Department of Primary Industries and Regional Development
GOVERNMENT OF WESTERN AUSTRALIA

Journal of the Department of Agriculture, Western Australia, Series 3

Volume 7
Number 2 *March-April, 1958*

Article 20

3-1958

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W. P. Cass Smith

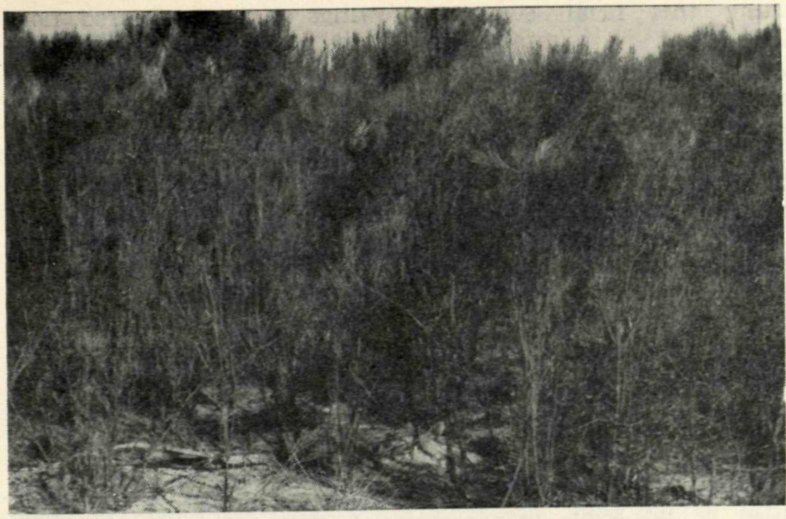

Fig. 1.—Showing the native vegetation, consisting of a moderately dense stand of tamma scrub (Casuarina humilis), on the experimental area at Wongan Hills Research Station. The site was cleared and prepared for planting by ploughing up the standing scrub with a heavy disc implement, and scrub-raking twice to remove the debris

THE EFFECT OF SOIL FUNGICIDES AND FUMIGANTS ON THE GROWTH OF SUBTERRANEAN CLOVER ON NEW LIGHT LAND

By W. P. CASS SMITH, Government Plant Pathologist and A. A. HOLLAND, Plant Pathologist

SUMMARY

A SUBTERRANEAN clover establishment difficulty is described, which occurs on new scrubplain soils in Western Australia, and is caused by a widespread seedling mortality, associated with faulty nodulation. During an exploratory investigation conducted on a "problem" lateritic podsolic soil at Wongan Hills Research Station, the following preliminary results were obtained:—

1.—In unsterilised soil very poor establishment of subterranean clover was obtained due to faulty nodulation and heavy losses from seedling mortality.

2.—In soil sterilised with vapam and formaldehyde fumigants before planting an excellent stand of well-nodulated plants developed.

3.—Partial soil sterilisation resulting from the use of certain fertiliser-fungicide mixtures also promoted good establishment with adequate nodulation.

4.—It is concluded that seedling mortality of subterranean clover, as it occurs in this area, is caused by indigenous soil micro-organisms which prevent nodulation

1. INTRODUCTION

In Western Australia, extensive areas of sandy and gravelly "scrubplain" country, within a rainfall of approximately 14-25 inches, have been brought under cultivation in recent years. As these light lands are inherently low in fertility, they cannot sustain a permanent and payable agriculture until they are farmed on a legume-based rotation. After clearing, cereal cash crops must be limited and legume pastures established at an early developmental stage if humus impoverishment, followed inevitably by soil erosion, is to be avoided.

Subterranean clover has been utilised very largely for this purpose, but under certain conditions, great difficulty is often

experienced in establishing this ideal legume on these light soils.

This difficulty in establishment is caused by a widespread seedling mortality which is invariably associated with faulty nodulation. The seed germinates normally and healthy plant growth occurs for the first few weeks but, thereafter, numbers of seedlings become unthrifty and severely stunted. These affected seedlings have no nodules on the extensive and apparently healthy root system and they develop purplish red foliage in contrast with odd healthy well-nodulated plants which may occur in close proximity. The majority of these stunted plants die progressively, but a few become sparsely nodulated on the deeper roots and survive until the end of the season, but with little or no seed formation. Seedling losses are variable, but in some instances, they have amounted to almost 90 per cent.

Attempts to overcome this faulty nodulation by inoculation combined with various sowing methods have been unsuccessful. (Cass Smith, Harvey & Goss unpublished data).

Many forms of rhizobial inoculum have been tested, including seed inoculation with agar and peat cultures, seed-cleaning residues and soil from established subterranean clover stands. The seed has also been sown mixed with, or separated from superphosphate or basic superphosphate fertiliser, and has been both non-pelleted or pelleted with calcium carbonate or other materials. However, none of these treatments has brought about any marked improvement in nodulation, or significant reduction in seedling mortality.

Extensive nutritional trials have also been conducted in these areas but the results indicate that this difficulty in establishing subterranean clover is not caused basically by deficiencies of major or trace elements. (Dunne & Shier, personal communication).

Information concerning the occurrence of seedling mortality has been obtained from investigations related to the development of light lands in W.A. (Shier & Dunne, personal communication.)

It has been found that the seedling mortality problem becomes less significant as the soil ages under cultivation. It is most serious in sowings on newly-cleared, non-fallowed ground and, under these conditions, losses are generally so great that the stand is virtually a failure. On new ground which has been well fallowed after burning off the native scrub, or on ground which has been cultivated for a number of years, seedling mortality is usually much lower and, in normal seasons, a satisfactory establishment of well-nodulated plants may be expected. In those cases nodulation often occurs in the absence of inoculation.

As a result of these findings and observations it was concluded that the difficulty in establishing subterranean clover might be caused by indigenous soil micro-organisms. In order to test this hypothesis an exploratory experiment was designed to study the effect of soil sterilisation treatments on the first and second year growth of subterranean clover in a newly cleared scrubplain problem area. In this paper some preliminary first year results are reported which support this hypothesis.

2. MATERIALS AND METHODS

Soil Sterilisation with Fumigants.

Soil fumigants with a "wide spectrum" if used at high dosages would eradicate or greatly reduce the population of indigenous soil organisms. Furthermore, when applied at a pre-planting stage, they would not be injurious subsequently to the rhizobia introduced by seed inoculation, or to the host plant. They would therefore serve as a measure of the importance of soil micro-organisms in relation to this problem. *Vapam* (Sodium N-methyl dithio-carbamate) and *formaldehyde* were selected for this purpose.

Partial Soil Sterilisation with Fungicides.

Available fungicides, with few exceptions, are highly toxic to rhizobial bacteria but, if mixed with the fertiliser and sown without contact with, and beneath the inoculated seed, they might provide zones of partially sterilised soil with a reduced content of micro-organisms. This might enable the inoculating rhizobia to colonise these zones at a later stage and bring about nodulation.

After some preliminary tests the following fungicides were selected for trial

and used in mixtures with the basal fertiliser at two dosage levels.

Thiram (Tetramethyl thiuram disulphide) and *spergon*, syn. *tetroc*, (Tetrachloro-para-benzoquinone) at 2 lb. and 20 lb. per acre.

P.C.N.B. (Pentachloronitrobenzene) at 14 lb. and 28 lb. per acre of a proprietary 60 per cent. formulation.

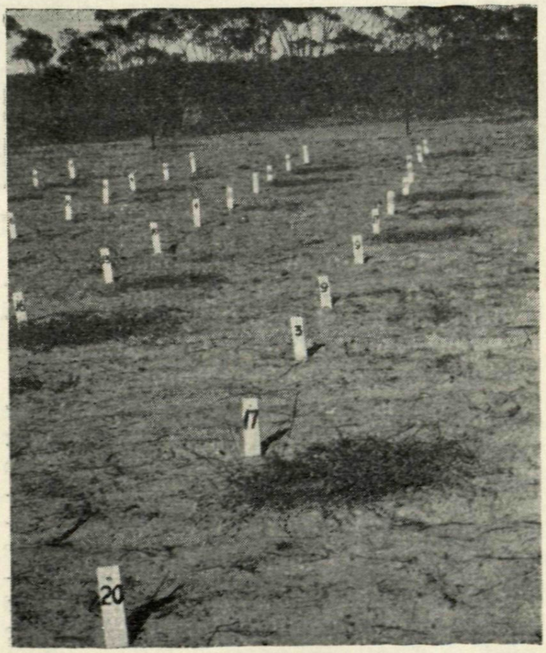

Fig. 2.—Portion of the experimental plots showing layout and differences in the growth of subterranean clover due to treatment. Plots numbered 16 and 17 were fumigated before planting with formaldehyde and vapam respectively

In addition to these fungicides *calcium cyanamide* was also selected for trial. This material is not only a nitrogenous fertiliser but possesses fungicidal properties which bring about a partial sterilisation of the soil (Collings, 1955). A granular commercial product containing 21 per cent. nitrogen was used at a single dosage of 28 lb. per acre mixed with the basal fertiliser. To check the value of calcium cyanamide as a fungicide, rather than a fertiliser, a treatment was also included in which nitrate of soda was mixed with the basal fertiliser at the rate of 1 cwt. per acre.

Seed Pelleting.

In all treatments involving the use of mixtures of fungicide and basal fertiliser the inoculated seed was pelleted with calcium carbonate by the method devised by Cass Smith & Goss (1958).

This technique was applied with the object of delaying the movement of the rhizobia into the rhizosphere and thereby minimising the risk of injury from toxic fungicide residues.

Other Treatments.

A number of other treatments* were also tested but as they were ineffective in the first year they have not been listed in the table in this article or mentioned subsequently except as required for purposes of comparison.

3. FIELD EXPERIMENT DETAILS

Site.

An area was selected at the Wongan Hills Research Station, on the hardpan phase of the Elphin series (Smith, personal communication) of a lateritic podsolic soil. (Stephens, 1953.) On newly cleared soils of this series poor nodulation of subterranean clover and severe seedling mortality have occurred, but good clover pastures are obtained after cultivation and cereal cropping.

A simplified profile consists of 4 in. of grey, gravelly, loamy sand, pH 6.0 = 6.5 overlying 9 in. of gravelly, sandy loam usually grey-yellow. The native vegetation consists of a moderately dense stand of tamma scrub (*Casuarina humilis*) between 5 ft. to 7 ft. in height with bare ground below (see Fig. 1).

The experimental area was cleared and prepared during February and March, 1957, by ploughing up the native vegetation with a heavy disc implement, and scrub-raking twice to remove the debris.

The ground was cultivated to a maximum depth of three inches during these operations and, as a seed bed, was much inferior to those usually prepared by farmers.

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* Seed treated with actidione; seed pelleted with calcium carbonate, calcium gluconate, and activated carbon, both with and without the addition of actidione; activated carbon @ 1 cwt. per acre (Hely *et al* 1957); nitrate of soda @ 1 cwt. per acre; pre-plant treatments comprising water drench, surface soil removal, and removal of organic matter by sieving.

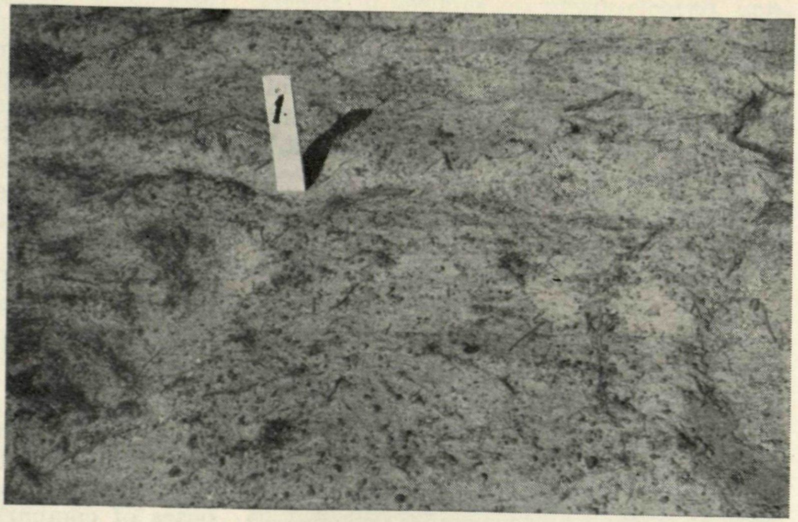


Fig. 3.—Showing very poor establishment of subterranean clover planted by the standard method in unsterilised soil

Layout.

A randomised block layout with six replications of each treatment was used. Plot size was, 4 links x 4 links with buffers of similar area between plots (see Fig. 2).

Pre-planting Soil Fumigation.

Vapam and formaldehyde fumigants were applied to the appropriate plots on April 9 and 10, 1957. Vapam was used at a concentration equivalent to 435 lb. active ingredient per acre, and formaldehyde as a 2 per cent. solution of commercial formalin.

Each material after dilution with water was applied as a soil drench at the rate of three gallons per square foot. As a check on the effectiveness of these fumigants for soil sterilisation purposes, a drench of water only, was applied at the same rate to other plots.

Planting Details.

The plots were planted on May 28 and 29, 1957. Plantings were made in drills, each plot containing three drills, 4 links long x 1 link apart. The basal fertiliser consisted of zinc-copper superphosphate at the rate of 180 lb. per acre. Both basal fertiliser and fertiliser-fungicide mixtures were sown in the drills at a depth of 1½ in. Unsterilised subterranean clover seed var. Dwalganup, inoculated with a pure culture of rhizobial strain NA.30 was sown uniformly in each plot at the calculated rate of 5 lb. of viable seed per acre. Both inoculated seed and inoculated pelleted seed were planted at a depth of ¾ in. directly above the fertiliser.

The standard method of planting mentioned later, refers to inoculated seed planted above basal fertiliser.

Plant Counts.

Three counts were made during the season namely (1) on June 25, after the completion of germination, (2) on July 17, by which time considerable seedling mortality had occurred, (3) on September 30 and October 1 at the late flowering stage when counts were confined to established plants which had more or less formed runners and set some seed. In all cases (see Table) plant numbers have been expressed as a mean percentage of the viable seeds sown.

Sampling.

When the second and third counts were made, sample plants were taken for nodule examination. However, the number of healthy plants removed for this purpose was limited, as second year establishment data is also required.

Rainfall.

The monthly rainfall during the growing period, compared with the 31 year average shown in brackets, was as follows:—June 643 points (254), July 81 (260), August 202 (194), September 46 (91), October 41 (69).

4. RESULTS

Plant counts together with other data are shown in the table.

THE EFFECT OF PRE-PLANTING SOIL FUMIGATION AND FUNGICIDE/FERTILISER MIXTURES ON THE FIRST YEAR GROWTH AND ESTABLISHMENT OF SUBTERRANEAN CLOVER ON NEWLY CLEARED, NON-FALLOWED LIGHT LAND AT WONGAN HILLS RESEARCH STATION.

Treatment	Germination (June 25th) Percentage	Plant Nos. (July 17th) Percentage	Plant Establishment (late flowering stage)		Calculated plot yield (air dried wt. gms.)
			Percentage	Visual rating	
Vapam	89	83	82	excellent	1760
Formaldehyde	89	74	74	excellent	1512
Thiram—high dosage	84	70	67	good	656
Thiram—low dosage	82	53	41	poor	....
Spergon—high dosage	82	43	33	poor	....
Spergon—low dosage	87	60	55	good	429
Calcium cyanamide	87	55	50	fair-good	157
P.C.N.B.—high dosage	82	43	26	poor	....
P.C.N.B.—low dosage	89	60	33	poor-fair	....
Standard method	88	20	14	very poor	....

Following the heavy opening rains in June, a good and reasonably uniform germination occurred in all treatments. When germination counts were made on June 25, plants in the vapam and formaldehyde treatments were mostly at the second trifoliate leaf stage and they were more vigorous and had a darker green colour than the remainder. In all other treatments growth was somewhat less advanced and the cotyledons and first trifoliate leaves of most plants were developing the reddish tinge which is associated as a primary symptom with seedling mortality.

By July 17, when the second plant counts were made, the superiority of the vapam and formaldehyde treatments had become even more apparent. The plants in these plots were all healthy and vigorous and they were more advanced in growth than the remainder, runner formation being general.

Plots planted with the standard method had become inferior to all others. Seedling mortality losses were very high and even the plants which had survived were with few exceptions unthrifty.

Of the plots planted with fertiliser-fungicide mixtures, the *thiram* high dosage and *spergon* low dosage treatments appeared most promising at this stage. In these, seedling mortality losses were low, and numbers of plants with purplish red leaves were beginning to recover and form healthy runners. To a lesser extent recovery and runner formation were also noted in the *calcium cyanamide* and P.C.N.B. low dosage treatments.

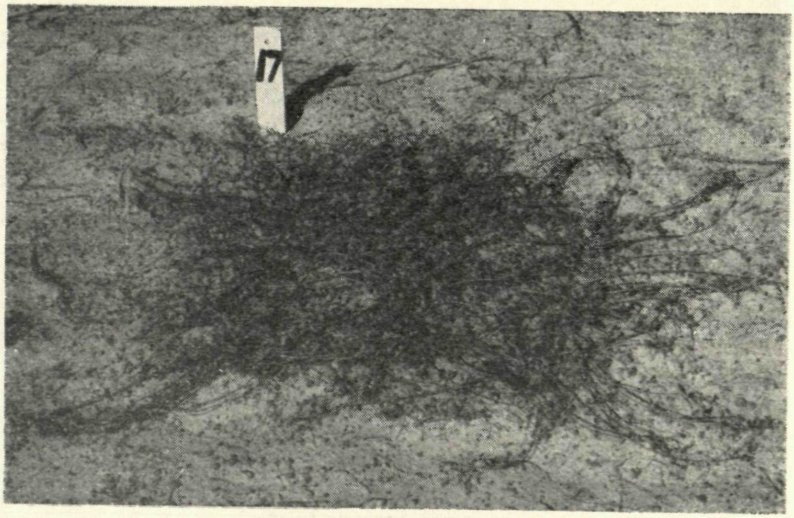
On September 30 and October 1 when final plant establishment counts were made at the late flowering stage, each treatment was rated visually with plant size, density and ground cover as criteria. Yield data based on a limited number of plants sampled for nodule examination, were also obtained from treatments which were obviously more effective than the remainder (see Table).

Plots planted by the standard method proved a failure (Fig. 3). The plants in this treatment were so poorly nodulated that only a small percentage had survived, and even the established plants had set little seed, and were very sparsely nodulated on the deeper lateral roots.

In marked contrast the plots fumigated before planting with vapam and formaldehyde were outstanding, the ground being covered over with a dense mat of vigorous plants which had set seed abundantly (Figs. 2 and 4). Nodulation was excellent and was largely confined to the upper tap-root area.

Of the fertiliser-fungicide mixtures, three were obviously effective and superior to the remainder namely—thiram high dosage (Fig. 5) spergon low dosage and calcium cyanamide. In these treatments establishment was good and the plants had formed strong runners and set quantities of seed.

Fig. 4.—Showing excellent establishment of subterranean clover planted by the standard method in soil sterilised with vapam



The plants were nodulated on the tap and lateral roots, but at a deeper level than the plants in the fumigated plots, and the nodules appeared to be confined largely to a root zone in proximity to the fungicide placement area.

It is noteworthy that the check treatments comprising a pre-plant soil drench with water, and the addition of nitrate of soda to the basal fertiliser, gave poor results and, were respectively, greatly inferior to the soil fumigation and calcium cyanamide treatments.

5. DISCUSSION

It has been shown in this experiment that, despite the use of a tested rhizobial culture strain and adequate nutrients, subterranean clover planted by the standard method failed to establish because of seedling mortality losses, which were associated with poor nodulation.

By sterilising this soil with *vapam* and *formaldehyde* fumigants and planting under otherwise identical conditions, seedling mortality was eliminated and an excellent stand of well-nodulated plants was obtained.

These results provide strong evidence that seedling mortality as it occurs on this newly-cleared light land is caused by indigenous soil micro-organisms which prevent nodulation.

Although the seedling mortality problem is of considerable economic importance its solution by this means is impracticable and cheaper methods must be sought. Partial soil sterilisation, obtained by mixing appropriate fungicides with the basal fertiliser, would appear to have promising possibilities both for this purpose and, as a technique for the determination of problem areas.

The effectiveness of such fungicides would depend largely on their fungicidal value, phytotoxicity to host plant and rhizobia, and residual properties, which in turn would be influenced by dosage.

Specific differences were demonstrated in this experiment, *thiram* fungicide being more effective than *spergon* at high dosage (20 lb. per acre) whereas at low dosage (2 lb. per acre) the reverse was true.

Each of these materials at its better level promoted an establishment of subterranean clover which would be regarded as very satisfactory if the ground had been fallowed and planted by the standard method.

Calcium cyanamide (28 lb. per acre) also gave a satisfactory establishment apparently because of its fungicidal properties, and as this material is available elsewhere as a standard nitrogenous fertiliser, its use in mixtures with basal fertiliser should be widely tested.

Although specific micro-organisms which hinder nodulation in this new light soil have not been identified it seems probable that they inhabit the rhizosphere of the subterranean clover plant and affect the

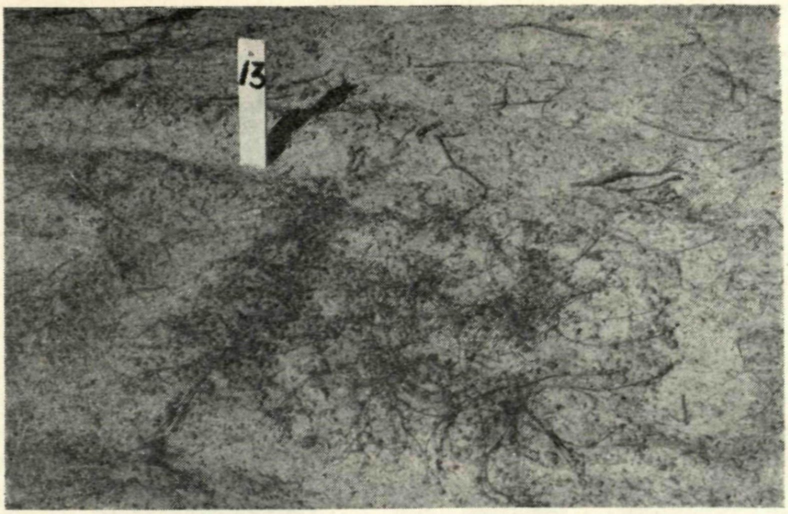


Fig. 5.—Establishment of subterranean clover obtained by mixing thiram fungicide with the basal fertiliser at a dosage of 20 lb. per acre

rhizobia adversely, either directly by antagonism (using this term in the widest sense) or indirectly by an influence on the host plant.

The results obtained in this experiment lend some support to the findings of Hely *et al* (1957) who have shown by laboratory techniques that poor nodulation of subterranean clover on a yellow podsolic soil in N.S.W. is caused by microbiological antagonism which results in failure of seed inoculation.

6. ACKNOWLEDGMENTS

The authors gratefully acknowledge the co-operation of colleagues in the West Australian Department of Agriculture, particularly Dr. T. C. Dunne and Mr. F. L. Shier who supplied details of their nutritional and cultural experiments, and Mr. S. T. Smith who described the soil type and profile. Thanks are also given to Associate Professor J. M. Vincent, School of Agriculture, University of Sydney for providing a culture of NA.30. Supplies of P.C.N.B. and calcium cyanamide were kindly made available by Henry H. York and Co. Pty Ltd., and the American Cyanamid Co. respectively.

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