PELLETING AND FERTILIZER EFFECTS ON NODULATION OF *GLYCINE WIGHTII*

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ABSTRACT

Two inoculation treatments (peat applied by normal water slurry, and peat applied with 5% Cellofas A sticker followed by lime pelleting) were compared when mixed with various forms of superphosphate before sowing in krasnozem soils at Lismore, New South Wales and Maleny, Queensland.

At Lismore, lime pelleting did not affect the proportion of plants with crown nodulation at six weeks, but at eight weeks, it increased the number of nodules per plant. At Maleny, a significant fertilizer-inoculant interaction ($P < 0.01$) was obtained, indicating that lime pelleting increased the percentage of nodulated plants when the seed was sown in contact with superphosphate or ammoniated superphosphate, and depressed nodulation percentage when it was in contact with ammoniated superphosphate plus lime.

The results from these experiments indicate that where germination can be expected soon after sowing the practice of lime pelleting immediately before sowing may have value if the seed is to be drill-sown in contact with superphosphate.

INTRODUCTION

When temperate legumes such as vetches (*Vicia sativa*) and subterranean clover (*Trifolium subterraneum*) are sown into grass-dominant pastures on krasnozem soils by sod-seeding, inoculation and mineral nutrition require special attention. Near Lismore a mixture of lime and superphosphate applied in the rows (Carter, Crofts and Jenkins 1956, Crofts 1957, Swain 1959) or lime pelleted seed mixed with superphosphate (Colman and Pulsford, unpublished) favoured nodulation and growth of these species. The object of both practices was to protect the inoculant from the fertilizer acidity when seed is in direct contact with the banded fertilizer.

The reversion of superphosphate by mixing with lime or by ammoniation to reduce its acidity is an expensive and technically troublesome operation; since 1964 the simpler process of lime-pelleting the vetch or clover seed and sowing it with superphosphate has become more popular. Perennial tropical legumes are of increasing interest for summer growth in these areas and poor nodulation has frequently been noted, but little work has been done to compare their reaction to banded fertilizer or lime pelleting with that of clovers and vetches. The field experiments reported in this paper were to investigate the effect of lime pelleting on nodulation of *Glycine wightii* (previously *G. javanica*) when sown in contact with banded fertilizers.

MATERIALS AND METHODS

For ease of experimentation and to permit sampling for nodulation without excessive loss of nodules the two experiments were done on cultivated seed beds prepared on level land dominated by kikuyu grass (*Pennisetum clandestinum*). Experiment 1 was at Lismore, New South Wales on soil of pH 5.2 (1:5 water), and Experiment 2 at Maleny, Queensland, on soil of pH 4.6 (1:2.5 water).

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Experiment 1, Lismore

G. wightii cv. Clarence was inoculated with *Rhizobium* strain QA922 (cowpea type), the then current commercial inoculant recommendation. All seed was inoculated on the day of sowing with peat culture, using 12 g per lb of seed (approximately three times the normal rate) to give an estimated 400,000 bacteria per seed. Simple inoculation was made by water slurry, and lime pelleted seed was prepared by mixing the peat inoculum with 5% water solution of Cellofas A which was then used to stick laboratory grade calcium carbonate to the seed (Goss and Shipton 1965a, 1965b).

The soil received a broadcast basal dressing of 1 cwt potassium chloride and 4 oz sodium molybdate per acre before planting. Rows were opened with a tine cultivator. Each treatment was sown into a subplot consisting of six adjacent rows 15 feet long and 1 foot apart. There were four replicates arranged in randomized blocks. Slurry inoculated seed and lime pelleted seed were both sown at 6 lb per acre with fertilizer at the equivalent of 2 cwt superphosphate per acre. Seed and fertilizer were thoroughly mixed immediately before planting, divided volumetrically between the six rows and hand sown on January 28, 1965. To check on natural nodulation uninoculated plots with and without superphosphate were included in the experiment. There were five fertilizer treatments:

1. No fertilizer
2. Granular superphosphate (pH 2.7; 1:10 water—AOAC method)
3. 3% ammoniated superphosphate (pH 4.8)
4. 4½% ammoniated superphosphate (pH 4.8)
5. 3% ammoniated superphosphate (60%) plus lime (40%) (pH 6.8)

The total rainfall for the period of establishment was 11.3 in. which was 38% of the average for this period (February-May inclusive).

Six weeks after sowing 30 randomly selected plants per plot were dug up and nodulation recorded either as crown nodulation (nodules on the roots within ¼ in. of the crown and 1 in. down the taproot), or lateral nodulation. Dry weight of tops was measured. Eight weeks after sowing some treatments (Table 1) were again sampled to count, dissect and weigh nodules in two of the four replicates by excavating 20 contiguous plants along a drill row at random. Dry weight of tops was again measured. Sixteen weeks after sowing a final dry matter yield estimate was made by cutting a quadrat at random from each subplot of six rows.

Experiment 2, Maleny

At this site *G. wightii* cv. Tinaroo was used. Basal fertilizer was broadcast at 200 lb potassium chloride and 15 oz sodium molybdate per acre before sowing. The same comparison was made of water slurry inoculation with lime pelleting, but in this case the lime-pelleted seed was commercially prepared 24 hours before sowing using commercial inoculant at normal rate of 70 g per 15 lb seed, Cellofas A as sticker, and plasterers' whiting as the coating. Slurry inoculated seed was prepared immediately before planting using 10 g peat per lb of seed, approximately twice the rate applied in the lime pellets.

In this experiment there were three replicates in randomized blocks, each replicate being a single row 5 feet long with 2 feet between rows. The mixed seed and fertilizer was hand sown on March 4, 1965, using 17 lb of slurry inoculated seed and 20 lb of lime pelleted seed per acre. (The extra weight was due to the lime in the pellet). One extra fertilizer treatment (3% ammoniated superphosphate (80%) plus lime (20%) of pH 6.7) was incorporated. Uninoculated rows without fertilizer were also included to observe natural nodulation.

Soil moisture was good at planting but only 1.3 in. of rain were received in the 50 day period between sowing and sampling.

Seven weeks after sowing at least 20 plants of the same size were dug at random from each row and nodulation was assessed. Yields were not measured.

Analyses of variance were carried out on the nodulation data after suitable transformation as follows:—

From Lismore: Percentage plants with crown nodules (arc sine transformation)
Mean nodules per 20 plants ($\log_{10}$ transformation)

From Maleny: Percentage plants with crown nodules (arc sine transformation)
Percentage plants nodulated (arc sine transformation)

RESULTS

Lismore

The data on mean percentage of plants with crown nodules and the data on number of nodules per 20 plants are summarized in Table 1. There was a significantly greater number of nodules with the lime pelleted treatment than with the slurry inoculated seed in the case of both the superphosphate and the ammoniated superphosphate + lime treatments. It should be noted that uninoculated seed without fertilizer produced 4.5 nodules per 20 plants which increased significantly to 23.5 by application of superphosphate alone. Slurry inoculation raised nodulation significantly in both the no-fertilizer and the superphosphate treatments.

Significant differences ($P < 0.05$) in percentage plants bearing crown nodules occurred at six weeks. It is noteworthy that 30% of plants in the control rows (no fertilizer, no inoculation) bore such nodules and that the application of superphosphate significantly increased this to 49%. Inoculation, whether by slurry or lime pellet, approximately doubled the number of plants bearing crown nodules, regardless of the fertilizer treatment applied in the row. There was no significant difference between lime pelleting and slurry inoculation.

Differences in yield between treatments at 6, 8 and 16 weeks were not significant. The data are not presented.

Maleny

Data on nodulation are summarized in Table 2. Both crown and total nodulation were poor, the best being 33% of the plants nodulated. In both criteria of nodulation there were highly significant fertilizer-inoculation interactions. In the case of superphosphate the mean percentage of nodulated plants was significantly greater for lime pelleting than for slurry inoculation, while the reverse occurred in the case of 3% ammoniated superphosphate (80%) + lime (20%). Thus lime pelleting improved nodulation with the most acid fertilizer (superphosphate) and as the acidity of the fertilizer was decreased by ammoniation the effect became less evident. When lime was added to the ammoniated superphosphate to produce a near neutral fertilizer lime pelleting was no longer advantageous and tended overall to depress nodulation.

DISCUSSION

Inoculated seed pelleted with lime and Cellofas A was superior to slurry inoculation in the presence of banded superphosphate irrespective of whether, as at Lismore, the mean number of nodules per 20 plants was taken, or as at Maleny, the mean percentage of plants with nodules or crown nodules was taken as the criterion.

TABLE 1

Nodulation of Clarence glycine resulting from slurry inoculation and lime pelleting under four fertilizer treatments at Lismore (Experiment 1)

Fertilizer treatments	Fertilizer pH (1 : 10 water)	Mean % of plants with crown nodules 6 weeks after sowing			Mean number of nodules per 20 plants 8 weeks after sowing		
		Method of seed inoculation			Method of seed inoculation		
		Uninoculated	Slurry	Lime pelleted	Uninoculated	Slurry	Lime pelleted
No fertilizer		30 (31.1*)	75 (60.9)	76 (61.3)	4.5 (0.650†)	35.0 (1.538)	‡
Granular superphosphate	2.7	49 (44.2)	83 (66.3)	88 (70.9)	23.5 (1.371)	46.0 (1.659)	75.0 (1.857)
3% Ammoniated superphosphate	4.8		69 (56.4)	82 (65.9)		41.0 (1.612)	‡
4½% Ammoniated superphosphate	4.8		70 (57.3)	82 (65.5)		‡	‡
3% Ammoniated superphosphate (60%) + Lime (40%)	6.8		87 (68.8)	82 (67.5)		28.0 (1.440)	76.0 (1.880)
Least significant difference P = 0.05			(11.5)			(0.166)	

* arc sine transformation in degrees.
† $\log_{10} \times$ transformation.
‡ no sample taken.

TABLE 2

Nodulation of Tinaroo glycine resulting from slurry inoculation and lime pelleting under five fertilizer treatments at Maleny (Experiment 2)

Fertilizer treatments	Mean % of plants with crown nodules 7 weeks after sowing				Mean % of plants with nodules in any position 7 weeks after sowing		
	Method of seed inoculation				Method of seed inoculation		
	Uninoculated	Slurry	Lime pelleted	Mean	Slurry	Lime pelleted	Mean
No fertilizer	0	4 (10.9)*			7 (14.4)		
Granular superphosphate		2 (4.0)	17 (24.1)	(14.3)	12 (12.1)	33 (34.7)	(23.4)
3% Ammoniated superphosphate		0 0	9 (16.6)	(8.4)	5 (12.5)	20 (25.8)	(19.1)
4½% Ammoniated superphosphate		4 (9.7)	8 (15.5)	(12.8)	6 (11.6)	9 (16.5)	(14.1)
3% Ammoniated superphosphate (80%) + Lime (20%)		8 (15.5)	3 (8.0)	(12.0)	26 (30.1)	5 (10.8)	(20.5)
3% Ammoniated superphosphate (60%) + Lime (40%)		15 (22.9)	10 (16.6)	(19.8)	29 (32.9)	26 (30.4)	(31.6)
	Mean	(10.6)	(16.3)		(19.8)	(23.6)	
Least significant difference		P = 0.05	P = 0.01		P = 0.05	P = 0.01	
Between fertilizer treatments for any one inoculation treatment or between inoculation treatments for any one fertilizer. (Mean of 3 reps)		(9.8)	(13.4)		(13.6)	(18.5)	
Between fertilizer treatments, i.e. both inoculation treatments combined. (Mean of 6 reps)		(7.0)	(9.5)		(9.6)	(13.1)	
Between inoculation treatments i.e. fertilizer treatments combined. (Mean of 15 reps)		(4.4)	(6.0)		(6.1)	(8.3)	

Fertilizer × inoculation interactions significant at P = 0.01.

* Figures in brackets are means of transformed data (arc sine, expressed as degrees).

Inoculation rates were heavier at Lismore, and there is evidence that the Lismore soil had a much higher population of indigenous *Rhizobium* than the Maleny soil. At Maleny, no crown nodulated plants were observed from uninoculated seed in unfertilized rows, but at Lismore 30% of the plants had crown nodules. The cultivar Clarence used at Lismore may be less specific in its *Rhizobium* requirement or inherently quicker to nodulate than cv. Tinaroo used at Maleny. Laboratory evidence of the latter has been noted by Russell and Coaldrake (1966), and field evidence at Lismore by Mears (unpublished). In spite of these site, cultivar and the inoculation differences, the effect of lime pelleting was similar in both experiments.

At Lismore there is clear evidence that the superphosphate stimulated nodulation by indigenous rhizobia. Nodulation by these rhizobia masked many differences between inoculation treatments. Despite this, a significant improvement in number of nodules at eight weeks resulted from lime pelleting compared with slurry inoculation. This improvement occurred in both the most acid and the least acid fertilizer.

More clear-cut effects were obtained at Maleny, presumably because the nodule situation reflected more completely the survival of inoculants applied to the seed under rigorous field conditions.

The evidence of these two field experiments indicates firstly that ammoniation of superphosphate without addition of lime offers no solution to the problem of protecting "cowpea" inoculant bacteria against fertilizer damage at or subsequent to sowing. This is in contrast to the beneficial effect of ammoniated superphosphate on clover nodulation reported by Loneragan *et al* (1955). Secondly the evidence indicates that lime pelleting of *G. wightii* is unnecessary when inoculated seed is mixed with certain fertilizers other than highly acid ones. In the interpretation of the results of both experiments it is not possible to isolate the influence of the sticker, Cellofas A, from the effect of the lime pellet.

The lack of yield response to improved nodulation at Lismore probably reflects the effectiveness of indigenous cowpea rhizobia, combined with variable availability of nitrate from soil cultivation and later, competition from kikuyu. If it is assumed that improved early nodulation is desirable and beneficial then the simplest way to obtain this for *G. wightii* sown in contact with superphosphate would appear to be to lime pellet the inoculated seed. However, in view of evidence that lime pelleting may shorten survival time of cowpea inoculant bacteria on certain seed (Norris 1967, Date 1968) it would be wise to do the lime pelleting as soon as possible before planting, and preferably sow into moist soil.

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