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Effect of Sowing Dates and Amount of Nitrogen Fertilizer at Sowing on Fixation Activity and Different Attributes of *Lupinus albus* L. cv. Kievskij Mutant

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ABSTRACT

*Lupinus albus.*cv. Kievskij Mutant was sown on two sowing dates, 17 March 1978 and 6 April 1978 respectively. The early-sown crop was shorter, less branched, senesced several weeks earlier and gave seed yield only half of the late-sown. The early-sown crop produced 33 per cent of its seed yield on the branches compared with 47 per cent in the late sown. Delaying in sowing more than doubled the seed yield of the branches whilst the main shoot seed yield increased by 50 per cent. These differences are probably due in large part to the 'excessive vernalization' which the early-sown crop experienced and which was greatly reduced by a three-week delay in sowing. The late-sown crop showed no significant response to nitrogen fertilizer although the 20 and 80 kg treatment yields almost certainly gave some advantage. The earlier senescence which the early-sown exhibited may have been a major factor in reduced nodule growth after the end of June and for the fact that fixation activity reached only a quarter of the level attained by the late-sown crop.

Senesced leaves contributed a small portion to total dry matter, they were 7.34 and 4.66 per cent for the early and late-sown crop respectively.

INTRODUCTION

It is postulated for several reasons that an early sown crop may have a greater demand for nitrogen fertilizer. Firstly, if the period of early crop growth is a period of low temperature, both the rate of nodule development and the rate of mineralization of organic nitrogen will be reduced. Of course the rate of plant growth and therefore the demand for nitrogen will also be slowed down, but soil temperatures commonly lag behind air temperature in the spring and nitrogen supply could be a limiting factor. Sowing dates studies have already indicated the importance of early sowing for high seed yield(Williams *et al.,* 1978)

The occurrence and extent of natural vernalization in lupin cultivation is dependent upon sowing date. If the crop is sown in the autumn, full vernalization will occur as a result of low winter temperatures and the crop will flower very early in the spring. But most of the available lupin cultivars are not winter hardy in Britain (Tayler and Sylvester-Bradley, 1977).

Many investigations indicated that lupins can be artificially vernalized to hasten flowering. Rahman and Gladstones (1972) successfully vernalized seeds of *L. angustifolius* cv. Uniharvest at 1-2°C for a period of three weeks. Sylvester-Bradley *et al.*,(1980) have also successfully vernalized seeds of *L.albus.*cv. Kievskij Mutant at 0-4° C for a period of four weeks. The work of Gladstones and Hill (1969) and other reports also indicated similar results as found by the above investigators.

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Pongkao (1980) found that high rates of nitrogen fertilizer in the seedbed reduced nodule dry weight and total fixation activity and also reduced seed yield in ***L.albus*** cv. Kievskij Mutant. There was some evidence that nitrogen at 20 to 40 kg/ha enhanced nodule production and fixation activity, and possibly seed yield. He also conducted an experiment after forage corn at a different point in a rotation which suggested a lower soil nitrogen environment. It confirmed previous results that small applications of nitrogen fertilizer in the seedbed may enhance seed yield, but applications greater than 40 kg/ha showed no advantage. A bi-modal pattern of fixation activity was found, the second peak was probably induced in part by rainfall following a dry period.

Stimulation of symbiotic fixation by small amounts of nitrogen fertilizer have been reported by many workers in soybeans, probably because the fertilizer supplies nitrogen needs prior to active nodulation and nitrogen fixation (Allos and Bartholomew, 1955; Orcutt and Wilson, 1935), or by delaying nodulation during the earlier stages of plant growth, and in the long run providing more sites for nodule formation (Mac Connel and Bond, 1957; Gibson and Nutman, 1960). It is also possible that later formed nodules may be deeper in the soil and so less exposed to fluctuations in temperature and water supply.

The present study was conducted to evaluate the effects of sowing dates and amount of nitrogen fertilizer at sowing on fixation activity, yield and other attributes of *L. albus.* cv. Kievskij Mutant.

MATERIALS AND METHODS

Fresh **Rhizobium lupini** str. 3211 in peat inoculum was obtained from Rothamsted Experimental Station. Seed was mixed with prepared inoculum and then air dried before planting. The rate of inoculum used was a mix of 0.5 g peat inoculum plus 2 ml of 1% Polycell[1] for one kg of seed. After land preparation P and K fertilizers were applied before sowing at the rate of 141 kg of available P and 282 kg of soluble K per hectare. Source of N was from ammonium nitrate (34.5% N). Different rates of N at sowing (0, 20, 40 and 80 kg per hectare) were applied by broadcasting at each sowing date one day before seed drilling and were then incorporated into the top 5 cm of soil by hoe. Each plot was 4 × 12 m, consisting of 20 rows of 20 cm apart with 10 cm between plants, to give a target of 50 plants per square meter. Germination was poor in spite of the fact that seeding rate was doubled, as a result plant population in each sub-plot was uneven, and sub-plots had to be thinned down to 32 plants per square meter, with 16 cm between plants in the row.

A spilt plot design was employed where sowing dates acting as main plot and N fertilizer at sowing as sub plot. The experiment was conducted at Sonning Farm of University of Reading UK. Seed was sown by Oyjord seed drill on 17 March 1978 for early sowing and 6 April 1978 for late sowing. One week after sowing Simazine (Gesatop WP 50) at the rate of 800 g active ingredient per hectare was applied by knapsack sprayer as a pre-emergence herbicide. Both early and late sowing were thinned on 17 May 1978 which was 61 and 41 days from sowing respectively. Sampling areas were 0.5m^2 for the intermediate harvests. made up of two adjacent rows 0.4 x 1.25 m^2. Four 0.5 m^2. areas per plot were used for the final harvest. Both sowing date treatments were sampled on the same dates (Table l).

Table 1. Dates of sampling and days from sowing of early and late sown.

Dates	Days from sowing	
	Early sown	Late sown
29 May 1987	73*	53*
5 June 1978	80	60
12 June 1978	87	67
19 June 1978	94	74
26 June 1978	101	81

1 Trade name of pelleted dried starch.

Table 1. (Cont.)

Dates	Days from sowing	
	Early sown	Late sown
3 July 1978	108	88
10 July 1978	115	95
24 July 1978	129	109
7 August 1978	143	123
21 August 1978	157	137
6 September 1978	173 +	
22 September 1978		169 +

*No acetylene reduction test

+ Final harvest

Intermediate Harvest

The first acetylene reduction test was done on 5 June 1978 when early sown and late sown plants were 80 and 60 days from sowing. From 5 June 1978 to 10 July 1978 (6 times) acetylene reduction test was done weekly and from 24 July 1978 to 21 August 1978 (3 times) fortnightly. The whole sample was dug, and 16 plants were used for acetylene reduction test. Roots were cut at ground level and adhering soil shaken off, then the roots were put in 1,000 ml sealed glass jar. Ten per cent air was removed from the jar with a 100 ml syringe and the same volume of acetylene gas was injected in. The jars were incubated for 30 minutes at field temperature away from direct sunlight, 10 ml of reduced gas samples were collected in vacutainers and the acetylene and ethylene content were measured by GLC (gas liquid chromatography). Full details of the techniques are described in Hardy *et al.*, (1968); Hardy *et al.,* (1971) and Hardy *et al.,* (1973) Acetylene reduction test was done from 8 a.m. onward and finished before noon. After gas sample collection nedules were removed and oven-dried at a constant 80°C for at least 24 hours to reach constant dry weight before they were finally weighed.

After nodule removal, except in the first harvest, root and tops were bulked and oven-dried at a constant 80° C for at least 24 hours to reach constant dry weight before they were finally weighed. Total dry weight is composed of nodules, roots and tops at early samplings; nodules, roots, tops, pod shells, seeds and senesced leaves at later samplings.

Nodules were bulked with corresponding root and top samples before grinding. They were ground and after grinding total introgen content was determined by Kjeldahl digestion.

Senesced leaves were collected after the 8th harvest onward. They were collected by hand every two days from each half square meter plot for the last two samplings and stored in cold room. At sampling time, leaves were lightly washed and oven-dried to reach constant dry weight before they were finally weighed. Then they were ground and digested for total nitrogen content.

Plant structure measurements were made on eight plants randomly chosen from outside the sample areas. Average figures from eight plants were calculated and analyzed statistically.

Dry matter determination of main shoot pod shells, main shoot seeds, branch pod shells and branch seeds were done on 6 September 1978 on the following treatments: early sowing, no nitrogen; early sowing, 80 kg N ha^{-1}; late sowing, no nitrogen; and late sowing, 80 kg N ha^{-1} Ten plants outside the sampled plot were randomly selected, with main shoot pod shells, mainshoot seeds, branch pod shells and branch seeds separated and weighed immediately for fresh weight. They were then oven-dried at 80° C to reach constant dry weight before they were finally weighed.

Final Harvest

Plants were harvested on 6 September 1978 for early sowing and on 22 September 1978 for late sowing which was 173 and 169 days from sowing respectively. Plants from four sample sites (64 plants) were dug and from each site four plants were randomly selected and separated into different parts for determination of yield components and other important characteristics. All samples were oven-dried at a constant 80° C for at least 24 hours before they were weighed seperately, then they were ground and ground samples were digested for total nitrogen content.

Senesced leaves were collected in the same way as described for intermediate harvests. They were deep frozen until final harvest then they were thawed, oven-dried, weighed, ground and digested for total nitrogen content.

Main stem seeds and branch seeds were measured using Nuclear Magnetic Resonance (NMR) machine (Newport Instruments Quantity Analyzer). Per cent oil in seeds was calculated as follows:

Per cent oil =

$$\left[\frac{\text{NMR of seed}}{\text{NMR of oil standard}} \times \frac{\text{Wt of oil standard}}{\text{Wt of seed}}\right] \times 100$$

RESULTS

Late sowing gave seed yields significantly higher than early sowing at all four rates of N fertilizer (Figure 1). There was no significant response to nitrogen in the late sown crop, but even though it was lower yielding the early sown treatment showed a significant response to 80 kg ha^{-1}. Yield level at early sowing ranged from 1.5 to 2.7 t ha^{-1} while that of the late sowing was from 3.2 to 3.9 t ha^{-1}.

The major components of yield are shown in Table 2. Late sowing increased yield by increasing seed size in both main shoots and on the branches, by some increase in the number of main shoot pods, and by a substantial increase in the number of branch pods. Thus the seed yields of both main stems and branches were increased. Late sowing increased the proporting of seed yield from the branches and also increased the oil percentage in main shoot and branch seeds.

The only significant difference in main effect of nitrogen was to increase seed size on the branches, although the data do suggest that pod number may have been increased on both main shoots and on the branches. There were no significant differences in sowing date x nitrogen interactions.

The appearance of the crop suggested that the late-sown treatments were slower in senescence and seed ripening. Samples for dry matter content were therefore taken from 6 September 1978, 173 and 153 days from sowing (Table 3). There was no significant effect of the nitrogen treatments but the late-sown treatments showed a much lower dry matter content in all components examined.

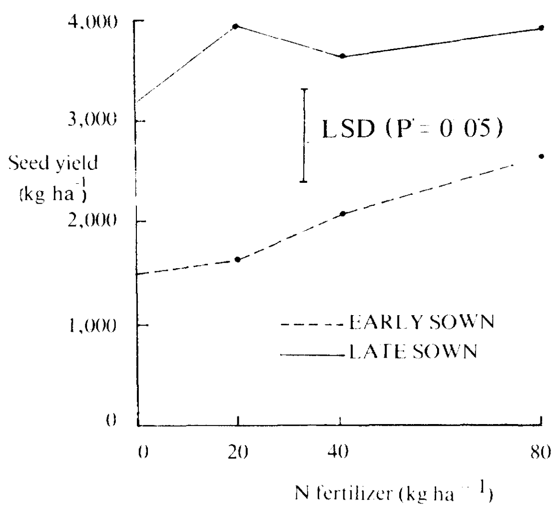

Figure 1 Effect of sowing dates and N Fertilizer at sowing on seed yield

Table 2 Effect of sowing dates and fertilizer at sowing on yield components

Treatments	Main shoots						Branches				
	% pod set	Seeded pods/ plant	Unseeded pods/ plant	Seeds/ pod	1,000 seed wt g	% oil M shoot seeds	Seeded pods/ plant	Unseeded pods/ plant	Seeds/ pod	1,000 seed wt g	% oil Br seeds
SOWING DATES											
Early	84	4.77	1.77	3.51	246	13	3.80	2.96	2.86	180	13
Late	67	5.67	0.78	3.68	296	15	7.42	4.24	2.81	256	15
Significance	—	*	*	—	*	**	*	**	—	**	**
LSD (P = 0.05)	18	0.55	0.87	0.43	30	0.78	2.29	0.65	0.54	10	0.90
C.V. (%)	3.15	7.96	41.01	10.23	1.13	3.54	15.12	12.55	14.53	0.81	3.80
N AT SOWING											
0	77	4.92	1.13	3.53	270	15	4.30	2.91	2.74	200	14
20	75	5.04	1.16	3.62	269	14	5.57	3.28	2.78	212	14
40	68	5.19	0.95	3.58	280	15	5.52	4.05	2.88	227	14
80	82	5.72	1.86	3.64	265	15	7.05	4.16	2.92	233	14
Significance	—	—	—	—	—	—	—	—	—	*	—
LSD (P = 0.05)	13	0.73	0.92	0.33	25	0.69	2.09	1.48	0.28	20	0.63
C.V. (%)	3.29	11.29	51.90	11.03	1.27	3.88	17.78	23.31	12.90	1.42	3.91

— Not significant

* Significance at P = 0.05

** Significance at P = 0.01

Table 3. Effect of sowing dates and N fertilizer at sowing on percentage of dry matter of pod shells and seeds

Treatments	Main shoots		Branches	
	Dry matter in pod shells %	Dry matter in seeds %	Dry matter in pod shells %	Dry matter in seeds %
SOWING DATES				
Early	71.10	66.43	57.44	52.67
Late	17.72	34.59	11.89	32.10
Significance	**	**	*	**
LSD (P = 0.05)	18.47	8.79	12.20	28.11
C.V. (%)	5.42	3.29	5.65	7.01
N AT SOWING				
0	48.62	51.54	35.02	42.47
80	40.20	49.47	34.31	42.30
Significance	—	—	—	—
LSD (P = 0.05)	14.87	6.28	17.31	17.31
C.V. (%)	5.99	3.42	8.28	6.77

Not significant

* Significance at P = 0.05

** Significance at P = 0.01

Nodule dry weight and total fixation activity rose to a peak during the growth of the crop and then declined. Sowing date had little effect on either attribute until the end of June, but thereafter the late sown showed highter levels and a later peak which for nodule weight differed by almost five weeks (Figures 2 and 3).

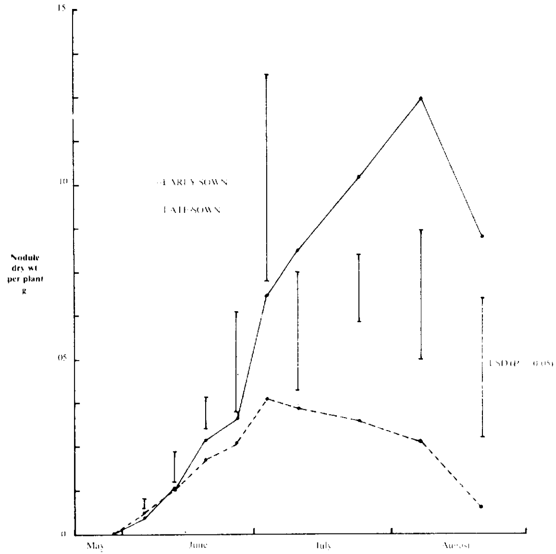

Figure 2. Effect of sowing dates on nodule dry weight

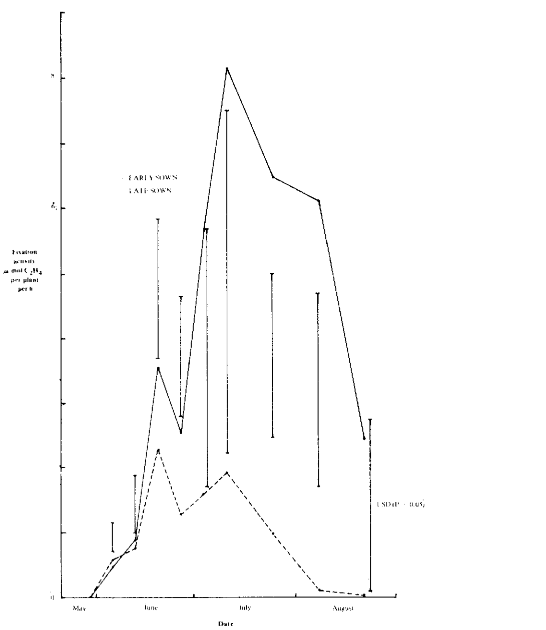

Figure 3. Effect of sowing dates on total fixation activity

Effects of fertilizer treatments of nodule dry weight and fixation activity are shown in Figures 4 and 5. Results are variable, but performance was poorest from 80 kg treatment and there was some indication of an advantage to nitrogen fertilizer use; the nil treatment showed a rapid decline in fixation after 10 July and was significantly poorer than the 20 and 40 kg treatments at the sampling in early August.

There were few significant interactions and only one instance where nodule dry weight was greater with nitrogen fertilizer than without (Table 4). This occurred with 40 kg N ha^{-1} in the late-sown crop at 137 days but was not significantly reflected in the concurrent measurement of fixation activity.

Table 4. Effect ðf N fertilizer at sowing on nodule dry weight per plant and total fixation activity of late-sown crop at 137 days from sowing

N at sowing kg ha^{-1}	Nodule dry weight (mg)	Total fixation activity μ mol C_2H_4 $plant^{-1}h^{-1}$
0	84.92	1.73
20	72.34	2.72
40	141.42	3.48
80	39.88	1.86
Significance	*	—
LSD (P = 0.05)	48.54	1.92
C.V. (%)	5.68	39.06

— Not significant

* Significance at P = 0.05

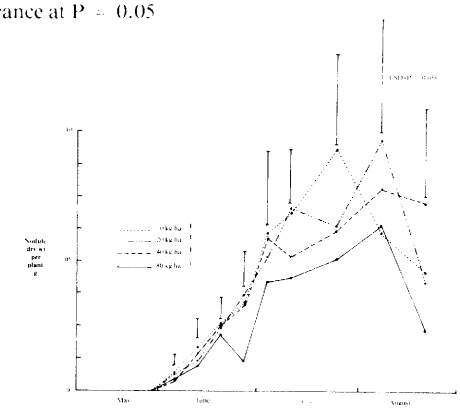

Figure 4 Effect of N fertilizer at sowing on nodule dry weight

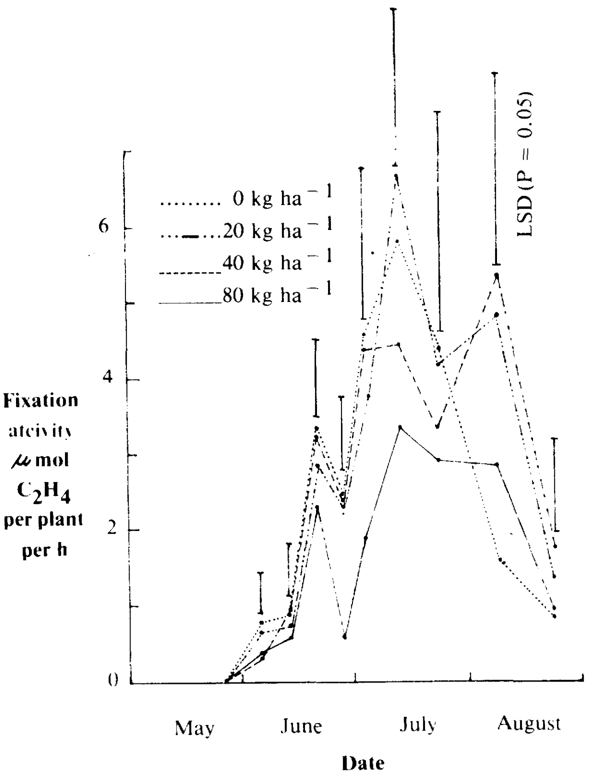

Figure 5 Effect of N fertilizer at sowing on total fixation activity

Effect of sowing dates on total dry matter and total N accumulation was similar. In both cases, late sowing was superior to early sowing especially in late samplings (Figures 6 and 7). Senesced leaves were a relatively small proportion of the total dry matter. It is noteworthy that leaf senescence was virtually complete by early August on the early-sown treatments but continued through September on the late sown.

Effect of sowing dates and N fertilizer at sowing on total dry weight and total N accumulation is shown in Figures 8 and 9. The pattern is similar for both attributes. Peak rates of accumulation were similar for both attributes. Peak rates of accumulation were similar for both sowing dates but convered a longer time span in late sown. Both dry weight and nitrogen accumulation were increased by N fertilizer application, particularly in the early-sown crop.

One of the effects of sowing date was to alter the structure of the plant (Table 5). Late sowing produced longer stems with more nodes and longer internodes and more primary and secondary branches of which a greater portion carried pods. Nitrogen treatments had no effect on the attributes measured except to lengthen the first primary branches (Table 6).

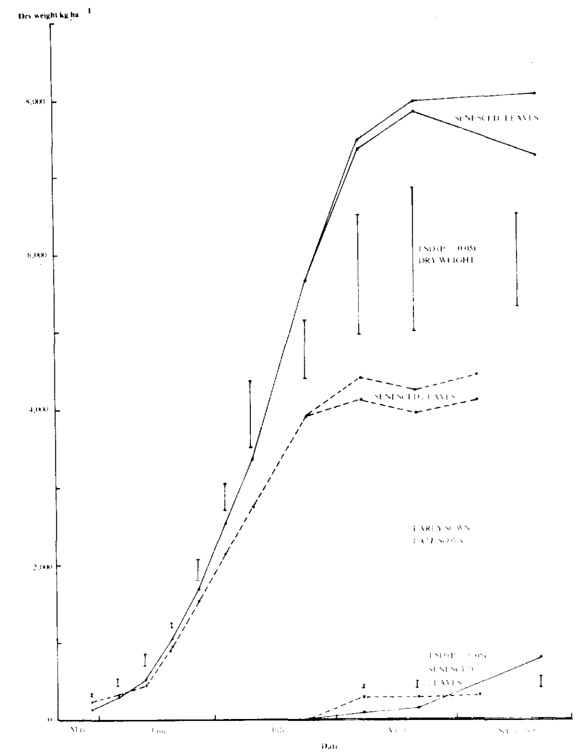

Figure 6 Effect of sowing dates on total dry matter and senesced leaves

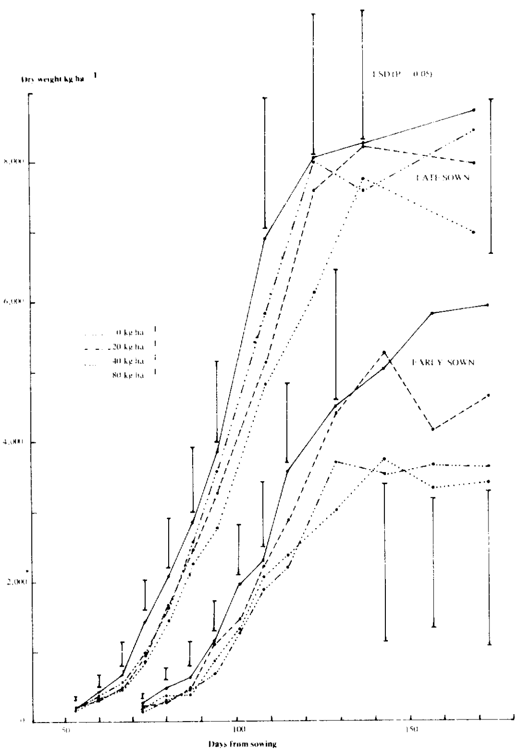

Figure 7 Effect of sowing dates on total N accumulation in total dry matter and seneseed leaves

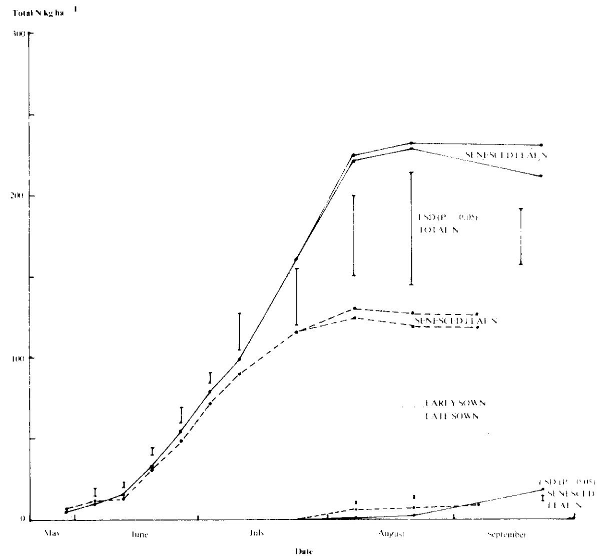

Figure 8 Effect of sowing dates and N fertilizer at sowing on total dry weight

Table 5 Effect of sowing dates on number of nodes and length of main shoots and first primary branches; and number of primary and secondary branches

Sowing dates	Main shoots		First primary branches		Primary branches		Secondary branches	
	No. of nodes per shoot	Length cm	No. of nodes per shoot	Length cm	Total number per plant	Branches with pods per plant	Total number per plant	Branches with pods per plant
Early	7.91	21.69	2.54	15.01	1.93	1.59	1.68	0.59
Late	8.91	28.92	2.88	18.18	2.62	2.34	3.30	1.77
Significance	*	*	*	—	**	**	**	**
LSD (P=0.05)	0.64	2.23	0.21	3.72	0.28	0.14	0.45	0.16
C.V. (%)	5.33	3.31	9.48	6.52	13.04	10.67	15.10	19.00

— Not significant

* Significance at P = 0.05

** Significance at P = 0.01

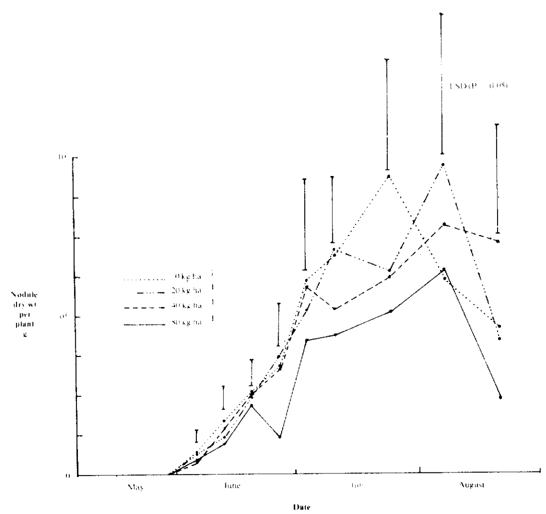

Figure 9 Effect of sowing dates and N fertilizer at sowing on total N accumulation

Table 6. Effect of N fertilizer at sowing on length of first primary branches

N at sowing kg ha^{-1}	Length of first primary branches cm
0	14.62
20	16.14
40	16.99
80	18.64
Significance	*
LSD (P = 0.05)	2.58
C.V. (%)	6.68

* Significance at P = 0.05

DISCUSSION

The three-week difference in sowing date treatments had a very marked effect on crop performance. The early-sown crop was shorter, less branched and senesced several weeks earlier than the late-sown, and gave substantially reduced seed yield, averaging 1.9 t ha^{-1} compared with 3.7 t ha^{-1}. The early-sown crop produced 33 per cent of its seed yield on the branches compared with a figure of 47 per cent in the late sown. Delaying in sowing more than doubled the seed yield of the branches whilst the main shoot seed yield increased by 50 per cent. These differences are probably due in large part to the vernalization influence which the early-sown crop experienced and which was greatly reduced by a three-week delay in sowing. Figure 10 gives the relevant temperature data which shows that although temperature remained low until mid-April shortly after the second sowing, they then rose rapidly. It is not the purpose of this study to quantify the effects of vernalization, but it is known that it advances the date of flowering and results in a more determinate habit (Rahman and Gladstone, 1972).

Studies of Williams *et al.*, 1978 on sowing dates have already indicated the importance of early sowing for high seed yield. The results of a 1977 experiment at Reading in which Kievskij Mutant was sown on twelve dates between February and May. Ignoring the last date in late May, seed yield varied from 1.2 t ha^{-1} to 3.0 t ha^{-1}. Declining seed yield from February to May was associated with an increase in plant height, with later flowering and with a reduction in the contribution of the branches to seed yield. Some of these effects are probably the result of decreasing vernalization with the delay in sowing. Later sowing may therefore reduce the demand for nitrogen because of a reduced seed yield, whilst the greater indeterminacy of the later sown crop may increase the ability of the crop to supply the energy demands of nitrogen fixation in the nodules. Thus, if a response to fertilizer nitrogen occurs in a nodulated crop, it may be affected by sowing date and is more likely to occur with early sowing.

At first sight, the effect of sowing date in this experiment appears to differ from that of Williams *et al.,* (1978) where seed yield declined in crops sown after mid-March. However, the data showed that yields were also reduced by sowing before mid-March. It is possible that the pattern of vernalization effects in 1977 differed from those in 1978 of this experiment and that what might be termed 'excessive vernalization' did not occur for March 1977 sowings. This is not apparent from the temperature data in Figure 11 but it is possible that none of these measurements correctly measures the vernalization effect of the environment; for example the 10 cm soil temperature records the temperature below the zone where the unemerged or emerging seedling is sited and therefore underestimates the temperature fluctuation to which it is exposed. Of course degree of vernalization is not the only factor which causes differences in lupin performance between years. In 1978 the crop experienced suitable temperature and moisture conditions during pod-setting and pod-growth to allow full expression of the yield potential per plant which a reduced vernalization effect had developed. Thus, the branches were able to produce pods which in other years might have been absent due to poor environmental conditions, particularly in later sown crops.

The late-sown crop showed no significant response to fertilizer nitrogen although 20 and 80 kg treatment yields almost certainly gave some advantage. The earlier senescence which the March sowing exhibited may have been a major factor in reduced nodule growth after the end of June and for the fact that fixation activity reached only a quarter of the level attained by the April sowing. In turn, poor fixation may have further hastened senescence; it is relevant to point out (Figure 8) that the early sown 80 kg treatment combination showed an increase in total dry weight until 170 days, whilst the other nitrogen treatments showed no increase after 140 days.

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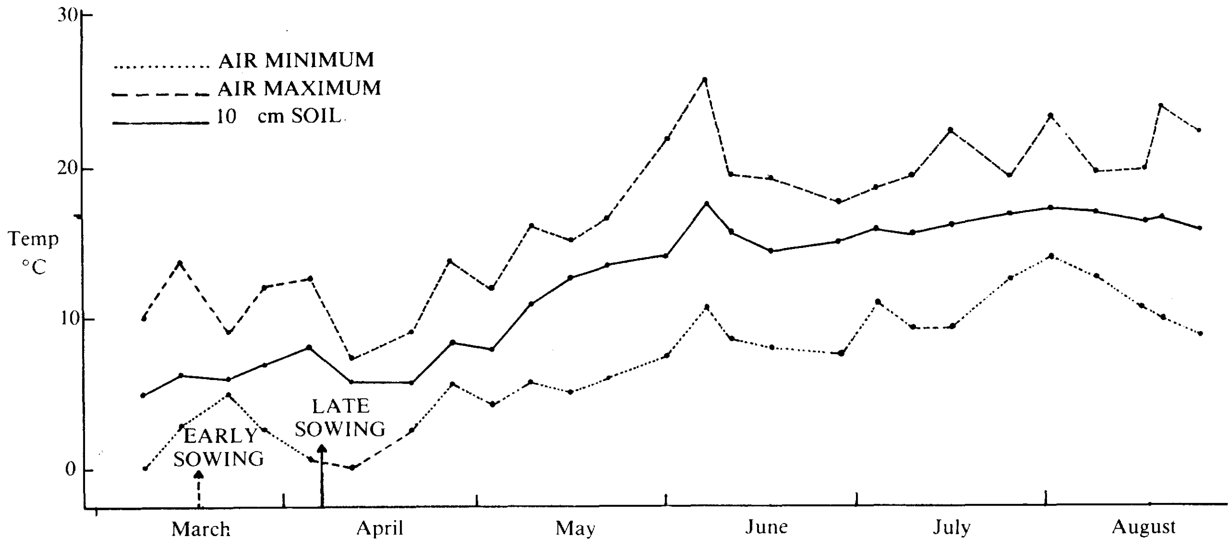

Figure 10 Weekly means of daily minium and maximum air temperatures and 10 cm soil temperatures at Sonning Farm, Berkshire in 1978

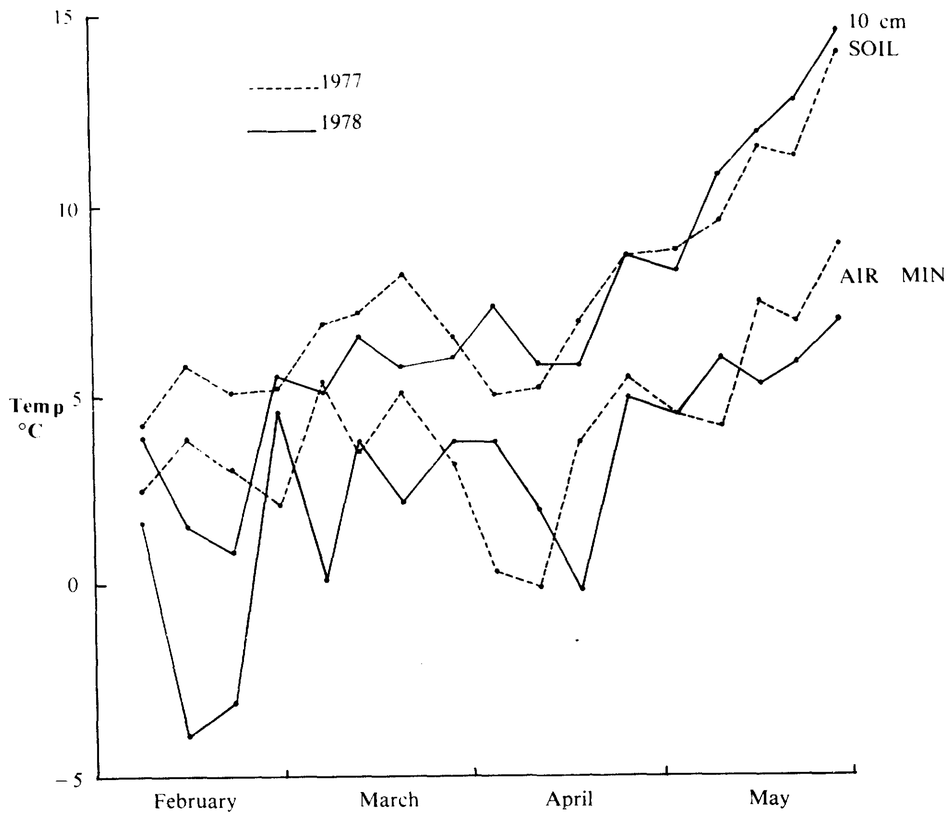

Figure 11 Weekly means of daily minimum air temperatures and 10 cm soil temperatures at Sonning Farm, Berkshire in 1977 and 1978

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