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Research solutions for the control of white rot

A report prepared for

New Zealand Onion Exporters Association

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CROP & FOOD RESEARCH
Mana Kai Rangahau

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CONTENTS

1 EXECUTIVE SUMMARY

1. White rot is a soil-borne disease that only affects onions and related crops such as garlic, leeks and chives (*Allium* spp.).

2. Once soil has been infested with white rot sclerotia (small, black survival structures) the disease is always present. White rot infects only living onion tissue and spreads from root to root in neighbouring plants.

3. The severity of white rot infection varies from year to year because of climatic variation, number of sclerotia in the soil and crop density.

4. Crop losses from white rot are variable. There has been no survey of the cost of losses due to white rot in the Pukekohe district.

5. White rot control measures are based on:

   - *Protecting the onion plant from infection.*
     These include fungicide treatment and biological control.

   - *Reducing the number of sclerotia in the soil.*
     These include germination stimulants such as diallyl disulfide DADS, biological control, soil sterilants and crop rotation and hygiene.

   - *Developing onion cultivars resistant to white rot.*
     There are no existing cultivars with resistance to white rot, but genetic engineering to incorporate antifungal genes into onions is promising.

6. Application of Sumisclex and/or Folicur is currently an economic and effective control measure for white rot. It costs $500-600/ha. However, it is prudent for the industry to invest in alternative control measures in case their efficacy is reduced as a result of problems such as enhanced degradation of Sumisclex in the soil, the chemical becoming ineffective (as happened with Rovral and Ronilan), or because of residue and/or phytotoxicity problems.

7. Applying DADS to the soil to reduce the numbers of white rot sclerotia has resulted in both successes and failures in other countries. Research is needed to determine the efficacy of the treatment for Pukekohe conditions.

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The cost of DADS will range from $100-200/ha depending on the required rates.

The cost of research will be $80 000-150 000, over three years.

8. Developing white rot-resistant cultivars through genetic engineering is a long term approach to white rot control.

   The cost of research will be $132 000 over six years.

   The cost of developing disease-resistant cultivars could partly be recovered in premium-value seed sales.

9. Alternative chemicals to Sumisclex and Folicur will continue to be developed by agricultural chemical companies internationally.

2 FEATURES OF WHITE ROT

1. White rot is a fungal disease that never goes away

White rot is a soil-borne disease of onions caused by the fungus *Sclerotium cepivorum* Berk. The 0.2-0.6 mm 'survival structures' of the fungus occur as spherical sclerotia in the soil. Sclerotia can survive in the soil for long periods - 18 years survival has been recorded. This means that once white rot is present in the soil it never goes away.

Controlling white rot involves:

- reducing the number of sclerotia in the soil,
- protecting the onion crop from infection, and
- developing onion cultivars resistant to white rot.

2. White rot is especially adapted to infect onions

White rot is especially adapted to infect only onions and related crops such as garlic, leeks and chives. The sclerotia germinate only in the presence of certain sulfur compounds produced in the roots of these crops. These same sulfur compounds also give rise to the pungency of onions.

The germinated sclerotia form a mass of white fungal growth on the roots, base plate and lower part of the onion bulb. The fungal mass on the infected plant darkens, and becomes transformed into large numbers of minute, black, rounded sclerotia. These new sclerotia remain in the soil and are able to infect onion plants in the following season.

3. White rot spreads slowly through root contact

White rot fungus cannot live alone in the soil. It can only live on onion roots and bulbs, and spreads from root to root of plants in close contact.

Plants can become infected at any stage of growth providing environmental conditions are favourable. In New Zealand, the disease affects plants throughout the growing season, but the disease is most prevalent in October-November. The fungus is also capable of affecting onions in storage, but this form of the disease is not a problem in New Zealand.

The first indications of white rot in the early part of the season are the death of unusual numbers of small seedlings in the 1-2 true leaf stage. Affected seedlings collapse; the fragile dead leaves quickly disintegrate or are blown away by wind. The characteristic tuft of white fungal growth is not usually seen on small seedlings.

As the plants approach early bulbing, the first symptoms of white rot infection are a subtle change in leaf colour from a bright green to a deep blue-green. The colour change to the leaves occurs quite rapidly following infection and is noticeable when only one or two roots are rotted. The outer leaves turn yellow and die back from the tips and the whole plant soon wilts and dies. The fungus causes an extensive soft rot of the stem plate and lower parts of the storage leaves of the bulb.

Other microorganisms and insects soon invade the rotted bulbs, rapidly reducing them to empty shells. Sometimes the growing point of an infected plant is not killed. In such cases the bulb is usually distorted and split, often with a shoot growing from the split.

White rot can be spread more widely by agricultural practices. Deep ploughing can re-distribute sclerotia within the soil and increase crop disease incidence. Sclerotia can also be spread from paddock to paddock on tractor wheels, boxes and implements.

4. White rot is universal

This disease is present in many areas of the world where onions are cultivated and environmental conditions are favourable to the pathogen.

White rot is a problem because of:

1. the loss of crop and the additional costs associated with harvest,

2. the uncertainty associated with the possible increased damage when onions are grown again in infested land, and

3. the possibility of new outbreaks in areas previously unaffected by the disease.

The life cycle of white rot is shown in Figure 1.

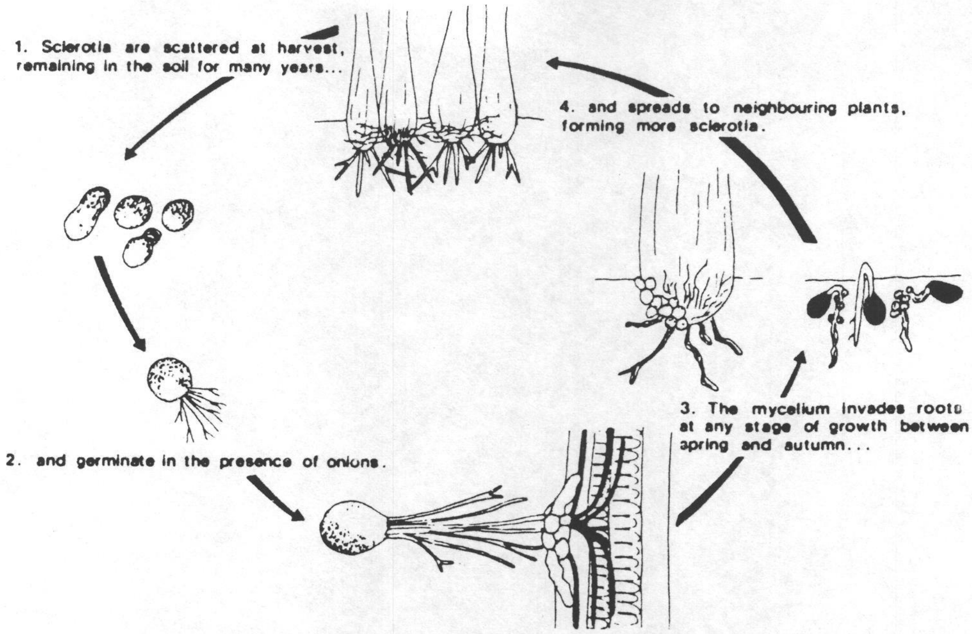

Figure 1: Life cycle of white rot, *Sclerotium cepivorum.*

White rot has been reported in the UK, Europe, India, Japan, Eastern Europe, Chile, Egypt, South Africa, Tasmania, Australia, New Zealand and in most of the onion-growing regions of the USA. In all of these regions the disease is always present, but varies in its severity from year to year depending on onion cropping rates and environmental conditions.

5. White rot varies in its severity

Although white rot sclerotia are always present in the soil of a region with a history of white rot, the severity of infection of onion crops varies from year to year because of:

- the number of sclerotia in the soil. The greater the number of sclerotia the greater the likelihood of infection in a crop,

- the survival of sclerotia in the soil. Sclerotia die in hot, flooded or waterlogged soils. After white rot infection of a crop the number of sclerotia will be high, but may decrease during a wet winter. In the following year white rot incidence may be low,

- weather conditions in the spring. White rot is a disease of cool, moist climates. Sclerotia germinate in cool soils, and spread from root to root in moist soils, and

- plant density in the crop. Because the fungus has to be in 1-2 cm contact with onion roots to grow, white rot spreads more easily in closely planted crops.

The interaction and variability of these factors mean firstly that it is difficult to predict the likely incidence of white rot, and, secondly, that the severity of the disease varies from paddock to paddock and year to year.

6. Crop losses from white rot

The disease can cause significant seedling losses early in the season, but most losses are caused later in the season when the plants approach early bulbing. Cosmetic damage to the appearance of the bulbs at harvest requires them to be graded out.

Although there has been no survey of losses due to white rot in the Pukekohe district, the disease varies in its impact across the district and from year to year. Severe losses of 70-80% in some paddocks have been reported in some years.

The variation in severity of white rot makes it difficult to analyse the cost of white rot and the benefits from developing new control measures.

3 CONTROLLING WHITE ROT

Control of soil-borne diseases is difficult, and onion white rot is no exception. Because the organism lives and infects belowground it is not easily reached by fungicides. It can also survive for long periods in the absence of an onion crop. At present, successful commercial control generally calls for the use of fungicides to provide direct protection to the plants, and the use of cultural practices to limit the numbers of sclerotia in the soil.

Two new research solutions, application of diallyl disulfide (DADS) and genetic engineering, offer new hope for white rot control. A further four disease control options are also described below.

3.1 Control by fungicides

The fungicides Rovral (active ingredient: iprodione) and Ronilan (active ingredient: vinclozolin), both belonging to the group of chemicals known as dicarboximides, were first used in white rot control trials in New Zealand in 1979. Dramatic results were obtained with Rovral when used as a seed dressing followed by a series of foliar sprays during the season. Foliar sprays of Ronilan were equally effective. These two fungicides, used in the seed treatment/foliar spray combination, became the mainstay of white rot control in the early 1980s. For a time they were highly effective. By 1983, however, growers became aware that in some paddocks white rot was not being adequately controlled. Studies by DSIR Plant Protection Division and the University of Auckland demonstrated that, with continued use, Rovral and Ronilan would break down very rapidly in the soil to the extent that they would have little effect on white rot.

Under the normal application regime of seed treatment followed by up to three foliar sprays, it is possible that in any particular area Rovral and Ronilan could have become ineffective after the first season of use. This phenomenon is known as "enhanced degradation" and has been recognised for a range of other soil-applied chemicals including fungicides, insecticides, nematicides, and herbicides. It is caused by soil microbes becoming conditioned to break down the chemical. With successive applications the chemical is degraded so rapidly that it is unable to control the target organism.

A related fungicide, Sumisclex (active ingredient: procymidone), also of the dicarboximide group, gave a high level of control of white rot. Sumisclex was subsequently registered for use on onions in 1987, and has since been routinely used for disease control.

Sumisclex is applied as a combination of seed dressing followed by a series of three foliar sprays. At high rates of application Sumisclex can cause mild phytotoxicity to seedlings. Although its related chemicals, Rovral and Ronilan, became ineffective because of enhanced degradation in the soil, the relative resistance of Sumisclex to enhanced degradation has been demonstrated under both field and laboratory conditions. Its stability is further borne out by the continued effectiveness of this fungicide after up to eight years of continuous use in the Pukekohe district.

However, the continued dependence on a single fungicide is a cause for concern and it is prudent to investigate alternative control strategies.

Folicur (active ingredient: tebuconazole), Cereous (active ingredient: triadimenol) and Alto (active ingredient: cyproconazole) have all been shown to be highly effective against white rot when applied as foliar sprays. Folicur and Cereous have full registration for use on onions as foliar sprays.

A common practice in the Pukekohe district at present is to apply Sumisclex as a seed treatment and/or granule applied at sowing followed by foliar application of either Sumisclex or folicur.

Cost of Sumisclex: Seed treatment = $13-17/ha
Foliar application (x 3 per season) = $540/ha

3.2 Control by DADS

DADS is an alternative approach to using fungicides to control white rot.

Sclerotia will germinate only in the presence of onions. Once germinated, the fungus must infect a plant or die. It cannot live freely in the soil, and rarely re-forms sclerotia in the absence of onion plants. These two features offer a novel opportunity to combat the fungus. By stimulating sclerotia to germinate in the absence of an onion crop and then to die, it is possible to achieve a substantial reduction in the numbers of sclerotia in the soil.

Applications of onion oil, garlic oil and a number of propyl and allyl sulfides have been used in an effort to stimulate germination of sclerotia. Of the various stimulants tried, the most effective have been DADS and n-propyl disulfide. These are the main ingredients of onion oil. These chemicals have the advantage over oils in that they can be chemically synthesized and readily produced in commercial quantities.

The potential for reducing subsequent disease depends on the initial density of sclerotia in the soil, the absorptive capacity of the soil and the efficiency of the germination stimulant. In the field, responses to onion oil and garlic oil have been in the order of 50-

70%. Recent research in the USA has demonstrated 99% reductions in sclerotia with a single application of DADS. A second application reduced numbers to undetectable levels in the soil. In one locality the infection rate in garlic was reduced from an expected 50+% to 0-2% by two applications of DADS applied 12 months apart. However, there are also reports that onion oil and DADS have been ineffective in the control of white rot. In Tasmania DADS was not effective. The inconsistency of the results points to the need to fine tune the chemical to local conditions.

A number of factors have to be considered when using these chemicals. Under normal circumstances, newly formed sclerotia undergo a period of dormancy before they will respond to germination stimulants (either natural or applied). To obtain the best results, application of the chemicals must be delayed until the dormancy requirement has been satisfied. In addition, it may take several weeks for maximum germination. Therefore, the treatments must be applied well before planting to avoid young onions being exposed to the actively germinating sclerotia. Applications are most effective when soil temperatures remain in the range 15-20°C for several weeks.

The chemicals smell strongly of garlic, but experience in the first series of applications has shown that they are not unduly offensive to handle. Otherwise, the chemicals are of low toxicity. Ideally, DADS would be applied in the period between lifting of the summer crop and before early winter sowing. The efficacy of this chemical to reduce numbers of sclerotia needs to be tested in the Pukekohe climate and on Pukekohe soil types.

Cost of DADS - in the region of $100-200/ha, depending on the results of trials to determine application rate/timing etc.

Cost of research - $80 000-150 000 over three years

3.3 Control by genetic engineering

Genetic engineering can be defined as the combining of DNA sequences (genes) from widely different organisms to produce a novel characteristic in a particular organism. This is now being routinely achieved in many agricultural crops.

Fungal resistance genes that control fungus infection in plants have been indentified and isolated. These genes interfere with fungal growth, interfere with host-pathogen recognition, or reduce levels of specific components required for fungal invasion. Introduction of these genes reduces fungal disease symptoms in other crops and could be applicable against white rot in onions.

In particular, a gene, oxalate oxidase, has been shown to increase resistance to *Sclerotinia* fungus in the tobacco and oil seed rape crops in which it has been tried in the USA.

Crop & Food Research is currently developing the technology for inserting new genes into onion plants, and its scientists are world leaders in this field.

Research would be required to put an anti-fungal gene into onions, and test the resistance of the engineered plants to white rot in the field.

This technology is long-term, and would require five to six years research to produce onion lines resistant to white rot. Cost of research = $132 000 over six years. Bulking up of seed from experimental to commercial volumes would take four to six more years. Seed of genetically engineered, white rot-resistant cultivars would fetch approximately 50% premium above other hybrid cultivars.

3.4 Biological control

Within the soil environment many microorganisms are parasitic on, or are otherwise antagonistic to, the onion white rot fungus. Dr Alison Stewart, Lincoln University, has been investigating the potential for biological control of white rot in a research programme which commenced in 1987.

A large number of different microorganisms has been isolated from onion paddocks in the Pukekohe district and tested in the laboratory for antagonistic activity against the white rot fungus. Microorganisms that either limited the growth of the fungus, inhibited production of sclerotia, or that directly parasitised the sclerotia, were selected. These fungi were then used in trials in the glasshouse to determine their ability to control the disease.

A number of fungi showed promise. *Chaetomium globosum* and *Trichoderma* species, when incorporated into the soil at planting, gave a greater level of control than the standard fungicidal seed treatment, Sumisclex. Both of these organisms were capable of degrading sclerotia as well as inhibiting growth of the fungus i.e. they demonstrated both parasitic and antibiotic action against white rot.

The *Trichoderma* species are currently being investigated in a TBG (Technology for Business Growth) grant by Agrimm Biochemicals and Lincoln University.

Cost of applying a biological control is anticipated to be $500/ha.

3.5 Control by breeding

There are no resistant cultivars or breeding lines currently available even though a lot of effort has gone into selecting breeding material for resistance, both in New Zealand and internationally.

A low incidence of white rot in some lines developed at the University of Wisconsin was interpreted as evidence of resistance. Their lines were subsequently trialled in California and in Tasmania, and in both locations they succumbed to the disease.

3.6 Control by soil treatments

3.6.1 *Solarization*

Several soil-borne plant pathogens have been controlled or partly controlled by mulching fields with polythene sheeting during summer prior to cropping, thus raising soil temperatures. This process is termed solarization. The effects are most noticeable in hot, sunny regions and when the soil is moist. In South Australia, solarization killed 96% of sclerotia at 25 cm depth when the mean maximum temperature was approximately 38°C; there was total kill at 5-15 cm where the temperatures were higher. Similar effects on sclerotial viability were observed in the Northern Territory, Australia at, a soil depth of 5 cm; at 10 and 15 cm the mean maximum soil temperatures were 29-34°C and had less effect on sclerotia. *S. cepivorum* sclerotia were killed by a diurnal regime of six hours at 45°C and 18 hours at 25°C maintained for 20 days. Some of the effects of solarization occurred at temperatures that were not directly lethal. Antagonistic fungi and bacteria were more abundant under these conditions and probably parasitised sclerotia that had been weakened but not killed. Solarization also reduced white rot incidence by 50 to 67% in Sohag, Egypt.

Although this technique has proved effective in these countries, and also in western USA, we calculate that summer temperatures in Pukekohe are not high enough for this control measure to be effective. However, there may be some potential for this method in the Canterbury region where soil temperatures in the summer are higher. This aspect is under investigation by Dr Stewart at Lincoln University.

3.6.2 *Soil fumigants*

Several soil fumigant compounds have the potential to kill sclerotia and thus to control white rot and reduce further dissemination. However, control using these substances can be unreliable. For instance, metham sodium was alternately very effective and ineffective. A similar variability in effect was noted with dazomet.

The efficacy of soil fumigants is likely to be governed by the duration and concentration of active ingredient to which sclerotia are exposed. For example, the continuous injection of metham-sodium into sprinkler irrigation, thus prolonging exposure of sclerotia to the active ingredient, was considerably more effective at killing sclerotia than a single injection of the chemical.

Soil absorptive capacity and soil structure affect the release of fumigants and their subsequent diffusion. Distribution of dazomet granules was improved by using a purpose-built rotovator ("spading machine") to incorporate the chemical. The use of polythene sheets to cover soil is proving increasingly necessary in the UK to optimise retention of fumigants that are rotovated or injected into soil.

Disadvantages of soil fumigants are their high cost, the fact that they are environmentally unfriendly chemicals and their variable effectiveness.

3.7 Control by cultural practices

3.7.1 *Crop rotation*

The use of crop rotation for disease control has two aims:

1. to prevent the progressive build up of sclerotia in the soil that will inevitably occur with successive crops of onions, even with commercially acceptable levels of chemical control, and

2. to provide an opportunity for numbers of sclerotia to decline naturally between crops.

Unlike many other soil-borne fungi, the onion white rot fungus cannot live freely in the soil on dead plant tissues. The long term survival of the organism, therefore, depends on the ability of the sclerotia to remain viable between crops.

Although there is a significant reduction in both numbers and viability of sclerotia within the first 12 months, if the survivors remain viable for an extended period e.g. three to five or 10 years, then short term rotations three to five years are probably of limited value. For example, a 100-fold reduction in numbers in a severely infested paddock (containing 1-10 sclerotia/10 g soil) would still leave sufficient numbers of sclerotia to initiate infection in a subsequent crop. Although numbers of infected plants may be small in the first year, numbers would quickly escalate in subsequent crops.

More information is needed on the long term survival of sclerotia under New Zealand conditions before advice can be offered on the appropriate length of rotations.

3.7.2 *Hygiene*

The sclerotia of onion white rot are readily carried in soil on machinery and bins. They can survive for long periods in the absence of a crop, are highly efficient at infecting onion plants, and the fungus has the ability to multiply rapidly within any new area. Preventing the spread of the fungus to new areas calls for extreme vigilance on the part of the grower. All machinery and bins to be used in the area should be free from soil and plant debris. This applies not only to operations relating to an onion crop, but to all situations where machinery is moved between the areas. For example, if soil is transferred between sites in the course of growing squash or leaf vegetables, the risk of transferring white rot sclerotia is just as high as during a year of onion production.

More information is needed on the relationship between numbers of sclerotia in the soil and the likelihood of disease incidence.

4 RECOMMENDATIONS

1. In the absence of surveys of the extent and frequency of the disease in Pukekohe it is difficult to estimate the cost of white rot to growers, either from loss of production or cost of prevention.

2. Sumisclex has proven ability to control white rot, and has so far shown no signs of losing its effectiveness either through the white rot fungus developing resistance or through enhanced soil degradation of the chemical. The cost of control by Sumisclex is estimated at $540/ha. If there are 3000 ha of onions grown in Pukekohe and 10% are affected by the disease then the cost of prevention becomes $162 000/year.

3. However, it is possible for Sumisclex to lose its effectiveness, as happened to Rovral and Ronilan, and so it is prudent to investigate alternative control strategies.

4. DADS has been shown to be effective against sclerotia of white rot. Application rates and timing need to be worked out for Pukekohe conditions and soil types. The effect of the chemical on populations of sclerotia in the soil needs to be monitored.

   Cost of DADS is $100-200/ha. Research to determine the effectiveness of this chemical in Pukekohe is estimated to cost approximately $80 000-150 000 (basic studies on efficacy and field evaluations).

5. *Trichoderma* fungi as biological agents are being tested by Dr Stewart, Lincoln University, under a Technology for Business Growth grant. A commercial biocontrol product is likely to be comparable in price to Sumisclex. Biological controls such as this offer promise because of their perceived environmental friendliness. However, they are unlikely to replace fungicides completely and will probably be used as part of an integrated control programme.

6. Genetic engineering of onion cultivars for white rot resistance is an interesting new opportunity. This approach, if shown to be feasible, would provide the industry with white rot-resistant cultivars. This is a long term approach. Feasibility would be demonstrated in six years, and, thereafter, it would take four years to bulk up seed for field release. However, since onion white rot will not go away, long term control measures undertaken should start sooner rather than later. The cost of demonstrating feasibility would be about $130 000 over six years.