VG012
Biological control of white rot in export onions

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VG012

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Summary

Onion white rot (*Sclerotium cepivorum*) infects onion roots by means of appresorial formation with infection associated with a watery soft rot and formation of oxalic acid. Infected roots showed a dull mottled appearance.

The fungicide Sumisclex used succesfully to control onion white rot was shown to retain 60% of active ingredient after 17 weeks in moist krasnozem soil indicating that the effectiveness of this fungicide was partly due to its greater stability in soil.

In field trials, terbuconazole satisfactorily controlled onion white rot but exhibited some phytotoxic effects. A biocontrol agent was tested against onion white rot and was found to be promising.

A) Introduction

Onions are a major vegetable crop produced in Tasmania, with growers producing about 60,000 tonnes in the 1989/90 season. This is an expanding and efficiently run industry with exports in the 1989/90 season being 20% over the previous season. Approximately ninety-five percent of all onions grown in Tasmania are exported.

White rot, caused by the fungus *Sclerotium cepivorum* is a serious disease of onions in Tasmania. This disease is present throughout the onion growing areas of the state and has the potential to cripple the industry, as it did at Colac in Victoria. Onion white rot has proven a difficult disease to control throughout the world.

Effective control is now obtained using the fungicide Sumisclex (procymidone), as a result of studies conducted by the Department of Primary Industry. Control of onion white rot by Sumisclex was first developed in Tasmania as a result of research conducted by Dr James Wong at the request of the onion industry. However, use of this fungicide remains the only economic method for control of onion white rot.

There is a need to investigate alternative control strategies to minimise the risks associated with sole reliance on this fungicide. Problems that could arise include: (1) accelerated degradation of the fungicide in the soil due to build-up of fungicide-degrading microorganisms; (2) development of fungicide resistance; (3) possible fungicide withdrawal at a future date.

Currently, fungicide is applied as a seed dressing and also applied in a band below the seed, mixed with fertiliser. Strategic application of fungicide in this way means that the amount of fungicide required is minimised and there is minimal fungicide residue in onion bulbs, which was shown to be 0.04ppm. Based on our data, I.C.I. has set a M.R.L. at 0.2ppm which is considerably lower than those set for other vegetable crops. Additionally, as there is no aerial spraying there is less risk to the growers and the surrounding environment.

Field trials conducted in 1988/89 indicated that application of fungicide mixed with lime-super around the seed was a promising alternative to application with fertiliser below the seed. However some phytotoxicity was observed when this was combined with seed treatment. It appeared that lower fungicide levels could be used with this method. Field trials were conducted to resolve these aspects.

The new strategies being investigated to reduce reliance on Sumisclex were to investigate new fungicides and to investigate biological control alternatives. Three new fungicidal chemicals were identified as having potential for controlling onion white rot. Glasshouse trials have established the toxicity limits of these on onion seedlings. Field trials were conducted to investigate the potential of these new fungicides for control of white rot under field conditions (Appendix 1).

A number of potential biological control microorganisms have been identified. One of these, *Trichoderma koningii* has been identified as having particular potential as a biological control agent. One promising *T.koningii* isolate (IMI 338777) has been subjected to laboratory and glasshouse tests and was also evaluated under field conditions.

Although biological control agents may prove not to be highly successful at controlling white rot when used alone, their integrated use with lower levels of chemical control agents could be possible. This would be environmentally more acceptable than use of chemicals alone.

In order to improve control systems it was seen to be necessary to have a greater understanding of the behaviour of the white rot fungus in onion roots, its interaction with other soil fungi and with chemical control agents.

B) Objectives

The objectives of this project were to conduct a number of laboratory and glasshouse studies aimed at gaining a greater understanding of the growth of the white rot fungus, its interaction with soil fungi, particularly *Trichoderma* and with fungicides, and to field test several fungicides and *Trichoderma* for the control of white rot.

Aspects to be studied included:-

1) Laboratory and Glasshouse Studies

1.1) The infection process of *S.cepivorum* on onion roots:

- growth of hyphae in and along roots

- whether root cells are killed in advance of the advancing hyphae

- detection of living and dead root cells, and mechanism of root death

1.2) The survival of plants in the presence of inoculum and soil mycoflora. In particular to determine the following:

- whether there is interception by soil fungi of the infection as saprophytic colonisation of dead or dying root tissues, killed ahead of the advancing white rot fungus

- whether soil fungi such as *Coniothyrium* and *Trichoderma* or other potential biocontrol agents could intercept and kill the *S.cepivorum* hyphae in roots.

- the effect of soil oxygen and carbon dioxide levels on germination of sclerotia, their infectivity and progress of infection through roots.

1.3) The way in which fungicides protect root tissues from infection:

- whether contact or translocation of procymidone provides control

- how long protection is afforded by procymidone placed in the soil

- whether procymidone, prochloraz and terbuconazole were toxic to roots and root tips

1.4) Biocontrol potential of *Trichoderma*. To determine:

- whether incorporation of *Trichoderma* into soil provided protection from infection by *S. cepivorum*

2) Field Studies

Several fungicides and *Trichoderma* to be tested under field conditions for the control of onion white rot.

C) Research Methodology, Results and Conclusions

Study of the infection of Onions by *Sclerotium cepivorum*

The infection process of *Sclerotium cepivorum* was studied under laboratory conditions. Onion seedlings were grown in sterile conditions on an agar medium consisting of water agar with 0.005% aquasol fetiliser. Two week old seedlings were inoculated with *S. cepivorum* by placing a small amount of inoculum adjacent to the end of the root. Seedlings were incubated at 15^{o}C in constant light. The progress of infection was studied using light microscopy and other means.

A number of staining techniques were investigated. Among the most useful of those stains used were Neutral Red, Lactophenol Cotton Blue and Congo Red. When infected roots were placed in 0.005% Neutral Red at pH 6, living hyphae of *S. cepivorum* stained bright red while root tissue remained mainly unstained. This allowed details of hyphal growth on the surface of roots to be viewed readily using living tissue. This method could not be used with preserved root tissue. Lactophenol Cotton Blue was a good general stain for hyphae in both fresh and preserved root tissue. Congo Red acted as a pH indicator and was useful in tracing a number of aspects of infections.

Approximately 200 onion plants were examined in this study.

Results and Discussion

Growth of hyphae on roots

Other researchers have noted that hyphae of *S. cepivorum* tend to follow the lines of longditudinal junctions of the epidermis. In these studies this pattern was generally not observed to occur. Hyphae appeared to grow at random across the root surface. Hyphae were generally closely appressed to the surface of the roots but also grew away from the surface.

Penetration of Onion Seedlings

There was a distinct difference in the methods of penetration of root and leaf tissue. This was observed by

light microscopy and also confirmed by scanning electron microscope.

Penetration of the root surface occurred by single hyphae. This appeared to occur via simple appressoria which were observed by s.e.m. as slightly swollen hyphal tips.

Penetration of the leaf surface occurred via dome shaped infection cushions. These infection cushions consisted of numerous closely packed hyphae, arranged at right angles to the leaf surface. Hyphal tips were seen to be swollen. The tissue underneath and surrounding these infection cushions was frequently collapsed. Infection cushions where infection had occurred were observed to be strongly attached to the leaf surface.

Development of Infection

Infection of onion tissue by *S. cepivorum* was associated with a soft watery rot. Healthy roots in comparison, had a glistening granular appearance when viewed with reflected light. Root hairs when present, were rigid.

Mode of advance of infection tended to vary, depending on whether hyphae were advancing within the root, advancing on the root surface or both. In the early stages of infection hyphal activity appeared to be mainly on the root surface. At this stage hyphae were generally closely appressed to the root surface and the roots appeared to be generally healthy. Within 24 hours the root surface developed a dull watery appearance in the region where hyphae were in contact.

As the infection became more established, hyphal growth tended to be more active and hyphae advancing along the root surface frequently grew away from the surface. In many cases, within 5mm in advance of advancing surface hyphae, the root became dull and mottled and became translucent. Plating out of sections from within this 5mm zone indicated the absence of *S. cepivorum* from much of this region. However, this altered zone in advance of surface hyphal growth was not always present, especially within the first 48 hours of infection. The presence of this altered zone in advance of surface hyphal growth appeared to be dependent on the presence of hyphal growth within the root which was not always well established early on in the infection process. When this altered zone was present, hyphal advance within the root could be expected to be slightly ahead of the surface hyphae.

After root tissue had become infected, hyphae on the surface continued to grow away from the surface, sometimes

reaching in excess of 1cm from the surface although 3mm or less was more common. Much of this hyphae had originated from within the root and had broken through the surface. The extent of hyphal growth away from the root surface appeared to be very much dependent on source of nutrient. At this stage, which is about two to four days after infection, collapse of root tissue also occurs. Within five days, infected tissue has normally collapsed totally and associated hyphae have also collapsed. No evidence of cuticle breakdown was observed. This was observed to remain intact, even after all other tissue had totally collapsed. Only this and the xylem tissue were observed to remain intact during the final stages of infection.

Advance of hyphae within cotyledons appeared to occur in a similar way, although at a faster rate. Infection of cotyledon leaf tissue could be traced by a distinct yellowing of newly infected tissue. Plating out of sections from infected cotyledons revealed that the infection had advanced a few millimetres beyond the zone of discolouration. Growth of hyphae away from the surface occurred to a greater extent on cotyledons than on roots.

Rates of advance of infection appear to be dependent on: 1) the level of nutrition; 2) how well the infection was esablished; 3) presence or absence of inhibitory chemicals or microorganisms on the root surface.

Measurements of the rate of spread of infection in roots varied between 3.3 and 13.2 millimetres per day at 15^{o}C. Rate of advance of hyphae was very much greater in cotyledons than in roots. A rate of 25 mm/day was measured for advance of an infection in a cotyledon.

When Congo Red dye applied to infected roots external hyphal growth was sometimes inhibited, although hyphal growth within the root still occurred. Bacterial contaminants were seen to inhibit the growth of *S. cepivorum* on water agar. In some cases these resulted in inhibition of growth of hyphae on root surfaces although hyphal growth within the root still occurred.

Evidence for Secretion of Oxalic Acid and Enzymes by *S. cepivorum*

Other researchers have found that pectolytic enzymes (Mankarios and Friend 1980) and oxalic acid (Stone and Armentrout 1985) are produced by *S. cepivorum* in the infection process. The soft watery rot observed with infected tissues is

consistent with this process. It is thought that oxalic acid acts synergisticly with pectolytic enzymes by chelating calcium ions and by lowering the pH to near the enzyme optimum (Maxwell and Lumsden 1970).

When treated with 1% Congo Red dye the surfaces of infected roots stained dark blue indicating a pH of 5 or less. This was noted to be patchy with only some cells affected. The point of most active hyphal growth appeared to be most strongly affected. No staining was observed on healthy roots. When the dye was applied to agar or incorporated into agar medium, this turned purple or very deep red near cultures of *S. cepivorum* again indicating a pH of 5 or less. This showed that acid was being produced by *S. cepivorum* when growing in PDA culture and when growing on onions. Using gas chromatography it was confirmed that oxalic acid was present at a concentration of at least 1% in PDA culture medium on which *S. cepivorum* was growing.

These studies on the infection process of *S. cepivorum* on onion seedlings resulted in a greater understanding of the infection process and allowed development of techniques for further study of this process. An objective which was not achieved was adequate definition of a technique for conclusively determining whether individual root cells were living or dead. This aspect will be further pursued in future studies.

Literature Cited

Mankarios, A.T. & Friend, J. (1980), Physiol. Pl. Pathol. 17:93-104
Maxwell, D.P. & Lumsden, R.D. (1970), Phytopathology 60:1395-1398
Stone, H.E. & Armentrout, V.N. (1985), Mycologia 77:526-530

Behaviour of *Trichoderma* on onion roots

A laboratory study was conducted to determine whether the *Trichoderma* isolate IMI 338777 was pathogenic to onions. This was done by inoculating twenty onion seedlings grown under sterile conditions in the laboratory with the *Trichoderma* isolate and observing the response. Inoculation was carried out by placing a small amount of culture on or near the

seedling. Microscopic examination was carried out. The seedlings were observed for a period of one month following inoculation.

Results and Discussion

Although the *Trichoderma* isolate was observed to quickly colonise the whole root surface of the onion seedlings no detrimental effects to the health of the seedlings were observed. One seedling was subsequently grown on in soil in the glasshouse to the bulb stage.

Interaction between *S. cepivorum* and *Trichoderma* on onion seedlings

A laboratory study was conducted to observe the interaction between *S. cepivorum* and the *Trichoderma* isolate IMI 338777. Onion seedlings grown under sterile conditions in the laboratory were treated by either:

(a) inoculating with both *S. cepivorum* and *Trichoderma* on the same day;
(b) inoculating with *S. cepivorum* two days before inoculation with *Trichoderma*;
(c) inoculating with *Trichoderma* alone;
(d) inoculating with *S. cepivorum* alone;
(e) untreated control.

Inoculum of *S. cepivorum* was placed 1cm below the base of the root, and inoculum of *Trichoderma* was placed onto the upper portion of the root. Seedlings were arranged in such a way that all fungal growth between the the upper portion and base of the roots must occur on or within the roots, (Fig. 1).

Results and Discussion

Seedlings were observed for a period of twelve days following initial inoculation. They were regarded as being irretievably infected when the stem plate containing leaf and root meristems had been colonised by *S. cepivorum*
Results at 11 days were as shown in Table 1.

Figure 1.

Setup for study of interaction between *Trichoderma* and *S. cepivorum* on onion seedlings

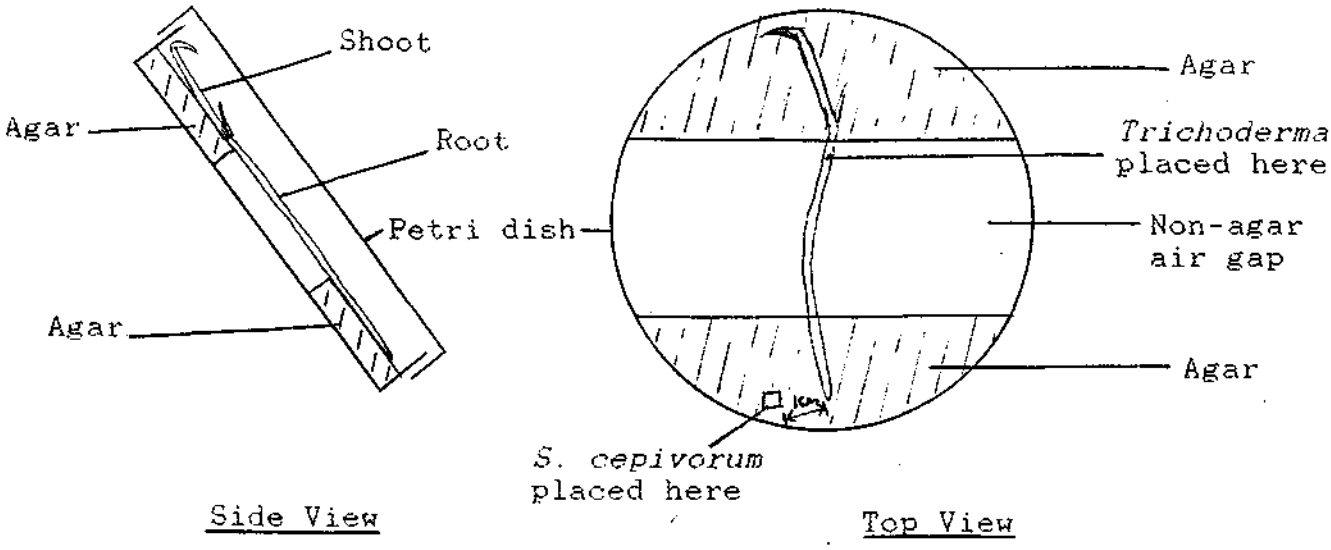


Table 1 Survival of Seedlings at 11 days

Treatment	No. of Seedlings	No. Infected	% Infected
(a)	29	14	48.2
(b)	28	21	75.0
(c)	20	0	0.0
(d)	20	20	100.0
(e)	20	0	0.0

Seedlings for which the meristem was not infected by day 12 generally remained healthy afterwards.

These results indicated that:

- the *Trichoderma* isolate was largely effective in halting the progress of infection by *S. cepivorum* on onion roots.
- inoculation with *S. cepivorum* two days before *Trichoderma* resulted in reduced effectiveness of *Trichoderma* at controlling the advance of the infection.

It was concluded that some form of interaction was occurring which resulted in protection of the seedlings from further infection by the white rot pathogen. The exact nature of this interaction remained to be determined. Further study was required to clarify the interaction process.

Survival of plants in the presence of disease inoculum and soil mycoflora

It was proposed to determine whether there is interception by soil fungi of the infection, as saprophytic colonisation of dead or dying root tissues killed ahead of the advancing white rot fungus. Successful accomplishment of this objective was dependent on developing adequate means for microscopic study of the relevant factors. Appropriate techniques were not developed during the term of this project. This study is continuing in the follow-up project, with emphasis being on the mode of interception of the infection by *Trichoderma*.

Effect of soil oxygen and carbon dioxide levels on growth of *S. cepivorum*

It was proposed that the effect of oxygen and carbon dioxide levels on germination and infectivity of sclerotia of *S. cepivorum* and on progress of infection through roots be examined. In order to obtain reproducible and reliable results it was considered necessary that specialised controlled atmosphere equipment would be required. No existing equipment of the required type was available. Although a gas chromatograph for gas analysis was already on site at New Town Research Laboratories other associated equipment needed to be constructed.

This aspect of the research project was not further pursued at this stage.

Study of the persistence of procymidone in the soil.

Experimental evidence indicated that Sumisclex remained effective in the soil for control of onion white rot of onions for a period of about three months. It was considered useful to gain a more precise determination of the rate of degradation in krasnozem soil of procymidone, the active ingredient of Sumisclex.

Sumisclex wettable powder was added to moist krasnozem soil giving a concentration of 41.5 ppm procymidone on a dry weight basis. Soil moisture content was 20.5%. This soil was then incubated at 10, 15 and 20^{o}C, layered to a depth of 6cm. in partially sealed containers. Samples taken at intervals for up to 17 weeks. Samples were oven dried at 50^{o}C. They were subsequently stored at -18^{o}C for the majority of the intervening before analysis.

Sample analysis was conducted by a separate laboratory with specialised facilities for this task (C.I.G. Pyrethum). Samples were extracted with ethanol. After evaporation of the ethanol and redissolution in N,N-dimethyl formamide, the extracts were analysed by gas chromatography for detection of procymidone.

For logistical reasons samples were analysed in two batches. Samples for the first three weeks were analysed at week six. The remaining samples were analysed 7 months after the final samples were taken.

Results and Discussion

Results are given in Table 2. These results showed a good stability of Procymidone in the soil regardless of temperature. procymidone content stabilised by week five and was constant for the following twelve weeks at just over 50% of the original concentration.

Some doubt exists on the reliability of data for the final 12 weeks due to uncertain storage conditions of the samples during the time elapsed before analysis. However this is not expected to greatly affect the conclusions drawn.

Further investigation into fungicide degradation was not pursued at this stage due to increased priority of biological control work.

Table 2. Procymidone Content of Krasnozem Soil Incubated at 10, 15 and 20°C Over a 17 week Period.

Treatment week.	Temperature (°C)	Procymidone (ppm)	Analysis date
0		41	Nov.89
0		32	Aug.90
1	10	39	Nov.89
2	10	35	Nov.89
3	10	32	Nov.89
5	10	25	Aug.90
8	10	26	Aug.90
12	10	25	Aug.90
17	10	23	Aug.90
1	15	34	Nov.89
2	15	32	Nov.89
3	15	30	Nov.89
5	15	24	Aug.90
8	15	24	Aug.90
12	15	21	Aug.90
17	15	25	Aug.90
1	20	33	Nov.89
2	20	33	Nov.89
3	20	32	Nov.89
5	20	28	Aug.90
8	20	22	Aug.90
12	20	24	Aug.90
17	20	23	Aug.90

Phytotoxicity Studies

Four new fungicide preparations were identified as having characteristics that made them suitable for testing for their capacity to control onion white rot. These were Folicur EC 250 , Raxil WP 25, Sportak EC 450 and Octave WP 462.

Preliminary to field testing of the fungicides for control of onion white rot, a series of trials were conducted to determine the toxicity of these fungicide formulations to onion seedlings when used as a seed dressing. Comparison was made to treatment with Sumisclex Flocol at 100 ml/kg seed, the currently recommended treatment for onion white rot. Previously this treatment has been shown to cause little phytotoxicity to onion seedlings. Active ingredient concentrations for each fungicide were: Folicur, 250g terbuconazole per litre; Raxil, 25g terbuconazole per kilogram; Sportak, 450g prochloraz per litre; and Octave, 462g prochloraz per kilogram. Sumisclex Flocol contained 275g procymidone per litre.

Onion seeds were treated with a range of levels of each fungicide including an untreated control. Seed had not been previously treated with any fungicide. Methyl cellulose was used as a sticking agent for the powdered fungicide formulations. Seed was sown in trays in either krasnozem soil or a potting mix containing 50% peat and 50% sand. Four replicates of 25 seeds were tested for each treatment in each soil type. Seedlings were grown in a temperature controlled glasshouse at 15 to 25^{o}C with soil moisture maintained at about field capacity.

Results and Discussion

Measurements were taken of the percentage seedling emergence over time. Seedling heights were also measured at four weeks after sowing. Fungicide rates at or below which little or no effect on seedling emergence was observed are given on Table 3. Rates at or below which little or no effect was observed on seedling size at four weeks are given in Table 4.

Table 3. Rates At or Below Which Little or No Effect on Seedling Emergence Was Observed.

Fungicide	Amount per Kilogram of seed	
	Krasnozem soil	Potting Mix
Folicur	14 ml	14 ml
Raxil	100 g	50 g
Sportak	4 ml	1.5 ml
Octave	10 g	10 g

Table 4. Rates At or Below Which Little or No Effect on Seedling Size was Observed at Four Weeks After Sowing

Fungicide	Amount per Kilogram of Seed	
	Krasnozem soil	Potting Mix
Folicur	10 ml	10 ml
Raxil	50 g	50 g
Sportak	1.5 ml	1.5 ml
Octave	4 g	4 g

Based on these results it was concluded that little or no phytotoxicity could be expected if onion seed was treated with the following fungicide levels alone and grown under optimal growth conditions:

Folicur	EC 250	10 ml/kg
Raxil	WP 25	50 g/kg
Sportak	EC 450	1.5ml/kg
Octave	WP 462	4 g/kg

It was also found that Sportak was more toxic than Octave at equivalent active ingredient levels greater than 1.0 g a.i./kg of seed. As a result Sportak was not field tested.

Figures 2 and 3 show the relationship between fungicide concentration and seedling emergence.

igure 2. Effect of Concentration on Emergence

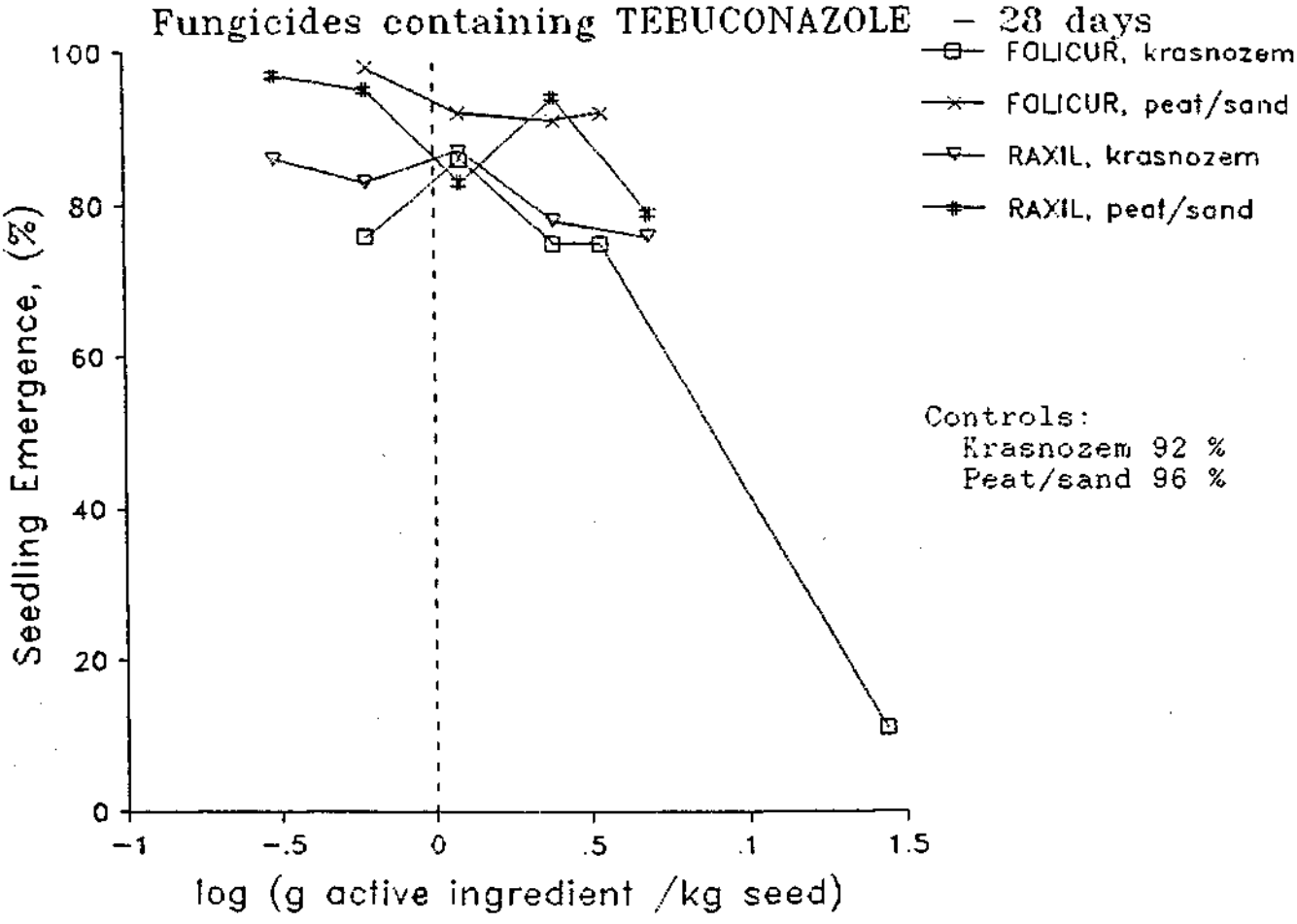
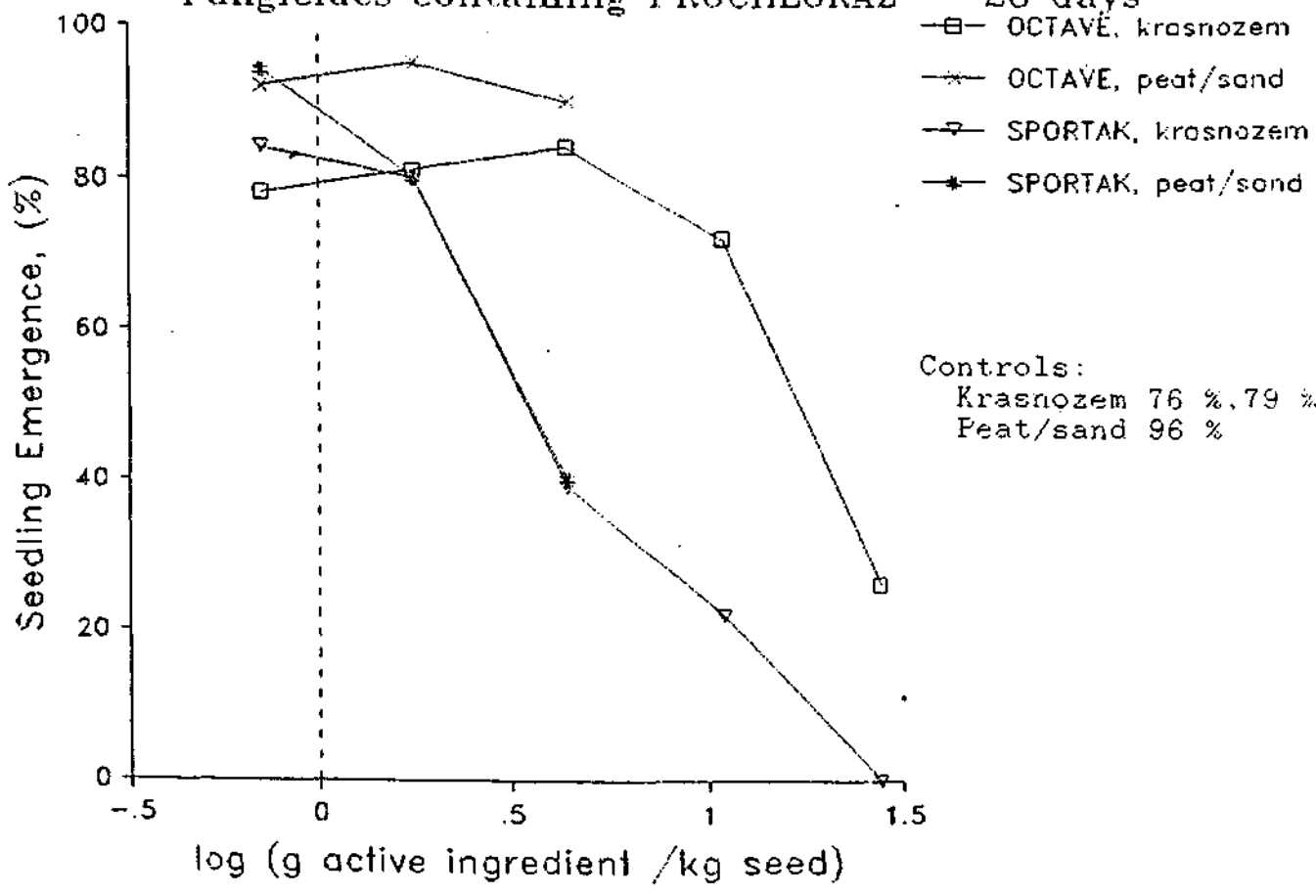


Figure 3. Effect of Concentration on Emergence



CONFIDENTIAL

Field Testing of *Trichoderma* and Fungicides with Different Fertiliser Carriers

Field testing was conducted to determine the effectiveness of an alternative fungicide carrier, new fungicides and a *Trichoderma* strain at control of onion white rot.

These field tests were an integral part of the overall project but were funded mostly by the Department. this work was carried out mainly by Dr Wong and his technical support group.

Details of these trials are given in Appendix 1.

Methods and results are summarised as follows for completeness and continuity of information

A replicated field trial on onion white root rot control was carried out in the 1989/90 season in a Tasmanian North west region property with a history of severe onion white root rot. This trial compared several fungicide treatments and a biocontrol treatment base don the use of a *Trichoderma koningii* strain (IMI 338777) that was isolated in Tasmania. Each treatment plot was 1.6m (6 rows) wide by 7m long and was replicated 6 times

The site was sown on 14th July, 1989, with a seeding rate of 130 seeds/m^2.

The *Trichoderma* inoculum used was a four week old culture grown on autoclaved white millet in two litre flasks. After inoculating the autoclaved millet (1 part millet : 1 part water), with a *Trichoderma* culture grown on potato dextrose agar, the flasks were incubated at 15^{o}C under fluorescent light. After four weeks the culture was spread out to dry. The dried culture inoculum was then introduced directly into the soil at sowing around the seed at the rate of 115kg dried fungus-millet clture per hectare.

Sampling plots consisted of 200 plants per plot, made in the four inner rows x 2.5m length of bed.

Disease development was monitored at intervals to observe emergence rates, and onset of disease. When the first sign of disease (wilt) was seen (29th August), observations of wilt in plots were made weekly thereafter.

Plant emergence rate in the biocontrol treatment was about 5% better than the untreated plots.

At commercial harvest on 7th February, 1990 (31 weeks after sowing) all plants were destructively sampled, and the disease incidence in addition to yields in bulb numbers and

bulb weight for each plot were determined. the data on disease incidence and yields are given in Table 5. The biocontrol treatment had increased bulb number by 2.6 fold and weight yield by 1.73 fold compared to the control.

Wilt progress curves were plotted up to 1 week before commercial harvest. Comparisons between the untreated control, the seed treatment with Sumisclex, best fungicide treatments and the biocontrol treatment are given in Figure 4.

The disease curves (Figure 4.) highlighted the following:

1) The bio-control agent reduced disease incidence from 72.1% to 29.2%, giving a reduction in the level of disease by about 60%

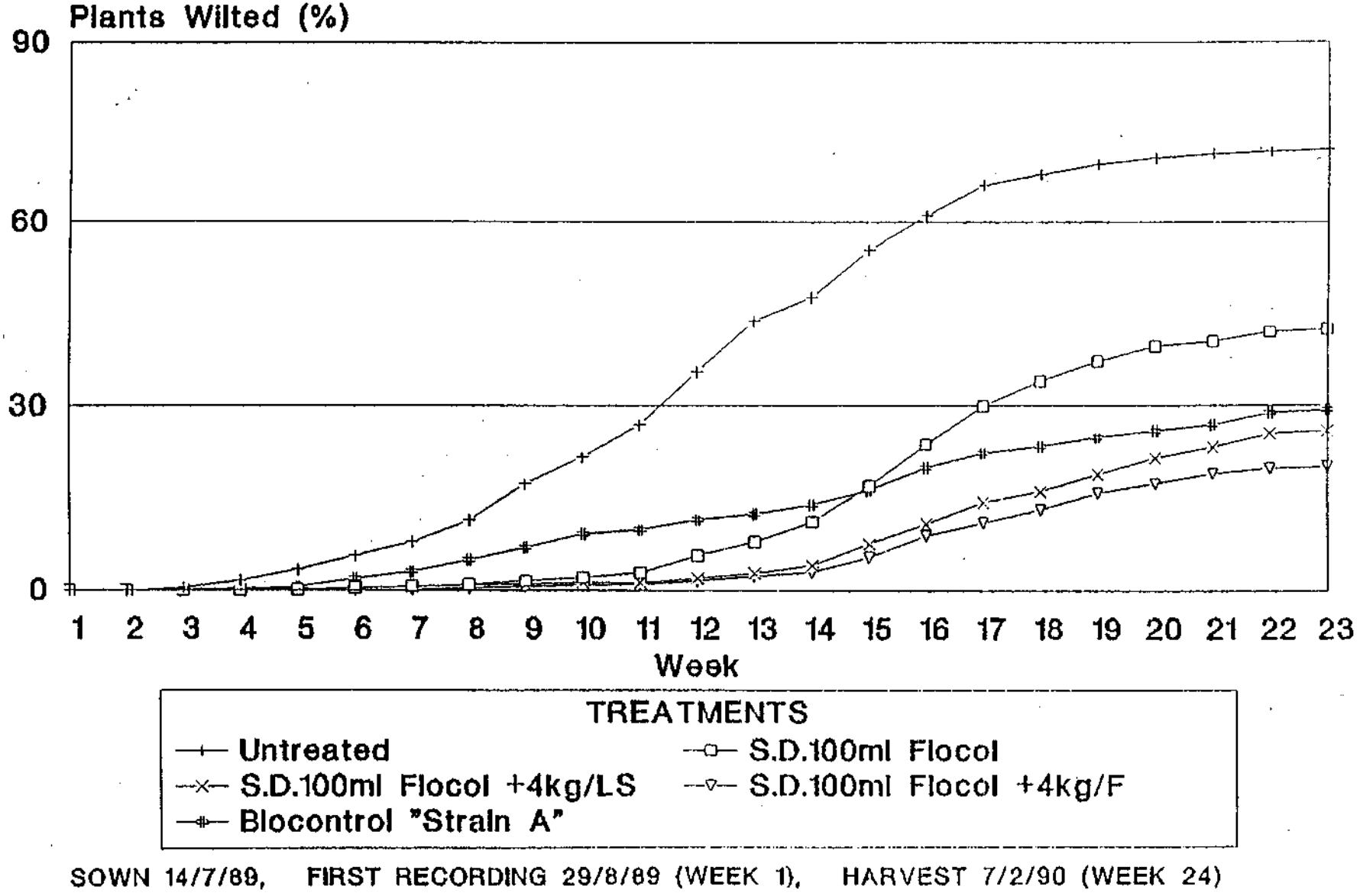
2) The curve for the changing incidence of wilt in the biocontrol treatment is a gentle linear slope. This contrasts sharply with the steep increase in the curve for the untreated control. The biocontrol curve indicates continuous disease control throughout the season

3) All of the chemical control curves for wilt incidence changes are sigmoidal in shape. All chemical treatments provided good disease control initially and up to 18 weeks (for Sumisclex-dressed seed treatment) or 21 weeks (for the best chemical control treatment). After these times the disease levels increased rapidly, indicating that the fungicides were no longer providing protection as the season progressed. This is probably due to the depletion (by microbial/chemical decomposition) of the fungicide in the soil.

4) A prediction from the behaviour of the disease curves is that, if biocontrol treatment is integrated with a minimal amount of fungicide dressed onto seeds, the disease control level will be dramatically improved, with the fungicide providing good initial disease control and the biocontrol agent providing continuing control after the fungicides have depleted.

Figure 4.

ONION WHITE ROOT ROT
MAIN TRIAL 1989/90, FORTH



SOWN 14/7/89, FIRST RECORDING 29/8/89 (WEEK 1), HARVEST 7/2/90 (WEEK 24)

Table 5. Disease incidence and Yields in Bulb Numbers and Weight

Treatment	% Disease	Bulb No's per plot ($2m^2$)	Weight in kg per plot ($2m^2$)
1. Untreated	75.7	51.3	5.0
2. Seed dressed with 100ml Sumisclex Flocol per kg of seed	46.5	111.8	6.8
3. Seed dressed with 50 ml Sumisclex Flocol per kg of seed	56.8	89.5	6.3
4. Seed dressed with 25ml Sumisclex Flocol per kg of seed	57.3	90.7	6.4
5. S.D. 100ml Flocol + 4kg Sumisclex W.P. with lime super	29.1	149.8	8.6
6. S.D. 50ml Flocol + 4kg Sumisclex W.P. with lime super	28.8	149.5	8.9
7. S.D. 25ml Flocol + 4kg Sumisclex W.P. with lime super	30.2	144.8	8.3
8. 4kg Sumisclex W.P. with lime super	25.5	135.7	8.5
9. S.D. 25ml Flocol + 4kg single super	43.8	119.2	7.7

(continued next page)

(table 5 continued)

10. S.D. 100ml Flocol + 4kg Sumisclex W.P. with N.P.K. fertiliser	25.0	160.2	9.5
11. S.D. 100g Raxil +4kg Raxil W.P. with lime super	46.3	111.0	6.7
12. S.D. 25g Octave +2.5kg Octave W.P. with lime super	52.2	100.3	7.1
13. Seed Dressing with 10ml Folicur per kg seed	38.4	102.0	8.7
14. Biocontrol with *Trichoderma*	34.1	133.8	8.7

Biocontrol Potential of Trichoderma - Glasshouse Trial

A pot trial was set up on 7 December 1989 with a krasnozem soil to test the biocontrol agent in various combinations, and especially to test the predicted enhancement in disease control when the biocontrol treatment was combined with Sumisclex - dressed seed treatment. Details of the treatments are given in Table 6.

Cultures of *Trichoderma* were grown as described for the field trial except that the growth medium consisted of seven parts white millet and three parts water. Cultures were grown for 8 weeks, air dried and then stored at 4^{o}C until needed.

Sclerotia of *S. cepivorum* were incorporated into the soil at the rate of 1000 sclerotia per kilogram of soil and mixed thoroughly before being potted out into 15cm pots.

A seperate lot of soil was mixed with the appropriate biocontrol treatment, and this biocontrol inoculated soil was then layered to 1.5cm thickness over the white rot inoculated soil.

Table 6. Pot Trial Treatments to Test Trichoderma spp. for Biocontrol of Onion White Rot, 1989/90

Treatment	Code	Rate
1. Untreated Control	Untreated	nil
2. Seed dressed with Sumisclex Flocol	S.D.	100mls Sumisclex Flocol/kg seed
3. Autoclaved millet control	Dead Millet	0.15g autoclaved millet only/pot
4. Autoclaved Trichoderma "A"-millet culture control	Dead Tricho A-millet	0.15g autoclaved Trichoderma "A"-millet/pot
5. Live Trichoderma "A"-millet	Tricho A	0.15g Trichoderma "A"-millet/pot
6. Trichoderma "A" spores only	Tricho A spores	Spores washed from 0.15g Trichoderma "A"-millet culture and drenched into soil/pot
7. Seed dressed with Sumisclex Flocol plus Trichoderma "A"-millet	S.D.& Tricho A millet	100mls Sumisclex Flocol/kg seed plus 0.15g Trichoderma "A"-millet/pot
8. Coniothyrium minitans-millet plus Trichoderma "A"-millet	Conio-Tricho A millet	0.075g Coniothyrium minitans-millet per pot plus 0.075g Trichoderma "A"-millet/pot

(table continued next page...)

(table 6. continued)

9. Trichoderma "B"-millet	Tricho-B millet	0.15g Trichoderma "B"-millet/pot
10. Trichoderma "C"-millet	Tricho-C millet	0.15g Trichoderma "C"-millet/pot
11. Trichoderma "D"-millet	Tricho-D millet	0.15g Trichoderma "D"-millet/pot

Trichoderma isolates: Trichoderma "A" = *T. koningii* IMI 338777
Trichoderma "B" = *T. koningii* IMI 338778
Trichoderma "C" = *T. koningii* IMI.338779
Trichoderma "D" = *T. koningii* IMI 338780

The amount of *Trichoderma*-millet culture was 0.15g per pot, calculated to give the equivalent of 115kg/ha (field rate). The *Trichoderma* "A" millet culture was determined to have in excess of 3.2×10^{10} spores per gram of culture, with a viability of 95%.

For *Trichoderma* spores-only treatment, a suspension of freshly prepared spores were obtained from the millet culture. This was filtered with a cheese cloth and 500mls spore suspension was drenched into soil at 55.5ml per pot.

Ten seeds were sown per pot, which was later thinned to 6 plants per pot. Each treatment was replicated 9 times to give a total of 54 plants per treatment. Observations were made weekly until 28th May 1990.

The results are given in Table 7.

Table 7. Effect of Trichoderma, Coniothyrium, Sumisclex and Combined Treatments in Control of Onion White Rot, 1989/90, (7th December, 1989 to 28th May, 1990

Treatment	% Disease	Sample size (No. of plants)
1. Untreated	20.4	54
2. S.D.	9.3	54
3. Dead millet	14.8	54
4. Dead Tricho A-Millet	18.9	54
5. Tricho A-Millet	9.3	54
6. Tricho A Spores	11.8	54
7. S.D.& Tricho A-millet	0.0	54
8. Conio-Tricho A-Millet	13.0	54
9. Tricho B-Millet	0.0	12
10. Tricho C-Millet	0.0	12
11. Tricho D-Millet	16.7	12

The above results support the field trial results showing that *Trichoderma* is an effective biocontrol agent against onion white rot when applied to the root zone areas in the soil as a fungus-millet preparation. Control obtained by use of *Trichoderma*-millet was comparable to use of seeds dressed with Sumisclex.

The most striking result is that combined use of *Trichoderma* with seeds dressed with Sumisclex reduced disease to nil. This also supports the prediction made that the combined effects of two treatments will enhance disease control over either of the treatments alone.

D) Implications and Recommendations

As a consequence of the promising results obtained with *Trichoderma* a new research project investigating the biocontrol potential of *Trichoderma* has been initiated. This three year project titled "Biological Control of White Rot in Export Onions" will be funded by the Tasmanian onion industry and H.R.D.C. This project commenced 1st July 1990.

E) Literature Arising from this Project

A Provisional Australian Patent covering aspects of this work has been lodged.

Appendix 1a

Onion White Rot Trial Forth - R Bonney 1989/90

Treatments	Method and Rates		
		Soil Treatment	
	Seed Dressing (per kg seed)	Fungicide/Biocontrol Agent per ha	Carrier Fertiliser
(A) Untreated Control			
(B) Seed Dressed (SD) 100 ml	100 ml Sumisclex Flocool	-	-
(C) SD 50 ml	50 ml " "	-	-
(D) SD 25 ml	25 ml " "	-	-
(E) SD 100 ml + 4 kg/LS	100 ml " "	4 kg Sumisclex WP	EZ Lime-Super
(F) SD 50 ml + 4 kg/LS	50 ml " "	"	"
(G) SD 25 ml + 4 kg/LS	25 ml " "	"	"
(H) 4 kg/LS	-	"	"
(I) SD 25 ml + 4 kg/SS	25 ml " "	"	EZ single Super
(J) SD 100 ml + 4 kg/F	100 ml " "	"	EZ NPK Fertiliser
(K) Raxil:SD 100 g + 4 kg/LS	100 g Raxil WP	4 kg Raxil WP	EZ Lime-Super
(L) Octave: SD 5 g + 2.5 kg/LS	5 g Octave WP	2.5 kg Octave WP	EZ Lime-Super
(M) Folicur: SD 10 ml	10 ml Folicur EC	-	-
(N) Trichoderma Biocontrol	-	115 kg Trichoderma millet culture	-

Details

14 treatments x 6 replicates

Row spacing: 20 cm

No. of rows per bed: 6

Replicate plot size: 1 bed (6 rows) x 7 m

Seedling rate m^2: 130, later to be thinned to 100/m^2

Sampling plot: 4 inner rows x 2.5 m (2 m^2) containing 200 plants/plot

Sowing date: 14 July 1989

Notes on Products Under Test

A) Fungicides

(1) Sumisclex 275 Flocol Liquid (275 g procymidone per litre)

(2) Sumisclex 50 WP (500 g procymidone per kg, as Wettable Powder)

(3) Raxil WP (25 g tebuconazole/kg, 4 g cypermethrin/kg, as Wettable Powder)

(4) Folicur EC (250 g tebuconazole per litre, as Emulsifiable Concentrate)

(5) Octave WP (462 g prochloraz per kg, as Wettable Powder)

B) Fertilisers

(1) Base Fertiliser - EZ NPK 14:16:11

(2) Granulated Lime-Super - EZ 50/50 Granulated Lime Superphosphate

(3) Single Super - EZ Granulated Single Superphosphate

C) Biocontrol Agent

(1) Trichoderma sp.

Use Rates

(1) Fungicides - as given in treatments

(2) Fertilisers - Base Fertiliser: 500 kg/ha

- Granulated Lime-Super: 125 kg/ha

- Single Super: 125 kg/ha

(3) Trichoderma Biocontrol - 115 kg/ha (includes millet carrier)

Seeds in all treatments pre-dusted with 2 gm Benlate and 5 gm Thiram per kg seed.

Layout - Onion Trial R. Bonney 1989.

Main Trial

	1 B	2 L	3 A	4 D	5 J	6 C	7 K	15 J	16 I	17 H	18 B	19 M	20 F	21 L
R1														R2
	8 N	9 M	10 E	11 H	12 I	13 F	14 G	22 C	23 K	24 D	25 N	26 G	27 A	28 E
	29 D	30 L	31 N	32 C	33 M	34 J	35 A	43 K	44 G	45 L	46 B	47 J	48 I	49 D
R3														R4
	36 E	37 G	38 F	39 K	40 H	41 B	42 I	50 N	51 A	52 F	53 H	54 E	55 M	56 C
	57 M	58 H	59 E	60 A	61 D	62 L	63 F	71 I	72 B	73 N	74 K	75 G	76 J	77 A
R5														R6
	64 N	65 J	66 I	67 C	68 G	69 K	70 B	78 E	79 M	80 C	81 F	82 L	83 H	84 D

1 2 3 4

Supplementary Trial

Garlic Trial