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NODULATION OF LEGUMES ON NEW LIGHT LAND

1. Survival of rhizobia on inoculated pelleted seed held for varying periods before sowing into dry soil

By OLGA M. GOSS and W. A. SHIPTON, Plant Pathology Branch

MOST of the light land areas being opened up in Western Australia are very sparsely populated with the rhizobial bacteria so essential for successful legume establishment. Seed inoculation is therefore a necessity. Farmers often have to resort to dry sowing because of the large acreages being planted or late opening rains.

A field experiment conducted at Badgingarra Research Station has shown that under the conditions of this experiment, lime pelleting of legume seed adequately protects the bacteria inoculated on to the seed for lengthy periods. Nodulation of legume seedlings and subsequent plant yield was the same when seeds were inoculated several days before planting as when they were inoculated and stored at room temperature for up to 12 weeks before sowing, and sown into dry soil.

The Experiment

Plant Species

The legumes used were Geraldton subterranean clover and Cyprus barrel medic.

Inoculum

Standard commercial peat cultures were used at the normal rates as recommended to farmers.

Soil Type and Land Preparation

The experimental area was a newly-cleared area of scrub plain, the soil type being a light grey sand over gravelly clay at 4 to 18 inches. The soil was slightly acid (pH 6.1-6.3). The scrub, which was only 3 to 4 feet high, was knocked down and raked in November, 1963. It was lightly ploughed and fire-harrowed in February, 1964, and then more deeply ploughed in April.

Fertiliser Treatment

A pre-planting dressing of zinc, copper and molybdenum superphosphate was given at the rate of one bag (180 lb.) per acre. At sowing, superphosphate at the rate of 100 lb. per acre was used for the subclover and basic superphosphate at 125 lb. per acre for the barrel medic.

Seed Treatments

At monthly intervals from mid-February to mid-May, seed lots were inoculated or inoculated and pelleted using either Cellofas A or Methocel adhesive. The control seed was neither inoculated nor pelleted.

After treatment all seed samples were stored at room temperature (up to 84° F.) until sowing in mid-May.

The pelleting method used was that of Cass Smith and Goss (1958, 1964).

Standard commercial peat cultures were used at recommended rates. All the cultures used came from a single batch, and were kept in cold storage, one packet being withdrawn at each inoculation period.
General view of experimental plots at Badgingarra Research Station, two months after germination, showing the effect of inoculation and pelleting on growth of Cyprus Barrel medic.

Centre plot—Seed inoculated only and held one month; Left.—Inoculated, pelleted and planted within a few days; Right.—Inoculated, pelleted and held three months.

The soil was dry at sowing and no rain fell until June 4. Hence there was a three-week dry sowing period in addition to the holding periods of one, two and three months.

**Sowing Method**

Seed was sown at a depth of 1 to 1½ inches through the seed box of a 12-run combine drill, using reducing cogs to ensure a seeding rate of 10 lb. of actual seed per acre. Contact of the seed with the fertiliser occurred only as the seed entered the soil.

**Experimental Design**

For each host the 13 treatments were randomised within each of two replications. Each plot measured 10.5 links wide x 300 links long and there was a buffer of 2 links between plots.

**Sampling**

**NODULATION ASSESSMENT**: Each plot was divided into 10 equal areas. Five consecutive plants were taken from one drill run in each area, the runs being selected in order across the plots. Samples were not taken from the outside drill runs or near the ends of the plots. Each plant was assessed for the position and amount of nodulation on the roots and points ranging from 0 to 4 were allotted as follows:

- 0—No nodulation.
- 1—Few nodules on the laterals only.
- 2—Nodules low down on the laterals and tap root.
- 3—Crown nodulation only.
- 4—Crown and lateral nodulation.

The points were totalled to give a reading for the plot as a whole. The maximum total for a plot would therefore be 200.

**YIELD OF TOP GROWTH**: Three random quadrat cuts (5 links x 2 links) were taken from each plot—near the centre and towards either end. The samples were oven dried and the dry weight recorded in grams.
Results

Detailed results are given in Tables 1 and 2. Major findings from this experiment were:

- There were no significant differences in plant nodulation and yield between any of the pelleting treatments. The only exception to this was the nodulation of the sub-clover seed which had been held for two months after being pelleted using the adhesive Cellofas A. Seed inoculated, pelleted

Table 1.—Yield and nodulation of subterranean clover

Nodulation and dry weight yields of Geraldton subterranean clover grown from seed inoculated or inoculated and lime pelleted at various times before dry sowing in mid-May. Both the adhesives Cellofas A and Methocel were used. Control plants were neither inoculated nor pelleted.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Period of Holding</th>
<th>Nodulation</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dry wt/quadrat (gm.)</td>
<td>Transformed figures (100 log₁₀)</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Cellofas A)</td>
<td>2-3 days</td>
<td>182.0</td>
<td>319</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Methocel)</td>
<td>2-3 days</td>
<td>183.5</td>
<td>292</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Cellofas A)</td>
<td>1 month</td>
<td>171.0</td>
<td>298</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Methocel)</td>
<td>1 month</td>
<td>176.5</td>
<td>307</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Cellofas A)</td>
<td>2 months</td>
<td>150.5</td>
<td>349</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Methocel)</td>
<td>2 months</td>
<td>173.0</td>
<td>259</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Cellofas A)</td>
<td>3 months</td>
<td>165.5</td>
<td>306</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Methocel)</td>
<td>3 months</td>
<td>162.5</td>
<td>303</td>
</tr>
<tr>
<td>Inoc. only</td>
<td>2-3 days</td>
<td>101.0</td>
<td>109</td>
</tr>
<tr>
<td>Inoc. only</td>
<td>1 month</td>
<td>96.0</td>
<td>119</td>
</tr>
<tr>
<td>Inoc. only</td>
<td>2 months</td>
<td>92.5</td>
<td>123</td>
</tr>
<tr>
<td>Inoc. only</td>
<td>3 months</td>
<td>48.5</td>
<td>105</td>
</tr>
<tr>
<td>Control (no treatment)</td>
<td></td>
<td>7.5</td>
<td>26</td>
</tr>
</tbody>
</table>

Differences for significance:

- P < 0.05 22.2 20.7
- < 0.01 30.1 28.1
- < 0.001 40.3 37.6

Figures linked by the unbroken lines are not significantly different from one another but are significantly different from all other figures at the 0.001 probability level.
Barrel medic plots showing the effect of inoculation, pelleting and holding the seed for varying periods before planting. The numbered plots are: 1.—Inoculated, pelleted, planted within a few days; 2.—Inoculated only, held three months; 3.—Inoculated, pelleted, held one month; 4.—Inoculated, pelleted, held two months; 5.—No inoculation; 6.—Inoculated only, held two months; 7.—Inoculated, pelleted, planted within a few days; 8.—Inoculated only, held one month; 9.—Inoculated, pelleted, held three months.

Pelleted plots are outstanding in each case.

Close-up view of typical subterranean clover drill runs, photographed in August: Left.—Inoculated only; Centre.—Inoculated and pelleted; Right.—Untreated.

The differences in plant size were related to nodulation differences.
and held for three months, gave results equal to those where the seed was sown within two to three days of inoculation and pelleting.

- Pelleted seed gave significantly better results than seed which was inoculated only, even when inoculation was done just before sowing.

- Nodulation was due mainly to the rhizobia applied to the seed, the natural soil content being very low.

Discussion

Nodule bacteria have been shown to survive on lime-pelleted legume seeds for up to 12 months when vacuum infiltrated into the seeds (Loneragan et al, 1981). However, with the methods normally available, inoculated and pelleted seed must be kept for much shorter periods if any advantage is to be gained.

Rather more complex pelleting materials than lime have been investigated in combination with different adhesives and some of these methods allow good survival of the rhizobia for up to five months (Brockwell, 1962; Hastings and Drake 1962). The New Zealand workers Hastings and Drake (1960, 1962) have shown that when using the method of Cass Smith and Goss (1958) the nodulation of inoculated and lime-pelleted seed was good after the seed had been stored for up to three months. However, particular soil conditions and other factors may have a decided influence on the numbers of rhizobia necessary to ensure good nodulation, and thus long storage of inoculated pelleted seed may not be advisable (Hastings and Drake, 1963).

Under the conditions of the experiment conducted at Badgingarra in 1964 no deleterious effect on either nodulation or plant yield was found after inoculated and lime-pelleted seeds had been held for up to 12 weeks and had also been dry sown three weeks before rain fell. Glasshouse trials and field experiments conducted over a number of years have given essentially similar results (unpublished data).

There is not a great deal of information available on the effects of dry sowing on the survival of rhizobia. Brockwell and Whalley (1962) showed that plant nodulation was good when freshly inoculated and lime pelleted seed was placed in soil which remained dry for 23 to 31 days. However, it is generally recognised that high soil

Table 2.—Nodulation of Cyprus barrel medic

Nodulation of Cyprus barrel medic plants grown from seed inoculated or inoculated and lime pelleted at various times before dry sowing in mid-May. Both the adhesives Cellofas A and Methocel were used. Control plants were neither inoculated nor pelleted.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Period of Holding</th>
<th>Nodulation</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoc. &amp; pell. (Cellofas A)</td>
<td>2-3 days</td>
<td>97.0</td>
<td>22.2</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Methocel)</td>
<td>2-3 days</td>
<td>110.0</td>
<td>20.1</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Cellofas A)</td>
<td>1 month</td>
<td>95.5</td>
<td>50.0</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Cellofas A)</td>
<td>2 months</td>
<td>113.0</td>
<td>30.1</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Methocel)</td>
<td>2 months</td>
<td>124.0</td>
<td>30.1</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Cellofas A)</td>
<td>3 months</td>
<td>94.0</td>
<td>30.1</td>
</tr>
<tr>
<td>Inoc. &amp; pell. (Methocel)</td>
<td>3 months</td>
<td>100.5</td>
<td>30.1</td>
</tr>
<tr>
<td>Inoc. only</td>
<td>2-3 days</td>
<td>43.5</td>
<td>10.3</td>
</tr>
<tr>
<td>Inoc. only</td>
<td>1 month</td>
<td>31.5</td>
<td>10.3</td>
</tr>
<tr>
<td>Inoc. only</td>
<td>2 months</td>
<td>43.0</td>
<td>10.3</td>
</tr>
<tr>
<td>Inoc. only</td>
<td>3 months</td>
<td>33.0</td>
<td>10.3</td>
</tr>
<tr>
<td>Control (no treatment)</td>
<td></td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

Difference for significance

- \( P < 0.05 \)
- \( < 0.01 \)
- \( < 0.001 \)

Representative yield assessments could not be made owing to the early haying off of plants in the experimental plots. Figs. 5, 6, 7, 8, indicate the differences apparent during growth.

Figures linked by the unbroken lines are not significantly different from one another but are significantly different from all other figures at the 0.001 probability level.
temperatures are deleterious to the survival of rhizobia (Bowen and Kennedy, 1959; Marshall, 1964). There is the additional risk that germinating rains may not be followed by sufficient rain to ensure continuation of plant growth.

**RECOMMENDATIONS**

- Farmers are advised to pellet all clover and medic seed before planting on new land, with a very finely ground limestone such as M.A.F. (actually used in this experiment) or S/LC.

- In this experiment satisfactory growth followed pelleting as early as February, but as there are a number of factors which adversely influence the survival of rhizobia on the seed, it is unwise to prepare the seed unnecessarily early. In most areas seed preparation in April should be suitable. The seed should be stored in a cool, dry place.

- Dry seeding more than about three weeks before the anticipated break of the season is unwise because of the danger of rains which germinate the seed but do not provide enough moisture for subsequent plant growth.

**REFERENCES**


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