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# Survey of fungi associated with diseased lupin roots

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# EXPERIMENTAL SUMMARY 1983

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1. Survey of Fungi associated with diseased lupin roots

2. Assessment of lupin root disease in Lupin Permanent Disease Nurseries

3. Effect of fungicide drenches on lupin root rots

4. Mini plot seed treatment trials on Lupins

    A. Solvent infusion of fungicides
    B. Comparison of methods of applying Rovral to lupin seed

5. Root disease investigations of the Wheat-Lupin rotation trial 82M26.

## 1. SURVEY OF FUNGI ASSOCIATED WITH DISEASED LUPIN ROOTS

### (a) Seedling root disease survey, June-July 1983

Isolations were performed from diseased lupin roots sampled from a wide range of locations throughout the wheatbelt. Water agar + 25 ppm aureomycin HCl, Peptone-PCNB (selective for *Fusarium*), and 3P (selective for Pythiaceous fungi) were employed for isolations from each sample. Table 1 shows the frequency of isolation of the most commonly encountered fungi on a district basis.

Not all *Fusarium* isolates were identified to species level, however, representative types were identified using the procedures outlined by Burgess and Liddell (1). *F. oxysporum*, *F. equiseti*, *F. acuminatum* and *F. solani* were the most frequently encountered species (Table 2).

Table 1 Fungal incidence - Seedling root rot survey June-July 1983

Fungal incidence

(% diseased root pieces cultured on appropriate medium from which fungi grew)

| District | n | Pleiochaeta setosa | Rhizoctonia solani | Rhizoctonia spp. | Pythium irreg. | Pythium acanth. | Fusarium | Cylindrocarpon didymum | Cylindrocarpon dest. |
|---|---|---|---|---|---|---|---|---|---|
| Geraldton | 6 | 79 | 4 | 13 | 10 | 0 | 100 | 0 | 0 |
| Moora | 7 | 66 | 2 | 18 | 16 | 1 | 96 | 0 | 0 |
| Albany | 4 | 22 | 0 | 2 | 38 | 2 | 92 | 15 | 2 |
| Esperance | 3 | 51 | 2 | 9 | 22 | 0 | 100 | 0 | 2 |
| Total | 20 | 60 | 2 | 12 | 20 | 1 | 97 | 3 | 1 |

n = number of paddocks sampled

Table 2 *Fusarium* species from seedling root rot survey June-July 1983

| District | n | Number of paddocks from which *Fusarium* species were confirmed | | | | |
|---|---|---|---|---|---|---|
| | | oxysporum | solani | acuminatum | equiseti | other |
| Geraldton | 6 | 4 | 1 | 1 | 5 | 1 |
| Moora | 7 | 7 | 1 | 5 | 4 | 0 |
| Albany | 4 | 3 | 1 | 2 | 3 | 0 |
| Esperance | 3 | 2 | 2 | 0 | 2 | 0 |
| Total | 20 | 16 | 5 | 8 | 14 | 1 |

n = number of paddocks sampled

*Pleiochaeta setosa* (Kirchn.) Hughes is the fungus responsible for Brown Leaf Spot of lupins which is widespread throughout lupin growing areas. The isolation of this fungus from diseased roots was particularly frequent from paddocks with a previous history of lupin cultivation (Table 3). Root disease levels also increase with increasing history of lupin cultivation (Section 2, Figure 1).

Table 3 Effect of paddock history on fungal incidence - seedling root rot survey June-July 1983.

| Paddock History | n | Fungal incidence (%) | | | | |
|---|---|---|---|---|---|---|
| | | Pleiochaeta | Rhizoctonia | Pythium | Fusarium | Cylindrocarpon |
| Lupins | 13 | 83 | 19 | 19 | 97 | 1 |
| No lupins | 7 | 4 | 4 | 24 | 97 | 8 |

n = number of paddocks sampled

At least two species of Rhizoctonia-like fungi were isolated in this survey. Multinucleate types conforming to descriptions of *R. solani* contributed to only 15% of these isolates.

Ninety six percent of all *Pythium* isolates were identified as *P. irregulare*, the remaining isolates being *P. acanthicum*.

Cylindrocarpon was only encountered on the south coast (Albany and Esperance districts). The isolates were identified as C. didymum and C. destructans.

At certain sites in the two northern districts (Geraldton and Moora) a distinct type of disorder, characterised by reddish-brown coloured lesions which develop on the below ground portion of the hypocotyl, was observed. This disorder is considered responsible for large losses in stand density on many farms, particularly those on deep white sands. Table 4 shows the frequency of isolation of the most commonly isolated fungi from the hypocotyl lesions.

Table 4 Fungi associated with the Hypocotyl disorder in Geraldton and Moora districts

| | Fungal Incidence (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| Paddock | Rhizoctonia solani | Rhizoctonia sp | Pleiochaeta setosa | Fusarium oxysp. | Fusarium solani | Pythium equiseti | Pythium irregulare |
| 1 | 60 | 0 | 60 | 0 | 0 | 100 | 0 |
| 2 | 40 | 40 | 0 | 40 | 60 | 0 | 0 |
| 3 | 11 | 0 | 78 | 0 | 0 | 100 | 11 |
| 4 | 60 | 0 | 63 | 80 | 0 | 0 | 0 |
| Total | 44 | 10 | 50 | 30 | 15 | 50 | 3 |

Rhizoctonia solani was the only fungus consistently isolated from all sites and was subsequently shown to reproduce the reddish brown lesions in pathogenicity tests.

(b) Root disease survey, September 1983

A second survey was conducted in the Merredin district in mid-September, when the crops were beginning to flower on the primary axis. The media used in the seedling root disease survey together with a new Rhizoctonia selective medium were employed. Table 5 shows the frequency of isolation of the fungi most commonly encountered from diseased roots.

Table 5 Fungal Incidence - Lupin root disease survey, Merredin District, September 1983

| Paddock | Lupin History | Fungal Incidence (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Pythium | Rhizoctonia | Pleiochaeta | Fusarium oxysp. | Fusarium acumin. | Fusarium equiseti |
| 1 | No | 0 | 21 | 36 | 73 | 0 | 0 |
| 2 | No | 25 | 19 | 0 | 40 | 20 | 0 |
| 3 | No | 25 | 56 | 0 | 46 | 34 | 0 |
| 4 | No | 22 | 40 | 0 | 73 | 0 | 27 |
| 5 | Yes | 28 | 7 | 94 | 93 | 7 | 0 |
| Total | | 21 | 33 | 21 | 61 | 16 | 5 |

As in the seedling disease survey, an increased frequency of isolation of Pleiochaeta setosa from paddocks with a previous lupin history was observed.

2. ASSESSMENT OF LUPIN ROOT DISEASE IN LUPIN PERMANENT DISEASE NURSERIES

(78WH20, 78BA51, 78C34, 78MT38, 80E21)

Aim:

To quantify levels of lupin seedling root disease and the population of Pleiochaeta setosa spores in soil. (P. setosa can cause root rot as well as Brown Leaf Spot).

Nursery Design:

Five (35 x 130 m) plots were sown in the following rotation:-

| Plot | '78 | '79 | '80 | '81 | '82 | '83 |
|---|---|---|---|---|---|---|
| 1 | L | C | L | L | C | C |
| 2 | C | L | C | L | L | L |
| 3 | L | C | L | C | L | L |
| 4 | L | L | C | L | C | L |
| 5 | C | L | L | C | L | C |

L = lupin, C= cereal

Results: Figure 1

Comments:

An overall trend of increasing root disease from first to second to third successive lupin crops is evident. Higher levels of root disease and soil *P. setosa* populations were present at Wongan Hills, Esperance and Mt Barker compared to Chapman Valley and Badgingarra. Considerable levels of *P. setosa* were found in Plot 1 soil demonstrating the ability of these spores to survive under a cereal for at least one season.

## 3. EFFECT OF FUNGICIDE DRENCHES ON LUPIN ROOT ROT

Experiments: 83MT17 and 83WH9

Aim:

To elucidate which soil fungi cause lupin root rot by examining the effect of soil treatment with semiselective fungicides on root disease development.

Fungicides:

Benlate wettable powder (50% benomyl) was used at 10 and 20 $g/m^2$.
Rovral wettable powder (50% iprodione) was used at 5 and 20 $g/m^2$.
Ridomil wettable powder (25% metalaxyl) was used at 1.0 and 3 $g/m^2$.
Benlate and Ridomil were also applied as a mixture at 20 and 3 $g/m^2$ respectively.

All fungicides were applied as a soil drench (4 $l/m^2$) using a wetting agent (WETTASOIL) at 5 ml/l. The Nil treatment received water and wetting agent at the appropriate rates.

83MT17 and 83WH9 were located on the third year lupin plots (Plot 2) of the Lupin Permanent Disease Nurseries, 78MT38 and 78WH20, respectively. Treatments were applied to 2.25 m x 1 m plots one week after the lupins were sown. In 83MT17 the treatments were applied a second time, 4 weeks later.

Measurements:

Quadrats samples were taken 4 and 16 weeks after the first treatments were applied in 83MT17. Whole plots were harvested 4 weeks after applying the treatments in 83WH9.

Root rot and hypocotyl rot were rated on a scale of 0-6 and 0-5 respectively. Plant counts and total dry matter production were determined. Diseased root pieces were cultured on a range of selective media to provide an estimate of the level and genera of fungi present. Brown leaf spot incidence was also assessed on the second sampling of 83MT17.

Results: See Tables 6-8

Table 6 83MT17, Effect of fungicide drenches on lupin root rot, 4 weeks after treatment

| Fungicide | Rate ($g/m^2$) | Emergence plants/$m^2$ | Hypocotyl Rot | Root Rot |
|---|---|---|---|---|
| Control | - | 55 | 0.08 | 0.52 |
| Benlate | 10 | 58 | 0.26 | 0.40 |
| Benlate | 20 | 55 | 0.08 | 0.68 |
| Rovral | 5 | 63 | 0.37 | 0.61 |
| Rovral | 20 | 55 | 0.42 | 0.34 |
| Ridomil | 1 | 60 | 0.32 | 1.27 |
| Ridomil | 3 | 55 | 0.34 | 0.58 |
| Benlate | 20 ) 1.5) | 48 | 0.34 | 0.65 |
| | | NS | NS | NS |

Table 7 83MT17, Effect of fungicide drenches on Brown Leaf Spot (BLS) (12 weeks after second treatment).

| Fungicide | Rate ($g/m^2$) | % plants "moderate" BLS | % plants "severe" BLS | Density ($plants/m^2$) | Total dry weight per $2.25m^2$ |
|---|---|---|---|---|---|
| Control | - | 12.3 | 20.9[A] | 38 | 132 |
| Benlate | 10 | 45.7 | 57.7[B] | 41 | 204 |
| Benlate | 20 | 31.6 | 26.8[A] | 45 | 185 |
| Rovral | 5 | 0.3 | 0.1[A] | 49 | 242 |
| Rovral | 20 | 0.0 | 1.7[A] | 50 | 246 |
| Ridomil | 1 | 34.7 | 27.2[A] | 49 | 189 |
| Ridomil | 3 | 37.6 | 30.6[A] | 32 | 158 |
| Benlate | 20 ) | 28.1 | 16.8[A] | 38 | 183 |
| Ridomil | 1.5) | | | | |
| L.S.D. (p = 0.5) | | NS | 30.6 | NS | NS |

Table 8 83WH9, Effect of fungicide drenches on lupin root rot

| Fungicide | Rate ($gm^{-2}$) | Hypocotyl Rot | Root Rot | FUNGAL INCIDENCE (% disease root pieces cultured on appropriate medium from which fungi grew) Rhizoctonia | Pythium | Pleiochaeta | Fusarium |
|---|---|---|---|---|---|---|---|
| Control | - | $1.63^{A}$ | $2.85^{DE}$ | 25 | $13.5^{A}$ | $93^{A}$ | $96^{A}$ |
| Benlate | 10 | $0.55^{B}$ | $2.63^{CDE}$ | 26 | $25.0^{B}$ | $53^{B}$ | $48^{B}$ |
| Benlate | 20 | $0.73^{B}$ | $2.35^{BC}$ | 14 | $11.8^{A}$ | $33^{C}$ | $29^{C}$ |
| Rovral | 5 | $0.58^{B}$ | $2.00^{AB}$ | 19 | $10.0^{AC}$ | $28^{C}$ | $96^{A}$ |
| Rovral | 20 | $0.58^{B}$ | $1.95^{A}$ | 15 | $8.25^{AC}$ | $22^{C}$ | $99^{A}$ |
| Ridomil | 1 | $1.40^{A}$ | $2.98^{E}$ | 26 | $0^{C}$ | $89^{A}$ | $96^{A}$ |
| Ridomil | 3 | $1.40^{A}$ | $2.93^{E}$ | 34 | $0^{C}$ | $91^{A}$ | $195^{A}$ |
| Benlate + Ridomil | 20 ) 1.5) | $0.68^{B}$ | $2.53^{CD}$ | 25 | $0^{C}$ | $43^{BC}$ | $20^{C}$ |
| L.S.D. (p= .05) | | 0.30 | 0.37 | NS | 10.4 | 18.2 | 10.1 |

Comments

Antifungal spectrum of the fungicides used:-

| Pathogens | Ridomil | Rovral | Benlate |
|---|---|---|---|
| Pythium spp | ++ | 0 | 0 |
| Phytopthora spp | ++ | 0 | 0 |
| Rhizoctonia spp | 0 | ** | ** |
| Fusarium spp | 0 | 0 | ++ |
| Pleiochaeta setosa | 0 | ++ | + |

++ Highly inhibitory
+ Moderately inhibitory
0 Not inhibitory
** Highly inhibitory to some strains of Rhizoctonia

At Wongan Hills Rovral and to a lesser extent Benlate caused a reduction in root disease. Ridomil had no effect. These results, together with the fungal isolation data, strongly implicate Pleiochaeta as the most likely causal organism.

The Mount Barker data is highly variable suggesting an insufficient number of plants sampled. The only statistically significant result was the reduction of plants with severe Brown Leaf Spot by the two Rovral drenches.

## 4. MINI PLOT SEED TREATMENT TRIALS ON LUPINS

### A. Solvent Infusion of Fungicides

The solvent infusion technique has shown promise as a more efficient and effective method of applying fungicides to seeds as compared with conventional methods (Locke et al, 1983; Shortt and Sinclair, 1980). Glasshouse experiments suggested that solvent infusion of certain fungicides may improve establishment and seedling vigour in lupin stands.

Aim:

Lupin seed (Illyarrie) was immersed for 2 hours in dichloromethane (DCM) containing 2.5% W/V (active ingredient) of the following fungicides:

1. Benlate
2. Vitavax
3. Rovral
4. Ridomil

The control treatments were:-

5. Untreated
6. DCM

Methods:

Exactly 100 seeds of each treatment were hand sown in 1 m x 1 m plots. There were five replicates.

Measurements:

Plots were harvested 5 weeks after sowing and assessed for root disease and Brown Leaf Spot (BLS).

BLS was assessed by rating all the leaflets of the first two leaves on a scale of 0-5.

0 - 0% leaf area affected
1 - 1-2 " " "
2 - 2-10 " " "
3 - 10-30% " " "
4 - 30% " " "
5 - leaflet defoliated

Results

83PE40: The trial was completely eaten out by the bean seedling maggot before the seedlings reached the 4 leaf stage. However, the Rovral treatments appeared more vigourous prior to maggot attack. No data could be collected.

83MD31: Unfortunately no root or hypocotyl rots developed at this site. Rovral was the only fungicide which reduced the severity of BLS (Table 9).

Table 9 Effect of solvent infusion of fungicides into lupin seeds on Brown Leaf Spot : 83MD31

| Treatment | Emergence (%) | Brown Leaf Spot (0-5) | Dry weight of tops per plot (g) |
|---|---|---|---|
| Control | 87.6 | 2.46[A] | 49.5 |
| DCM | 90.6 | 2.25[A] | 54.0 |
| Benlate/DCM | 85.4 | 2.35[A] | 52.1 |
| Vitavax/DCM | 88.2 | 2.52[A] | 51.3 |
| Rovral/DCM | 90.0 | 1.04[B] | 57.4 |
| Ridomil/DCM | 87.2 | 2.35[A] | 52.8 |
| L.S.D. (p = .05) | NS | 0.60 | NS |

Comments:

No treatment affected nodulation

References:

Shortt, B.J. and Sinclair, J.B. (1980) Efficacy of polyethylene glycol and organic solvents for infusing fungicides into soybean seeds. Phytopathology 70 : 971-973

Locke, J.C., Papavizas, G.C., Lewis, J.A. and Lumsden, R.D. (1983) Control of Pythium blight of Snap Beans by seed treatment with systemic fungicides. Plant Disease 67 : 974-977.

3.B. Comparison of methods of applying Rovral to lupin seed.

Aim:

To compare solvent infusion with conventional methods of applying Rovral to lupin seed for the control of root rot and early Brown Leaf Spot (BLS).

Methods:

Rovral was applied at 1.25 and 2.5 g/kg as a dust and a slurry. Solvent infusion, over 2 hours, was performed with 2.5% and 5.0% active ingredient in dichloromethane (DCM) and with 2.5% active ingredient in acetone (AC).

Exactly 100 seeds of each treatment were hand sown in 1 m x 1 m plots. There were five replicates.

Plots were harvested 5 weeks after sowing and assessed for root disease and BLS. BLS was assessed as outlined for 83MD31.

Results:

83PE41 was completely eaten out by the bean seedling maggot and no data was recoverable.

83MD32: Unfortunately no root or hypocotyl rot developed at this site. All Rovral treatments reduced BLS. (Table 10).

Table 10 83MD32, Effect of different methods of applying Rovral to lupin seed for control of BLS

| Treatment | Emergence (%) | Brown Leaf Spot (0-5) | Dry weight of tops per plot (g) |
|---|---|---|---|
| Control | 84.5 | 3.63[A] | 118 |
| Dust 1.25 g/kg | 84.0 | 1.50[B] | 105 |
| Dust 2.5 g/kg | 89.2 | 1.28[B] | 120 |
| Slurry 1.25 g/kg | 89.2 | 1.40[B] | 126 |
| Slurry 2.5 g/kg | 89.8 | 1.28[B] | 143 |
| DCM 2.5% | 88.0 | 1.48[B] | 129 |
| DCM 5.0% | 90.5 | 1.20[B] | 132 |
| AC 2.5% | 91.3 | 1.25[B] | 121 |
| L.S.D. (p = .05) | NS | 0.43 | NS |

Comments:

No treatment affected nodulation

The use of solvent infusion procedures can theoretically result in the use of much less fungicide per kg of seed compared with the traditional methods of application. However, I am unaware of any system developed to treat large quantities of seed economically.

## 5. ROOT DISEASE INVESTIGATIONS OF THE WHEAT-LUPIN ROTATION TRIAL 82M26

Aim:

To quantify the amount of root disease in wheat and lupins and determine the fungi present in the different rotation treatments.

Treatments:

The main treatments are:-

1. Wheat-Lupin (1:1) rotation
2. Wheat-Lupin (2:1) rotation
3. Continuous Wheat
4. Continuous Lupins

There are 5 different levels of nitrogen applied to the wheat.

As the trial has only been in progress two years, the main rotation treatments are effectively reduced to:-

| | '82 | '83 |
|---|---|---|
| 1. | Lupins | Wheat |
| 2. | Wheat | Lupins |
| 3. | Lupins | Lupins |
| 4. | Wheat | Wheat |

Measurements:

Lupins were sampled at first flowering on the primary axis. Wheat was sampled at anthesis. Samples were visually rated for root disease and cultured on a range of media to determine the fungi present.

Results:

a) Lupin root disease

The yield of lupins after wheat was double that of lupins after lupins. Hypocotyl rot was lower in the lupin after wheat although root rot was not statistically significant (Table 11).

Table 11 Lupin Root Disease and Yield data 82M26

| Rotation '82 | Rotation '83 | n | Hypocotyl Rot (0-5) | Root Rot (0.6) | Stand Density (plants/$m^2$) | Yield (kg/ha) |
|---|---|---|---|---|---|---|
| L | L | 3 | 0.47 | 0.91 | 32 | 278 |
| W | L | 6 | 0.13 | 0.54 | 38 | 572 |
| Significance | | | * | NS | NS | ** |

n = number of plots
L = lupin
W = wheat

Isolation data and pathogenicity tests suggested Pleiochaeta setosa to be responsible for most of the lupin root rot.

b) Wheat root disease

Cursory examination of the root systems suggested that levels of the typical wheat root diseases were very low (Actual rating will be performed by Dr MacNish upon his return from study leave).

Isolation data is summarized in Table 12. Lower levels of Cochliobolus sativus (the Common Root Rot pathogen) and a Fusarium of the Roseum group (could not be identified to species as it failed to sporulate) were found on wheat grown after lupins.

Table 12 Fungi isolated from wheat crown roots and subcrown internodes

| | | | FUNGAL INCIDENCE (% root pieces cultured on appropriate medium) from which fungi grew | | | |
|---|---|---|---|---|---|---|
| Rotation '82 | Rotation '83 | n | Fusarium oxysporum | Fusarium Roseum | Cochliobolus sativus | Rhizoctonia |
| W | W | 9 | 51 | 19 | 16 | 8 |
| L | W | 6 | 50 | 2 | 3 | 11 |

W = wheat
L = lupin

There was no significant yield difference between the wheat grown after wheat and that grown after lupins.

Comments:

The failure of the wheat to yield better after lupins compared with continuous wheat may reflect the very low levels of wheat disease present.

Pleiochaeta setosa was isolated from 10% of root pieces of wheat sown after lupins. It was isolated from less than 1% root pieces of wheat sown after wheat. Saprophytic survival of this fungus on wheat roots may be significant mechanism by which it survives in soil.

A larger number of samples needs to be taken to assess lupin root rot and they should also be sampled much earlier after sowing.