# VG146
# Management strategies for Rhizoctonia of processing beetroot

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VG146

This report is published by the Horticultural Research and Development Corporation to pass on information concerning horticultural research and development undertaken for the beetroot industry.

The research contained in this report was funded by the Horticultural Research and Development Corporation with the financial support of the Cowra Vegetable Growers Association.



Cover Price $20.00

HRDC ISBN 1 86423 485 7

Published and Distributed by:

Horticultural Research and Development Corporation
Level 6
7 Merriwa Street
Gordon NSW 2072

Telephone: (02) 9418 2200
Fax: (02) 9418 1352

HORTICULTURAL RESEARCH & DEVELOPMENT CORPORATION
The Research Arm of the Australian Horticultural Industries

# FINAL REPORT

## Management strategies for Rhizoctonia Disease of processing beetroot

PROJECT V/0146


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1 June 1993

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**FINAL REPORT** **PROJECT V/0146**

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**PROJECT TITLE:** Management strategies for Rhizoctonia Disease of processing beetroot

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**COMMENCEMENT DATE:** October 1991

**ANTICIPATED COMPLETION DATE:** October 1992

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**TOTAL PROJECT COST IN 1991/92:** $6480

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## INDUSTRY FINANCIAL SUPPORT:

Cowra Vegetable Growers Association
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Edgell-Birds Eye
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Shell Australia and Bayer Australia supplied the specific fungicides used for efficacy experiments.

Growmix Pty Ltd provided the composted arboreal and garden waste material for the field experiment

## SUMMARY:

The closing of the Edgell-Birds Eye factory at Cowra decimated the processing beetroot industry in the Lachlan Valley. Although the reasons for the closure were complex, this act serves as a potent reminder that any of our agricultural industries face extinction unless they can achieve the production efficiency and standards required in the marketplace. Results and information gathered in this project are not lost, however, as they can be applied to the remaining beetroot growers and mixed cropping enterprises of the Lachlan Valley. There is also some relevance to the processing beetroot industry of the Lockyer Valley in Queensland.

Beetroot formed an important part of a rotation cycle with other processing vegetables (sweet corn, tomatoes & brassicas), grain and oil crops (cereals & canola), lucerne, mixed pastures and fallow. Disfigurement, hollow heart, cavities and cankers of beets were a major source of economic losses for several years. Cannery quality control standards were breached when 5-6% of beets showed external symptoms and where as little as 1% had hollow heart. Additional labour required in the cannery to maintain quality control added significantly to the costs to production.

This project identified Rhizoctonia Rot as a primary cause of crown cavities and rots on processing beetroot. A survey of crops in the 1990/91 season had detected significant infections of the fungus *Rhizoctonia* associated with field symptoms. The hypothesis that this fungus was the primary cause of field symptoms was tested in glasshouse and field experiments, while the influence of other factors such as boron deficiency, other fungi and insect pests were assessed. Boron applied as a foliar spray treatment in the field experiment actually reduced the number and weight of marketable beets at harvest. Leaf analyses showed that boron was not limiting in any of the samples tested. Extensive laboratory examination of affected plants found no evidence of insect attack and isolations from affected tissue yielded only the fungus, *Rhizoctonia*. Selective isolation media for other fungi (*Aphanomyces* and *Pythium*) failed to yield any positive results. Some lateral cracks on maturing beets were not shown to be due to any of the above factors. They appear to be a physiological condition

and are possibly due by cyclic extremes in soil moisture that are brought about by irrigation and subsequent drying.

Laboratory studies further characterised the *Rhizoctonia* fungus. Mark Sweetinghan, Plant Pathologist, WA Department of Agriculture used the pectic zymogram procedure. This procedure involves electrophoresis on polyacrylamide gels to separate particular enzymes from the fungus that exist in a number of forms. The patterns formed are compared with those of standard cultures, thereby characterising the group to which unknown fungi belong. Dark bands correspond to pectin depolymerase and white bands to pectin methyl esterase activity. The beetroot *Rhizoctonia* was similar, but not identical, to reference isolates belonging to zymogram group 4–2 (ZG4–2). The ZG4 group are thought to be legume pathogens and may also attack certain vegetables such as potatoes and cauliflowers (based on earlier experimental work done in WA).

Glasshouse assays determined alternative host crops for this *Rhizoctonia* isolated from beetroot. Plant species were chosen that formed part of the rotation cycle in the Lachlan Valley. Assays in field soils confirmed that beetroot were clearly the most susceptible species tested. Brassicas (cabbage and canola) and two of the grasses (barley and corn) were the next most susceptible species. Of the legumes tested, only lupins was moderately susceptible although the results were quite different when a pasteurised soil medium was used. Possible reasons for such differences will be discussed later in this report. This information could be useful for future cultural management strategies to minimise inoculum levels of *Rhizoctonia* in the crops still grown in the district.

Three fungicides were assessed for efficacy in glasshouse and field experiments. They were pencyuron (Monceren®, Bayer), tolclofos–methyl (Rizolex®, Shell) and ipridione (Rovral®, Rhone–Poulenc). All chemicals were applied as soil drenches. Monceren® performed the best in both the field and glasshouse. It was the only treatment to significantly increase plant establishment and the number of marketable beets at harvest compared to the untreated controls. Rovral® showed some promise in the field but Rizolex® failed in all experiments at the rates applied. It should be noted that there is evidence in the published literature that the efficacy of chemicals to *Rhizoctonia* may depend upon which strain of the fungus that is used. It would not be prudent to extrapolate these efficacy results to other districts without firstly characterising the fungus.

A composted organic matter treatment was compared to chemical treatments in the field experiment. It showed some promise in its ability to suppress *Rhizoctonia* but was highly variable between replicates. Laboratory bioassays conducted by Dr Peter Fahy, BCRI Rydalmere, showed that it was only mildly suppressive to *Rhizoctonia*. No attempt was made to measure soil physical changes from the addition of compost on the field trial at Cowra, but observations suggested that the soil did not compact and crack after heavy irrigation and drying out as was the case where the compost was not applied. One further observation in the canola crop that followed the beetroot trial was a marked growth response only in the areas where the compost treatments had been applied. Further experimental work is required to define the potential benefits of composts in these alluvial soils. There is clearly great potential for a compost with better disease suppressive qualities.

**OBJECTIVES:**

i. Confirm that *Rhizoctonia* is the principal cause of field symptoms and evaluate the role of boron deficiency, other pathogens and insect damage

ii. Identify which strain (or strains) of *Rhizoctonia* are responsible for field symptoms and investigate which other plant species act as alternative hosts in rotation crops

iii. Devise and implement disease management strategies based on cultural and chemical treatments

**METHODS:**

1. Initial Investigations

Beetroot crops were surveyed during the 1990–91 season for evidence of rots, cracks and cavities. Samples were examined by microscopy and tissue was plated to a range of culture media [acidified potato dextrose agar (PDA/LA), potato carrot agar with 30 ppm rifampicin and 10 ppm pimaricin (PCA/RP), a selective medium for *Aphanomyces* (PDA/PNP), water agar (WA) and a semi-selective medium for *Rhizoctonia* (WA/KHT)]. Recipes, references and selectivity are summarised in Table 1.

Isolates of *Rhizoctonia* were sent to Mark Sweetingham, Plant Pathologist, WA Department of Agriculture for characterisation using pectic zymogram analysis. Fungal enzymes were separated on polyacrylamide gels. The patterns formed were compared with those of standard cultures, thereby characterising the group to which unknown fungi belong. Dark bands corresponded to pectin depolymerase and white bands to pectin methyl esterase activity (Sweetingham *et. al.*, 1986; Sweetingham & MacNish, 1991).

TABLE 1. Media used for fungal isolations

| MEDIUM | SELECTIVITY | RECIPE/REFERENCE |
|---|---|---|
| PDA/LA | All | Johnston & Booth, 1983 |
| PCA/RP | *Pythium/Phytophthora* | Jeffers & Martin, 1986<br>Tesoriero, 1990 |
| PDA/PNP | *Aphanomyces* | Hutton & O'Brien, 1986 |
| WA | All | 15g/l Davis Agar |
| WA/KHT | *Rhizoctonia* | Ferris & Mitchell, 1976 |

2. Glasshouse Experiments

Two glasshouse experiments were conducted to determine the host range and relative pathogenicity of beetroot isolates of *Rhizoctonia*. Seedling trays (30 x 25 x 5 cm) were filled with field soil that had been inoculated with *Rhizoctonia* (230 propagules/tray). *Rhizoctonia* inoculum consisted of colonised oat grains (Gaskill, 1968). In the first experiment 100 seeds each of fifteen plant species were used (see Table 4). There were three replicates of each treatment. Trays were placed in a controlled temperature glasshouse with a diurnal range of 20° C (night) and 30° C (day). Trays were watered daily to soil moisture holding capacity. Establishing plants were scored over the next fortnight. Plants that died were collected, examined and the cause of death confirmed by isolation procedures described above. The second experiment used pregerminated seeds (100 seedlings of 12 plant species) and trays were kept at a constant temperature of 24° C. All other conditions and methods were as in the first experiment.

Separate experiments were conducted to compare fungicide efficacy. Ten pregerminated beetroot seed (cv. Garnet) were sown into 10 cm pots containing either field soil or pasteurised UC potting mix that had been colonised by *Rhizoctonia*. Inoculum was prepared as described above and 16 propagules used per pot. One set of control pots were kept uninoculated. Chemical drenches were applied after sowing at the rates shown in Table 6. Establishing plants were scored and the cause of death determined as described above. Five replicates were used in a complete replicated block design.

3. Field Experiment

A replicated complete block field experiment was designed to determine the importance of Rhizoctonia Rots of processing beetroot and to compare some different chemical and biological control treatments. Each treatment plot was 12 rows wide and 10 metres long. Buffer rows separated treatment blocks within the trial (6 rows) and from the adjacent crop (3 rows). The site was inoculated with a grain inoculum of *Rhizoctonia* (50kg/ha) to ensure an even density of disease pressure (Gaskill 1968). One set of control treatment plots was left uninoculated. The compost treatment was applied (500 cubic metres/ha) with a front end loader, raked evenly and worked into the top soil with a rotary hoe 24 hours prior to sowing. Chemical drenches were made in 200 litre drums and 40 litres were applied to each treatment plot with watering cans 24 hours prior to sowing. Treatments are summarised in Table 2.

TABLE 2. Treatments applied to the field experiment

| Treatment | Rate of Active Ingredient kg active ingredient/ha | Rhizoctonia Inoculum |
|---|---|---|
| Nil | - | - |
| Nil | - | 50 |
| Monceren® | 7.5 kg | 50 |
| Rovral® | 2.5 kg | 50 |
| Rizolex® | 1.12 kg | 50 |
| Compost | 500 cu m | 50 |

Boron (Solubor) was applied to six rows of plants in each treatment as beets were beginning to fill (50 days after sowing). Leaf petioles were sampled 7 days after Boron application and again at harvest from plots. A composite of twenty petioles were bulked. Samples were washed, dried and processed for ICP analysis.

Treatments and cultural operations for the field experiment are summarised in Tables 2 and 3 below.

TABLE 3. Summary of cultural operations applied to the field experiment

| Treatment | Details |
|---|---|
| Previous rotations | Fallow 1991, cereal 1990 |
| Fertiliser | 60 kg N/ha, 45 kg P/ha, 100 kg K/ha |
| Sowing Rate | Cultivar "Garnet" sown 29/1/92<br>410 mm between rows<br>25 mm in row (aiming for 70 mm plant spacing) |
| Herbicides | Pre emergent Pyramin[R] and Ramrod[R] |
| Establishment | Watered every 4 days until emergence thence weekly |
| Cultivation | Cultivated between rows at 4 true leaf stage<br>Chipping continued until row closure in the canopy |
| Sidedress | 125 kg/ha urea (63 kg N/ha) on 1/3/92 |
| Pest & Disease Control | No other insecticides or fungicides used |

Measurements and Statistical Analysis

Data was analysed by Dr I. Barchia (Biometrician), BCRI, Rydalmere.

(i) Plant Establishment Rating (6 weeks after sowing)

Plots were scored independently by the three investigators using the following rating system:

| | | |
|---|---|---|
| 1 | <40 % | plants established |
| 2 | 40-60 % | " " |
| 3 | 61-75 % | " " |
| 4 | 76-90 % | " " |
| 5 | >90 % | " " |

The scores of plant establishment were converted to a rating that ranged from 1 to 5, implying that the data follows a multinomial distribution. However, we used a poisson distribution as an alternative to the multinomial distribution to underline the error structure. A log link function was used to estimate the parameters.

(ii) Estimate of damping–off (6 weeks after sowing)

Counts of damped–off plants were made from 4 rows per treatment plot. Examination of data suggested that distribution was not normal since the skewness of residual values was 1.102 +/- 0.427. This indicated that the data were significantly skewed to the right. Variances within each treatment were found to be dependent on the treatment means with the coefficient b = 0.46 where b=1–B/2; B is the regression of log (variance) on log (mean). The value b = 0.5 suggests that data are Poisson distributed. A generalised linear model was used to relate the treatments to the number of damped–off plants and a log link function was used to estimate the parameters.

(iii) Marketable yield estimates

Two 8 metre rows from treatment plots were harvested and scored for the number and weight of marketable beets (sizes and quality acceptable for processing). The data was normally distributed and an analysis of variance carried out and treatment means compared with using the least significance difference test.

(iv) Effect of foliar Boron application on marketable yield

An 8 metre row from treatment plots were harvested and scored for the number of marketable beets and weights. Since the application of boron was not random, the analysis of beet number and weight was made separately for plots with and without boron. Examination of data suggested that they were within normal distribution ranges. Variances of the two variables were independent of means. The analysis of variance technique was used to analyse these variables and the least significant difference test used for testing pair treatment mean differences.

## RESULTS & DISCUSSION

### 1. Initial Investigations

*Rhizoctonia* was consistently isolated from beetroot with rots, cavities and cankers. These symptoms are illustrated in Figures 1 to 4. Fungal threads of *Rhizoctonia* were found associated with crown tissue of young plants (2–3 leaf growth stage) that already showed deterioration of surface tissue. No other pathogenic fungi were isolated despite the use of selective and non selective isolation media. Figures 5 and 6 show the typical fungal threads of *Rhizoctonia* growing on an isolation medium. This suggests that *Rhizoctonia* was a primary cause of these symptoms. Some lateral cracks (Figure 7) were shown not to be due to any biological factors. The cause of such symptoms was not determined but were possibly caused by the cyclic extremes in soil moisture or simply mechanical damage.

Pectic zymogram analysis determined that the beetroot *Rhizoctonia* was similar, but not identical, to reference isolates belonging to zymogram group 4–2 (ZG4–2). The ZG4 group are thought to be legume pathogens and may also attack certain vegetables such as potatoes and cauliflowers (based on earlier experimental work done in WA).

FIGURE 1. External rots and cankers of beetroot caused by *Rhizoctonia*

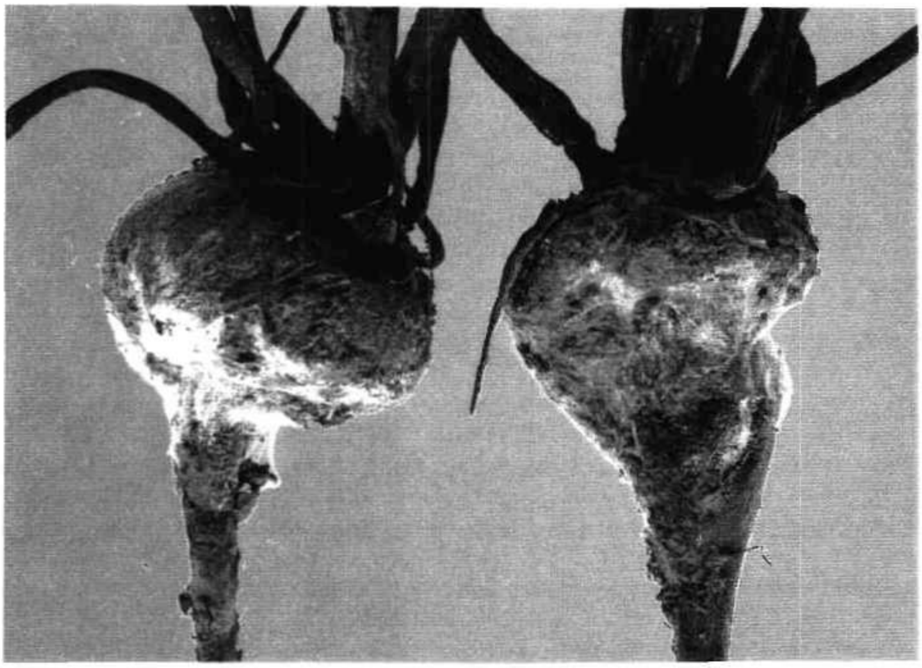
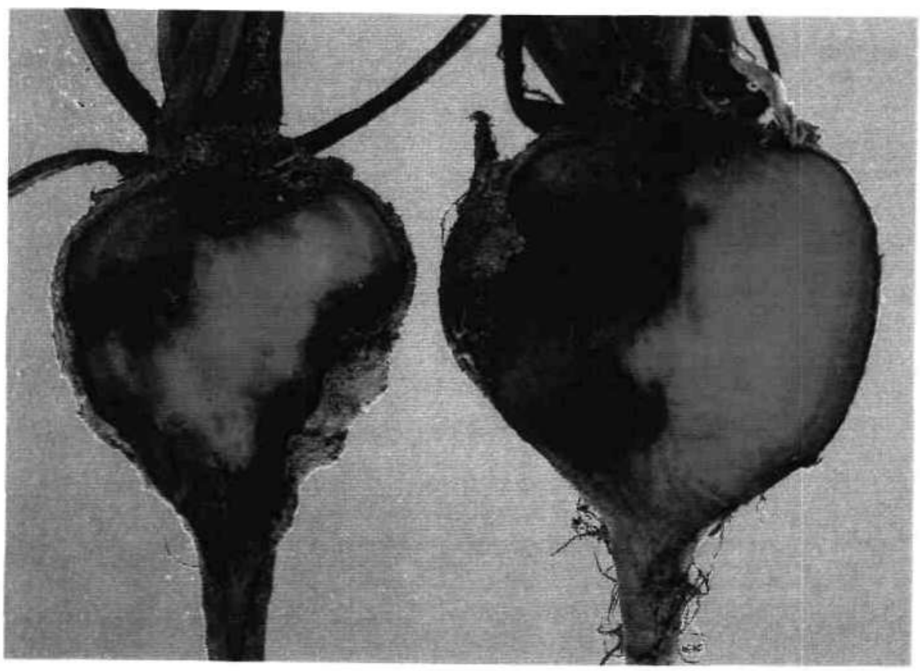
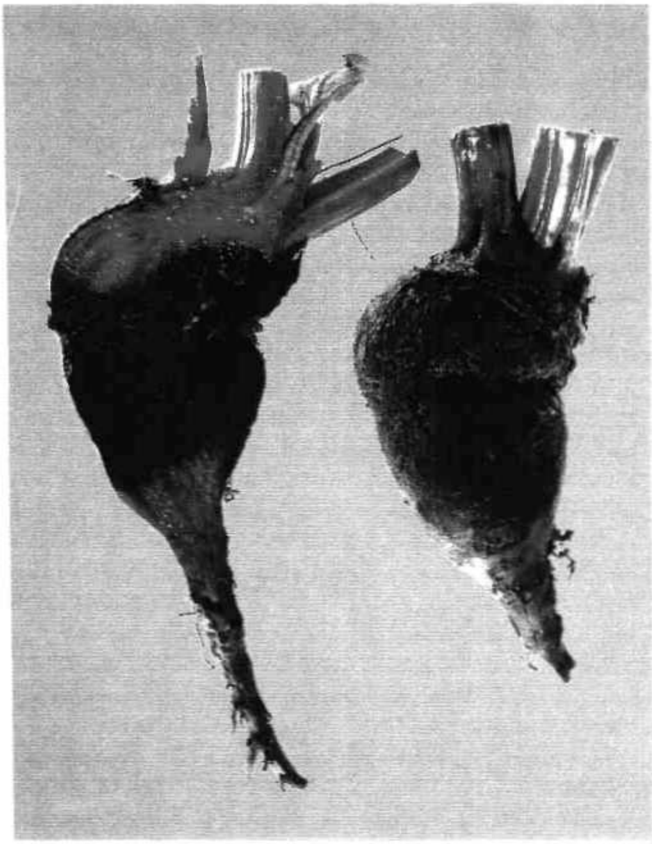


FIGURE 2. Internal rots of beetroot caused by *Rhizoctonia*



FIGURES 3 & 4. Internal and external rots and cavities of beetroot caused by *Rhizoctonia*



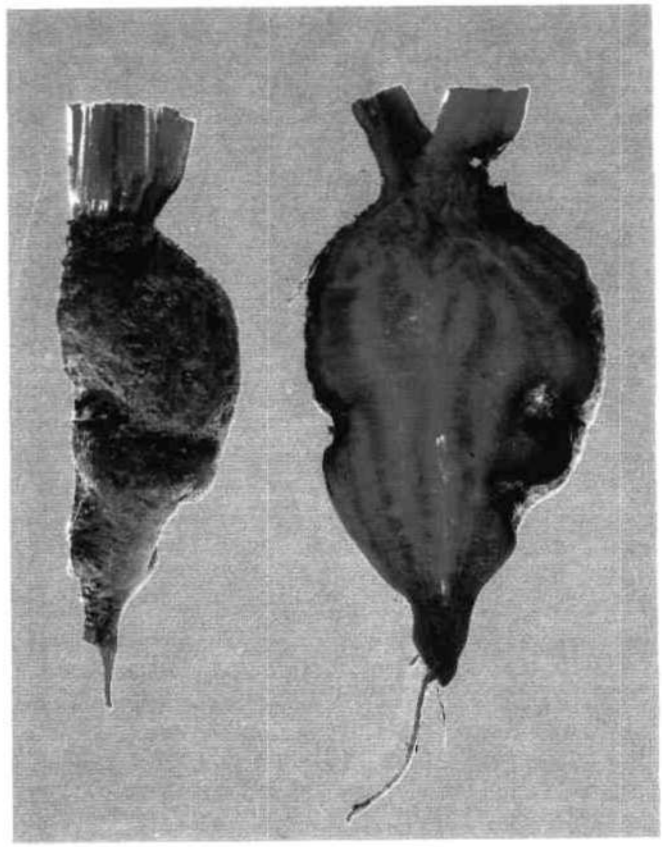


FIGURE 5. *Rhizoctonia* growing from infected beetroot tissue on an isolation medium

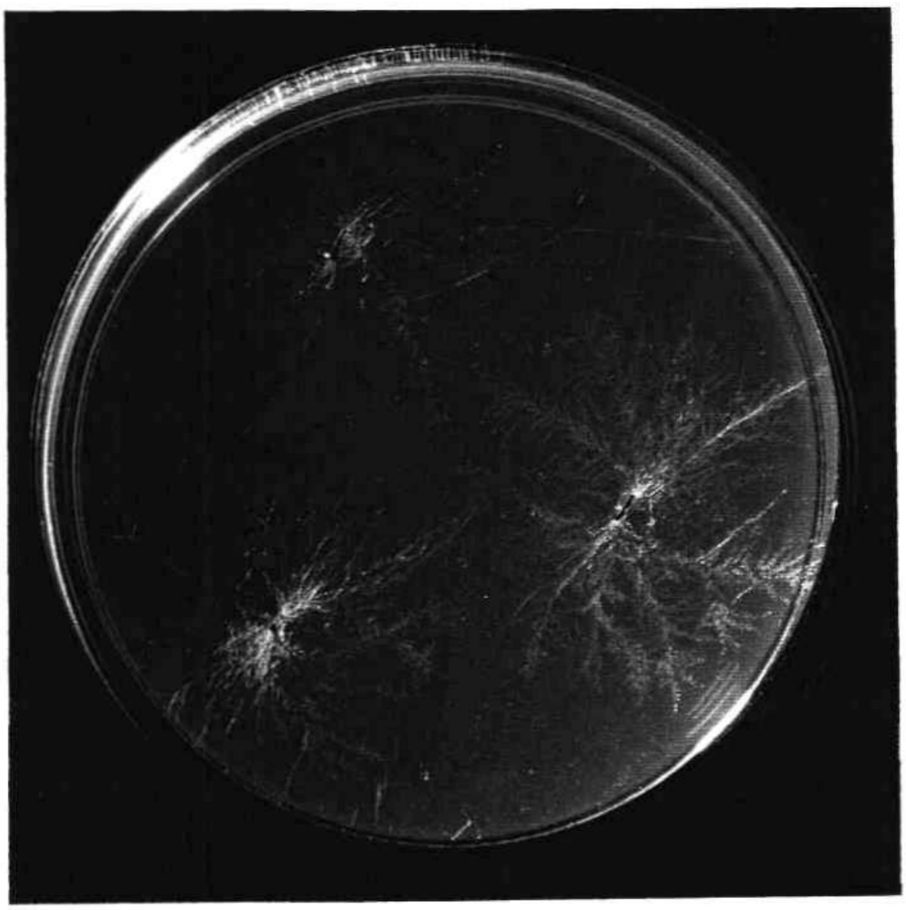


FIGURE 6. Typical fungal threads of *Rhizoctonia* on isolation medium (magnified)

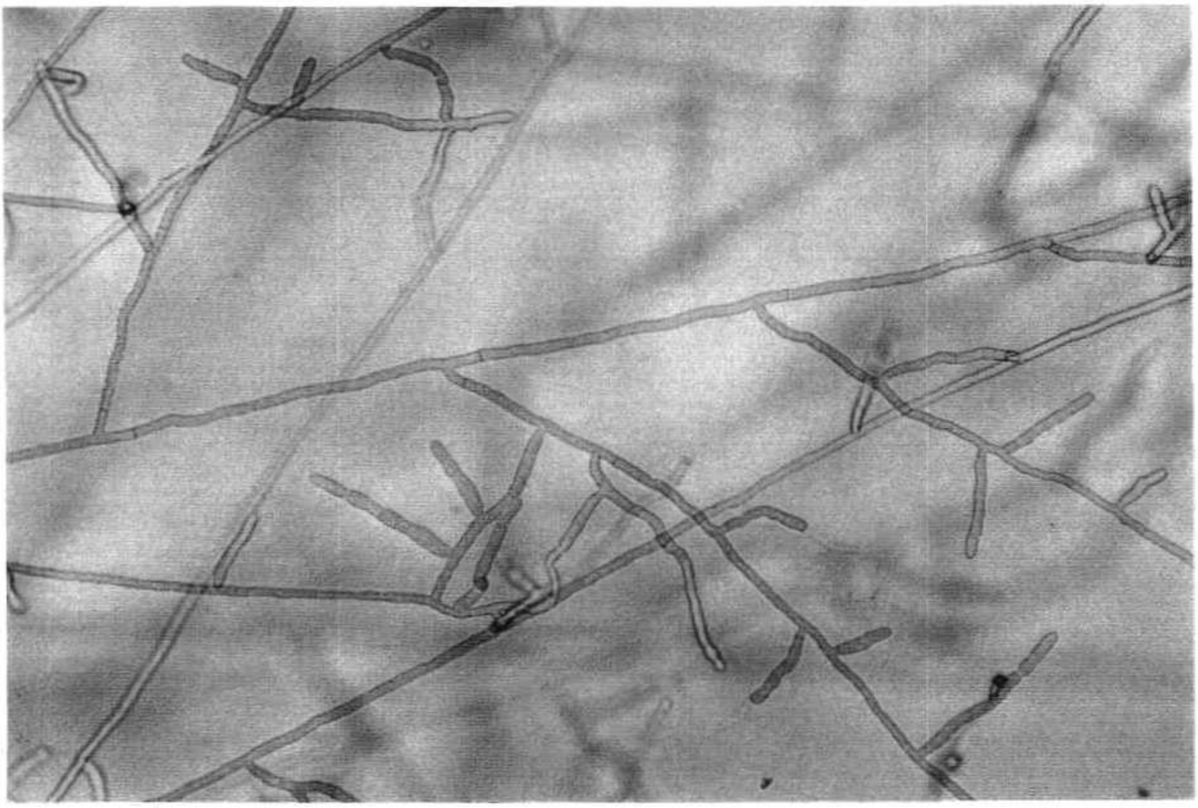
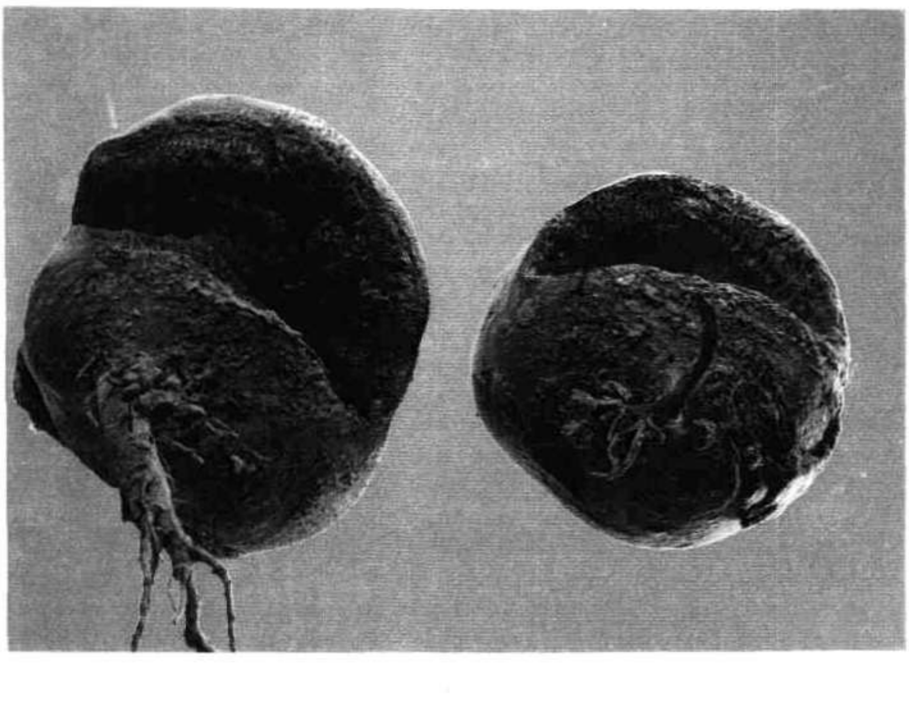


FIGURE 7. Lateral cracking of beetroot (cause undetermined)



2. Glasshouse Experiments

Susceptibility of various host plants to the *Rhizoctonia* isolate from beetroot differed depending on the experimental procedure used. Results are summarised in Tables 4 and 5. Pasteurised potting mix generally caused much greater plant mortality while constant temperature and artificial light conditions gave a greater mean survival for all host species. Beetroot was clearly the most susceptible host in all assays. The Brassicas were moderately susceptible as were lucerne and lupins in field soils. Other legumes were less susceptible except in pasteurised potting mix. Sunflowers and tomatoes were also moderately tolerant in field soils but were again much more susceptible in pasteurised soils. The grasses were generally tolerant except for corn and barley which were more susceptible at 14 days in field soil. Both these plant species were more tolerant in pasteurised soils.

TABLE 4. Host Susceptibility to Beetroot strain of *Rhizoctonia* – Glasshouse Conditions

| Crop Group | Crop | Mean % Seedling survival | | |
|---|---|---|---|---|
| | | Cowra field soil | | UC mix |
| | | 7 days | 14 days | 14 days |
| Beet | Beetroot | 8 | 8 | 1 |
| Aster | Sunflower | 100 | 86 | 9 |
| Solanaceous | Tomato | 100 | 78 | 20 |
| Brassicas | Cabbage | 71 | 57 | 11 |
| | Canola | 77 | 39 | 60 |
| Grasses | Barley | 43 | 31 | 68 |
| | Corn | 86 | 50 | 81 |
| | Ryegrass | 99 | 99 | 91 |
| | Wheat | 91 | 91 | 83 |
| Legumes | Lucerne | 100 | 87 | 43 |
| | Lupins | * | 72 | 6 |
| | Red Clover | 100 | 96 | 27 |
| | Sub. Clover | 97 | 88 | 8 |
| | White Clover | 100 | 81 | 24 |
| | Peas | | | 5 |

* slow to emerge – no data collected

TABLE 5. Host Susceptibility to Beetroot strain of *Rhizoctonia* – Constant temperature (24° C)

| Crop Group | Host | Cultivar | Mean % Survival |
|---|---|---|---|
| Beet | Beetroot | Garnet | 50 |
| Aster | Sunflower | var AG 5640 | 100 |
| Solanaceous | Tomato | UC 82C | 98.3 |
| Brassicas | Cabbage | Green Salad | 94.9 |
| | Canola | Barossa | 82.3 |
| Grasses | Oats | Blackbutt | 100 |
| | Corn | GP 12678 R | 96.8 |
| | Ryegrass | Concord | 100 |
| | Wheat | Dollarbird | 98.5 |
| Legumes | Lucerne | Aurora | 75.3 |
| | Sub. clover | Woogenellup | 98.9 |
| | White clover | Haifa | 95.3 |

Overall these experiments should be used as a guide to the relative susceptibility of the various crop species. There are clearly some inconsistencies in the data that are not readily explained. The use of pasteurised potting mix probably allowed the fungal inoculum to reach a greater potential because of the lack of competitive organisms. This can explain the trend to greater pathogenicity for most of the crop species but does not explain why canola, barley and corn were less affected by these conditions. A field assessment is really required to confirm the trends shown in these experiments. Furthermore, as *Rhizoctonia* will grow as a saprophyte on plant residues of any crop, management strategies should endeavour to precede a beetroot crop with bare fallow. Mixed pastures, brassicas, lucerne and possibly corn may harbour *Rhizoctonia* as relatively minor infections and their residues would provide a source of nutrients for the survival of fungal propagules. A cereal (oats or wheat) could be used as a break crop after more susceptible crops to minimise inoculum potential. Again a period of bare fallow would enable crop residues to breakdown before beetroot are sown.

Pot experiments testing chemical efficacy showed that a soil drench with Monceren® significantly increased beetroot seedling establishment in the presence of *Rhizoctonia*. The Rizolex® treatment was significantly better than the nil chemical control only at the highest application rate (9.0 kg ai/ha). This level of establishment was still significantly less than the equivalent rate of Monceren®.

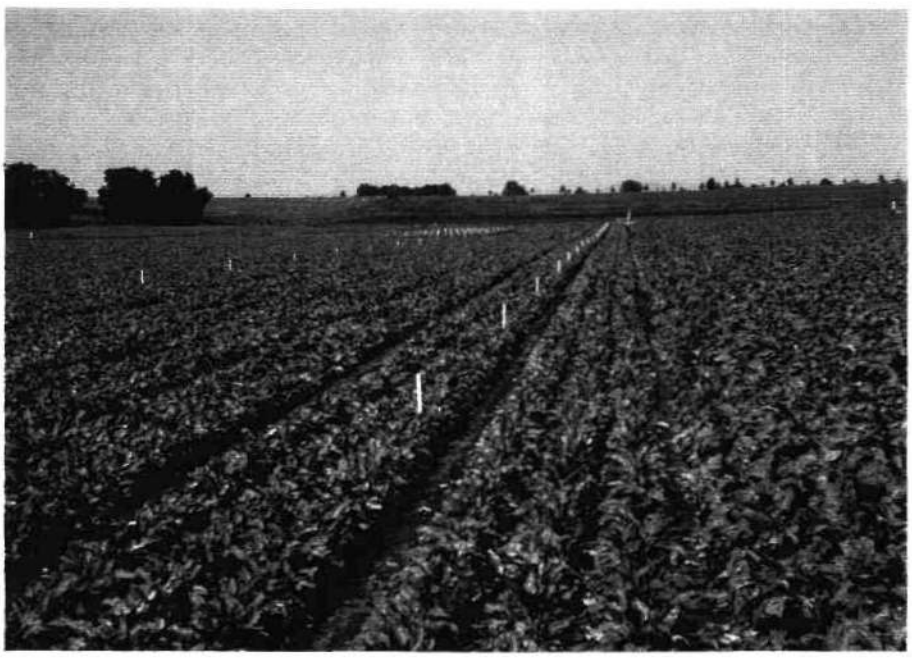
TABLE 6. Chemical Efficacy to *Rhizoctonia* in field soil

| **Treatment** | **Rate of application kg ai/ha** | **% Mean Establishment of Beet Plants** | |
|---|---|---|---|
| | | **7 days** | **14 days** |
| Rizolex[R] | 18.0 | 97.7 a | 66.0 b |
| Rizolex[R] | 9.0 | 89.2 a | 37.9 c |
| Rizolex[R] | 4.5 | 85.2 ab | 36.7 c |
| Rizolex[R] | 2.24 | 73.5 b | 34.2 c |
| Monceren[R] | 7.5 | 100 a | 98.3 a |
| Nil Chemical | 0 | 36.0 c | 35.5 c |
| Nil *Rhizoctonia* inoculum | 0 | 97.5 a | 92.8 a |

3. Field Experiments

Figure 8 shows an overview of the trial site at Cowra.

FIGURE 8. Field experiment at Cowra (treatments marked with white pegs)



(i) Plant establishment

Plot ratings for establishment at six weeks after sowing are shown in Table 7. Only the Monceren[R] and nil *Rhizoctonia* inoculum treatments had significantly better establishment ratings than control plots. Compost and Rovral[R] treatments were intermediate, while Rizolex[R] performed relatively poorly.

TABLE 7. Mean Plot Rating for plant establishment 6 weeks after sowing

| Treatment | Predicted values | approximate standard errors |
|---|---|---|
| Monceren[R] | 3.733 a | 0.499 |
| Nil *Rhizoctonia* inoculum | 3.600 a | 0.490 |
| Compost | 2.800 ab | 0.432 |
| Rovral[R] | 2.667 ab | 0.422 |
| Nil Chemical | 2.267 b | 0.389 |
| Rizolex[R] | 2.200 b | 0.383 |

(ii) Estimate of damping-off

Counts of damped-off plants six weeks after sowing (Table 8) show a similar trend to the plot establishment ratings. However, the greater variability in the data resolved fewer significant differences between treatments. Plots treated with a Rizolex[R] drench had significantly more damped-off plants than the Monceren[R] and nil *Rhizoctonia* inoculum treatments. Rovral[R] and the nil chemical treatments also had significantly more damped-off plants than the control while the compost treatment was intermediate and not significantly different from any other treatment. Figure 9 shows damping off symptoms in the field.

TABLE 8. Mean number of damped-off plants scored 6 weeks after sowing

| Treatment | Predicted values for damping-off | approximate standard errors |
|---|---|---|
| Rizolex[R] | 78.6 a | 18.03 |
| Nil Chemical | 70.0 ab | 17.02 |
| Rovral[R] | 63.2 ab | 16.17 |
| Compost | 39.8 abc | 12.82 |
| Monceren[R] | 27.0 bc | 10.56 |
| Nil *Rhizoctonia* inoculum | 20.0 c | 9.06 |

(iii) Estimates of marketable yield

Only Monceren[R] and the nil *Rhizoctonia* treatments significantly increased the number of marketable beets over the control plots. All other treatments were intermediate, while there were no significant differences between the weights of any treatments.

TABLE 9. Number and Weight of Marketable Beets at Harvest (per 16 metre row)

| Treatment | Number of beet | Weight of beet (kg) |
|---|---|---|
| Monceren[R] | 202.4 a | 32.6 a |
| Nil *Rhizoctonia* inoculum | 178.2 ab | 28.8 a |
| Rovral[R] | 161.2 abc | 32.0 a |
| Compost | 155.2 abc | 32.6 a |
| Rizolex[R] | 133.0 bc | 28.1 a |
| Nil Chemical | 114.0 c | 26.1 a |
| L.S.D. (p=0.05) | 52.2 | |

(iv) Effect of foliar Boron on Marketable yield

The foliar Boron treatment caused some phytotoxicity and significantly reduced the number and weight of marketable beets (Table 10 and Figure 10). Foliar analysis of plants from Boron treated and untreated plots failed to show any significant differences in levels of Boron. These levels were within the normal range expected to beetroot crops (G. Cresswell, Plant Nutrition Chemist, BCRI, personal communication).

TABLE 10. Effect of foliar boron on marketable yield

| Measurement | Weight | | Number | |
|---|---|---|---|---|
| Treatment | Boron | No Boron | Boron | No Boron |
| mean | 13.50 b | 16.58 a | 72.07 b | 82.76 a |
| se | 0.627 | 0.544 | 3.92 | 3.30 |

FIGURE 9. Damped-off beetroot in field trial caused by *Rhizoctonia*

FIGURE 10. Boron application to foliage caused phytotoxicity (yellow plants on right)

## RECOMMENDATIONS

* *Rhizoctonia* has a broad host range and therefore the best management practice is a period of bare fallow that allows trash from previous crops to decompose. Cereal crops appear to be the most resistant to attack by this fungus and would be the best choice for the crop preceding beetroot.

* Chemicals showed some potential to control this disease, but further research is required to determine the most effective form and rate of application. Pencyuron (Monceren®) clearly had the best efficacy under the experimental conditions in this project.

* Composts that have disease suppressive qualities could be used both to control diseases and improve soil fertility. The cost effectiveness of such products should be further investigated, particularly if there if following crops are assisted.

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