of the Physiology of Symbioses Formed by *Rhizobium japonicum* with *Glycine max*, *Vigna unguiculata*, and *Macroptilium atropurpurem*¹

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HAROLD H. KEYSER, PETER VAN BERKUM², AND DEANE F. WEBER

United States Department of Agriculture, Agricultural Research Service, Plant Physiology Institute,
Cell Culture and Nitrogen Fixation Laboratory, BARC-West, Beltsville, Maryland 20705

ABSTRACT

Although *Rhizobium japonicum* nodulates *Vigna unguiculata* and *Macroptilium atropurpurem*, little is known about the physiology of these symbioses. In this study, strains of *R. japonicum* of varying effectiveness on soybean were examined. The nonhomologous hosts were nodulated by all the strains tested, but effectiveness was not related to that of the homologous host. On siratro, compared to soybean, many strains reversed their relative effectiveness ranking. Both siratro and cowpea produced more dry matter with standard cowpea rhizobia CB756 and 176A22 than with the strains of *R. japonicum*. Strains USDA33 and USDA74 were more effective with siratro and cowpea than with soybean. The strain USDA122 expressed high rates of hydrogenase activity in symbiosis with the cowpea as well as the soybean host. The strains USDA61 and USDA74 expressed low levels of hydrogenase activity in symbiosis with cowpea, but no activity was found with soybean. Our results indicate host influence for the expression of hydrogenase activity, and suggest the possibility of host influence of nitrogenase for the allocation of electrons to N₂ and H⁺.

Rhizobium japonicum forms a N₂-fixing symbiosis with *Glycine max* L. Merr. (soybean), but these bacteria also nodulate *Vigna unguiculata* (cowpea) and *Macroptilium atropurpurem* (siratro) (12, 20, 22). However, symbiotic characteristics with these nonhomologous hosts have been investigated in detail with only few strains of *R. japonicum*. Strain-dependent hydrogen evolution and hydrogenase activity by *R. japonicum* in symbiosis with soybean has been reported (18, 19), but these characters have not been studied with other legumes as hosts. Our objectives were to investigate the legume host-*R. japonicum* strain interactions for nodulation, effectiveness, acetylene reduction, hydrogen evolution, and hydrogenase activity in soybean, cowpea, and siratro.

MATERIALS AND METHODS

Plant Material. Seed of soybean (*Glycine max* L. Merr.) cv 'Bragg', 'Clark', 'Hood', 'Kent', 'Lee', and 'Roanoke'; cowpea

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(*Vigna unguiculata*) cv 'Cardinal', 'Black Eye-5', 'Pinkeye Purple Hull', 'Queen Anne', and 'Suneto'; and siratro (*Macroptilium atropurpurem*) were surface sterilized with acidified 0.1% (w/v) HgCl₂ (24) for 3 min and washed 10 times with sterile distilled H₂O. Surface sterile seed was sown in sand or vermiculite moistened with 100 ml of a N-free nutrient solution (14) in modified Leonard jars (12) sterilized with steam for 4 h. Each jar was inoculated with approximately 10⁹ cells of *Rhizobium*, which had been grown in yeast-mannitol-salts broth (24) for 7 d. The strains of *R. japonicum* used were obtained from the USDA *Rhizobium* Collection at Beltsville. These strains were originally isolated from soybean root nodules and have been described (11). These strains were chosen for their wide range in effectiveness on soybean. Strains of *Rhizobium* 'cowpea miscellany' CB756 and 176A22 were used as controls with siratro and cowpea. Soybean and cowpea (four per jar) and siratro (six per jar) were grown in a greenhouse without artificial lighting from April to June in 1979 and 1980. Each legume cultivar-*Rhizobium* strain combination was tested in triplicate. Experiments comparing symbioses of siratro with Clark, and cowpea with Lee, were harvested at 55 and 40 d after sowing, respectively. Measurements of symbiotic characteristics with five cultivars each of soybean and cowpea were made 47 d after sowing. The tests for hydrogenase activity with cowpea were done 28 d after sowing and repeated 7 d later. The data of these two experiments were combined because the results were similar.

Measurements. Effectiveness of host-*Rhizobium* strain combinations was determined from measurements of plant dry weight (24). Effective combinations were determined as having significantly higher dry weight than the uninoculated control. Plant tops were removed at the cotyledon from each jar and dried at 60°C for 2 d before weighing. The excised root systems from each replicate jar were removed and divided equally between separate containers used to measure hydrogen evolution or acetylene reduction. The roots were briefly washed in water to remove most of the sand or vermiculite and were placed into 225-ml Mason jars, which were sealed with lids provided with rubber serum stoppers. Hydrogen evolution was determined at room temperature by incubating roots in air for 60 min in sealed jars and removing 0.2-ml samples of the gas phase with hypodermic syringes. Concentrations of H₂ were determined with a Varian model 1520 gas chromatograph³ fitted with thermal conductivity detectors set at 150 mamp and using a 0.3 × 115 cm molecular sieve 5A column (45–60 mesh) at room temperature and N₂ as carrier gas

³ Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

at 15 ml/min. Acetylene reduction was determined at 21°C by incubating roots in jars with 10% (v/v) acetylene for 60 min and removing 0.5-ml samples of the gas phase of each jar with hypodermic syringes. The analyses of acetylene and ethylene were as described by van Berkum and Sloger (21). The nodules were removed from the roots and their fresh weight for each sample determined after the gas analyses had been completed. Hydrogenase activity was determined in 10-ml vials by placing 0.2 to 0.3 g fresh nodules in 1.0 ml buffer consisting of 1 mM K_2HPO_4 , 2.5 mM $MgCl_2$, 50 mM Hepes (pH 7.5) (6). The nodules were crushed with clean copper rods, the contents were mixed, the vials were sealed with serum stoppers, and H_2 to a concentration of 1% (v/v) was injected into each vial. The samples for hydrogenase measurements were incubated at 30°C for 1 or 2 h and 0.2 ml of the gas phase of each vial was removed for H_2 analysis by GC. Hydrogenase activity was determined by measuring the rate at which the injected H_2 was consumed in the test vials in relation to control vials without nodule tissue. In each experiment, randomly selected nodules from each legume host-*Rhizobium* combination (except CB756 and 176A22) were frozen. Subsequently, the bacterial contents of the nodules were determined using the direct fluorescent antibody method (16), or tube agglutination (24) when fluorescent antibody for a strain was not available. These tests verified that the nodules in each treatment had been formed by the inoculated strain.

RESULTS

All 14 strains of *R. japonicum* tested on siratro produced nodules, but only USDA33, USDA46, USDA74, and USDA120 were symbiotically effective determined by plant top dry weights (Table I). The four effective strains produced significantly more nodule mass and had significantly higher rates of acetylene reduction and H_2 evolution than the 10 ineffective strains. Although plant dry weight production by 176A22 on siratro was higher than that of CB756, nodule mass, rates of acetylene reduction, and H_2 evolution were similar. The rate of H_2 evolution (\pm SE) by USDA74 on siratro ($1.40 \pm 0.15 \mu\text{mol } H_2/\text{plant}\cdot\text{h}$) was higher than with 176A22 and CB756 (combined mean of $0.77 \pm 0.09 \mu\text{mol}/H_2\text{plant}\cdot\text{h}$) although the rate of acetylene reduction with this strain was significantly lower ($4.71 \pm 1.90 \mu\text{mol } C_2H_4/\text{plant}\cdot\text{h}$) than the rate of the symbioses with the 'cowpea miscellany' strains (combined mean of $7.32 \pm 0.60 \mu\text{mol } C_2H_4/\text{plant}\cdot\text{h}$). The rate of acetylene reduction by USDA74 on siratro was similar to that by USDA46, but the rates of H_2 evolution differed significantly with relative efficiencies (17) calculated to be 0.70 and 0.93, respectively. Rates of acetylene reduction by strains USDA33 and USDA120 on siratro were similar, while significantly different rates of H_2 evolution were observed with relative efficiencies of 0.61 and 0.85, respectively. The same 14 strains of *R. japonicum* tested on siratro all nodulated soybean cv Clark with a varying degree of effectiveness.

Ten strains of *R. japonicum* were effective on cowpea cv Pinkeye Purple Hull (Table I) and all 20 strains tested produced nodules. Nodule mass ranged from 0.12 g fresh weight/plant for USDA117 to 0.68 g fresh weight/plant for USDA136. Those plants nodulated with the ineffective strains produced light green foliage in contrast to the yellow leaf color of the uninoculated controls suggesting low rates of N_2 fixation, although plant dry weights were similar. Plant dry weight production with the two *Rhizobium* cowpea miscellany was higher than plants nodulated with the 10 effective strains of *R. japonicum*. This effect probably was not mediated by a delay of N_2 fixation during the development of the *R. japonicum*-cowpea symbioses compared to the *Rhizobium* cowpea miscellany combinations, because the foliage in each treatment turned green after approximately the same time interval. The rates of acetylene reduction by USDA31 and USDA136 (15.5 ± 1.50 and $15.9 \pm 5.10 \mu\text{mol } C_2H_4/\text{plant}\cdot\text{h}$, respectively) were higher than with

Table I. Effectiveness Rating of *Rhizobium* with Siratro, Cowpea, and Soybean

Rhizobium Strain	Plant Top Dry Wt ^a			
	<i>M. atropurpureum</i>	<i>G. max</i> cv Clark	<i>V. unguiculata</i>	<i>G. max</i> cv Lee
	g/jar			
USDA6	0.50 f	5.86 abc	2.29 b-e	2.49 c-f
USDA31	ND ^b	ND	2.40 bcd	2.18 def
USDA33	2.31 d	2.83 e	2.40 bcd	1.33 fg
USDA46	2.50 cd	5.86 abc	2.32 b-e	2.45 c-f
USDA59	0.22 f	4.48 cd	1.87 d-g	2.45 c-f
USDA61	ND	ND	2.73 b	2.32 c-f
USDA74	3.00 bc	3.95 de	2.46 bc	2.29 c-f
USDA76	ND	ND	2.21 b-e	2.65 cde
USDA94	ND	ND	1.83 d-g	2.00 def
USDA110	0.37 f	6.17 abc	2.38 bcd	3.46 ab
USDA117	ND	ND	1.21 h	1.85 ef
USDA120	1.71 e	5.95 abc	2.29 b-e	2.89 b-e
USDA122	0.29 f	7.01 ab	2.04 c-f	3.44 abc
USDA123	ND	ND	2.01 c-f	2.89 b-e
USDA125	0.18 f	5.50 bcd	2.06 c-f	3.08 a-d
USDA136	0.42 f	7.28 a	2.39 bcd	2.82 b-e
USDA138	0.53 f	6.54 ab	1.81 d-g	2.44 c-f
USDA140	0.33 f	5.56 a-d	1.96 c-g	2.63 cde
USDA141	0.21 f	6.07 abc	1.44 ab	2.41 c-f
USDA142	0.23 f	5.99 abc	1.76 e-h	4.04 a
176A22	3.75 a	ND	4.03 a	ND
CB756	3.12 b	ND	4.17 a	ND
Uninoculated	0.15 f	1.31 f	1.56 fgh	1.13 g

^a Numbers in the same column flanked by the same letter are not significantly different at the 5% level of probability as determined by Duncan's new multiple range test.

^b ND, not determined.

CB756 or 176A22. We observed that strains USDA6, USDA110, USDA122, and USDA136 did not evolve H_2 with cowpea cv Pinkeye Purple Hull, suggesting that these bacteria expressed hydrogenase as has been reported with soybean as host (2, 13).

The 20 strains of *R. japonicum* tested on cowpea all nodulated soybean cv Lee, and with the exception of USDA33, formed effective symbioses as determined by plant top dry weight (Table I). Strain USDA33 was effective on cowpea cv Pinkeye Purple Hull. Nodule mass on soybean with the effective strains ranged from 0.20 fresh weight/plant for USDA117 to 0.42 g fresh weight/plant for USDA125, and for the ineffective strain USDA33 was 0.05 g fresh weight/plant. We also observed significant differences among the effective strains for acetylene reduction and H_2 evolution. The mean values for acetylene reduction and H_2 evolution by the 19 effective soybean symbioses were 7.54 ± 0.64 and $3.96 \pm 0.56 \mu\text{mol}/\text{plant}\cdot\text{h}$, respectively, and for the 10 effective cowpea-*R. japonicum* symbioses were 9.00 ± 1.28 and $0.72 \pm 0.23 \mu\text{mol}/\text{plant}\cdot\text{h}$, respectively. Acetylene reduction and H_2 evolution by soybean nodulated with the strains of *R. japonicum* determined to be effective with cowpea had mean values of $6.56 \pm 1.04 \mu\text{mol } C_2H_4/\text{plant}\cdot\text{h}$ and $2.76 \pm 0.74 \mu\text{mol } H_2/\text{plant}\cdot\text{h}$, respectively.

We chose four strains of *R. japonicum* to further investigate their symbiotic characteristics with five cultivars each of soybean and cowpea. The strains USDA110 and USDA122 were selected because they form efficient N_2 -fixing symbioses with soybean, and USDA61 and USDA74 were used because of their effectiveness on cowpea (Table I). Acetylene reduction by the cowpea and soybean cultivars nodulated with USDA61 and USDA74 were similar, but hydrogen evolution with the cowpea cultivars was significantly lower than with the soybean cultivars (Tables II and III). We calculated the overall relative efficiencies to be 0.92 and

Table II. Growth, Nodulation, Nitrogenase Activity, and Hydrogen Evolution by Five Cultivars of Cowpea in Symbiosis with *R. japonicum*

Rhizobium Stain	Cultivar	Plant Top Dry Wt	Nodule Fresh Wt	Nitrogenase Activity	H ₂ Evolution
		g/jar	g/jar	μmol C ₂ H ₄ /plant·h	μmol H ₂ /plant·h
USDA61	Black Eye-5	5.05 ± 0.74 ^a	3.66 ± 0.33	8.10 ± 4.13	0
	Cardinal	6.70 ± 0.66	3.66 ± 0.19	2.00 ± 0.45	0.1 ± 0.1
	Pinkeye Purple Hull	5.29 ± 0.66	2.95 ± 0.33	3.85 ± 1.05	0
	Queen Anne	6.06 ± 0.40	3.81 ± 0.09	4.87 ± 1.01	0.1 ± 0.1
	Suneto	2.67 ± 0.35	2.32 ± 0.31	2.48 ± 0.63	0
USDA74	Black Eye-5	4.90 ± 0.65	3.66 ± 0.15	1.40 ± 0.45	0
	Cardinal	7.62 ± 0.51	3.53 ± 0.19	4.03 ± 0.90	0.54 ± 0.13
	Pinkeye Purple Hull	5.76 ± 0.41	2.86 ± 0.10	4.96 ± 1.63	1.41 ± 0.43
	Queen Anne	6.97 ± 0.96	3.37 ± 0.17	5.37 ± 1.96	0.59 ± 0.31
	Suneto	3.51 ± 0.19	1.73 ± 0.14	5.15 ± 1.76	1.10 ± 0.26
USDA110	Black Eye-5	5.29 ± 0.11	4.78 ± 0.13	2.60 ± 1.45	0
	Cardinal	4.18 ± 0.55	3.78 ± 0.15	4.10 ± 1.79	0
	Pinkeye Purple Hull	2.82 ± 0.54	1.79 ± 0.59	2.07 ± 1.41	0.30 ± 0.30
	Queen Anne	5.10 ± 1.00	3.17 ± 0.60	3.65 ± 1.89	0
	Suneto	1.42 ± 0.12	1.21 ± 0.07	3.00 ± 1.18	0
USDA122	Black Eye-5	4.11 ± 0.73	2.89 ± 0.20	19.63 ± 2.04	0
	Cardinal	4.65 ± 0.47	4.25 ± 0.46	10.72 ± 3.29	0
	Pinkeye Purple Hull	4.92 ± 0.45	4.17 ± 0.33	4.13 ± 0.43	0
	Queen Anne	4.07 ± 0.62	4.37 ± 0.82	6.85 ± 3.96	0
	Suneto	0.93 ± 0.07	0.88 ± 0.06	0.09 ± 0.04	0

^a Results are means of three replicates ± SE.

0.35 for these two strains on the cowpea and soybean cultivars, respectively. A strain-host cultivar interaction for ineffectiveness was observed for the USDA122-cowpea cv Suneto combination, which had very low nodule mass and nitrogenase activity (Table II).

The strain of *R. japonicum* USDA122 expressed hydrogenase activity on three cultivars each of soybean and cowpea (Table IV). The mean rate of hydrogenase activity by USDA122 on the cowpea cultivars was significantly lower than on the soybean cultivars ($P = 0.05$). We observed low levels of hydrogenase activity by the cowpea cultivars nodulated with USDA61 and USDA74, but these strains did not express hydrogenase activity when in symbiosis with the soybean cultivars (Table IV).

The soybean cultivars used in this study when nodulated with USDA74 produced symptoms characteristic of the production of rhizobitoxine (23). However, siratro and all the cowpea cultivars tested with USDA74 did not exhibit the symptoms of rhizobitoxine production.

DISCUSSION

Siratro and cowpea are legumes commonly used in studies of symbiotic evaluation of the cowpea miscellany rhizobia, and soybean is used to rate strains of *R. japonicum*. In our study, all the strains of *R. japonicum* nodulated siratro and the five cultivars of cowpea. We report that several of the *R. japonicum*-siratro and *R. japonicum*-cowpea combinations form effective symbioses, which reduced acetylene and evolved hydrogen at diverse rates. Both rhizobia groups nodulate the representative host of the other group (9, 12, 20, 22), but details of the physiology of these symbioses have not previously been described.

Van Rensburg et al. (22) reported that five strains of *R. japonicum*

changed their relative ranking for effectiveness in symbiosis with siratro when compared to soybean as host. We observed similar host influence on effectiveness of *R. japonicum* when we compared 14 strains with soybean cv Clark and siratro. Strains USDA6, USDA59, USDA110, USDA122, USDA125, USDA136, USDA138, USDA140, and USDA141 were effective on soybean cv Clark, but ineffective on siratro. In contrast, strains USDA74 and USDA33 ranked last for dry matter production with soybean cv Clark, but with siratro they were ranked first and third, respectively. Plant dry matter production by siratro in symbiosis with USDA74 was similar to that of plants nodulated with CB756.

As with siratro, we observed that the ranking of symbiotic effectiveness of strains of *R. japonicum* with cowpea is not related to that with soybean as host. However, more of the strains of *R. japonicum* formed effective N₂-fixing symbioses with cowpea than with siratro. We detected acetylene reduction by cowpea cv Pinkeye Purple Hull when nodulated with all 20 strains of *R. japonicum* tested, and all but four host-strain combinations had detectable H₂ evolution. It has been suggested that H₂ evolution by nodulated legumes represents an energy loss for N₂ fixation which could reduce yield of dry matter (17). However, dry matter yield by cowpea cv Pinkeye Purple Hull in our study was significantly higher with the cowpea miscellany than with the soybean rhizobia, although in terms of acetylene reduction and H₂ evolution, N₂ fixation by *R. japonicum* USDA31 and USDA136 was more efficient than by CB756 and 176A22. This observation suggests that factors other than efficiency of N₂ fixation in terms of H₂ metabolism are important for significantly higher yield of cowpea when nodulated with the cowpea miscellany rhizobia. This inference is consistent with that of Dilworth (4), who suggested that the fraction of the energy used by nitrogenase to reduce protons

Table III. Growth, Nodulation, Nitrogenase Activity, and Hydrogen Evolution by Five Cultivars of Soybean in Symbiosis with *R. japonicum*

<i>Rhizobium</i> Strain	Cultivar	Plant Top Dry	Nodule Fresh	Nitrogenase Ac-	H ₂ Evolution
		Wt	Wt	tivity	
		g/jar		$\mu\text{mol C}_2\text{H}_4/\text{plant}\cdot\text{h}$	$\mu\text{mol H}_2/\text{plant}\cdot\text{h}$
USDA61	Bragg	2.94 ± 0.48 ^a	1.06 ± 0.08	6.52 ± 3.16	3.31 ± 0.14
	Clark	4.13 ± 0.17	1.65 ± 0.09	7.15 ± 1.29	3.62 ± 0.88
	Hood	4.39 ± 0.06	1.49 ± 0.33	2.88 ± 1.90	3.16 ± 0.55
	Kent	4.41 ± 0.13	1.63 ± 0.06	5.60 ± 0.83	3.20 ± 0.24
	Roanoke	4.74 ± 0.31	1.76 ± 0.05	6.33 ± 0.12	4.01 ± 0.51
USDA74	Bragg	3.45 ± 0.51	1.11 ± 0.06	7.78 ± 0.18	3.11 ± 0.86
	Clark	4.06 ± 0.53	1.78 ± 0.22	10.06 ± 0.81	6.11 ± 0.53
	Hood	3.13 ± 0.77	0.97 ± 0.38	3.74 ± 1.79	2.42 ± 0.65
	Kent	3.42 ± 0.24	1.45 ± 0.14	7.11 ± 0.63	6.06 ± 0.72
	Roanoke	2.66 ± 0.24	0.88 ± 0.07	2.59 ± 0.33	1.91 ± 0.17
USDA110	Bragg	3.19 ± 0.26	1.15 ± 0.16	6.30 ± 1.81	0.25 ± 0.02
	Clark	5.59 ± 0.25	1.85 ± 0.16	11.65 ± 3.24	0.20 ± 0.09
	Hood	4.98 ± 0.64	1.89 ± 0.56	11.39 ± 1.45	1.02 ± 0.35
	Kent	6.02 ± 0.25	1.92 ± 0.12	13.32 ± 0.84	0
	Roanoke	6.37 ± 0.17	1.93 ± 0.03	8.38 ± 1.12	0.66 ± 0.26
USDA122	Bragg	4.51 ± 0.15	1.91 ± 0.10	12.85 ± 2.03	0
	Clark	4.96 ± 0.22	1.75 ± 0.20	7.94 ± 1.81	0
	Hood	5.11 ± 0.17	1.83 ± 0.11	10.52 ± 2.99	0.13 ± 0.10
	Kent	5.39 ± 0.10	1.92 ± 0.07	11.96 ± 1.45	0
	Roanoke	4.63 ± 0.53	1.09 ± 0.78	6.23 ± 1.29	0

^a Results are means of three replicates ± SE.

Table IV. Hydrogenase Activity (Hydrogen Uptake) by *R. japonicum* Bacteroids from Nodules of Soybean and Cowpea

Host		Hydrogenase Activity		
		USDA122	USDA61	USDA74
		$\mu\text{mol H}_2/\text{g fresh wt}\cdot\text{h}$		
<i>G. max</i>	Clark	9.1 ± 1.8	<0.2	<0.2
	Hood	9.7 ± 1.5	<0.2	<0.2
	Kent	11.3 ± 0.8	<0.2	<0.2
<i>V. unguiculata</i>	Cardinal	6.8 ± 0.6	2.2 ± 0.4	0.6 ± 0.1
	Pinkeye Purple Hull	7.4 ± 0.4	2.3 ± 0.2	1.5 ± 0.3
	Black Eye-5	6.6 ± 0.4	1.5 ± 0.2	1.0 ± 0.4

may not be a growth-limiting factor in legumes. Alternatively, the disparity in yield may be explained by a delay in N₂ fixation by cowpea in symbiosis with *R. japonicum* relative to *Rhizobium* cowpea miscellany. However, we observed no evidence that a delay in N₂ fixation was a factor which contributed to the difference in yield between cowpea plants nodulated with cowpea miscellany or soybean rhizobia.

It has been suggested that soybean yield may benefit from hydrogenase activity, because H₂ produced from nitrogenase activity may be recycled by this process to conserve carbon substrates, protect nitrogenase from O₂ damage, and produce energy (1, 7, 8, 19). It has been concluded that the strain of *R. japonicum*, rather than the plant cultivar, plays the major role in controlling hydrogen loss from soybean nodules (2, 7, 13). Evans *et al.* (7) suggested that some nodules that evolve H₂ may contain hydrogenase activity in bacteroids, but at levels insufficient to recycle all the H₂ produced by nitrogenase. We observed low rates or no H₂ evolution by cowpea and soybean in symbiosis with USDA6, USDA110, USDA122, and USDA136, which probably was due to the presence of hydrogenase activity. In the case of USDA122,

we substantiated that hydrogenase activity was expressed by the bacteroids in the nodules of both soybean and cowpea. Furthermore, we detected significantly lower rates of H₂ evolution with cowpea than soybean cultivars when nodulated by USDA61 and USDA74 and subsequently observed that these strains expressed low rates of hydrogenase activity when in symbiosis with cowpea but not with soybean. Similarly, Dixon (5) and Gibson *et al.* (10) have demonstrated host influence for the expression of hydrogenase activity in *R. leguminosarum* and *Rhizobium* cowpea miscellany, respectively. Although the nature of the host influence on the expression of hydrogenase activity by bacteroids is not known, Evans *et al.* (7) have suggested that the environment within the nodule may play a crucial role.

Schubert and Evans (17) reported that in the nodules of many legumes approximately half of the total electron flux to nitrogenase is diverted to form H₂. Similarly, the rate of H₂ evolution by USDA61 and USDA74 with soybeans in our study suggested that at least 50% of the electrons allocated to nitrogenase were lost by H₂ evolution. In contrast, the rates of H₂ evolution by these two strains were small when in symbiosis with the cowpea cultivars.

The rates of H₂ uptake by the cowpea-USDA61 or cowpea-USDA74 combinations were low and probably were insufficient to recycle most of the potential for H₂ evolution by these two strains as observed with soybean as host. It is possible that the cowpea host influenced the proportion of electrons allocated to the substrates N₂ and H⁺ of nitrogenase in nodules formed by *R. japonicum* USDA61 and USDA74. Allocation of electrons to N₂ in favor of H⁺ may represent an alternative mechanism to hydrogenase by which legumes conserve energy and reductant for more efficient N₂ fixation.

Host control for nodule development and N₂ fixation among soybean cultivars has been determined with *R. japonicum* USDA33, USDA61, and USDA74 (3, 23). USDA61 and USDA74 have been reported to cause chlorosis induced by rhizobitoxine production when in symbiosis with soybean (15). However, we did not detect chlorosis in cowpea or siratro nodulated with these strains of *R. japonicum*, and to our knowledge no published reports have indicated *Rhizobium*-induced chlorosis in species other than soybean. USDA33 was described as a 'type B' soybean rhizobia, which nodulates poorly and is less effective for N₂ fixation when compared to 'type A' (25). We observed that symbioses formed by USDA33 and USDA74 are more effective with siratro and cowpea than soybean. Nevertheless, these strains have been classified as *R. japonicum* because they were originally isolated from soybean nodules. The designation of isolates as *R. japonicum* or *Rhizobium* cowpea miscellany based on their source is arbitrary because both groups of rhizobia nodulate soybean and several hosts associated with the cowpea cross-inoculation group. Our results indicate that a strain may be more effective with another legume, although it was initially isolated from a soybean root nodule.

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