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Variation in Preference for *Rhizobium meliloti* Within and Between *Medicago sativa* Cultivars Grown in Soil†

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Variation in nodulation preferences for *Rhizobium* strains within and between *Medicago sativa* cultivars was assessed in the greenhouse with plants grown in Leonard jars and two soils of diverse origin (Lanark and Ottawa), using inocula consisting of effective individual or paired strains of *R. meliloti* which could be recognized by high-concentration antibiotic resistance. The results indicated considerable variability in host preferences for *R. meliloti* among plants within cultivars but not between cultivars. The implications of this variation are discussed from the point of view of possible improvement of symbiotic nitrogen fixation. With one exception, the differences in nodulation success between inoculant *R. meliloti* strains were consistent in Leonard jars and both soils. All introduced strains formed significantly more nodules in Renfrew soil containing few native rhizobia than in Ottawa soil with a large resident *R. meliloti* population. Plants grown in Lanark soil without inoculation were ineffectively nodulated by native rhizobia and yielded significantly less growth than those receiving inoculation. In contrast, the yield of inoculated plants in Ottawa soil did not significantly differ from those without inoculation due to effective nodulation by native *R. meliloti*. The data indicated synergistic effects on yield by certain paired strain inocula relative to the same strains inoculated individually in Lanark but not in Ottawa soil or Leonard jars.

The relative success of different *Rhizobium* strains in producing legume root nodules, the influence these rhizobia have on the introduction of other strains, and the factors affecting this process (nodulating competitiveness) are of considerable practical importance with respect to legume inoculation. Evidence for specific legume host effects in determining the nodulation success of *Rhizobium* strains in mixed inocula have been given by Labandera and Vincent (14), Pinto et al. (17), Robinson (18), and Vincent and Waters (24) for various *Trifolium* and *Medicago* spp. Other reports indicate that this host selection or preference for *Rhizobium* strains in nodule formation differs between certain species or cultivars of *Trifolium* (13, 20, 24), *Glycine max* (5), and *Medicago sativa* (9, 10) and between individual plants within cultivars of *Trifolium repens* and *Trifolium pratense* (13, 20). However, little attention has been given to the possible influence of genotypic variation within cultivars of *M. sativa* on nodulating competitiveness. The main objective of the present investigation was to assess the effect of inter- and intracultivar variation in *M. sativa* on the nodulating success of *Rhizobium meliloti* with plants grown in soils containing native rhizobia and, for comparison, under axenic conditions in Leonard jars. Strains of *R. meliloti* resistant to high concentrations of antibiotics were used in this work to facilitate identification.

MATERIALS AND METHODS

Rhizobium strains. *R. meliloti* 2011 (originally SU47) and 2001 were from the Rothamsted collection, Harpenden, England, and MB10 was from the Chemistry and Biology Research Institute collection; all strains were symbiotically effective on the cultivars of *M. sativa* used in this investigation. *R. meliloti* MB10 differed from the other two strains by forming large, gummy colonies (ca. 5 to 8 mm in diameter after 4 days at 28°C) on the yeast extract-mannitol agar (YEM) medium used. The method of Schwinghamer and Dudman (21) was used to mark strain 2011 with 500-μg ml^{-1} streptomycin resistance (Str^r), strain MB10 with 500-μg ml^{-1} spectinomycin resistance (Spc^r), and strain 2001 with 200-μg ml^{-1} kanamycin resistance (Kan^r). Assays of acetylene reduction (6) showed no significant differences between the antibiotic-resistant strains and their respective wild types.

Media. *R. meliloti* was routinely grown on YEM modified from that of Fred et al. (8) by use of 1 g of dehydrated yeast extract (Difco Laboratories) per liter and omission of $CaCO_3$. Tryptone-yeast extract agar (TY) medium (1) was used when it was required to reduce gum formation by certain isolates of *R. meliloti*.

Leonard jar experiment. The influence of *M. sativa* cvs. Apollo, Saranac, and Vernal on the nodulation success of *R. meliloti* under axenic conditions was investigated with modified Leonard jars (23) containing quartz sand and supplied with nitrogen-free nutrient solution (16). Seedlings from surface-sterilized seed (23) were planted at a rate of three of each cultivar per Leonard jar. Immediately after this operation, seedlings of each cultivar were inoculated with 1 ml of cell suspension in YEM broth adjusted turbidimetrically to provide ca. 10^9 cells ml^{-1}. Inocula consisted of *R. meliloti* 2011 Str^r, MB10 Spc^r, and 2001 Kan^r both individually and in intended 1:1 mixtures. The actual proportions of strains in the mixed inocula were estimated from colony counts on YEM (23). After emergence in a greenhouse at 22 ± 5°C (day) and 17 ± 2°C (night) with supplemental fluorescent lighting for a 16-h photoperiod, the seedlings were thinned to one of each cultivar per Leonard jar. The experimental design was a randomized complete block with 10 replicates of each inoculum and cultivar combination; uninoculated controls were included. Shoot dry weight and nodule numbers were determined 6 weeks after planting, and the roots were stored at −20°C for assays of strain identity at a convenient time.

Soil experiment. Two soils were used. One, a Renfrew gray podzol (pH 6.1, water), was from Ramsay Township, Lanark County, Ontario, Canada, and had no previous

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history of *M. sativa*. The other was a loam (pH 7.5, water) from the Central Experimental Farm, Ottawa, Ontario, on which *M. sativa* had grown for several years. Both soils were collected from the surface 15 cm, and each consisted of a composite of 10 samples taken at random from a 100-m^2 area; soils were stored at 4°C for 3 weeks before use. Soil nitrate and ammonium nitrogen levels determined by specific ion electrode were, respectively (mg $liter^{-1}$), 36 and 8 (Lanark soil) and 73 and 6 (Ottawa soil).

The experiment was carried out at the same time as the Leonard jar experiment, using the same conditions, inocula, and seedlings from surface-sterilized seed. The experimental design and procedure were identical to those described above except that seedlings of cultivars Apollo, Saranac, and Vernal were grown in the two soils in 10.5-cm-diameter pots (500 g of moist soil per pot). To prevent cross-contamination by drainage water and splashing, the base of each pot was sealed to a smaller pot (7 cm in diameter) which acted as a drainage trap, and each assembly was enclosed in a polyethylene sleeve extending 10 cm above the soil surface. The pots were supplied with nitrogen-free nutrient solution (16) once every 7 days for the duration of the experiment and with distilled water for the remainder of the time. The experiment was terminated after 6 weeks when the plants were harvested and treated as described above. The number of indigenous *R. meliloti* in each soil was estimated immediately before planting by the most-probable-number method (23) with fivefold dilutions and *M. sativa* cv. Saranac as the test plant.

Strain identification. As many nodules as possible (generally not less than 10) were typed from each plant. Excised nodules were surface sterilized by 20 s of exposure to sodium hypochlorite (14% [wt/vol] available chlorine), and isolations were made by a modification of the method of Franco and Vincent (7) such that each nodule was crushed in ca. 0.1 ml of sterile water between watchmakers' forceps which were sequentially inserted into plates of TY without antibiotics and YEM with streptomycin (300 $\mu g\ ml^{-1}$), spectinomycin (300 $\mu g\ ml^{-1}$), or kanamycin (200 $\mu g\ ml^{-1}$). TY medium without antibiotics was selected for use because *R. meliloti* MB10 and all indigenous isolates from the Ottawa soil produced copious gum on YEM. To inhibit fungal growth, cycloheximide (150 $\mu g\ ml^{-1}$) was incorporated in all media. Plates were incubated for 4 days at 28°C, when they were scored for the presence or absence of the inoculant strains.

RESULTS

Table 1 shows data for strain identification in nodules of *M. sativa* cvs. Apollo, Saranac, and Vernal grown in Leonard jars and inoculated with paired strains of *R. meliloti*. When cases of double-strain occupancy of nodules are excluded, it is evident that in all inoculant and cultivar combinations the most nodules were formed by strain MB10 Spc^r (between 70 and 90%) and the least by strain 2001 Kan^r (between 10 and 23%). *R. meliloti* 2011 Str^r was intermediate in nodulation success, forming between 24 and 81% of the nodules. Chi-square analyses relating strain representation in each inoculum with that in nodules (excluding double infections) were significant in each instance ($P < 0.001$), indicating that cultivar preferences for *R. meliloti* strains were MB10 Spc^r > 2011 Str^r > 2001 Kan^r. Analyses of variance on the percentage of nodules formed by the most successful strain in each inoculation treatment (data transformed to angles) showed no significant differences between cultivars in their preferences for *R. meliloti* in any of the mixed inocula. However, there was considerable variation in *R. meliloti* strain representation in nodules of individual plants of cultivars Apollo, Saranac, and Vernal, with some plants within each cultivar predominantly nodulated by a particular strain in an inoculum mixture and others by the alternative strain.

Data for shoot dry weights and nodule numbers of cultivars Apollo, Saranac, and Vernal grown in Leonard jars and inoculated with *R. meliloti* individually and in mixtures are given in Table 2. All inocula were symbiotically effective on the three cultivars, which formed more ($P < 0.01$) shoot dry matter than the uninoculated controls. *R. meliloti* MB10 Spc^r was the least effective strain, causing less ($P < 0.05$) shoot dry matter to form on all cultivars than 2011 Str^r and 2001 Kan^r. The shoot dry matter production of cultivars with double-strain inocula did not differ significantly from that of cultivars inoculated with the individual strains which formed the majority of nodules in each respective mixed inoculum. Nodule numbers per plant did not differ significantly between inocula or cultivars.

Table 3 shows summarized data for strain identity of nodules of cultivars Apollo, Saranac, and Vernal grown in soil and inoculated with *R. meliloti* strains individually and in mixtures. In Lanark soil containing few indigenous *R. meliloti* ($5 \times 10^1\ gm^{-1}$), all single-strain inocula formed more than 90% of the nodules on the three cultivars. For all cultivar and mixed inoculation treatment combinations with this soil, MB10 Spc^r formed between 60 and 95% of the nodules; 2011 Str^r formed between 17 and 97%; and 2001 Kan^r, the least successful inoculant strain, formed between 1 and 3%; indigenous isolates were not encountered in more than 1% of the nodules typed. Cultivar preferences for introduced strains in these inoculation treatments were similar to those in the Leonard jar experiment.

The inoculant strains in Ottawa soil (5×10^5 indigenous *R. meliloti* per g) formed fewer nodules than in Lanark soil and accounted for between 20 and 77% of the nodules on all cultivars in the single-strain inoculation treatments. The remaining nodules were due to indigenous isolates. Nodulation success and cultivar preferences for *R. meliloti* in these inoculation treatments were consistent with those in Lanark

TABLE 1. Strain identity of nodules of *M. sativa* cvs. Apollo, Saranac, and Vernal grown in Leonard jars and inoculated with mixtures of *R. meliloti* strains

Inoculum	% Strain representation in inoculum[a]	% Strain representation in nodules of[a,b]:		
		Apollo	Saranac	Vernal
2011 Str + MB10 Spc^r	59, 41	23, 54 (23)	18, 57 (25)	23, 56 (21)
2011 Str^r + 2001 Kan^r	45, 55	63, 16 (21)	63, 23 (14)	68, 16 (16)
MB10 Spc^r + 2001 Kan^r	36, 64	77, 18 (15)	81, 10 (9)	74, 14 (12)

[a] Values are for each strain in the listed order. Data represent 1,334 nodule isolates assayed for strain identity.
[b] Values in parentheses denote percentage of double infections.

TABLE 2. Response of *M. sativa* cvs. Apollo, Saranac, and Vernal grown in Leonard jars to inoculation with *R. meliloti* strains individually and in mixtures

Inoculum	Shoot dry wt (mg/plant)[a]				Nodule no./plant[a]			
	Apollo	Saranac	Vernal	Mean	Apollo	Saranac	Vernal	Mean
2011 Str^r	64.0	41.4	85.5	63.63	29.5	25.4	30.6	28.50
MB10 Spc^r	42.4	38.0	45.7	42.03	29.8	26.4	26.3	27.50
2001 Kan^r	76.0	55.3	58.8	63.37	28.2	26.3	23.1	25.87
2011 Str^r + MB10 Spc^r	50.8	43.2	51.0	48.36	22.1	28.5	40.3	30.30
2011 Str^r + 2001 Kan^r	64.3	48.8	45.8	52.97	30.0	27.1	22.7	26.60
MB10 Spc^r + 2001 Kan^r	41.0	31.9	40.5	37.79	24.0	31.8	23.9	26.57
Uninoculated	11.9	9.2	10.2	10.42				
Least significant difference ($P < 0.05$)[b]				17.89				NS

[a] Values are means of results from 10 plants.
[b] Least significant difference value for comparison of inoculum means. NS, Not significant.

soil and Leonard jars for mixed-strain inocula. Data for the double-strain inoculation treatments in Ottawa soil indicated modification of the relationship between introduced strains in terms of cultivar preferences, with cultivars Apollo, Saranac, and Vernal tending to show no preference for either MB10 Spc^r or 2011 Str^r. However, 2001 Kan^r remained the least successful inoculant strain, forming 5% or less of the nodules in all mixed inoculation treatments. Indigenous isolates accounted for between 31 and 53% of the nodules in all double-strain inoculum and cultivar combinations.

The frequency of doubly infected nodules (between 1 and 14%) involving inoculant strains in both soils was generally less than that in the Leonard jar experiment (between 9 and 25%). There were considerable differences in the morphology of *R. meliloti* growth on YEM between indigenous isolates from Lanark and Ottawa soils. All isolates from the former soil produced growth which was nongummy, opaque, and white in appearance, whereas indigenous isolates from Ottawa soil invariably formed growth which was translucent, misty in appearance, and accompanied by copious gum formation. The differences in growth morphology between 2011 Str^r or 2001 Kan^r (nongummy) and indigenous isolates from Ottawa soil (copious gum formation) permitted detection of double infections between either of these inoculant strains and native isolates. Such doubly infected nodules were encountered in most cultivar and inoculum combinations involving 2011 Str^r or 2001 Kan^r but generally at frequencies of <2% (data not presented).

In both soils there were no significant differences among cultivars Apollo, Saranac, and Vernal in preferences for *R. meliloti* in each inoculum as detected by analyses of variance

TABLE 3. Strain identity of nodules of *M. sativa* cvs. Apollo, Saranac, and Vernal grown in soil and inoculated with *R. meliloti* strains individually and in mixtures

Inoculum[a]	% Strain representation in nodules from[b]:														
	Apollo					Saranac					Vernal				
	I	Str	Spc	Kan	D	I	Str	Spc	Kan	D	I	Str	Spc	Kan	D
Lanark soil															
2011 Str^r	0	100	0	0	0	5	95	0	0	0	0	100	0	0	0
MB10 Spc^r	3	0	97	0	0	2	0	98	0	0	2	0	98	0	0
2001 Kan^r	7	0	0	93	0	4	0	0	96	0	3	0	0	97	0
2011 Str^r + MB10 Spc^r	0	23	66	0	11	0	26	60	0	14	1	17	71	0	11
2011 Str^r + 2001 Kan^r	1	90	0	2	7	0	97	0	1	2	0	96	0	3	1
MB10 Spc^r + 2001 Kan^r	0	0	96	1	3	0	0	95	3	2	1	0	94	3	2
Uninoculated	100	0	0	0		100	0	0	0		100	0	0	0	
Ottawa soil															
2011 Str^r	49	51	0	0	0	50	50	0	0	0	32	68	0	0	0
MB10 Spc^r	23	0	77	0	0	30	0	70	0	0	31	0	69	0	0
2001 Kan^r	77	0	0	23	0	80	0	0	20	0	76	0	0	24	0
2011 Str^r + MB10 Spc^r	31	31	30	0	8	40	20	33	0	7	34	23	36	0	7
2011 Str^r + 2001 Kan^r	53	43	0	2	2	45	52	0	0	3	46	49	0	3	2
MB10 Spc^r + 2001 Kan^r	38	0	54	5	3	38	0	60	1	1	38	0	59	2	1
Uninoculated	100	0	0	0		100	0	0	0		100	0	0	0	

[a] Percent strain representation in mixed inocula as for Table 1.
[b] Values are means of results from 10 plants. I, Indigenous isolates, Str = 2011 Str^r, Spc = MB10 Spc^r, Kan = 2001 Kan^r; D, double infections involving inoculant strains. Data represent 4,494 nodule isolates assayed for strain identity.

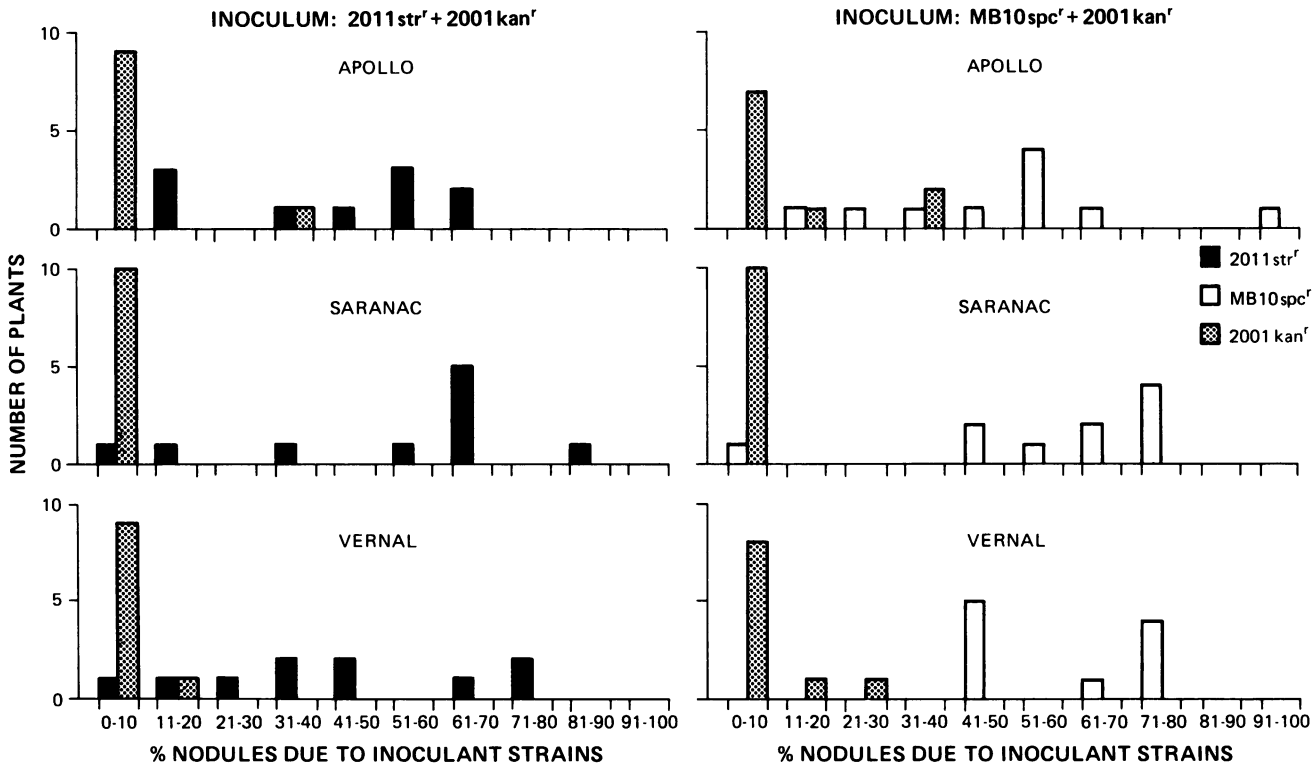

FIG. 1. Frequency distributions for two mixed inoculation treatments showing interplant variation in *R. meliloti* strain representation in nodules of cvs. Apollo, Saranac, and Vernal grown in Ottawa soil.

on the percentage of nodules formed by the most successful introduced strain (data transformed to angles). However, consistent with results of the Leonard jar experiment, individual plants within each cultivar showed considerable heterogeneity in preferences for *R. meliloti* in a particular inoculum. An example of this variation is given in Fig. 1 for two inoculation treatments in Ottawa soil.

Table 4 shows data for shoot dry weights and nodule numbers of *M. sativa* grown in soil and inoculated with *R. meliloti* individually and in mixtures. In Lanark soil all inoculum and cultivar combinations formed more ($P < 0.01$) shoot dry matter than uninoculated cultivars, indicating that indigenous *R. meliloti* in this soil were symbiotically ineffective. All double-strain inocula in this soil generally caused the plants to form more ($P < 0.05$) shoot dry matter than that formed by plants inoculated with the individual strains which produced the majority of nodules in each respective mixed inoculum. The exception was mixed inoculum 2011 Str^r plus 2001 Kan^r; shoot dry matter production by plants inoculated with this mixture did not differ significantly from shoot dry matter production by plants inoculated with 2011 Str^r. The shoot dry weights of all cultivar and inoculum combinations in Ottawa soil did not differ significantly from those of the uninoculated cultivars, indicating that indigenous *R. meliloti* in this soil were as symbiotically effective as the inoculant strains. There were few significant differences between inoculation treatments in this soil, although plants inoculated with 2001 Kan^r formed more ($P < 0.05$) shoot dry matter than all except plants inoculated with 2011 Str^r and the uninoculated cultivars. Overall, shoot dry matter production was greater in Ottawa than Lanark soil ($P < 0.01$), mainly due to ineffective nodulation by native *R. meliloti* in the uninoculated treatment of the latter soil. The interaction of inoculum and soil on shoot dry matter production indicated that the various inocula were generally more effective in Ottawa than Lanark soil ($P < 0.01$), although some mixed-strain inocula were less effective in the former soil.

Data for nodule numbers per plant showed that native *R. meliloti* in the uninoculated treatment of Lanark soil formed fewer nodules ($P < 0.01$) than all inocula except for 2011 Str^r and 2001 Kan^r. In contrast, uninoculated plants in Ottawa soil formed more ($P < 0.05$) nodules than those inoculated with 2011 Str^r, 2011 Str^r plus 2001 Kan^r, and MB10 Spc^r plus 2001 Kan^r. Nodule numbers for double-strain inocula in both soils generally did not differ significantly from those for the individual strains which formed the majority of nodules in each respective mixed inoculum. The exception was mixed inoculum MB10 Spc^r plus 2001 Kan^r, which formed more ($P < 0.01$) and fewer ($P < 0.05$) nodules than MB10 Spc^r in Lanark and Ottawa soils, respectively. Overall, fewer nodules were formed in Lanark than in Ottawa soil ($P < 0.01$), probably due to the small number of native *R. meliloti* in the former soil (5×10^1 g^{-1}). However, the interaction of inoculum and soil on nodule number ($P < 0.01$) indicated that some mixed-strain inocula formed more nodules in Lanark than Ottawa soil.

DISCUSSION

The cultivars of *M. sativa* used in this investigation and grown axenically or in soils containing indigenous rhizobia consistently showed the same preferences for *R. meliloti* in each particular inoculum. This contrasts with reports involving *Trifolium* spp. (13, 20, 24) and *M. sativa* (9, 10) which indicate different preferences by species or cultivars for specific strains of *Rhizobium* in mixed inocula. However, the present data for *M. sativa* showed considerable variation between individual plants within each cultivar in preferences for *R. meliloti* in a particular inoculum. Such interplant

variability was to be expected, since the cultivars of *M. sativa* used are cross-pollinated and as such each consists of a distinct population of genetically heterogeneous plants. This heterogeneity would tend to reduce differences between cultivars and may to some extent account for the similarity in preferences by cultivars Apollo, Saranac, and Vernal for *R. meliloti* in a particular inoculum. Similar intracultivar variation in preferences for strains of *R. trifolii* by cross-pollinated clover species have been reported (13, 20). A reduction in this type of variation would permit more uniform nodulation by a highly effective strain applied as inoculum and might be expected to enhance the ultimate efficiency of the symbiosis. Such uniformity within cultivars might possibly be achieved by means of an appropriate breeding program involving simultaneous selection of *M. sativa* and *R. meliloti* genotypes.

Johnson and Means (12) reported that relative success in nodulation of several strains of *Rhizobium japonicum* was similar under axenic conditions and in field soils containing few native rhizobia. With one exception, this is in agreement with present data for *R. meliloti*, for which the differences in nodulation success between inoculant strains (i.e., MB10 Spc^r > 2011 Str^r > 2001 Kan^r) were consistent in Leonard jars and soil. The exception involved one mixed inoculation treatment in Ottawa soil, in which 2011 Str^r tended to be as successful in nodulation as MB10 Spc^r on all cultivars. This may indicate that in this case the soil type or nature of the native *R. meliloti* population influenced *M. sativa* host preferences for introduced strains. van Rensburg and Strijdom (22) similarly reported that several paired strains of *R. meliloti* reacted consistently in different autoclaved soils, although a few varied in their reactions in some of the soils.

It is well known that the nodulation success of inoculated *Rhizobium* strains in soil is influenced by the size of the natural population (11, 19, 25, 26). Despite the use of a high inoculation rate, this effect was evident in the present investigation, in which the inoculant strains formed significantly more nodules in Lanark soil with few native *R. meliloti* than in Ottawa soil containing a large indigenous population. The absence of significant yield differences between inoculated and uninoculated treatments in Ottawa soil, due to effective nodulation by indigenous *R. meliloti*, emphasizes the importance of both qualitative and quantitative characteristics of the naturalized *Rhizobium* population in determining the requirement for inoculation and the ultimate benefit obtained from this practice.

Brockwell and Hely (3), in a survey of the symbiotic characteristics of *R. meliloti* isolated from effective nodules of different *Medicago* and *Melilotus* spp., found that although all isolates were able to nodulate *M. sativa*, several were poorly effective on this host. In the present work, the ineffective nodulation of uninoculated cultivars Apollo, Saranac, and Vernal in Lanark soil with no previous history of *M. sativa* may have been due to indigenous *R. meliloti* originating from naturally occurring *Medicago* or *Melilotus* spp., both of which were present in the area where the soil was collected.

Bromfield and Jones (4) reported that double-strain occupancy of nodules occurred rarely on *Trifolium* spp. grown in soil, accounting for less than 1% of the nodules typed. The present results for *M. sativa* grown in soil show much greater frequencies of nodules inhabited by two strains (up to 14%) and may be due, at least in part, to the rate of inoculation, which in this work exceeded 10^9 cells per pot (15). However, the present data for proportions of nodules containing two strains of *R. meliloti* on axenically grown

TABLE 4. Response of *M. sativa* cvs. Apollo, Saranac, and Vernal grown in soil to inoculation with *R. meliloti* strains individually and in mixtures

Inoculum	Shoot dry wt (mg/plant)[a]						Nodule no./plant[a]					
	Lanark soil			Ottawa soil			Lanark soil			Ottawa soil		
	Apollo	Saranac	Vernal	Apollo	Saranac	Vernal	Apollo	Saranac	Vernal	Apollo	Saranac	Vernal
2011 Str^r	62.9	53.7	48.1	71.2	61.3	56.1	11.1	9.8	11.5	14.6	17.7	15.5
MB10 Spc^r	49.6	39.6	44.0	62.6	59.2	53.8	10.7	14.5	14.0	25.4	24.1	17.5
2001 Kan^r	48.0	58.5	49.7	80.9	72.8	52.4	5.0	11.2	9.0	17.9	22.4	16.2
2011 Str^r + MB10 Spc^r	60.6	52.7	46.1	68.7	56.2	47.2	14.8	16.8	13.2	18.4	22.3	17.7
2011 Str^r + 2001 Kan^r	65.4	59.3	64.1	58.5	53.0	57.5	11.9	15.5	14.4	11.7	15.6	14.0
MB10 Spc^r + 2001 Kan^r	65.6	61.5	50.8	52.1	58.3	55.7	20.0	21.2	18.9	13.1	18.3	17.6
Uninoculated	20.4	13.9	17.2	63.4	59.8	60.6	8.5	9.4	5.5	18.0	22.0	21.6
Soil means	49.12			60.06			12.71			18.17		

Inoculum	Nodule no./plant[a]					
	Lanark soil			Ottawa soil		
	Apollo	Saranac	Vernal	Apollo	Saranac	Vernal
2011 Str^r	14.6	17.7	15.5	15.93		
MB10 Spc^r	25.4	24.1	17.5	22.33		
2001 Kan^r	17.9	22.4	16.2	18.83		
2011 Str^r + MB10 Spc^r	18.4	22.3	17.7	19.47		
2011 Str^r + 2001 Kan^r	11.7	15.6	14.0	13.77		
MB10 Spc^r + 2001 Kan^r	13.1	18.3	17.6	16.33		
Uninoculated	18.0	22.0	21.6	20.53		
Soil means	18.17					

[a] Values are means of results from 10 plants. The following are the least significant differences (LSD) ($P < 0.05$) for comparison of: (i) inoculum means, dry wt LSD = 8.58, nodule no. LSD = 2.64; (ii) cultivar means, dry wt LSD = 4.28, nodule no. LSD = 1.73; (iii) soil means, dry wt LSD = 3.49, nodule no. LSD = 1.41; (iv) inoculum means for each soil, dry wt LSD = 9.24, nodule no. LSD = 3.74.

plants (up to 25%) are in accord with those reported for other species of *Rhizobium* under similar conditions (4, 15).

Bordeleau and Antoun (2) reported increases in yield of *M. sativa* inoculated with certain paired strains of *R. meliloti* relative to *M. sativa* inoculated with each strain individually. The present data indicated similar significant synergistic effects by certain double-strain inocula on shoot dry matter production and nodule numbers in Lanark soil. Further evidence for this effect was provided by the interactions of inoculum and soil on yield parameters (shoot dry weight and nodule number) which indicated that certain double-strain inocula formed higher yields in Lanark than Ottawa soil ($P < 0.01$), although all remaining inoculation treatments were lower yielding in the former soil. Such additive effects in Lanark but not in Ottawa soil or the Leonard jar experiment suggest that the use of specific combinations of *R. meliloti* strains in the field inoculation of *M. sativa* might be beneficial only under certain circumstances.

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