1983

Summary of Medic-Rhizobium field experiments

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Summary of Medic-*Rhizobium* Field Experiments 1983/84

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Trials

83 ME 9 - Nutrition of *R. meliloti* in acid soil.

- Meaningful results only available in 1984/85

83 ME 87 - Effect of pH of culture conditions on survival in, and colonisation of, an acid soil by *R. meliloti*.

- Meaningful results available 1984/85.

82 ME 22)
82 ME 22A) Survival and colonisation of acid soils by 18 strains of *R. meliloti* in association with 6 *Medicago* hosts.
82 ME 23) - Results discussed in this summary.

Method

The 1982 trials were sown in 2 m rows with *Medicago* - *R. meliloti* treatments in factorial combination. This procedure merely served to introduce the bacteria into the soil in a manner similar to drill sown pastures. In 1983 inoculated seed of the same *Medicago* species and cultivar was sown along the original lines of 1982 to facilitate an estimate of survival of the bacteria at the point of inoculation. To estimate colonisation of the soil by the bacteria three cross rows were sown at right angles to, and across, the original line. Percentage of nodulated plants was then assessed as an indication of the presence of *R. meliloti*, at distances from the original row.

Details of the origin of the 18 R. meliloti strains used in this experiment are available in the 1982/83 summary. The trial was designed to develop field techniques for assessing the acid tolerance of a range of bacterial isolates. The soil was a brown-yellow, loamy-sand of pH 5.8 (1:5 aq).

In the second year, uninoculated Medicago seed was sown at 0 cm, 1-10 cm and 11-20 cm from the point of inoculation in the original year.

TABLE 1: The effect of R. meliloti strains on second year nodulation of Medicago sp. averaged over all hosts at 0, 1-10, and 11-20 cm from the initial point of inoculation (crown nodulation in parenthesis).

Strain	0 cm***		1-10 cm***		11-20 cm***	
Nil	11	(7)	8	(5)	4	(4)
U 45*	80	(65)	42	(32)	16	(11)
Na 2290	73	(56)	21	(12)	15	(10)
CC 169	73	(53)	32	(22)	12	(6)
WSM 244	77	(61)	24	(18)	9	(9)
NA 39**	73	(69)	32	(27)	9	(10)
WSM 232	58	(43)	27	(19)	9	(4)
241	69	(54)	22	(14)	8	(3)
303	66	(47)	37	(29)	17	(12)
324	78	(61)	35	(27)	9	(8)
329	77	(60)	38	(27)	15	(10)
383	74	(59)	37	(24)	15	(7)
397	76	(62)	43	(35)	27	(22)
407	68	(60)	40	(29)	22	(17)
413	89	(80)	48	(35)	30	(18)
419	78	(67)	46	(32)	36	(24)
429	78	(70)	40	(31)	27	(16)
441	77	(63)	40	(26)	15	(10)
LSD P < .05	15	(17)	17	(14)	12	(10)

* Corrected for Serena
** Corrected for Serena and Murex
*** Significant at P < .001

TABLE 2: The effect of host *Medicago* sp. on nodulation by *R. meliloti* averaged over all strains in the second year at 0, 1-10, 11-20 cm from the point of inoculation (crown nodulation in parathesis).

Host	0 cm***		1-10 cm***		11-20 cm***	
*M polymorpha** cv. serena	81	(75)	50	(40)	35	(25)
M tornata cv. swani	68	(46)	26	(15)	10	(4)
M tornata cv. tornafield	64	(45)	26	(17)	10	(6)
M littoralis cv. harbinger	65	(54)	21	(16)	4	(3)
M truncatula cv. cyprus	76	(63)	32	(23)	12	(7)
*M murex*** cv. murex	74	(63)	48	(36)	29	(21)
L.S.D. $P < .05$	8	(10)	10	(8)	7	(6)

* Corrected for U45 and NA39
** Corrected for NA39
*** Significant at $P < .001$

TABLE 3: Selected data for nodulation of hosts M. polymorpha, M. murex and M. tornata by R. meliloti in the 0, 1-10 and 11-20 cm sampling areas*

Strain	Nil	U45	Na 2290	CC 169	WSM 244	WSM 232	WSM 303	WSM 324	WSM 383	WSM 397	WSM 413	WSM 419	WSM 429
Sampling area													
				(a) M. polymorpha cv. Serena									
0 cm	29	-	85	51	100	98	98	80	47	91	100	91	67
1-10 cm	7	-	29	29	49	43	89	60	27	42	69	58	71
11-20 cm	2	-	29	5	38	22	44	25	27	31	78	60	62
				(b) M. murex									
0 cm	17	95	80	87	49	36	69	58	76	69	91	89	93
1-10 cm	10	47	27	40	32	36	40	58	64	42	71	89	71
11-20 cm	4	20	36	39	11	11	29	22	22	40	44	71	51
				(c) M. tornata cv. Swani									
0 cm	0	38	71	58	93	73	58	82	76	84	93	69	73
1-10 cm	0	18	10	20	13	24	22	31	39	42	38	44	22
11-20 cm	0	25	2	9	0	4	2	2	18	20	11	30	11

*L.S.D. $P < 0.1$ for 0 cm 31
1-10 cm 34
11-20 cm 25

Comments

1. Both strain and host differences in ability to form a successful symbiosis in this soil were identified.

2. Hosts M. polymorpha cv. Serena and M. murex showed a highly significant ability ($P < .001$) to nodulate away from the original source of inoculum more successfully than M. truncatula cv. Cyprus, M. tornata cv's Tornafield and Swani and M. littoralis cv. Harbinger. This ability to nodulate successfully when a minimum number of root nodule bacteria are present has important consequences if medics are to be established on soils in which R. meliloti has difficulty surviving.

3. Strain differences in ability to colonise the soil were also apparent, with isolates WSM 397, WSM 413, WSM 419 and WSM 429 showing a significantly greater saprophytic competence ($P < .001$) in acid soil than the commercially available inoculant strains U45 and CC169. A significant host-strain interaction ($P < 0.1$) with some isolates, eg. CC169 on Serena and Murex, indicates that certain symbiotic

relationships within the Medicago - R. meliloti association may be more tolerant of soil acidity than others. Hence, testing for acid tolerance of bacteria should be carried out with more than one host, and, when testing medics, more than one strain of R. meliloti should be used.

This experiment was designed to investigate whether amelioration of acid conditions by liming would enhance colonisation of the soil by R. meliloti. The strains and hosts used essentially comprised a subset of those in 82ME22, and the techniques of assessment were also the same. Results are presented in Table 4.

TABLE 4: Second year nodulation at distances 0 cm, 1-10 cm and 11-20 cm from the initial point of inoculation in lime treated soil.

	Nil	U45	WSM 244	WSM 397	WSM 413	WSM 429
(i) 0 cm						
Cyprus	65 (40)	65 (45)	100 (100)	97 (89)	100 (99)	100 (93)
Serena	47 (22)		100 (90)	99 (83)	100 (89)	93 (72)
Murex	70 (40	93 (65)	97 (93)	99 (90)	100 (95)	100 (100)
(ii) 0-10 cm						
Cyprus	29 (15)	37 (19)		79 (33)	87 (70)	85 (42)
Serena	9 (2)		73 (37)	60 (39)	85 (55)	67 (33)
Murex	60 (32)	90 (49)	59 (33)	100 (93)	93 (83)	70 (70)
(iii) 11-20 cm						
Cyprus	33 (15)	29 (19)	47 (27)	67 (43)	72 (37)	65 (45)
Serena	27 (17)		55 (30)	50 (30)	37 (13)	50 (25)
Murex	62 (25)	62 (37)	80 (70)	80 (49)	83 (50)	90 (45)

Comments

1. Nodulation of all hosts in the 11-20 cm region was rarely below 50%, which indicates that liming will overcome the problems with survival and colonisation of R. meliloti in this soil. The excellent nodulation obtained following liming implies that soil acidity is the factor most limiting proliferation of R. meliloti populations in this soil (as opposed to an effect of acidity on the nodulation process as such).

2. Despite amelioration of soil acidity, host Murex still showed a trend towards greater nodulation in the sampling areas more distant from the point of inoculation than M. truncatula (as in 82ME22) and, surprisingly, M. polymorpha.

3. Isolates WSM 413 and WSM 429 appeared to have colonised the 11-20 cm sampling region to a far greater extent than the commercial inoculant strain, even when acidity should not have been limiting. This suggests that the superior performance of isolates WSM 413 and WSM 429 in acid soils is due to a mechanism somewhat more fundamental than a tolerance of hydrogen ions.

82 ME 23

This trial was essentially the same as 82ME22, except that it involved a reduced number of Medicago hosts, and was carried out on a different soil type. The soil here was a red loam of pH 6.0 (1:5 aq). The results are presented in Table 5, and once again were gathered with the same techniques as used in trial 82ME22.

Comments

1. The superior saprophytic competence of the Sardinian isolates of R. meliloti identified in 82ME22, particularly strains WSM 397, WSM 413, WSM 419 and WSM 429, was again in evidence in this experiment. Nodulation in the acid soil away from the initial point of inoculation was particularly poor in the plots sown with commercial inoculant strains. Strain WSM 244 seemed to perform better in ths soil than in the lighter soil of experiment 82ME22.

2. M. polymorpha cv. Serena again displayed a greater ability to locate bacteria, and hence nodulate, than M. truncatula cv. Cyprus.

3. This trial, importantly, corroborates the results obtained in 82ME22, on a different soil type.

The set of experiments, as a whole, establish the technique used to differentiate acid tolerant strains of R. meliloti as one which can return highly significant results. The major drawback with the technique is that two growing seasons are required before any useful data can be collected.

TABLE 5. Nodulation of Serena and Cyprus in the second year at points 0 cm, 1-10 cm, and 11-20 cm from the source of inoculation in the first year (% of plants nodulated)

Strain of *R. meliloti*	0 cm		1-10 cm		11-20 cm	
	Serena	Cyprus	Serena	Cyprus	Serena	Cyp
Nil	27	10	20	7	14	0
U45	10	83	7	10	7	7
Na2290	85	53	24	10	10	0
CC169	90	70	90	23	7	0
WSM244	97	67	67	27	34	0
Na39	20	77	14	35	10	10
WSM232	77	67	80	13	24	0
WSM241	80	40	13	10	7	0
WSM303	83	44	67	17	44	10
WSM324	90	54	50	20	14	7
WSM329	90	74	37	33	14	7
WSM383	97	54	50	10	17	4
WSM397	100	70	47	24	44	0
WSM407	83	83	80	43	34	17
WSM413	94	77	60	34	33	34
WSM419	97	90	64	30	54	27
WSM429	87	93	54	40	40	20
WSM441	97	63	67	10	33	0