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PLANT RESEARCH DIVISION

1970 RESULTS OF FIELD EXPERIMENTS

D.L. CHATEL - RESEARCH OFFICER

SOIL MICROBIOLOGY

SUBTERRANEAN CLOVER INOCULATION EXPERIMENTS

Badgingarra and Newdegate

Comparisons between Rhizobium trifolii strain WU290 and possible replacement strains were continued. The work was conducted for the following reasons :

1. The need for information on "back-up" strains, particularly since the inoculant manufacturers had experienced difficulty in producing high-count peat cultures of WU290.
2. Strain CC2480a had proved very satisfactory in the Eastern States. Although it did not show up to advantage under our conditions in 1968, further testing was indicated.
3. The need for information on inoculation needs on old land.

LOCATIONS : Badgingarra and Newdegate Research Station.

1. Badgingarra - new land. 70BA16.
Grey sand overlying gravel, fallow since 1967.
2. Badgingarra - old land. 70BA17.
Similar to new land site, less gravel than above. Cleaned 65/66; fallow 1966, cereal 1967, serradella 1968. There was considerable self sown rose and sub clovers in 1968 and 1969.
3. Newdegate - new land. 70N18.
Grey sand and gravel over clay. Cleaned 1968.
4. Newdegate - old land. 70N19.
Grey sand over clay. Old Dwalganup country (about 10 years to 1966). Kondinin rose and barley 1968, barley 1969.

TREATMENTS :

Host plants - Daliak, Geraldton, Seaton Park and Woogenellup subterranean clovers on all sites. Yanninicum 39314YA and 39314YB at Newdegate new land site.

Inoculation - Nil and strains WU290, WA67 (current commercial strains), CC2480a and WU95.

RESULTS AND DISCUSSION :

Dry weight and nodulation data are presented in Tables 1-4.

New land : There was a significant response to inoculation on all hosts at both sites. WU290 was the superior strain overall; being the best or equal best on all hosts other than Woogenellup. At Badgingarra strains WU95 and CC2480a were superior on Woogenellup at both harvests. It is interesting to note that WU290 had replaced WU95 as the superior strain on Woogenellup at Newdegate by the second sampling occasion. Naturally, Woogenellup is not considered a starter at Newdegate; nevertheless this result introduces the need to consider more carefully the influence of sampling time on treatment effects.

Old land : These experiments gave markedly different results to those on new land. Small responses were obtained with CC2480a and WU95 on Woogenellup at Newdegate and with WA67 and WU95 on both Seaton Park and Woogenellup at Badgingarra. WU290 was not particularly promising in these competitive sites and consideration will have to be given to finding better strains for old clover country. As expected, the control nodulation was much better at Newdegate than at Badgingarra.

These experiments provided little insight into possible replacement strains. Strain WU95 could be used if ever a reserve strain is required for the sub clovers. (Unfortunately it is not very effective on the other Trifoliums.)

There is no urgency to replace WU290 since the manufacturers have largely overcome their problems with that strain.

3rd March, 1971.
DLC:LMM.

TABLE 1.

70BA16/2682 Ex

Inoculation of Sub. Clover - New Land
Badgingarra Research Station

(a) Top Yields (oven dry wt, 4 reps)First Sampling - Aug. 6 (mg/plant)

Host	Inoculum Strain					LSD	
	WU290	WA67	CC2480a	WU95	Nil	.01	.001
Daliak	184.5	165.3	142.3	160.8	47.8	35.1	49.7
Geraldton	168.3	142.0	131.0	115.0	46.3	43.7	61.8
Seaton Park	222.8	191.3	189.3	195.8	54.5	77.8	110.0
Woogenellup	123.3	117.5	150.8	171.0	59.8	46.4	65.6

Second Sampling - Sept. 21 (g/plant)

Host	Inoculum Strain					LSD	
	WU290	WA67	CC2480a	WU95	Nil	.05	.01
Daliak	3.592	3.655	2.624	2.685	0.887	1.230	1.725
Geraldton	3.240	3.035	2.637	2.059	0.734	0.624	0.875
Seaton Park	3.805	3.269	2.940	2.332	1.053	1.364	1.912
Woogenellup	2.110	2.127	2.763	3.066	0.898	1.118	-

(b) Nodulation - Aug. 6 (ranking from 1 = poor to 5 = best, based on nodule number and size; each value mean of 80 observations - 20 plants from each of the 4 reps.)

Host	Inoculum Strain					LSD	
	WU290	WA67	CC2480a	WU95	Nil	.01	.001
Daliak	3.96	3.36	3.72	3.64	1.22	1.0	1.4
Geraldton	3.91	3.70	3.85	4.03	1.23	0.5	0.7
Seaton Park	3.89	3.79	3.84	4.20	1.99	1.0	1.5
Woogenellup	3.77	3.69	4.12	3.68	1.70	0.8	1.1

TABLE 2.

70N18/2682 Ex

Inoculation of Sub. Clover - New Land
Newdegate Research Station

(a) Top Yields (oven dry wt, 4 reps)First Sampling - July 23 (mgm/plant)

Host	Inoculum Strain					LSD	
	WU290	WA67	CC2480a	WU95	Nil	.05	.01
Daliak	21.0	21.2	18.8	19.3	13.4	2.4	3.4
Geraldton	20.8	18.4	20.6	18.6	13.3	3.4	4.8
Seaton Park	31.7	24.3	25.9	24.8	15.5	3.3	4.6
Woogenellup	16.7	15.4	17.8	21.4	15.2	3.1	4.3
Yanninicum YA	30.3	25.8	24.2	22.8	20.7	N.S.	
Yanninicum YB	22.0	15.6	16.5	16.8	16.3	N.S.	

Second Sampling - October 1 (gm/plant)

Host	Inoculum Strain					LSD	
	WU290	WA67	CC2480a	WU95	Nil	.05	.01
Daliak	1.994	1.713	1.527	1.804	0.683	0.312	0.437
Geraldton	1.768	1.197	2.143	1.352	0.762	0.543	0.762
Seaton Park	2.020	1.185	2.199	2.078	1.019	N.S.	
Woogenellup	2.582	2.219	2.155	2.033	0.789	0.94	-

(b) Nodulation - July 23 (ranking from 1 = poor to 5 = best, based on nodule number and size; each value mean of 80 observations - 20 plants from each of the 4 reps.)

Host	Inoculum Strain					LSD	
	WU290	WA67	CC2480a	WU95	Nil	.01	.001
Daliak	3.45	3.24	2.71	3.22	1.12 (2.31)	0.64	0.90
Geraldton	3.00	3.07	3.09	3.05	0.95 (1.84)	1.01	1.43
Seaton Park	3.40	3.09	3.29	3.19	1.15 (2.47)	1.02	1.44
Woogenellup	1.95	2.13	2.96	2.77	0.84 (2.47)	0.75	1.07
Woogenellup	(3.66)	(3.43)	(3.44)	(3.76)	(2.47)	-	-
Yanninicum YA	2.70	2.60	3.20	2.60	0.81	0.62	0.88
Yanninicum YB	2.30	2.20	2.80	2.60	0.61	0.70	0.99

Figures in parentheses refer to second sampling occasion.

TABLE 3.
70BA17/2682 Ex
Inoculation of Sub. Clover - Old Land
Badgingarra Research Station

(a) Top Yields Aug. 19 (oven dry wt, mg/plant; 4 reps)

Host	Inoculum Strain					LSD .05
	WU290	WA67	CC2480a	WU95	Nil	
Daliak	43.0	52.6	42.9	54.2	47.1	N.S.
Geraldton	41.8	51.8	49.0	56.8	53.9	N.S.
Seaton Park	36.5	48.5	40.0	50.1	38.5	9.6
Woogenellup	27.5	44.2	36.2	45.3	35.4	11.7

(b) Nodulation (ranking from 1 = poor to 5 = best, based on nodule number and size; each value mean of 80 observations - 20 plants from each of the 4 reps).

Host	Inoculum Strain					LSD
	WU290	WA67	CC2480a	WU95	Nil	
Daliak	3.32	3.37	3.35	3.66	3.25	N.S.
Geraldton	3.81	3.74	3.62	3.97	3.82	N.S.
Seaton Park	3.48	3.57	3.27	3.91	3.47	N.S.
Woogenellup	2.87	3.04	2.75	3.38	3.04	N.S.

TABLE 4.
70N19/2682 Ex
Inoculation of Sub. Clover - Old Land
Newdegate Research Station

(a) Top Yields - August 27 (oven dry wt, mg/plant; 4 reps)

Host	Inoculum Strain					LSD	
	WU290	WA67	CC2480a	WU95	Nil	:05	:01
Daliak	237.7	225.2	243.6	263.3	241.5	N.S.	
Geraldton	227.6	251.0	246.2	289.5	249.0	N.S.	
Seaton Park	224.4	265.5	304.8	298.5	287.2	N.S.	
Woogenellup	184.6	220.6	243.5	255.0	198.1	34.7	48.6

(b) Nodulation (ranking from 1 = poor to 5 = best, based on nodule number and size; each value mean of 80 observations - 20 plants from each of 4 reps).

Host	Inoculum Strain					LSD .05
	WU290	WA67	CC2480a	WU95	Nil	
Daliak	4.80	4.60	4.68	4.75	4.64	N.S.
Geraldton	4.47	4.46	4.33	4.50	4.55	N.S.
Seaton Park	4.69	4.72	4.79	4.80	4.87	N.S.
Woogenellup	4.08	4.45	4.25	4.18	4.68	0.39

LUPIN INOCULATION

Badgingarra

A number of poorly nodulated lupin stands examined since 1968 have characteristically been made up of a mixture of healthy, nodulated plants and poorly nodulated plants (including no nodules).

This situation could, in addition to other things, be an expression of low numbers of rhizobia, which might result from either faulty inoculation techniques, poor rhizobial survival, or poor quality commercial peats.

Past inoculant quality surveys have indicated the possibility that the populations of rhizobia in peats purchased by farmers vary from 10^9 per gram to below 10^6 per gram. Those farmers fortunate enough to purchase a high-count peat are more likely to grow healthy lupins than those with low-count peats.

An experiment was conducted in which lupins were inoculated at different rates; the highest being equal to the highest any farmer could hope to obtain.

LOCATION :

Badgingarra - new land. 70BA18.
Grey sand overlying gravel, fallow since 1967.

TREATMENTS :

Hosts - Lupinus cosentini (W.A. Blue)
L. angustifolius (Uniwhite)
L. luteus (Weiko)

Rhizobia - WU425 and W72 (Commercial peat obtained from the manufacturer).

Rates -

- (1) = R = Highest rate, approx. 10^5 per seed;
- (2) = R/10;
- (3) = R/1,000;
- (4) = R/10,000;
- (5) = Nil.

Sowing - Lime pelleted (including uninoculated) seed sown into rows following hand broadcasting and raking in of fertiliser.

Fertiliser - Copper 2 lb/ac; plain super 500 lb/ac;
zinc 0.75 lb/ac; muriate potash 50 lb/ac.

RESULTS AND DISCUSSION :

Dry weights of plant tops and nodulation data are presented in Table 5.

Yields : There was an inoculation response with both rhizobia on all host plants. This was generally confined to the highest inoculation rate.

Nodulation : Virtually all the W.A. Blue plants nodulated very early, irrespective of inoculation treatment. However, this was not reflected in yield and serves to indicate that even on the R. lupini ridden West Midland sandplain inoculation responses can be obtained - with W.A. Blue.

The nodulation patterns of both Weiko and Uniwhite were similar, with a tendency for the former to be superior. Nodulation was close to optimal at the highest inoculation rate; from which point there was a dramatic decline. It is interesting to note the apparent effect of rate of inoculation at the lowest rates - particularly with Weiko. This was not reflected in top yields.

.. /2.

(These lupins were sown quite late, June 16. Perhaps an earlier sowing would have resulted in higher yields and a better chance of nodulation differences being reflected in top yields.)

If rate R/10 and lower rates typify average farmer practice, then it is not surprising that nodulation problems are being encountered.

The quality standards for peat cultures are being more closely checked these days. It is possible that more attention to reliable and improved inoculation techniques for lupins might give less troublesome lupin stands.

3rd March, 1971.
DLC:LMM.

TABLE 5.

70BA18 - 2682 Ex

Rates of Inoculation on Lupins
Badgingarra Research Station

(a) Top Yields (oven dry weights, g per plant)

Rate	Host Plant and Inoculum Strain					
	W.A. Blue		Weiko		Uniwhite	
	WU425	W72	WU425	W72	WU425	W72
R	1.80	1.70	1.20	1.60	1.60	1.60
R/10	1.10	1.60	0.68	1.10	1.00	1.10
R/1,000	1.60	1.10	0.82	0.80	1.40	1.10
R/10,000	1.00	1.10	0.70	0.72	0.81	0.77
Nil	1.10		0.57		0.95	
LSD - 0.05	0.42	0.28	0.32	0.25	0.48	0.49
0.01	0.58	0.39	0.45	0.35	-	-

(b) Nodulation (per cent.; each observation based on examination of 80 plants)

Inoc. Strain	Rate Inoc.	Host Plant and Nodulation Category *								
		W.A. Blue			Weiko			Uniwhite		
		Early	Late	None	Early	Late	None	Early	Late	None
WU425	R	100	0	0	97.5	11.2	11.2	88.7	11.2	0
	R/10	97.5	2.5	0	43.3	26.7	30.0	28.7	52.5	18.7
	R/1,000	100	0	0	33.7	30.0	43.7	42.5	47.5	10.0
	R/10,000	100	0	0	36.2	12.5	51.2	26.2	61.2	12.5
W72	R	100	0	0	100	0	0	97.5	2.5	0
	R/10	96.2	3.7	0	65.0	20.0	15.0	41.2	53.7	5.0
	R/1,000	98.7	1.2	0	56.2	16.2	27.5	41.2	57.5	1.2
	R/10,000	98.7	1.2	0	40.0	10.0	50.0	25.0	58.7	16.2
Nil		95.0	3.7	1.2	22.5	7.5	70.0	23.7	65.0	11.2

* Early = nodules on upper tap-root (0 - 4 in.).
Late = lower tap-root nodules.
None = not nodulated.

LUCERNE INOCULATION

Esperance

Little is known of the need to inoculate lucerne sown on to old lucerne country at Esperance. An experiment was conducted during 1970 with the objective of investigating the requirement of lucerne sown on old land for inoculation, lime and superphosphate. This report refers mainly to inoculation responses; the fertiliser aspects will be covered by Mr Nicholas.

LOCATION :

Esperance Downs Research Station.
On Fleming gravelly sand (grey fine sand over gravel).
Vegetation - Chittick and blue mallee. Sown to lucerne 1959. Cereal trials 1969.

TREATMENTS :

Fertiliser - Nil (sand), superphosphate (50, 100 lb/ac),
lime (50, 100 lb/ac), 50/50 lime super (100, 300).

Sowing -

- (1) Seed and fertiliser mixed.
- (2) Seed sown through small seed box.

Inoculation - with and without.

Sampling - 2 x 10 plants from each of three reps.

RESULTS AND DISCUSSION :

Results are presented in Table 6.

There was a marked response to inoculation (based on nodulation date - not on plant size). The very poor nodulation of the uninoculated plants showed that there were very few R. meliloti in the soil. This is surprising for an old stand.

The nodulation of the inoculated plants varied with the treatment. The poor nodulation (per cent. plants nodulated and numbers of nodules) of the super-seed mixture was surprising. A major objective of a lime pellet is that it protects the rhizobi from the acid superphosphate. It would seem that such was not the case in this experiment.

The results suggest that the seed was not adequately pelleted. Sample fertiliser and seed mixtures sent to Perth from Esperance verify this conclusion. It is interesting to note the improved nodulation when seed was sown through the small seeds box - away from the fertiliser. The best nodulation was with the lime-super mixtures.

The nodulation of even the best nodulated plants left much to be desired. The nodules were quite small (some appeared to have dried). The numbers of nodules were quite small (ranging from virtually none to a "high" of around 2.5 per plant). The reasons for this poor performance are not clear - even when allowance is made for the poor recovery of root material. Perhaps the faulty pelleting also reflected faulty inoculation.

Nodulation differences were not reflected in top growth, whether measured by weighing dried tops or estimated visually. Top growth was more a reflection of superphosphate differences.

3rd March, 1971.
DLC:LMM.

TABLE 6.

70E17/2948 Ex

Lucerne Establishment

Treatment ^a	Yield ^b mg/pl. (O.D.)	Visual ^c rank- ing	Plants Nodulated (%)			Number of Nodules			
			Total	Early	Late	Total	Tap-root	Inner Lateral	Outer Lateral
(1) <u>Inoculated</u>									
Sand	296	2.93	73	40	65	124	45 (36.3)	65 (52.4)	14 (11.3)
Super - low	-	3.39							
Super - high	397	3.67	43	25	37	61	19 (31.1)	22 (36.1)	20 (33.3)
Lime - low	-	2.78							
Lime - high	267	2.67	70	45	57	95	38 (40.0)	37 (38.9)	20 (21.0)
Lime/super - low	406	4.00	82	60	62	145	64 (44.1)	62 (42.7)	19 (13.1)
Lime/super - high	423	4.11	82	35	82	142	33 (23.2)	89 (62.7)	20 (14.1)
Super - high (S.B.)	435	4.39	68	38	42	103	36 (34.9)	53 (51.4)	14 (13.6)
Lime/super - high (S.B.)	471	4.27	67	38	48	71	33 (46.5)	33 (46.5)	5 (7.0)
(2) <u>Not Inoculated</u>									
Sand	347	2.55	15	5	12	13	6	3	4
Super - high	449	3.67	25	0	25	31	0	11	20
Lime - high	317	2.55	3	1	1	3	1	1	1
Lime/super - high	447	4.17	17	0	17	11	0	4	6

a. Seed mixed with fertiliser (or sand) prior to sowing.
S.B. = sown through small seeds box - hoses left off.

b. The weights are the means of 60 plants.

c. Based on a ranking of 1 - 5 (1 = poorest, 5 = best). Three observers.

Sown September 18, examined November 11.

Figures in parentheses are percentages of the total nodules (each value based on 60 plants).

1. COATING MATERIALS

The results of a pelleting experiment with sub. clover are presented below. Commercial peat inoculum and methofas were used. Dried pellets were kept in capped jars.

Numbers of Rhizobia on Seed

Treatment	Days from inoculation				
	0	7	14	21	52
MAF lime	2,400	750	79	135	31
Local lime 1	79	-	38	38	17
2	585	1,000	58	59	58
3	1,000	79	66	58	-
Kaolin (pH 7.2)	1,840	135	170	135	17
Dry peat	3,450	455	170	380	324
No coating	1,705	1,840	79	135	135

The three local limes were not particularly promising, neither was the neutral kaolin. Dry peat (sterile, as used in peat cultures) is not a practical proposition.

Since this experiment was conducted an additional lime has been tested. It is a grey colour and will be sold during the 1971 season. It has proved the equal of MAF lime in a number of small laboratory experiments.

2. STICKING MATERIALS

It is well known that lucerne is particularly difficult to pellet well. Quite often the pellets disintegrate when bags of pelleted seed are moved - or when the seed is mixed with fertiliser.

In a pilot experiment, using recommended rates, only 25 per cent. of the lime pellet remained on methofas treated lucerne after very harsh treatment (shaking a few seed vigorously in an empty bottle). The corresponding value for gum arabic treated seed was 73 per cent. Unfortunately gum arabic has become too expensive for general use.

It appears that the differential sticking of the gums is related to their "wettability".

Further work will be done with alternative gums (5 per cent. gelatine is promising), and wetting agents.

3. GUM CONCENTRATION

Lime pelleting is a costly business. It is to be hoped that cheaper/quicker sowing techniques will be developed that do not require lime pellets.

If lime is not required but inoculation is, then it appears that weaker gum solutions can be used to stick the peat to the seed (peat dusting or water-slurry inoculation is very inefficient).

Some success has been achieved using half strength methofas in half quantities, requiring a quarter the normal gum. The peat adheres very strongly to the seed and the dried seed readily separate. Full strength gum without lime results in a seed-germ conglomerate.

3rd March, 1971.
DLC:LMM.