1984

Summary of Rhizobium experiments

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Summary of Rhizobium experiments in 1984/85

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Introduction

The main emphasis for rhizobial work in 1984 was on the acid tolerance of medic *Rhizobium*. The experimental programme in the field was greatly expanded to include testing of some 160 isolates collected from Sardinia in May 1984. Broadacre sowings of acid tolerant strain WSM 419 were undertaken as a preliminary step to its commercial release in 1985. Glasshouse work in 1984 was dedicated to a more intensive investigation of the factors contributing to the enhanced nodulating ability of *M. polymorpha* and *M. murex* on acid soils. As well, a simple technique for identifying acid tolerant strains of rhizobia in soil in the glasshouse was developed in a joint project with UWA.

Field Trials

Field trials discussed in this summary:

83 ME 9	-	Nutrition of *R. meliloti* in acid soil
84 ME 33	-	Isolation of *R. meliloti* from acid soils onto low pH media
84 ME 34	-	Colonisation of acid soil by *R. meliloti*
84 ME 35	-	Effectiveness of CC 169, WSM 419 and WSM 244 on *Medicago* sp.
84 ME 32) 84 WH 24) 84 N 18)	-	Persistence in acid soils of four acid tolerant strains of *R. meliloti*
84 AL 42		Delayed nodulation of south coast lupins

83 ME 9 Nutrition of *R. meliloti* on acid soil

Background

Nutritional stress of *R. meliloti* in acid soils may limit reproduction and survival of the bacteria. The literature indicates calcium, magnesium, phosphorus and molybdenum to be essential for rhizobial growth. These elements are commonly less abundant in acid soils than neutral soils.

Aim

To assess the growth response of *R. meliloti*, as identified by plant nodulation, to soil applications of Ca, Mg, Mo and P.

Treatments

$CaCO_3$ (2.5 t/ha), $CaCO_3$ + Mo (400 g/ha), $CaCO_3$ + low P (100 kg/ha), $MgSO_4.7H_2O$ (300 kg/ha), $CaSO.2H_2O$ (300 kg/ha), $CaCl_2.2H_2O$ (256 kg/ha), MoO_3 (400 g/ha), low P (100 kg/ha), Ca + Mg (50 kg div. cations/ha), Nil (super 300 kg/ha).

Results

Data were collected for six nodulation parameters, vis;

(i) number of small nodules/plant
(ii) number of medium nodules/plant
(iii) total number of nodules/plant
(iv) percentage of plants with lateral nodules
(v) percentage of plants with tap root nodules
(vi) percentage of plants nodulated.

	Percentage of plants nodulated			No. of nodules/plant		
	tap	lateral	total	small	medium	total
$CaCO_3$	79.0	70.2	96.5	3.67	0.9	4.64
$CaCO_3$ + MoO_3	67.2	76.9	95.3	2.71	1.2	3.86
$CaCO_3$ + low P	78.2	66.7	93.6	2.86	0.5	3.34
$MgSO_4.7H_2O$	24.6	65.5	77.2	1.12	0.5	1.62
$CaSO_4.2H_2O$	33.3	58.7	74.2	1.61	0.6	2.20
$CaCl_2.2H_2O$	22.8	61.2	72.6	1.04	0.4	1.45
MoO_3	22.5	63.6	72.1	2.0	0.5	2.51
low P	25.0	48.1	68.1	1.41	0.4	1.78
Ca + Mg	27.8	68	80.0	1.28	0.7	1.97
Nil	19.5	45.4	62.2	0.79	0.4	1.15
LSD (P = 0.05)	19.5	22	19.5	1.24	0.66	1.39

Comments

Application of lime to the soil raised the pH from 5.4 to 6.7 and markedly stimulated nodulation in the second year. Four of the six parameters used to assess nodulation indicated that rhizobial survival was greater in the limed plots than in the nil treatments ($P < 0.05$). The greatest effect of adding lime can be seen in the tap nodulation, where the percentage of plants which formed tap root nodules increased from around 20 to 30% in unlimed plots to approximately 70-80% in limed plots. Lime did not stimulate lateral nodulation where the low P treatment was imposed which indicates that rhizobial growth may be restricted when the soil level of P is low.

Application of Ca and Mg to the soil together increased the percentage of plants which formed lateral nodules, although neither Ca nor Mg increased nodulation where applied individually. This result supports the hypothesis that a low level of divalent cations under conditions of moderate soil acidity may reduce rhizobial survival. Application of MoO_3, which produced a yield response in the first year at this site, tended to give an increase in nodule number, although this was not statistically significant at the 5% level.

It is apparent from these results that soil acidity, more so than deficiencies of Ca, Mg, Mo and P limits rhizobial survival in this soil. The data indicates, however, that nodulation may be reduced by a low soil level of P, and increased by application of Ca, Mg and Mo to moderately acid soils. This could be further investigated in the glashouse. The low level of tap root nodulation in all but the plus lime treatments gives strong evidence that nodulation in regenerating medic pastures on this soil type will not be improved by soil application of calcium and magnesium.

84 ME 33 Isolation of *R. meliloti* from acid soils onto low pH media

Background

Isolation of rhizobia from nodules is conventionally undertaken with media of neutral pH. This process may inadvertently select against rhizobial genotypes with intrinsic acid tolerance.

A collection of *Rhizobium* from Sardinia in 1984 resulted in some 160 strains being isolated and grown at low pH. These will be field evaluated for their acid tolerance and compared to strains from the same collection isolated on neutral media.

Aims

1. To assess the acid tolerance of strains of *R. meliloti* isolated and grown on low pH media.

2. To compare the acid tolerance of strains of *R. meliloti* originating from the same nodule but isolated and grown at low or high pH.

Treatments

Thirty-two strains of *R. meliloti* (WSM 528-559), WSM 419, uninoculated control x 2 *Medicago* hosts - Serena and *M. murex*. Treatments to be sown in 2m rows, followed by cross rows in 1985.

	pH of isolating medium			
	5.4	5.7	6.1	7.0
Number of isolates	6	12	10	3

Strains WSM 544/534, WSM 549/533 and WSM 558/559 represent combinations of strains grown from the same nodule but at high/low pH. Ten strains were inoculated from agar slopes, and 23 from sterile peats impregnated with the rhizobia.

Results

First year data gives an indication of the effectiveness of the isolates in acid soil. The table below gives dry weights for 0.5 m of row, plus the pH of the medium used for isolation, for those strains which produced a yield similar to that of control strain WSM 419. Unfortunately, those strains inoculated from agar slopes did not survive to nodulate in the acid soil of this experiment.

Strain	Wt (g)	Origin (Site)	pH
Nil	7.4	-	-
WSM 419	12.7	-	neutral
WSM 531	12.6	JGH 67.B	6.1
WSM 532	11.8	" 58	5.7
WSM 534	10.0	" 57	5.7
WSM 535	11.2	" 44	5.7
WSM 537	12.6	" 46	6.1
WSM 538	11.5	" 44	6.1
WSM 541	13.1	" 39	6.1
WSM 542	11.4	" 49	6.1
WSM 543	12.3	" 49	5.7
WSM 547	12.8	" 63	5.7
WSM 548	10.0	" 63	6.1
WSM 549	15.2	" 58	neutral
LSD (P = 0.05)	4.4		

The lateral movement of strains in the soil will be assessed in 1986 as the major indication of the saprophytic competence of these isolates.

84 ME 34 Colonisation of acid soil by *R. meliloti*

Background

The pre-1984 collection of *R. meliloti* stored at the WA Department of Agriculture was screened for acid tolerant strains during 1983 and 1984 using a low pH artificial medium. Few strains gave any indication of being superior to WSM 419, however it was felt necessary to assess the acid tolerance of some strains in the field to verify laboratory results.

Aim

To assess the field acid tolerance of 22 strains of *R. meliloti* pre-screened in the laboratory on an acidic medium.

Treatments

Twenty-two strains x 2 hosts (Serena, *M. murex*) to be sown in 2 m rows, followed by cross rows in 1985.

Results

Sampling for yield and nodulation in 1984 has provided an estimate of effectiveness of the isolates under conditions of soil acidity. Cross rows will be sown in 1985 to measure the lateral spread of the rhizobia.

Dry weight (g/metre)

Strain	Nil		CC169	419	72	207	261	286	288	292	307	377
Murex	10.6		13.4	14.1	8.7	7.7	11.7	11.6	9.3	7.1	10.9	15.1
Serena	9.9		12.3	15.2	8.5	8.2	13.6	11.6	10.7	8.4	6.7	15.0

Strain	379	386	387	393	395	403	407	411	413	421	483	419 str.
Murex	14.6	9.3	14.7	15.6	14.3	15.9	18.7	14.4	17.9	9.6	11.0	17.1
Serena	12.0	11.9	15.5	17.4	12.0	16.2	17.4	11.7	20.0	16.9	11.5	12.8

LSD (P = 0.05) 6.2

Nodulation

Assessment was made of total, tap and lateral nodulation patterns.

Total nodulation (nodules/plant)

Strain	Nil	CC169	419	261	377	379	387	393
Murex	2.3	100	96	117	91	125	118	126
Serena	0.2	61	69	73	62	63	82	61

		395	403	407	411	413	421	419 str.
Murex		103	109	93	71	109	67	81
Serena		43	81	110	115	99	92	100

LSD ($P = 0.05$) 42

Comment

Several associations were ineffective on one or both hosts (WSM 72, WSM 207 and WSM 307). Strain WSM 413 produced a greater yield of Serena than did CC169, and WSM 407 produced a greater yield of M. murex than did CC169 ($P < 0.05$).

When averaged over all strains, M. murex produced a greater number of nodules in the acid soil than did Serena (98:70, $P < 0.001$). The difference in number of nodules is largely accounted for in the lateral nodulation where M. murex averaged 38 nodules per plant whilst Serena averaged 12 nodules per plant ($P < 0.001$).

Strain WSM 413 produced a greater number of nodules per plant than did CC169 on Serena ($P < 0.05$). This correlates well with the yield advantage of Serena inoculated with WSM 413 over CC169. Strain WSM 403 also produced more nodules per plant on Serena than did CC169.

84 ME 35 Effectiveness of CC 169, WSM 419 and WSM 244 on *Medicago* sp.

Background

There is doubt surrounding the effectiveness of the 1984 Group A commercial strain on *M. littoralis*. Several strains of *Rimeliloti* which show potential as replacement strains for commercial users should be assessed for their effectiveness on a range of *Medicago* species.

Aims

1. To assess the nitrogen fixing ability of the commercial medic inoculant strain, CC169, on *M. littoralis*.

2. To compare the effectiveness of three morphologically distinct types of strain WSM 244, and several potential commercial strains, on a range of *Medicago* hosts.

Treatments

Seven strains of *R. meliloti* x 6 *Medicago* hosts.

Strains:
- (i) CC169
- (ii) WSM 419
- (iii) WSM 413
- (iv) WSM 244A
- (v) WSM 244B
- (vi) WSM 244C
- (vii) Nil

Hosts:
- *M. polymorpha* cv. Serena
- *M. murex*
- *M. littoralis* cv. Harbinger
- *M. tornata* cv. Swani
- *M. littoralis* ecotype 1
- *M. littoralis* ecotype 2

Results

1. Yields

Spring cut dry wt. (g/metre row)

	Serena	Murex	Harbinger	Swani	M. litt(1)	M. litt(2)
Nil	24	32	24	21	13	21
CC169	64	61	69	65	14	17
WSM 419	84	68	87	70	23	22
WSM 413	79	77	76	54	32	24
WSM 244A	58	58	106	66	20	14
WSM 244B	92	53	55	70	17	18
WSM 244C	63	54	71	58	14	10

LSD (P = 0.05) 24.

Winter cut dry wt. (g/metre row)

	Serena	Murex	Harbinger	Swani	M. litt(1)	M. litt(2)
Nil	4.0	5.7	5.2	3.2	3.5	4.5
CC169	9.1	9.1	9.0	4.9	3.4	6.2
WSM 419	7.7	9.9	9.6	6.8	7.3	4.9
WSM 413	9.8	11.6	10.0	3.9	6.3	4.9
WSM 244A	6.4	8.9	11.6	8.6	6.1	5.7
WSM 244B	9.6	8.9	9.6	5.8	3.6	5.2
WSM 244C	7.6	8.7	7.1	8.1	6.4	6.5

LSD (P = 0.05) 4.1

2. Nodulation

Strains	Hosts (No. nodules/plant)					
	Serena	Murex	Harbinger	Swani	M. litt(1)	M. litt(2)
Nil	3	8	3	0	0	0
CC169	79	102	62	66	16	25
WSM 419	57	105	66	55	40	29
WSM 413	66	99	67	70	42	39
WSM 244A	86	97	74	74	42	20
WSM 244B	165	130	40	43	33	21
WSM 244C	60	84	42	41	33	30

LSD (P = 0.05) 47

Comments

The M. littoralis ecotypes formed ineffective associations with all R. meliloti strains tested. No strain produced a greater yield of these medics than was obtained in the uninoculated treatment. The commonly grown medic Harbinger, which is a M. littoralis x M. truncatula cross, formed sub-optimal associations with all strains except WSM 244A ($P < 0.05$). This indicates that Harbinger is not yielding to its full potential with the present commercial inoculant.

Serena yielded better with strain 244B than with CC169 ($P < 0.05$) at the spring cut, a result that is echoed in the nodulation data. There was a low correlation ($r^2 = 0.34$) between the number of nodules and dry matter yield. Acid tolerant strain WSM 419 proved to be effective and promiscuous on a wide host range.

The hosts can be grouped into three pairs based on the level of nodulation achieved in this acid soil (pH 5.4). Serena and *M. murex* formed more nodules than Harbinger and Swani, which themselves formed more nodules than *M. littoralis* ecotypes ($P < 0.05$). This is additional evidence of the suitability of *M. polymorpha* and *M. murex* to acid soils. The ability to nodulate freely in acid soil may be critical in regenerating stands of medics where the number of rhizobia is likely to be low relative to the number found on inoculated seed.

84 ME 32, 84 WH 24, 84 N 18 Persistence in acid soils of four acid tolerant strains of *R. meliloti*

Background

Four strains of *R. meliloti* were identified as possessing enhanced saprophytic competence in an acid soil following 1983 trial work. These bacteria need to be assessed over a range of soil types of varying acidity so the Department of Agriculture may formulate recommendations for future medic sowings.

Aim

To assess the suitability of a range of soil types for regenerative medic pasture sown with four strains of acid tolerant *R. meliloti*, in comparison with the commercial inoculant strain.

Treatments

Strains: WSM 419, WSM 413, WSM 397, WSM 429 and CC169

Hosts: *M. polymorpha* and *M. murex*

Soil pH

84 ME 32 - 4.9
84 WH 24 - 4.6
84 N 18 - 5.1

Assessment

Nodulation of 20 randomly selected plants in 1984. Quadrat cuts for spring yield, quadrat samples for nodulation (intensive) and seed yield in 1985.

Results

84 N 18: Trial decimated by aphids when at the seedling stage. Plots may be resown in 1985.

84 ME 32: Plots unevenly sown. Carry-over of seed (and rhizobia) into adjacent plots, placement of seed outside plot markings.

84 ME 24: 25% of plots poorly sown. Large diversity of established plants per square metre.

Comments

Establishment of these trials was poor, mainly due to problems with the cone seeder. Data collected in 1985 will be of limited value, however, some indication should be gained of the relative performance of the strains on these soil types. Nodulation assessment in 1984 of the Merredin trial showed some 40% of Murex plants and 30% of Serena plants in the nil uninoculated treatments to be nodulated. This probably indicates carry-over of rhizobia from adjacent plots.

84 AL 42 Delayed nodulation of south coast lupins

Background

Lupin yields on some southern soils are poor. Late nodulation has been implicated as possibly contributing to this problem. A collection of Br. lupini isolates was made in 1982 from wild serradella plants found growing well on problem soils in the Albany region. Following assessment of their effectiveness, eight strains were used to inoculate lupins and serradella for examination of nodulation patterns in the field.

Aim

To investigate the rate of nodulation induced by eight strains of Br. lupini on lupins and serradella grown on the south coast.

Treatments

Nine strains of Br. lupini : WSM 467, WSN 468, WSM 469, WSM 470, WSM 471, WSM 473, WSM 477, WSM 479 and WU 425.

On two hosts: L. angustifolius cv. Chittick
O. compressus cv. Pittman

Results

Establishment of the trial was poor because of problems with the cone seeder. This precluded yield assessment being obtained from the lupin plots, and any assessment of the serradella plots (sown 15 cm deep).

Figure 1 below illustrates the nodulation patterns obtained with four of the isolates. WSM 479 and WSM 471 produced earlier nodulation of the lupins, although differences are not significant. These promising results prompted further investigation of strains under controlled conditions in the phytotron. Figures 2 and 3 illustrate the rate of nodulation of serradella achieved at temperatures 7-14°C (Fig. 2) and 12-18°C (Fig. 3). Strains WSM 470, WSM 473 and WSM 479 induced the formation of nodules in a shorter amount of time after inoculation than did the commercial inoculant strain WU 425. At 7-14°C, when all nine plants inoculated with strain WSM 473 had nodulated, only three plants inoculated with WU 425 had nodulated. Complete nodulation by strain WU 425 was achieved some eight days later. At 12-18°C, 90% of WSM 479 inoculated plants had nodulated when only 20% of WU 425 plants had. A further week was required before all WU 425 plants had nodulated. Dry weights of plants harvested at 25 days in th 12-18°C treatment are given below.

Strain	Dry weight (mean of 9 plants, µg)
WSM 425	63
WSM 469	52
WSM 470	80
WSM 471	83
WSM 473	89
WSM 479	97
LSD (P = 0.05)	17

FIGURE 1: 84AL42 RATE OF LUPIN NODULATION

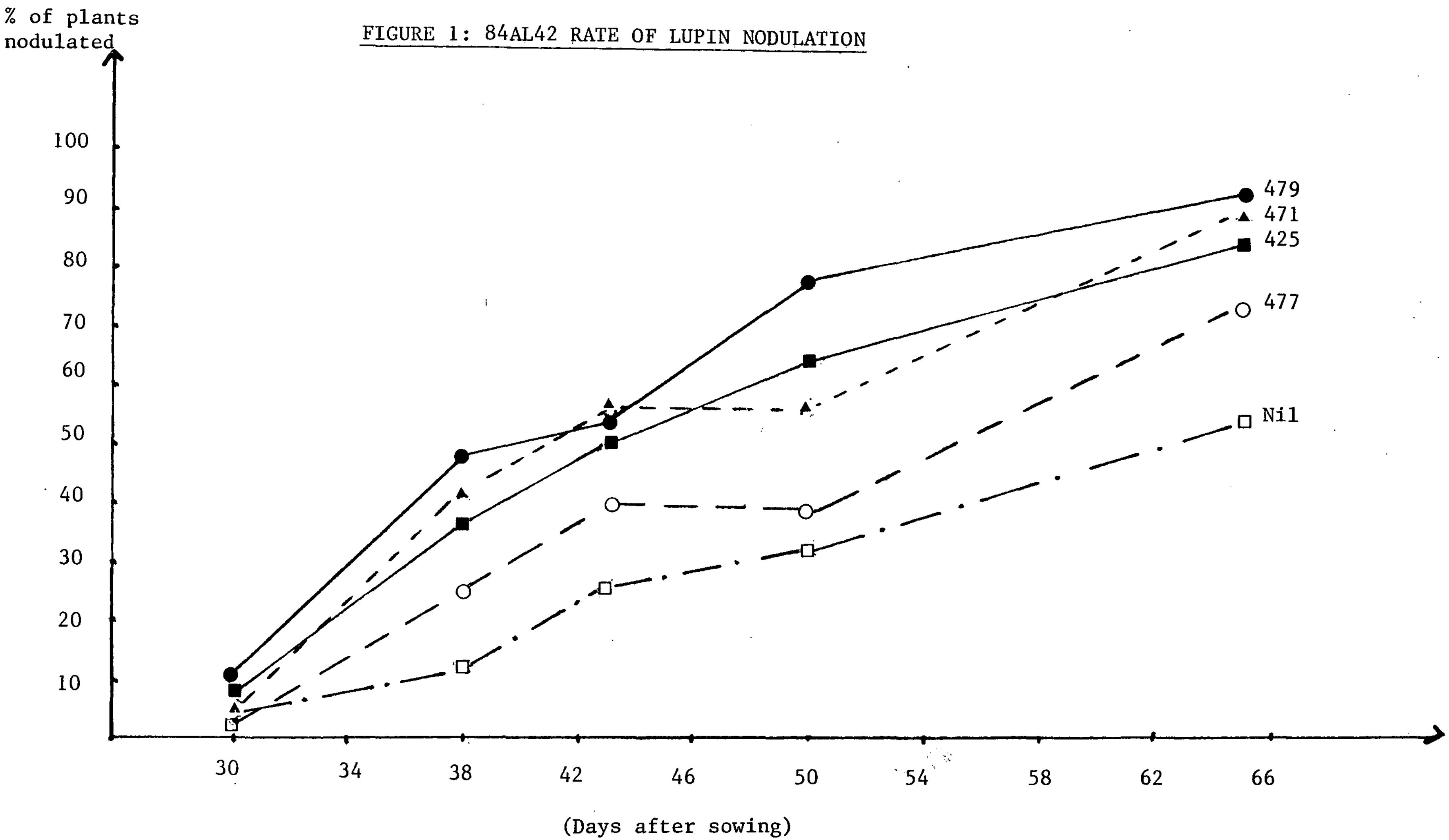

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FIGURE 2: SERADELLA NODULATION AT
7°C (NIGHT) - 14°C (DAY)

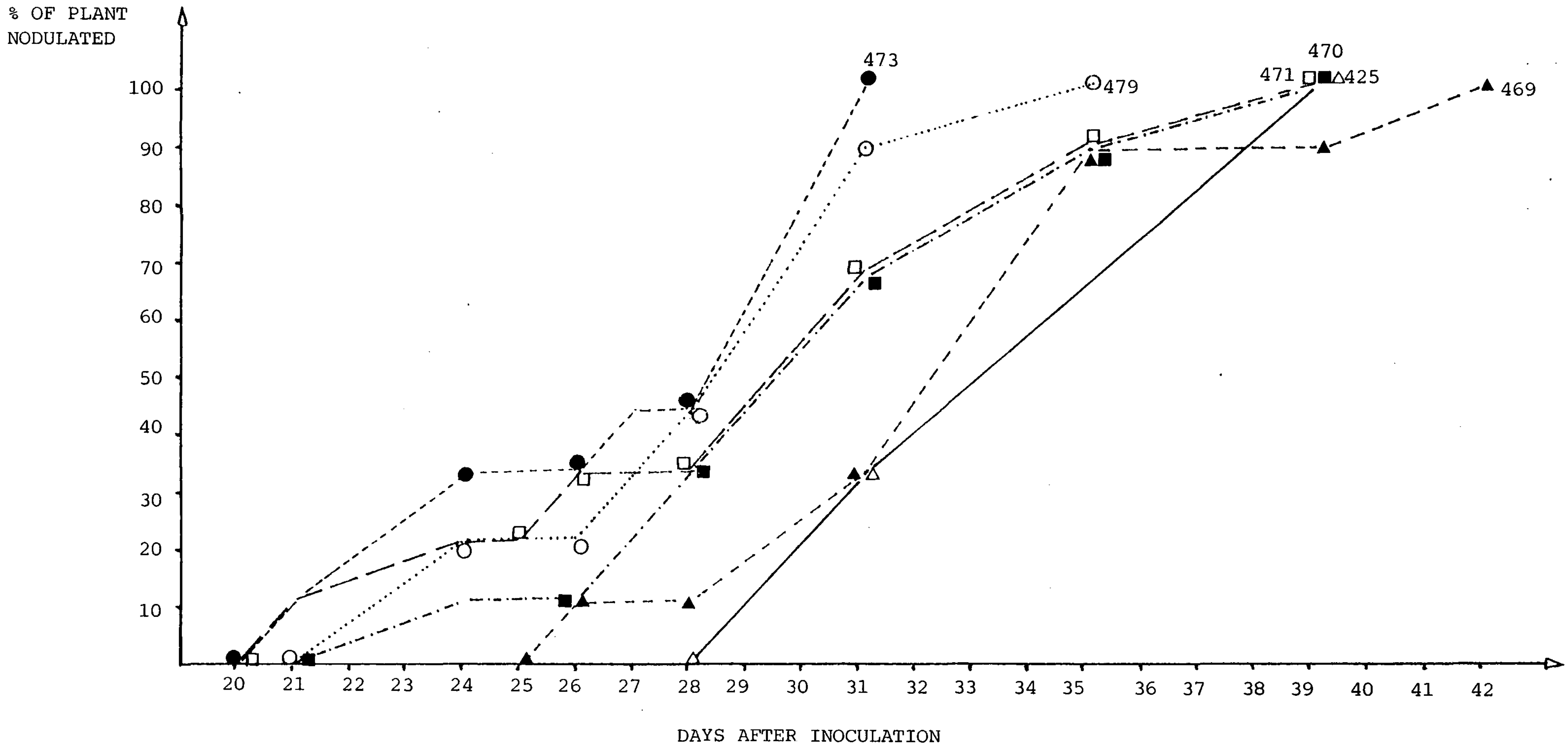
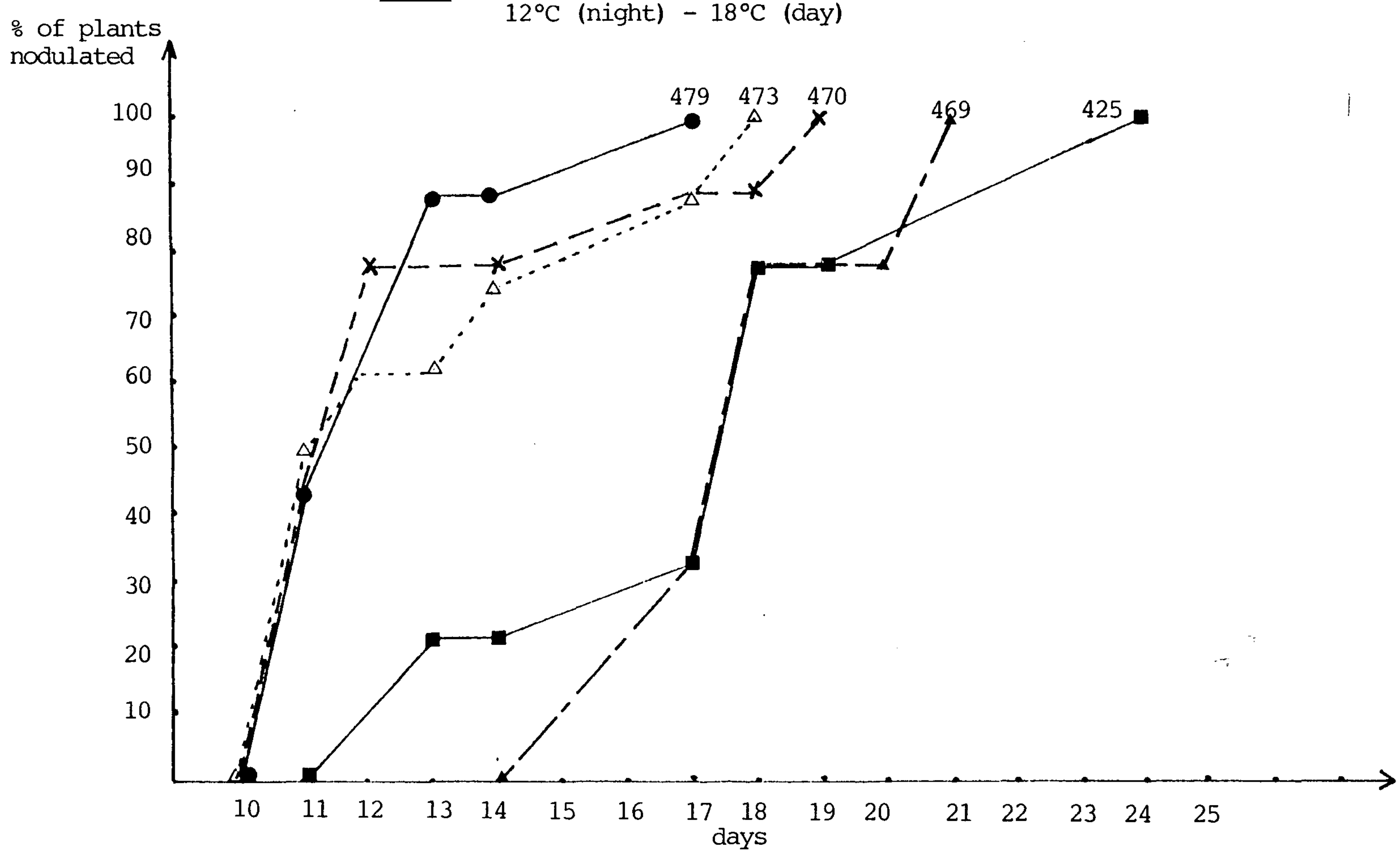


Fig 3: Serradella nodulation at 12°C (night) - 18°C (day)



Strains WSM 471, WSM 473 and WSM 479 all produced a higher yield than did WU 425 (and WSM 469) indicating that nitrogen was limiting the growth of the WU 425 inoculated plants during the four weeks.

Comment

The nodulation results obtained in the phytotron strongly reflected field results; it is problematical whether earlier nodulation by approximately one week would confer a potential yield advantage upon the lupin plants. The promising isolated identified in these experiments will be field tested in 1985 to answer this question.