AP97005
A study of Apple Replant Syndrome

Gordon Brown et al
Tasmanian Institute of Agricultural Research

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A Study of Apple Replant Syndrome

HRDC Final Report

Project Number AP 97005

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HRDC Final Report of Research Findings

August 2000

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Media summary

Research on apple replant problems, which affects apples planted back into old apple orchards, has been studied for the past 3 seasons. This research has identified that:

- Apple replant problems occur in all of the apple orchards sampled in the south of Tasmania.
- The problem on average, leads to a 50% reduction in tree growth rates and hence, potential yield per tree.
- All apple replant orchards tested responded to soil sterilisation indicating a biological cause to the problem.
- All apple replant orchards tested responded to antibiotics indicating that bacteria are the main cause of apple replant disease in Tasmania.
- Nematodes are also a minor problem in 40% of the soils tested.

Traditionally this disease has been controlled with methyl bromide soil fumigation. However, due to the destructive nature of this material on the environment this compound is being banned for soil applications. This project has identified two potential alternative chemicals to control this disease:

- Chloropicrin soil fumigation – currently not registered.
- Dazomet – needs careful application to be effective.

Limited data also suggests that the fertiliser mono ammonium phosphate may also be of some benefit against this disease. Other chemicals and cultural practices provided little control of the disease or provide long term benefits that were not detected in this project.

Recommendations for future research:

- Effectiveness of other chloropicrin materials currently undergoing registration against the disease.
- Application methods for dazomet to improve its reliability.
- Application methods for mono ammonium phosphate to improve its reliability.
Technical summary

Seven glasshouse pot trials and one field trial were conducted over 3 seasons to study the nature and control of specific apple replant disease (SARD) in Tasmania. The pot trials utilised ‘mm26’ apple rootstock plantlets derived from tissue culture.

The extent and Severity of apple replant disease
It was found that all soils with a history of apple production from the Huon Valley of Tasmania were infected with SARD. This disease on average led to a 50 percent reduction in the growth rate of apple rootstocks. The only soil where SARD was not detected was a soil with no history of apple production demonstrating the pot test did not detect SARD when it was not present. This result also highlighted that there is no growth promotion with soil sterilisation due to release of nutrients from the soil microbiota. Hence, sterilising soil in the absence of apple replant disease has no beneficial effects on tree performance.

The cause of apple replant disease
It was found that 40 percent of the soils tested had a limited response to the addition of nematicide indicating the presence of nematodes in these soils. Nematodes are non specific such that care should be taken when replanting these soils to other crops, such as cherries. In all of these nematicide responsive soils SARD was also present and was far more damaging than the nematodes.

Three different fungicides (Shirlan®, metalaxyl and thiram) registered for use against a range of soil borne fungal pathogens were ineffective against SARD indicating that the cause of SARD is not of fungal origin.

The role of bacteria as an agent of SARD was investigated by the incorporation of the antibiotic streptomycin into the soil. This treatment was found to be as effective and in one instance more effective, than the sterilisation of the soil with heat suggesting that bacteria are responsible for SARD in Tasmania, as has been found in Europe.

Cultural and Biological control of apple replant disease
The pot trials also identified that the addition of Trichoderma spp, calcium hydroxide and mono ammonium phosphate (MAP) had potential to reduce the impact of SARD. In the case of MAP the response was greater than that of nutrition alone indicating that this fertiliser is active against the disease. There was also some evidence that streptomycin and mycorrhiza added to the tree roots may be beneficial for trees planted in sterile soil due to elimination of SARD on the planting material (streptomycin) and providing the tree with long term protection from SARD (mycorrhiza). It was found that the symptoms of SARD were not reduced by increased quantities of irrigation water.

Field trial results
After the first season of growth it was found that trees growing in the methyl bromide treatments out performed all the other treatments while the MAP and dazomet gave equivalent and intermediate results. The Trichoderma and calcium hydroxide treatments provided no protection to the trees against SARD in this field trial. There was no growth response to the root applied streptomycin or the mycorrhiza (both applied after methyl bromide) although these responses may become evident in later seasons.
Results have also been obtained from three field trials not funded by this project. These trials have shown that chloropicrin is highly effective against SARD and that dazomet is as effective as methyl bromide. Soil applied thiram had no effect in these field trials.

**Recommendations to growers**
Chloropicrin and Dazomet both appear to be as effective as methyl bromide against SARD, however there are major concerns that prevent the commercial use of both these products. Unfortunately chloropicrin is not registered, and is not likely to be registered, for this use. A new product containing 35 percent chloropicrin, Telone 35C®, from Dow, is currently undergoing registration and may prove to be extremely useful against this disease although no trials have been conducted to confirm this.

Dazomet (Basamid®, BASF), in a field trial funded by BASF, but not reported here has also shown similar results to methyl bromide against SARD. Unfortunately this material, as for other MIT compounds such as Vapam, has a history of unreliability. This unreliability is usually associated with incorrect application methods.

The MAP plus nematicide treatment also looks promising and in the field trial reported here this material was as effective as the dazomet treatment and may prove as effective as methyl bromide with improved application techniques.

**Recommendations for possible future research.**
The effectiveness of Telone 35C® against SARD
Application technology for dazomet against SARD
The effectiveness and application technology of MAP against SARD
Long term monitoring of the existing field trials.
Introduction and literature review

Many tree and vine crops are subject to growth problems when they are replanted on sites within several years of removing the previous orchard or vineyard (McKenry, 1999). This phenomenon is referred to as the replant problem, and occurs in many crops including apples, pears, plums, cherries, peaches and grapevines. Although it is present in all countries where these crops are grown, it occurs at varying intensity and in some regions is entirely absent. From overseas studies it is evident that at least 50% of existing pome fruit orchards are growing on soils with replant problems (Kallay et al., 1996; Szczygiel and Zepp, 1996a; and Sewell, 1984). The replant problem includes symptoms such as poor tree establishment, low growth rates, reduced fruit yields, patchy leaf yellowing and shortened life of the orchards (McKenry, 1999). All these symptoms have a major detrimental effect on the commercial viability of newly planted orchards.

One of the major dilemmas facing orchardists is that replant problems are extremely hard to identify. Often the problem goes undiagnosed and hence untreated. Subsequently, the yields and quality of the crop are lower than anticipated. For decades, scientists have tried to identify the fundamental cause of this problem but even today, they are only able to provide partial and usually discipline-biased theories on the source of the problem. The complex and diverse range of elements that must be considered when looking at this problem include degraded soil structure, nutritional problems, soil toxins, over-watering, wind damage, nursery problems, rootstock selection, root diseases and root pests such as nemetodes (McKenry, 1999). In any one orchard several of these potential components may be contributing to the problem.

Apple replant problems

Commercial apple orchards in many parts of the world are prone to replanting problems. The problem can be very severe, as evidenced by pot trials which have shown a reduction in growth of up to 76% in apple seedlings affected by apple replant disease (Hoestra, 1968; Sewell, undated; and Szczygiel and Zepp, 1996b;). These results are reflected in field trials conducted in Holland, where it was found that replant disease caused a 50-90% reduction in growth of 'James Grieve' apples in the first year after planting (Hoestra, 1968). In England it was found that 'Queen Cox' apples grown in treated soils had a total shoot growth 5 metres per year more in the 6 years following planting than trees planted in untreated soils (Hipps, in press). Similar figures are apparent for the subsequent yield of fruit from trees affected by replant (Hipps, in press; Hoestra, 1968). In Tasmania a non replicated field study showed that the trunk cross sectional area of 15 year old 'Hi Early' apple trees was reduced from 85cm$^2$ to 35cm$^2$ if planted on old apple rows. A second trial, where methyl bromide was not applied to a small length of row, showed that the shoot length of 1-year-old 'Jonagold' trees was reduced from 940mm to 5mm (Brown, unpublished data).

The above data shows that apple replant problems have major detrimental effects on tree growth and yield for the entire life of the orchard. Modern intensive apple production requires early high yields to maximise yield and hence profits to growers. Any unpredictability in these yields will undermine the economic viability of the orchards, deterring further orchard development.
The causes of apple replant disease

There are two major classifications of replant problems in apple orchards. These are non-specific and specific apple replant disease (SARD). The non-specific problems are usually associated with large numbers of root-feeding nematodes, such as occur in Queensland. This type of problem can be controlled through the application of a nematicide prior to planting. Other causes of non-specific replant disease include soil structure degradation, pH and toxic compounds such as herbicides, heavy metals and biological products.

SARD is defined as the onset of growth problems only when an apple orchard is replanted after another apple orchard. This may occur both in nursery and orchard situations, and is of increasing concern due to economic constraints demanding quick turn around of crops. The cause of SARD has never been fully characterised, and it is probable that there are many factors involved, including soil pathogens. The soil is a complex environment that contains a large array of potential plant pathogens and pests. Unfortunately, the identification of a soil-based pathogen is difficult, since once the pathogen has weakened the plant, many others are able to invade, often out-competing the original organism. Many soil pathogens including *Phytophora*, *Pythium* and *Cylindrocarpon* have been implicated with SARD. However, it is possible that these are only secondary invaders and not the causal agent.

There is mounting evidence in many locations that SARD is the result of an actinomycete (a filamentous bacterium) attack. These bacteria are not usually associated as plant pathogens, but they are the cause of common scab in the potato. Otto and Winkler (1996) used histological techniques to record the presence of these bacteria in apple roots within 28 days of planting into replant soil. Unfortunately the research station in Germany where these studies were being conducted has been closed, and this research terminated. However, there are several other lines of evidence that support these initial indications that actinomycetes are responsible for SARD.

- In many locations fungicides have little impact in reducing the incidence of replant disease. These findings suggest that the causal agent is often not a fungus such as *Phytophthora* or *Pythium*.
- Chloropicrin and methyl bromide are fumigants that destroy soil borne fungi, actinomycetes and other bacteria. These are the only effective commercially available agents against SARD.
- It has been clearly demonstrated that the addition of large quantities of organic matter to the soil eliminated the symptoms of SARD (Szczygiel and Zepp 1996a and b). Actinomycetes are weak pathogens, and many organisms associated with the breakdown of organic matter produce antibiotics against them.
- Utkhede (1994), showed that *Bacillus subtilis* (strain EBW4), an antibiotic producing bacterium, controlled SARD.
- It has been noted that the presence of both potato scab and SARD are reduced in acid soils that have a moisture content close to field capacity (Hoestra, 1968; Szczygiel and Zepp 1996a) and that the application of large quantities of organic matter ameliorates the effects of both potato scab and SARD. Hence, this data suggests a similar casual agent between these two diseases.
The elimination of specific apple replant disease (SARD)

The only established effective chemical remedies for SARD is soil fumigation with either methyl bromide or chloropicrin. In Australia only methyl bromide is registered for use on apples, although chloropicrin is added as a odour to alert operators to poor fumigation practices. Both these fumigants are broad spectrum in activity and protect crops against a wide range of soil borne pest and disease problems. However they also kill beneficial soil organisms. There are currently no other commercially viable treatments that reliably eliminate apple replant disease from affected soils. In addition, other benefits, such as weed control, positive growth response of crops, and uniform yields have led to the widespread use of methyl bromide even in situations where the presence of SARD is not known (Methyl bromide consultative committee, 1998).

The dilemma for the Australian apple industry is that methyl bromide is being phased out of use by the year 2005, under the Montreal protocol on substances that deplete the ozone layer (a 50% reduction by 2001). As mentioned above, chloropicrin, is not registered for use by itself in Australia. Further, as much of the toxicological data for the registration of chloropicrin has not been determined, it is doubtful that this product will be registered by itself under modern registration procedures.

There are, however, two pesticides with potential to assist in the control of SARD. Dazomet, a methyl isothiocyanate (MIT) producing soil fumigant (as are metham sodium containing pesticides such as Vapam), has been shown to have excellent activity against SARD in some, but not all trials. This unreliability is often explained by incorrect application procedures. Dazomet is currently registered for use in apples and as such is already available to growers although its effectiveness against SARD in Australia, when correctly applied, needs to be confirmed and correct application methods need to be developed and promoted to growers. In addition, the chloropicrin containing pesticide, Telone C, is currently undergoing registration and, if successful, may prove to be useful against apple replant disease although this needs to be confirmed.

Many cultural methods of overcoming SARD have been proposed including the removal of all tree roots, the use of organic matter, new soil in the planting hole, placement of the new rows in the old inter row space, biofumigation, improved irrigation, use of more vigorous rootstocks etc. These procedures have been summarised in a poster by Jill Campbell and Paul James and published by NSW agriculture and Primary Industry and Resources South Australia. Used in combination these procedures reduce the impact of SARD but they do not eliminate the problem and the only successful cultural control is the use of extremely long fallow or new land.

The Huon Valley in southern Tasmania is the states largest apple-growing district. Prior to this study SARD was known to exist in the valley resulting in growers usually fumigating old orchard soil prior to replanting. The extent and severity of the disease was unknown, as were alternative control strategies available to growers. The aim of this project was to document the extent and severity of the problem in the valley and to test potential alternative treatments to control it. Much of the data collected also aids in the identification of the causal agent of SARD in Tasmania. A series of strategies are outlined that may provide orchardists with some alternatives to methyl bromide for the control of SARD in Tasmania and possibly throughout Australia.
1. The incidence and severity of specific apple replant disease and its potential control in the Huon Valley of Tasmania

Introduction

Apple replant disease results in decreased overall tree growth, reduced fruit yields and chlorosis of the leaves. That this problem occurs in both orchards and nurseries is of increasing concern, as economic conditions are demanding a quick turnaround of crops. There are two forms of replant problems, non-specific replant disease and specific apple replant disease (SARD). The former results in the new crop experiencing growth problems regardless of the previous crop and is generally caused by root-feeding nematodes. This form of replant disease can usually be controlled with the application of nematicides. Other causes of non-specific replant problems include degradation of the soil structure, pH and toxic compounds such as herbicides, heavy metals and biological products.

SARD presents only when an apple orchard is replanted after another apple orchard. Extrapolating from European research, up to 50% of Australian apple orchards could be affected by SARD although the extent and severity is likely to be regionally specific. Unlike non-specific replant disease the cause of SARD is still unknown, however scientific evidence does indicate that one causal agent may be filamentous actinomycetes.

The only effective and commercially available treatment for SARD in Australia is methyl bromide, which is a broad-spectrum soil fumigant. The dilemma for apple orchardists is that this product is being phased out of use by the year 2005 under the Montreal protocol on substances that deplete the ozone layer. Therefore an effective and commercially viable alternative must be found.

The following trial was designed to ascertain the extent of apple replant disease in the soils of the Huon Valley of Tasmania, and whether these soils were affected by apple specific or non-specific replant problems. One sample was left entirely untreated, as a control, complete soil sterilisation using heat was included to indicate whether any apple replant problems were present in the soils and a nematicide and air drying for 21 days were included to determine the extent and severity of non-specific replant disease. Shirlan was also included in the trial to determine whether this fungicide could be a potential control of SARD. It is registered for use only against basidiomycete root diseases, however, initial trials using this product in petri dishes have been successful in controlling the actinomycetes which cause common scab in potato (Wilson, pers comm).

The overall aim of this project was to examine the frequency, severity and nature of apple replant disease in the Huon Valley of Tasmania.

Materials and Methods

In October 1997 soil was collected from 10 different apple orchards and one pasture paddock with no history of apple production. These 11 sites were all located within a 15km radius in the apple-growing district of Southern Tasmania. At each orchard a transect in the shape of a ‘W’ was used as the sampling system, soil was collected at five equidistant positions along each arm of the ‘W’. Grass was removed and soil sampled to a depth of 150mm with approximately 2 litres of soil being collected.
at each of the twenty positions. These samples were mixed together to form a total of 40 litres of soil for each site, this was stored in plastic, but not airtight containers.

In November 1997 ten litres of perlite was mixed with each soil to improve the physical characteristics for use in pot trials. There were five replicates for each treatment, the treatments were an untreated control, air drying in the glasshouse for 21 days before planting, heat sterilisation (90° C for 48 hours), 28 μL/L of soil Shirlan® and 28 μL/L of soil Nemacur 400® (Bayer). The apple plants used were tissue cultured 'MM26' rootstocks established in pine bark/sand media in 200ml pots. These were obtained during the winter of 1997 and stored at 1°C till 5 December 1997. Shoot growth was initiated at room temperature and at planting (15 December) the seedlings had between 10 and 30 mm of new growth. Plants were planted individually into 1.7 L of soil and placed in the glass house in a 5 x 5 latin square design. An additional 1.7L of soil from each treatment without a plant was placed beside the experimental pots to provide an estimate of water loss from the soil surface.

The glass house was set to a minimum temperature of 15° C, although day temperatures commonly exceeded 30° C. Watering was by hand, to field capacity on Mondays and Wednesdays and with 200 ml/pot on Fridays. Weeds were removed, and aphids controlled with gentle digital force. Powdery mildew was controlled with Benlate® as required.

Pots were weighed on Mondays during March to determine the daily water use of the plants for the previous 5 days. This assumed that there was only a minor change in plant weight. Shoot heights from the soil surface were recorded 6 weeks after planting (2 February). This measurement was repeated on 6 April, prior to destructive sampling of the plants, and subsequent measurement of the leaf areas. Roots were extracted by soaking and gentle agitation in water. Root colour (in Lab colour notation) was determined using a Minolta chroma 200b meter taking 2 readings on each of the two sides of the flattened root mass. Root volume was measured using Archimedes principal of measuring the change in weight of a beaker of water when the roots were suspended in it.

The data was analysed using Genstat, as a split plot latin square ANOVA, with the sites as whole plots with each site containing a 5x5 latin square. Mean separation was by LSD $p = 0.05$. This analysis allowed for a comparison of sites (10 d.f.), treatments across sites (4 d.f.) and treatments within sites (Treatment by site interaction 40 d.f.).

Results and Discussion

In this research project soil 2 had no previous history of apple production and was included as a system check. This soil was found to have the highest shoot growth of all the untreated soils and was also found to be the only soil where the heat treatment failed to cause an increase in shoot growth (Table 1.1). Hence, the pot test did not diagnose apple replant syndrome for a soil where the problem was not present. The failure to detect any heat treatment effects for this site also indicates that there is no growth stimulation with soil sterilisation in the absence of SARD due to the release of plant nutrients from the soil microbiota. This has also been observed by growers who have observed no improvement in tree growth after the use of methyl bromide on new soil (Smith, pers comm).
Table 1.1
The effect of soil sterilisation for 11 sites on shoot growth of apple rootstocks after 4 months of growth

<table>
<thead>
<tr>
<th>Site #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>223 b</td>
<td>312 a</td>
<td>131 b</td>
<td>78 b</td>
<td>90 b</td>
<td>199 b</td>
<td>129 b</td>
<td>244 b</td>
<td>186 b</td>
<td>145 b</td>
<td>266 b</td>
</tr>
<tr>
<td>Sterile</td>
<td>521 a</td>
<td>275 a</td>
<td>491 a</td>
<td>242 a</td>
<td>214 a</td>
<td>286 a</td>
<td>415 a</td>
<td>441 a</td>
<td>489 a</td>
<td>483 a</td>
<td>570 a</td>
</tr>
</tbody>
</table>

* Soil collected from pasture paddock with no history of apple production.
Means in a column with the same letter not different by LSD at p=0.05

In all ten of the orchard soils there was a significant growth stimulation due to soil sterilisation relative to untreated samples. This growth stimulation ranged from a 1.4 fold increase in shoot height (soil 6) to a 3.75 fold increase (soil 3). Averaged across all 11 soils it was found that treating soil for apple replant syndrome caused a 2.2 fold increase in shoot height and a 2.8 fold increase in root volume of ‘MM26’ rootstocks (Table 1.2). As a result, this set of pot experiments indicates that SARD was present in 100 percent of the apple orchard soils tested and that heat sterilisation was very effective in controlling the effect of the disease. Although the obvious source of SARD is the soil from the orchards, there is a possibility that SARD was present on the rootstocks used in this pot trial. This is an unlikely scenario however, since soil 2 would be expected to indicate similar differences in growth between the sterilised and unsterilised treatments if the rootstock was the causative factor in the occurrence of SARD in these trials. The reduction in shoot height due to apple replant disease was severe and it is therefore considered necessary to treat soil prior to replanting new orchards in the Huon region of Tasmania. This is supported by grower observations and formal field trials where methyl bromide fumigation of old apple soils always leads to improved growth and tree performance.

Table 1.2
Treatment effects, averaged across all 11 soils, on the growth variables of ‘MM26’ apple rootstocks after 4 months of growth.

<table>
<thead>
<tr>
<th>Shoot height (mm)</th>
<th>Leaf Area (cm²)</th>
<th>Root Volume (mL)</th>
<th>Root to Leaf Brightness (x1000)</th>
<th>Root Leaf Yellow (b)</th>
<th>Water Use (*ml/week/cm² of leaf)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>182 a</td>
<td>151 a</td>
<td>7.5 a</td>
<td>56 a</td>
<td>29.6 a</td>
</tr>
<tr>
<td>Nemacur</td>
<td>201 bc</td>
<td>165 ab</td>
<td>9.1 b</td>
<td>57 a</td>
<td>29.9 a</td>
</tr>
<tr>
<td>Shirlan</td>
<td>192 ab</td>
<td>163 ab</td>
<td>9.6 b</td>
<td>64 b</td>
<td>31.6 b</td>
</tr>
<tr>
<td>Air Dry</td>
<td>212 c</td>
<td>179 b</td>
<td>11.3 c</td>
<td>66 b</td>
<td>32.3 b</td>
</tr>
<tr>
<td>Sterile</td>
<td>402 d</td>
<td>389 c</td>
<td>21.0 d</td>
<td>54 a</td>
<td>37.6 c</td>
</tr>
</tbody>
</table>

* Means in a column with the same letter not different by LSD at p=0.05.
The results of the ten orchard sites are combined. There were five replicates per treatment

The results of the ten orchard sites were combined, and the data is presented in Table 1.2. Nemacur and air drying the soil are effective means of controlling nematodes (non-specific apple replant disease). In this experiment it was found that these caused a small, but significant, increase in shoot height and root volume indicating the presence of non-specific replant disease in these soils (Table 1.2). Both these treatments are incomplete, however, as some nematodes survive the treatments and multiply in the soil after planting. Hence the nematicide effect is of a short duration. In this trial significant growth responses to the nematicide for individual soils were recorded at the first assessment date only. At this date four of the ten orchard soils responded significantly to the nematicide (Table 1.3). The level of control obtained with both these treatments was, however, not large and inadequate to recommend to industry for use as a standard replanting treatment. This result demonstrates that tests should be carried out on apple soils to be replanted with other crops, such as
cherries to determine if soil treatments against non-specific replant disease are needed. As non-specific replant disease (nematodes) may exist in soils with no history of apple production care should also be taken to ensure that new orchard soil is not affected with non-specific replant disease prior to planting.

The air drying of the soil is reported to control root nematodes (Szczygiel and Zepp, 1996a) although this treatment would also be detrimental to a range of soil fungi. In these tests, however, the soils that responded to this treatment were overlapping, but different to the ones that responded to the nematicide (data not shown). Furthermore, plants growing in this treatment had superior root colour to those growing in the nematicide treatment (table 1.2). These results suggest that this treatment is effective against a different spectrum of soil microbiota to the nematicide. A combination of these two treatments may therefore result in a significant improvement in plant growth.

Table 1.3
Nematicide effects on the shoot height of ‘MM26’ apple rootstocks.

<table>
<thead>
<tr>
<th>Site #</th>
<th>1</th>
<th>7</th>
<th>8</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>146 b</td>
<td>128 b</td>
<td>128 b</td>
<td>164 b</td>
</tr>
<tr>
<td>Nemacur</td>
<td>212 a</td>
<td>169 a</td>
<td>210 a</td>
<td>237 a</td>
</tr>
</tbody>
</table>

Means in a column with the same letter are not different by LSD at p=0.05

There were five replicates for each treatment.

The Shirlan treatment had no impact on the shoot growth of the trees (Table 1.2) although there was an increase in the volume of the roots that resulted in an increase in the root to leaf ratio. This increase in root volume with no corresponding increase in shoot growth cannot be explained from this experiment. One possible explanation however, is that the material adversely affected the root mycorrhiza (which are basidiomycetes) necessitating a larger root volume to extract soil nutrients. If this was the case then extra photosynthate would be required in the roots at the expense of shoot growth. Hence, the slight, but insignificant increase in shoot growth observed is encouraging although this treatment cannot be recommended to industry. There has also been recent data to indicate that Shirlan is not an effective control of the actinomycete disease, common scab of potato, when applied in the field (Wilson pers Comm), as a result this treatment was omitted from further studies.

Apple roots growing in the absence of replant syndrome and other soil microbiota, as occurred in the sterile soil, were larger and light in colour with a yellow tinge (Table 1.2). Roots of plants growing in untreated apple soils were dark with a brown/red tinge (Table 1.2). The Shirlan and air drying treatments both resulted in an improvement in root colour over the untreated controls while the nematicide, which improved shoot growth, had no effect on the colour of the roots (Table 1.2).

The plants growing in the sterile soil had the least water use (Table 1.2). Plants in the air-drying and Shirlan treatments also appeared to use significantly less water than the untreated control. The nematicide had only a small effect on the water use of the plants. This finding that apple trees suffering from SARD use more water suggests that trees planted in untreated orchard soils will require comparatively large volumes of water, for the size of the plants. The reason for this inefficient water use was not determined, however, the poor growth and high water use suggests a disruption in the physiology of the trees growing in apple replant soils.
In these pot experiments three possible alternatives to Methyl Bromide were tested, these were a nematicide (Nemacur), air drying for 21 days and a fungicide (Shirlan). None of these alternatives were found to overcome SARD. Both the nematicide and air drying the soil provided some improvement in plant growth although this was probably due to their control of non-specific apple replant syndrome. Shirlan, a compound that is used against soil borne fungal diseases of crucifer crops, was included as it was shown, in initial laboratory cultures, to have some activity against the actinomycete that causes common scab of potatoes (Wilson, pers comm.). In these pot tests it was found that this product improved the colour and volume of apple roots with no improvement in shoot height. Further, it was found that this material, while controlling powdery scab of potatoes in laboratory cultures, failed to control this disease in field trials.

Conclusions

Severe SARD was present in all apple orchard soils tested from the Huon Valley of Tasmania. On average this disease causes greater than 50 percent growth reduction in the trees. This finding highlights the importance of treating all apple orchard soils in the Huon Valley for SARD prior to replanting. No alternative treatment to methyl bromide fumigation of the soil for the control of SARD was identified in this set of experiments.

Non-specific replant syndrome was also found to be present in 40 percent of these orchards. The current fumigation treatment for the control of SARD also controls non-specific replant disease such that no additional treatments are required against this problem when methyl bromide is used. If an old apple orchard is to be replanted to another crop, such as cherries, however, it is important to test for non-specific replant syndrome and treat accordingly if a problem is found.
2. The effect of antibiotics on growth of ‘MM26’ apple rootstocks in soils with specific apple replant syndrome.

Introduction

The experiments conducted in the first set of pot trials examined the effect of four treatments independent from each other. From these it was ascertained that nematodes have a small impact on shoot growth in 40 percent of the soils tested, presumably due to the control of nematodes and that the only treatment effective in controlling SARD (other than Methyl bromide) in the orchard soils studied was heat treatment. There has also been recent data to indicate that Shirlan is not an effective control of the common scab of potato (actinomycete disease) when applied in the field (Wilson pers. Comm.). As a result this treatment was omitted from further studies.

Since it appears that Shirlan is ineffective against actinomycetes and may have detrimental effects on mycorrhiza in the field, a second set of pot experiments was undertaken using an antibiotic known to be effective against actinomycetes. Gillham (pers. comm.) advised that in veterinary environments streptomycin is an extremely effective antibiotic against actinomycetes. This trial is specifically designed to ascertain the effectiveness of the antibiotic streptomycin, in controlling SARD. In preparation for field trials and recommendations to orchardists, it was essential to understand the longer term effects of the treatments, therefore half the trial was harvested in the first growing season and the other half was harvested in the following growing season.

Materials and methods

In February 1998 soils were obtained from 2 sites identified in the earlier study as having severe apple replant disease. A transect in the shape of a ‘W’ was used as the sampling system, soil was collected at five equidistant plots along each arm of the ‘W’. Grass was removed and soil sampled to a depth of 150mm at each plot, between 4-6 litres of soil was collected at each of the twenty plots. These samples were mixed together to form a total of 100 litres of soil for each site, this was stored in lidded plastic, but not airtight containers.

All soil samples were mixed with perlite (to make 20% perlite in the final mixture) to improve the physical characteristics of the soil for use in pot trials. There were four replicates for each treatment repeated twice, to allow for destructive sampling of one set of four treatments in the first year. The treatments were an untreated control, heat sterilisation (90° C for 48 hours in 10 litre moisture proof bags), 28 μL/L of soil Nemacur 400® (Bayer) an antibiotic (streptomycin at 1 g/L of soil) and a mixture of the antibiotic and the nematicide. The apple plants used were tissue cultured ‘MM26’ rootstocks established in pine bark/sand media in 200ml pots. Plants were planted individually into 1.7 L of soil and placed in the glass house in a randomised complete block design. The glass house temperatures varied from a minimum of 16° C to a maximum of 35° C. Aphids were removed with gentle digital force, and powdery mildew was controlled with Benlate® as required.

Shoot heights from the soil surface were recorded on the 9/6/98 prior to destructive sampling of half the plants, and subsequent measurement of the leaf areas and leaf colour. Roots were extracted by soaking and gentle agitation in water. Root colour (in Lab colour notation) was determined using a Minolta chroma 200b meter taking 2 readings on each of the two sides of the flattened root mass.
Root volume was measured using Archimedes principal of measuring the change in weight of a beaker of water when the roots were suspended in it (in a metal cage suspended by string).

The second set of treatments that were not harvested in the initial treatment assessment were pruned back to 100mm above the soil surface in the winter of 1998 and moved outside the glasshouse to overwinter. In the spring they were moved back into the glasshouse and on 6 January 1999, after 3 months of growth, the shoot heights of these plants were measured.

The data was inspected for normality and uniformity of variances. Due to a relationship between the mean and variance the data for root volume was transformed using square root transformation prior to data analysis. The entire data set was analysed using analysis of variance with mean separation by LSD $p = 0.05$.

\section*{Results and Discussion}

\begin{table}[h]
\caption{Soil treatment effect on growth variables of ‘mm26’ apple rootstocks}
\begin{tabular}{lcccccc}
\hline
 & 1998 & & & & & 1999 \\
Treatment & Shoot height (mm) & Leaf area (mm\(^2\)) & Root volume* (ml\(^*\)) & Root brightness (%reflection) & Root redness (a) & Root yellowness (b) & Shoot height (mm) \\
\hline
Untreated control & 136 a & 16130 a & 6.48 a & 27.0 a & 5.28 bc & 15.5 a & 291 a \\
Nematicide & 138 a & 14101 a & 6.10 a & 30.6 b & 6.03 c & 17.7 b & 289 a \\
Antibiotic & 251 b & 32678 b & 5.59 a & 26.2 a & 4.75 ab & 14.9 a & 309 a \\
Heat Sterile & 343 c & 38088 bc & 9.88 b & 37.5 c & 4.23 a & 21.5 c & 357 b \\
Nematicide + antibiotic & 370 c & 42025 c & 5.56 a & 28.6 ab & 4.56 ab & 15.4 a & 388 b \\
\hline
\end{tabular}
\end{table}

Means within a column with the same letter not different (LSD $p = 0.05$).

\* Untransformed data

A full set of data was recorded for the first growing season (1998). This included shoot length, leaf area, root volume and root colour. As expected the untreated control had the lowest mean shoot height and leaf areas (1998) of all the treatments. In this set of pot experiments the nematicide also had no effect on the shoot height or leaf area of the plants, whereas the antibiotic resulted in a substantial increase in shoot height and leaf area, but the greatest increase, for any treatment alone, was in the heat sterilisation treatment. Interestingly the combined effect of the nematicide and the antibiotic was significantly greater than the effect of the antibiotic alone and similar to the heat treated soil indicating an interaction between SARD and nematodes. One possible explanation for this observation is that both nemetodes and bacteria were present in the soils tested. It is known that there was a growth response to nematicide in these soils in the earlier pot trials supporting this hypothesis. Hence in this pot trial the bacteria may have been more dominant a problem than nematodes such that the application of the nematicide did not overcome the major limitation to growth. When the bacteria were controlled, however, the control of the nematodes present became significant.

The data recorded in the second growing season (1999) was shoot height. This illustrated that neither the nematicide or the antibiotic resulted in a significant increase in shoot height, the heat sterilisation still resulted in a higher shoot height, as did the combined effect of the nematicide and the antibiotic.
The only treatment that caused a difference in the root volume from the untreated control was the application of heat sterilisation this resulted in a root volume one third greater than that of the untreated control. Roots that were growing in sterile soil were found to be of a substantially different colour to the other treatments. These roots were brighter and yellower but not quite as red as the untreated roots such that they appeared to be healthier. The nematicide treatment also slightly improved the root brightness and yellow indicating a small effect of this treatment on root colour while the antibiotic appeared to have no effect on root colour.

To have achieved such a large increase in plant growth in the first growing season with the antibiotic treatment implicates bacteria as a major component of specific apple replant syndrome in Tasmania. These results confirm the original hypothesis that specific apple replant syndrome is due to a bacterial pathogen such as an actinomycete. The lack of an increase in shoot height in the second growing season for antibiotics alone, may have been due to the short life of streptomycin in the soil. Regardless of this, the use of antibiotics is not a viable alternative for the control of SARD due to the cost and environmental issues that would restrict its registration for commercial use. However, antibiotic treatments remain an important research tool as they provide a clearer understanding of the type of soil microbiota that are responsible for apple replant disease.

**Conclusions**

This trial has clearly demonstrated that the effects of SARD in the soil can be substantially reduced with the addition of streptomycin to the soil provided nematodes are also controlled. This finding strongly suggests that this debilitating disease is due to soil borne bacterium.
3. The effect of calcium hydroxide application on growth of ‘mm26’ apple rootstocks in soils with specific apple replant disease

Introduction

Both Szczygiel and Zepp (1996 a and b) and Hoestra (1968) noted that apple replant disease was more severe in apple soils with a neutral pH. Hoestra observed that while on average, apple replant disease was twice as severe in soils with a pH of 7.0 compared to 5.0 this effect was “not always very marked”. Hoestra (1968) attempted to study this relationship in pot experiments by adding calcium hydroxide or sulphuric acid to the test soil. In this study the initial soil pH was 6.5 and the calcium hydroxide treatment raised the pH to 7.4 while the sulphuric acid treatment lowered it to 6.1. Trees growing in the soil treated with sulphuric acid were found to out-perform those growing in the other two treatments. However, in a later experiment Hoestra demonstrated that the trees responded to the sulphur in the sulphuric acid confounding the earlier conclusions.

It is well documented that the optimal soil pH for the growth of apple trees is between 6.0 and 7.5 (Janick, 1972). For this reason growers prefer their soils to be close to neutral and as apple soils to be replanted are often acidic they commonly need pH correction. The application of agricultural lime (calcium carbonate) to the soil is the simplest and most effective material for increasing the pH of soil in field situations. This treatment is commonly performed during soil preparation and prior to tree planting. As SARD is affected by pH in the field, this raises the possibility that this liming treatment has an impact on apple replant disease.

In this study hydrated lime (calcium hydroxide) which is faster acting than calcium carbonate and hence more suited to short term pot trials, was added to the soil to explore this common treatment and its effect on SARD. Lime affects a wide range of soil parameters, such as soil pH, availability of soil nutrients, calcium content of the soil, soil structure, soil drainage and the ratio of divalent to univalent cations in the soil. Therefore this lime treatment may have effects on plant growth and SARD such that treatment effects cannot be attributed to the effects on soil pH alone. There were two pot trials in this study and both examined the effects of lime application on sterilised and unsterilised soils.

Materials and Methods

Pot trial 1

Only one soil was used in this trial. This soil was sampled on 4 October 1998 from a mature apple orchard in the Huon Valley of Southern Tasmania. This orchard had been abandoned for about 12 years. An in-field pH measurement indicated it was extremely acidic (pH 4.5). Approximately 80 L of soil was collected from multiple locations around the site and split into two equal sub samples after mixing. One sub sample was left untreated while the other was heat treated in 10 litre moisture proof bags at 90°C for 48 hours. After heat treatment the pH of both samples was measured, on 12 October 1998, using a 1 in 4 water extract. The natural pH of this soil was found not to be as acidic as first determined at a pH of 5.7. Heat treatment had reduced this to a pH of 5.0.

On 14 October 1998 ten litres of perlite and 28 μL/L of nematicide (Nemacur®) was added and mixed into each pile. The perlite improves soil structure for the pot trials and the nematicide was added to eliminate the effect of any non-specific replant disease in the soil due to nematodes. Soils from the
two heat treatments were then further divided and four calcium hydroxide treatments allocated to each of the soil sub samples as outlined in table 3.1. Note that due to the lower initial pH of the heated soil proportionally more lime was added to the heat treatments.

Six days after calcium hydroxide addition tissue cultured ‘MM26’ rootstocks were planted individually into 1.7 L of soil and placed in the glass house in a randomised complete block design. At planting a sample of soil was taken from each treatment (pooled across replicates) and the pH determined. The plants were watered every day with an overhead irrigation system.

The plants were allowed to grow for 10 weeks before their final height was measured. In addition a small sample of soil was taken from each pot, pooled across the replicates, and the final soil pH was measured.

<table>
<thead>
<tr>
<th>lime application (grms/12.5L soil)</th>
<th>unheated</th>
<th>heated</th>
</tr>
</thead>
<tbody>
<tr>
<td>initial pH (21/10/98)</td>
<td>6.11</td>
<td>5.84</td>
</tr>
<tr>
<td>final pH (11/1/98)</td>
<td>5.79</td>
<td>5.58</td>
</tr>
</tbody>
</table>

As different rates of calcium hydroxide were used in the two heat treatments data was analysed by the analysis of variance utilising single degree of freedom comparisons (orthogonal comparisons) to identify significant heat effects and linear responses of lime application within the two heat treatments. The data was inspected for normality and equal variances prior to analysis.

**Pot trial 2**
This pot trial used soil from two commercial apple orchards in the Huon Valley of Southern Tasmania which had been identified from earlier studies as expressing SARD in an acid soil environment. On 13 January 1999 approximately 80 L of soil was collected from each site. To collect this soil a transect in the shape of a ‘W’ was used and soil collected from five equidistant plots along each arm of the ‘W’. Six days later both soil samples were mixed with perlite (to make 20% perlite in the final mixture) and half of each soil was heat treated in moisture proof bags at 90 °C for 48 hours.

On 29 January 1999 the pH of both samples were measured and the natural pH was found to be 5.3 (soil 1) and 6.0 (soil 2). In this experiment the heat treatment had little effect on the pH of the two soils. As for the first trial soils from the two heat treatments were divided and 4 calcium hydroxide treatments allocated to each of the soil sub samples as outlined in table 3.2. Due to only a small shift in soil pH with heat treatment, equal rates of calcium hydroxide were added to the two treatments. At this date 28 μL/L of nematicide (Nemacur®) was also added and mixed into each pile to eliminate the effect of any non-specific replant disease in the soil due to nematodes.

Twelve days after calcium hydroxide addition (10 February 1999) tissue cultured ‘MM26’ rootstocks were planted individually into 1.7 L of soil and placed in the glass house in a randomised complete...
block design. At planting a sample of soil was taken from each treatment and the pH determined (table 3.2). The plants were watered daily with an overhead irrigation system.

<table>
<thead>
<tr>
<th>lime application (grams/12.5 L soil)</th>
<th>unheated (pH)</th>
<th>heated (pH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil 1</td>
<td>5.39 6.67 6.64 7.05 5.70 6.53 6.89 7.34</td>
<td></td>
</tr>
<tr>
<td>Soil 2</td>
<td>5.76 6.95 7.22 7.49 5.59 6.88 7.20 7.30</td>
<td></td>
</tr>
</tbody>
</table>

After 7 weeks of growth, on 29 March 1999, the final shoot heights of the plants were measured.

Due to a relationship between the means and variances a square root transformation was necessary before analysis with this shoot growth data. The treatments were analysed by the same procedure as the first pot trial using a one-way analysis of variance, utilising single degree of freedom comparisons (orthogonal comparisons) to identify significant heat effects and linear responses of lime application within the two heat treatments.

**Results and Discussion**

With the exception of the heated soil in pot trial 2 there was a significant linear response to added lime. There was, however, an interaction between the heat treatment and lime treatments such that lime application improved plant growth in the absence of heat but reduced growth in the presence of heat (figures 3.1 and 3.2).

![Figure 3.1](attachment:image.png)

Pot trial 1, The effect of soil applied calcium hydroxide on shoot growth of 'mm26' apple rootstocks. For final soil pH values see Table 3.1.
In pot trial 1 the severity of SARD was not as great as in pot trial 2. Of significance is that in this first pot trial the maximum application rate of lime in the unheated soil increased growth to a level equivalent to that of the heat treated (sterilised) soils with no added lime (figure 3.1). Hence, this treatment negated the affect of apple replant disease in this soil. The results were not as clear in the second pot trial, possibly due to the more serious SARD problem or the increased delay between lime application and planting (12 versus 6 days), the same interaction was present in both sets of experiments indicating that it was real. The commercial significance of this finding is that different optimal soil pH levels may be required in sterile and non sterile soils. This finding has some serious practical ramifications in that the results suggest that it is unwise to add lime to soil that has been sterilised (standard industry practice) while it is beneficial to add lime if the soil has not been sterilised. The reason for this interaction was not determined from this study, however, these results indicate that lime addition to non sterile soil may help overcome the effects of SARD.

![Figure 3.1](image)

**Figure 3.1**
Pot trial 1, The effect of soil applied calcium hydroxide on shoot growth of ‘mm26’ apple rootstocks.

**Figure 3.2**
Pot trial 2, The effect of soil applied calcium hydroxide on shoot growth of ‘mm26’ apple rootstocks.

**Conclusions**

This trial has identified that in two sets of pot trials the addition of lime to non-sterile soil resulted in improved growth of ‘mm26’ apple rootstocks. In the case of one of trials the growth of the trees in this lime treated soil was similar to the growth of the trees in the heated treatment with no added lime.

The addition of lime to heat treated soil, however, was found to be detrimental to plant growth in one of the pot trials suggesting that the addition of lime to soil that has been treated with methyl bromide may not be a wise decision. The reason for this interaction was not determined from this pot trial, it does, however, raise the question as to the optimal soil pH for apple production in soil that has been sterilised.
4. The effect of mono ammonium phosphate (MAP) and micro element soil nutrition on specific apple replant disease

Introduction

Apple replant disease is a soil based disease that results in poor root development where roots characteristically have low numbers of fine roots and hairs and the roots are typically red in colour. This lack of fine root network would be expected to lead to poor nutrient and water uptake from the soil and there is some evidence that this is the case. There is, however, little sound information as to whether some nutrients are more adversely affected than others or if this lack of nutrition is a general lack of uptake of all nutrients from the soil. Hence it is difficult to determine the correct balance of macro and micro nutrients in soils affected by SARD. It is often claimed that soil sterilisation increases soil nitrogen due to the death of the soil microbiota and the subsequent release of the nutrients they contain to the soil. If true then this makes research in this area particularly difficult, however, data collected in an earlier experiment indicated that this is not the case where a plant pathogen was not present.

Mono ammonium phosphate (MAP) is a macro element soil nutrient often applied to apples prior to planting. There are several reports that ammonium based fertilizers, such as MAP, are beneficial in soils affected by SARD (Utkhede, 1996). For this reason it is appropriate to utilise this fertilizer in this study. The dominant soil nutrients in this fertilizer are both nitrogen and phosphorus.

The effect of SARD on the availability of micronutrients is unknown. Many micronutrients, such as copper, can be captured and tightly held by the cell walls and lignins in plant roots. As a result SARD may result in a reduction in the quantity of micro elements available to the foliar portions of the plants. Therefore the effect of soil applied micronutrients was also included in this trial.

This experiment studied the effect of increased quantities of soil applied nutrients on the severity of SARD. If successful recommendations regarding the optimum rates and types of nutrient applications appropriate for both sterilised soils and soils that have not been treated for SARD may be initiated.

Materials and Methods

This pot trial used soil from two apple orchards in the Huon Valley of Southern Tasmania. To collect this soil a transect in the shape of a ‘W’ was used and soil collected from five equidistant plots along each arm of the ‘W’. Approximately 60 L of soil was collected from each site and 15 litres of perlite was added to both soil samples to improve the physical characteristics for use in pot trials. Half of each soil remained untreated; the other half was heat treated in 10 litre moisture proof bags at 90°C for 48 hours to eliminate SARD. The heated and unheated soils from both sites had four replicates for each of four treatments; these were an untreated control, 0.75 g/L MAP, 1.5 g/L MAP and 2 g/L micro-nutrients.

Tissue cultured ‘MM26’ rootstocks were planted individually into 1.7 L of soil and placed in the glass house in a randomised complete block design. The plants were well watered through an automatic overhead irrigation system. To each pot a nematicide (Nemacur®) was watered onto the pots at a
concentration of 2.5ml/litre of water. This should have ameliorated the effect of any non-specific replant disease caused by nematodes in the soil. Shoot height (mm) was recorded for all plants after 3 months of growth.

Data was studied for normality and equal variances then analysed by a 2 factor analysis of variance. Mean separation was by LSD $p = 0.05$.

**Results and Discussion**

The data analysis showed a significant effect of heat, nutrients, and the interaction between heat and nutrients necessitating the presentation of all treatment means.

<table>
<thead>
<tr>
<th>nutrient application</th>
<th>soil treatment</th>
<th>unheated</th>
<th>heated</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated</td>
<td></td>
<td>176.3 a</td>
<td>595.0 c</td>
</tr>
<tr>
<td>MAP (0.75g/l)</td>
<td></td>
<td>400.0 b</td>
<td>660.0 d</td>
</tr>
<tr>
<td>MAP (1.5g/l)</td>
<td></td>
<td>386.9 b</td>
<td>717.5 d</td>
</tr>
<tr>
<td>micronutrient (2.0g/l)</td>
<td></td>
<td>213.8 a</td>
<td>598.1 c</td>
</tr>
</tbody>
</table>

Treatments with the same letter not significantly different (LSD $p = 5\%$)

All plants growing in heat-treated soils outperformed those growing in the unheated soil (Table 4.1). For both of the heat treatments the addition of the micronutrients to the soil had little or no impact on the growth of the trees indicating that micronutrients cannot be used alone to overcome the effect of SARD. This result also indicates that, providing nutrient uptake is not impaired, SARD affected trees are not deficient in micronutrients.

Similarly, for both the heat treatments the MAP significantly increased shoot growth. There were no differences noted between the two MAP application rates within either of the heat treatments. This indicates that the trees responded to MAP in both of the heat treatments but there was a difference in their relative responses as indicated by the significant heat by nutrition interaction. In order to study this interaction in more detail it is necessary to study the difference in shoot heights between the control and the treatments within a heat treatment. For the unheated soil the low rate of MAP application caused a 224 mm improvement in shoot growth compared with only a 65 mm improvement in the heated soil. This is more clearly shown in table 4.2 where these differences are expressed as a percentage of the untreated control.
Table 4.2 shows that the MAP, at both rates of application, had a dramatic effect on the performance of the trees growing in the non sterile orchard soil when compared with the same treatments in the sterilised soil. It was concluded in an earlier trial that soil sterilisation, in the absence of a specific plant pathogen, does not lead to improved tree growth. Therefore, the magnitude of the growth response to MAP in the sterile soil is an indication of the tree response to the added nutrients in the absence of SARD. Hence, the increased growth response to MAP observed in the unheated soil was larger than expected due to improved free nutrition which strongly suggests that the MAP was having a controlling effect on SARD. How MAP was exerting this control could not be determined from this experiment, however, it is known that ammonium compounds are very effective against bacteria. Further evidence of MAP control of soil microbiota is provided from the field trial results described in section 7.

Conclusions

In this trial heat treating the soil gave a large increase in growth due to the control of SARD. There was a small, but insignificant, improvement in tree growth with the addition of soil micronutrients. The addition of MAP to the soil, however, had a major effect on tree growth that was greater than that of nutrition alone. Although the data strongly suggests that this material is exerting some control over SARD the nature of this control is not known.

**Introduction**

Mycorrhizae are naturally occurring fungi that live on the roots of plants in a symbiotic relationship. In return for food they improve the plant's ability to obtain nutrients and water from the soil. These associations are especially useful on poor soils where the plant would normally suffer from nutrient deficiencies. In addition it has been shown that mycorrhizae also protect the roots of host plants from pathogenic fungal infections such as *Phytophora spp.* (Marx, 1970 and Davis and Menge, 1980). In natural environments almost all plants exist with mycorrhizal associations on the roots. Beneficial mycorrhizal associations lead to larger and healthier trees with a superior root structure. It has even been shown that mycorrhizal associations lead to improved water movement within the tree (Safir et al. 1972).

Vaminoc is a commercially available source of endomycorrhizae produced in the United Kingdom and marketed in Australia by Brooke Horticultural Products, Wandiligong, Victoria. This product contains selected strains of *Glomus* species which have a wide host range. While it is probable that nursery stocks are infected with mycorrhizae in the stool beds, these are uncontrolled infections and may not involve associations with productive mycorrhizae.

*Trichoderma* species, while not providing the host plant with nutrients, are effective antagonists of many plant diseases including soil-borne pathogens. Antagonists use a variety of mechanisms such as occupying infection sites and predation of pathogenic fungi to reduce the pathogenicity of the disease organism. In the case of *Trichoderma* some species also produce antibiotics which are active against a range of bacteria. As such *Trichoderma spp* may prove to be useful against the effect of SARD on apple trees. *Trichoderma spp* are currently commercially available and are sold under the trade name Trichopel-P (Brooke Horticultural Products, Wandiligong, Victoria).

Two separate applications of antibiotics were used in this trial, the soil application was to confirm previous data relating to the effectiveness of antibiotics in ameliorating the effects of SARD. If successful in pot trials the root application of antibiotics may be acceptable for implementation in the field.

Metalaxyl is a fungicide that has been shown to be very effective against *Pythium spp* (Mazzola, pers comm), one of the fungi often associated with apple replant disease. As streptomycin is also active against *Pythium spp* (Mazzola, pers com) the addition of metalaxyl as a treatment in this trial will help to identify the role of *Pythium spp* in apple replant disease in Tasmania.

**Materials and Methods**

This pot trial used soil from two apple orchards in the Huon Valley of Southern Tasmania. To collect this soil a transect in the shape of a ‘W’ was used and soil collected from five equidistant plots along each arm of the ‘W’. Approximately 60 L of soil was collected from each site; 15 litres of perlite was added to both soil samples to improve the physical characteristics of the soil for use in pot trials. Seven treatments were applied to each soil; an untreated control, 1.5g/pot Vaminoc (Brooke
Horticultural Products, Wandiligong, Victoria), 1.5g/pot Trichopel – P (Brooke Horticultural Products, Wandiligong, Victoria), 1g/L soil streptomycin applied to the soil, 1g/1 water streptomycin applied to roots (roots immersed for 60 seconds), 2g/L soil metalaxyl (Ridomil®), and heat sterilisation in 10 litre moisture proof bags at 90 °C for 48 hours. There were five replicates for each treatment.

Tissue cultured ‘MM26’ rootstocks were planted individually into 1.7 L of soil and placed in the glass house in a randomised complete block design. The plants were automatically watered daily using an overhead irrigation system. After planting, the pots were watered to field capacity with a nematicide (Nemacur® at 2.5ml/litre of water) to ameliorated the effect of nematodes associated with non-specific replant disease in the soil.

Shoot height (mm) was measured on 29 March 1999 for all plants, after 3 months of growth. Data was studied for normality and equality of variances then analysed by split plot analysis of variance with the soil source as the whole plots. Mean separation was by LSD/? = 0.05.

Results and Discussion

The treatment responses were similar between the two sources of soil (an insignificant site by treatment interaction) so a study of the individual sites was not necessary. Over the 3 month period of the trial the plants in the untreated soil grew to a height of 274mm while those in the heat treated soil grew 44 percent taller to a height of nearly 400mm (table 5.1). This result demonstrates that SARD was present in these test soils. Of interest is that plants growing in the streptomycin treatment applied to the soil substantially outperformed those growing in the heat treatment. This result suggests that either the heat treatment was incomplete in this trial or that the disease, as well as being present in the soil was also established on the planting material.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot height</th>
<th>Growth %*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control (UTC)</td>
<td>274.0 ab</td>
<td>0</td>
</tr>
<tr>
<td>Mycorrhiza (Glomus spp)</td>
<td>240.5 a</td>
<td>-14</td>
</tr>
<tr>
<td>Trichoderma spp</td>
<td>362.5 c</td>
<td>32</td>
</tr>
<tr>
<td>Metalaxyl</td>
<td>265.5 ab</td>
<td>-3</td>
</tr>
<tr>
<td>Streptomycin (root dip)</td>
<td>301.0 b</td>
<td>10</td>
</tr>
<tr>
<td>Streptomycin (soil applied)</td>
<td>557.0 d</td>
<td>100</td>
</tr>
<tr>
<td>Heat (90°C 48 hours)</td>
<td>394.5 c</td>
<td>44</td>
</tr>
</tbody>
</table>

* values with the same letter are not significantly different

In this trial the Trichopel – P (Trichoderma spp) treatment, while not as effective against SARD as the streptomycin to the soil, performed as well as the heat treatment. Therefore this treatment has potential for use in the field against apple replant disease.
The mycorrhizal treatment, root applied streptomycin and metalaxyl treatments all had no impact on shoot growth, when compared with the untreated controls, indicating that these materials are not effective against SARD when applied by the methods used here. The result for metalaxyl indicates that *Pythium spp*, and other fungi sensitive to metalaxyl such as *Phytophthora spp*, are not a major component of SARD in the soils examined. The failure of the root applied streptomycin treatment was a potential outcome of this treatment, and as such was not unexpected. It is interesting to note, however, that this treatment did result in taller plants when compared with the mycorrhizae treated plants. This result, combined with the finding for soil applied streptomycin indicates that this treatment may prove useful when applied to trees being planted into sterile soil to eliminate any SARD already existing on the planting material. The results for the mycorrhizae were disappointing. However, since mycorrhizae can take several months to establish this treatment may prove beneficial if applied several months before exposure to SARD. This may entail application in the nursery beds or prior to planting into sterile orchard soil where the treatment effect may become apparent after several years of growth.

**Conclusions**

This trial confirmed the previous finding that soil applied streptomycin is very effective against SARD in Tasmania. Since metalaxyl was ineffective against SARD, therefore SARD is not due to *Pythium spp* which have been reported to be also sensitive to streptomycin. This result also eliminates *Phytophthora spp* as the cause of SARD in the soils studied.

Of the materials tested the Trichopel – P treatment (*Trichoderma spp*) was the most commercially effective treatment and more studies with this material are needed. There is also a possibility that mycorrhizae applied several months prior to exposure to the disease and the use of streptomycin dips for plantings into SARD free sites may also provide a long term benefit to new orchards.
6. The effect of soil water availability on the growth on ‘mm26’ apple rootstocks growing in soils with specific apple replant disease.

Introduction

Schander (1958) observed that apple replant disease in Germany was not as severe in fields where there was a high water table. This led Hoestra (1968), to conduct a pot trial to study this aspect of apple replant disease in more detail. In this study Hoestra grew apple seedlings in 3 levels of soil moisture in either chloropicrin treated or untreated soil. It was found that the level of soil moisture had a major impact on tree performance in both sterile and non sterile soil with SARD being more severe in soil kept in a drier condition. In this trial no account appeared to be made of nematodes so the effects could not be attributed to either specific or non specific apple replant disease.

A second, and more recent experiment studying irrigation was reported by Utkhede (1996), who found that apple tree growth and fruit yield of trees planted in apple replant soil was significantly improved with high volume sprinkler systems as opposed to low volume sprinklers and drippers. While a pot test was conducted to confirm the presence of apple replant disease in the test orchard the disease was not partitioned into the component attributable to nematodes and that attributable to SARD.

In the trials reported here soil was collected from 5 apple orchards, known from previous pot experiments to be affected by SARD. These soils were then maintained at 4 levels of soil moisture after half of the samples had been heat sterilised.

Materials and Methods

In May of 1998 soil was collected from 5 different apple orchards from the Huon Valley of Southern Tasmania. These 5 sites were all located within a 5km radius of Huonville. At each site a transect in the shape of a ‘W’ was used as the sampling system, soil was collected at five equidistant positions along each arm of the ‘W’. Grass was removed and soil sampled to a depth of 150mm with approximately 3 litres of soil being collected at each of the twenty positions. These samples were mixed together to form a total of 60 litres of soil for each site and this was stored in lidded plastic, but not airtight containers.

On 26 May 12 litres of perlite was mixed with each soil to improve the physical characteristics for use in pot trials and half of the soil was then sterilised in 10 litre heat and moisture proof bags with heat at 90°C for 48 hours.

Prior to planting the soils were treated with 28 µL/L Nemacur® to eliminate any potential treatment responses due to nematodes and to restrict treatment responses to those caused by SARD alone. The apple plants used were tissue cultured ‘MM26’ rootstocks established in pine bark/sand media in 200ml pots. These trees were planted on 7 September 1998 into the soils (16 into heat treated and 16 into non heat treated for each of the 5 orchards). After planting the trees were watered daily until established. After 15 days, on 22 September, the soil field capacity of each pot was determined by weighing the pots after excess irrigation water had drained out. Four treatments were then allocated to each of the sterilised and unsterilised soils as outlined below.
For the following 2 months the pots were placed on a balance and watered to the predetermined weight every Monday, Wednesday and Friday. For the 2 weeks prior to the final destructive harvest the pot weight prior to re-watering was recorded to determine the average water consumption per pot over that period. At the termination of the trial (18 November 1998) the shoot heights were recorded, leaf water potentials measured using a pressure bomb on plants that had been left in the dark for the previous 18 hours and the leaf surface area determined.

Data was analysed by a factorial analysis of variance using Genstat where the treatment sum of squares were divided into soil effects (4 degrees of freedom), Heat (1), Moisture (3), Soil by Heat (4), Soil by Moisture (12), Heat by Moisture (3), and Soil by Heat by Moisture (12). Means were separated by Duncans Multiple Range (DMR) *p* = 0.05.

**Results and Discussion**

For all measured parameters there was a significant heat effect and, with the exception of the pressure bomb data, there was no soil site by heat interaction indicating that SARD was present in all of these orchards (table 6.1).

<table>
<thead>
<tr>
<th>Shoot height (mm)</th>
<th>Total foliar area (cm²)</th>
<th>Water use (g/week/cm²)</th>
<th>Tree water potential (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Heat</td>
<td>159 a</td>
<td>165 a</td>
<td>2.62 a</td>
</tr>
<tr>
<td>Heat</td>
<td>334 b</td>
<td>347 b</td>
<td>1.68 b</td>
</tr>
</tbody>
</table>

Means in a column with a different letter considered different (*p* = 0.05)

Hence, soil sterilisation resulted in larger trees with a greater foliar surface area that were using less water per cm² of leaf area. This reduction in water use indicates that the soil sterilisation led to more efficient water use, possibly through better stomatal control. Of interest is that although these trees were using less water per unit area of foliage they were, in fact using more water per plant (430 vs 580 mls per week - column 3 x column 4 of table 6.1). As these trees used more water from the soil it would be expected that the soil sterilisation treatment would result in a lower tree water potential after a dark period which it did not. The reason for this unexpected result cannot be explained from this experiment.
In addition to the significant heat treatment effect on tree performance, with the exception of the water use data there was a significant response in the data to the moisture level treatments (table 6.2).

### Table 6.2
The effect of soil sterilisation with heat and soil water potential on 'mm26' apple rootstock performance.

<table>
<thead>
<tr>
<th>Heat treatment</th>
<th>Moisture level</th>
<th>Shoot height (mm)</th>
<th>Total foliar area (cm²)</th>
<th>Water use (g/week/cm²)</th>
<th>Tree water potential (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fc</td>
<td>402 a</td>
<td>417 a</td>
<td>1.58 a</td>
<td>-0.42 a</td>
<td></td>
</tr>
<tr>
<td>Heat</td>
<td>fc-33g</td>
<td>360 b</td>
<td>376 a</td>
<td>1.62 a</td>
<td>-0.38 a</td>
</tr>
<tr>
<td></td>
<td>fc-66g</td>
<td>306 c</td>
<td>309 b</td>
<td>1.79 a</td>
<td>-0.31 b</td>
</tr>
<tr>
<td></td>
<td>fc-100g</td>
<td>266 c</td>
<td>286 b</td>
<td>1.73 a</td>
<td>-0.29 b</td>
</tr>
<tr>
<td>No heat</td>
<td>fc</td>
<td>163 d</td>
<td>175 c</td>
<td>2.65 b</td>
<td>-0.18 c</td>
</tr>
<tr>
<td></td>
<td>fc-33g</td>
<td>163 d</td>
<td>169 c</td>
<td>2.59 b</td>
<td>-0.17 c</td>
</tr>
<tr>
<td></td>
<td>fc-66g</td>
<td>160 d</td>
<td>160 c</td>
<td>2.58 b</td>
<td>-0.16 c</td>
</tr>
<tr>
<td></td>
<td>fc-100g</td>
<td>149 d</td>
<td>156 c</td>
<td>2.66 b</td>
<td>-0.16 c</td>
</tr>
</tbody>
</table>

Means in a column with a different letter considered different \((p = 0.05)\)

For all the measured parameters there was no soil moisture level treatment effects for the non sterilised soils, demonstrating that apple replant disease cannot be overcome with increasing the rate of water application to the trees. Contrary to this finding is that in the heat sterilised soil there was a significant reduction in growth, measured as both shoot length and foliar area, with reducing quantities of available water in the soil. While these smaller plants growing in the drier sterilised soil were using similar quantities of water per unit foliar area, they were using less water per tree due to the smaller tree size (500 ml/tree compared with 660 ml/tree – column 4 x column 5 of table 6.2). This reduced water use per tree was reflected in the tree water potential data where the larger trees had a greater water deficit. This implies that the trees growing in the soils maintained at field capacity were cycling through greater variations in tree water potential over the watering cycle than the slightly water stressed trees.

**Conclusions**

This trial has clearly shown that increased quantities of irrigation water has no impact on the performance of trees growing in apple soil affected with SARD in the trials undertaken. Therefore if soil has not been treated to control SARD there is no improvement in growth by the use of large quantities of irrigation water. This is in contrast to soils which have been treated to control SARD. In these soils increased quantities of irrigation water resulted in improved tree growth and performance resulting in a return to growers for their capital investment in irrigation systems.

These results differ to those obtained by Hoestra (1968) in the Netherlands and Utkhede (1996) in Canada. This difference in response cannot be explained from this trial, however, may have been due to the treatment overcoming the effect of nematodes in the soil which were controlled in this trial by the use of a nematicide.
7. The control of specific apple replant disease in the field

Introduction

The glass house pot trials conducted in 1997, 1998 and 1999 revealed several treatments with potential to reduce the problems of apple replant disease in the field. Specific treatments identified that were suitable for field application included the use of calcium hydroxide (hydrated lime), mono ammonium phosphate fertiliser, and *Trichoderma* spp.. In addition, dazomet, (a registered soil sterilant) that showed promise in preliminary field tests was included in this trial. This material was not investigated in the glass house studies.

The antibiotic treatment of the soil, while proving to be a useful treatment in the glass house studies, is considered a research tool and not a practical solution for growers. As a result this treatment was not included in the field trial. While the field application of antibiotics is not practical the dipping of tree roots in an antibiotic solution prior to planting may be feasible. This treatment may control SARD on the planting material thereby avoiding inoculating the sterilised field soil with the disease. Therefore, this treatment was included in the field trial.

The glass house studies also revealed that the inoculation of trees with mycorrhizae at planting time did not alleviate the symptoms of apple replant disease. However, as mycorrhizae, once established, have potential to protect roots against soil borne diseases it was felt that a mycorrhizae treatment in sterilised soil should be included in this trial to study any long term beneficial effects.

For the treatments where nematode control was not expected a nematicide was also added to the soil. A nematicide alone treatment was included to investigate any non-specific apple replant disease problems due to nematodes at this site.

Materials and Methods

An apple replant field trial was established in the winter of 1999 near Huonville in the Huon Valley of Tasmania, Australia. The experimental site has a long history of apple production and the previous apple orchard was removed in the winter of 1998. A bulldozer was used to push the trees over and to pile them up off the site for burning. After orchard removal the site was cultivated on several occasions and the exposed residual roots were removed by hand. In the spring of 1998 a crop of ryecorn (*Secale cereale*) was sown to add organic matter to the soil. This cereal crop was cultivated into the soil in the autumn of 1999 and the soil was hilled into the new tree rows. These hills were 3.5m apart and approximately 400mm high.
Initial treatment application (methyl bromide, MAP, calcium hydroxide, Dazomet and nematicide) was on 17 May 1999 to 15 metre long plots. Four rows of trees were used as 4 replicates and the treatments were randomly assigned to the plots within the replicates. Nine treatments were applied as outlined below;

1. Untreated Control
2. Methyl Bromide (50g/m² of Fungafume®, K & B Adams)
3. Dazomet (60g/m² of Basamid®, BASF)
4. Methyl Bromide + mycorrhiza (5g/tree of Vaminoc®, Brooke Horticultural Products, Wandiligong, Victoria)
5. Methyl Bromide + antibiotic (60 second dip in 1g/L streptomycin)
6. Nematicide (11 g/m² of Mocap®, May and Baker)
7. Nematicide + Trichoderma (30 g/tree Trichopel-P®, Brooke Horticultural Products, Wandiligong, Victoria)
8. Nematicide + MAP fertilizer (200 g/m²)
9. Nematicide + Calcium hydroxide (hydrated Lime 500 g/m²)

After treatment, but prior to methyl bromide injection, all plots were cultivated with a single pass of a rotary hoe to 150mm depth to incorporate the treatments. Methyl bromide was injected into the soil by a commercial soil fumigator. The soil temperature was 10.5°C and after methyl bromide injection all plots were covered with plastic although breaks were made between the plots for the methyl bromide and Dazomet treatments. These plastic covers were removed after 15 days, on June 1.

As dazomet activity is soil moisture sensitive, the soil moisture was measured with a portable tensiometer on May 26. At this date the average soil moisture tension of the dazomet treatment was 16.2 centibar (kPa), close to field capacity. Cress germination tests on soils collected to 150mm depth revealed no residual dazomet activity by July 1.

On July 27 soil samples were collected from treatments 1, 2, 3, 6, 8 and 9 and measured for ‘soil microbial activity’ by measuring dehydrogenase activity using triphenyl-tetrazolium (Line, 1999).

The trial site was planted to ‘Smoothie Golden Delicious’ trees on ‘mm26’ rootstocks at 1 metre spacings on 5 August 1999. These trees were about 1.8m tall and had about 15 ‘feathers’ (branches). The trees in the antibiotic, mycorrhizae and Trichoderma treatments were removed from their planting site and the treatments applied prior to replanting on August 6. The antibiotic treatment was applied by dipping the roots in a solution containing 1 g/L streptomycin for 60 seconds. A new streptomycin solution was used with each replicate. The Trichoderma and mycorrhizae were applied by removing the trees and placing 30g or 5g respectively of the material into the bottom of the planting hole of each tree prior to replanting.

Soil from the untreated control and nematicide treatment were collected on 17 December 1999 and assessed for nematodes by a commercial laboratory. A further sample of soil from the untreated control, nematicide, methyl bromide and dazomet treatments was collected on 30 March 2000 and also assessed for nematode numbers. Data presented are for Pratylenchus spp. only.

Tree performance was visually assessed on a 0 – 10 scale (0 – trees dead) on two occasions (29 November 1999 and 21 January 2000). After the first seasons growth three measurement of tree performance had been made. Butt diameters and shoot lengths of the apical dominant shoot were measured on 24 March 2000. Tree size was determined using image analysis of digital photographs taken at night with a flash to eliminate background interference.
Data was inspected for normality and equal variance and analysed by the analysis of variance using 5% LSD values for mean separation. Data for the visual assessment of the trees was analysed by the split plot in time analysis of variance.

Results and Discussion

Data for microbial activity and nematode numbers in the soil (table 7.1) indicate different responses between the treatments. It was found that the methyl bromide treatment reduce the microbial activity the greatest, although both the dazomet and nematicide plus MAP also significantly reduced the activity and were not statistically different to the methyl bromide. The finding that the nematicide alone and nematicide plus calcium hydroxide did not reduce microbial activity indicates that the response for nematicide plus MAP was due to the MAP alone and that this ‘fertiliser’ has features that are detrimental to soil organisms. This supports the findings from the pot trials where the evidence suggested that the MAP had effects greater than that of nutrition alone.

Table 7.1
Treatment effect on soil microbial activity using microbial dehydrogenase activity as an indicator of soil microbial life and nematode counts.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Microbial dehydrogenase activity*</th>
<th>Pratylenchus nematodes*</th>
<th>Number per 200 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Untreated Control</td>
<td>0.367 a</td>
<td>273</td>
<td>30/3/2000</td>
</tr>
<tr>
<td>2 Methyl Bromide</td>
<td>0.078 b</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3 Dazomet</td>
<td>0.165 b</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>6 Nematicide</td>
<td>0.362 a</td>
<td>62</td>
<td>112</td>
</tr>
<tr>
<td>8 Nematicide plus MAP</td>
<td>0.194 b</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>9 Nematicide plus calcium hydroxide</td>
<td>0.368 a</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

* Soil from plots mixed prior to assessment prohibiting statistical analysis

While the data for nematodes could not be statistically analysed the results are clear (table 7.1). At the first sampling date the nematicide substantially reduced Pratylenchus spp by 77 percent. Of note is that this treatment did not totally control of the nematodes at this date. Pratylenchus penetrans, a known nematode pathogen of apples were present in the sample. At the second date the number of nematodes in the nematicide treatment had nearly doubled from the first date while the number in the untreated control had slightly decreased indicating that the nematicide was losing its effect. At this date there were no nematodes in the methyl bromide treatment and only minor numbers in the dazomet treatment demonstrating the superior qualities of these two materials in controlling these plant pathogens.

The split plot analysis of variance for the visual assessments of tree performance failed to detect any difference between the observation dates or any observation date by treatment interaction. There was, however, a highly significant treatment effect.
Of the treatments tested it was found that those containing methyl bromide out performed the other treatments (table 7.2). These trees had a higher visual score, larger and heavier leaves, thicker stem diameters, longer terminal shoots and larger tree cross sectional areas.

Table 7.2
Treatment effects on newly planted apple trees in apple replant affected soil.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Visual assessment (0-10)</th>
<th>Leaf area (mm²)</th>
<th>Leaf shape (L/B)</th>
<th>Dry weight (mg/leaf)</th>
<th>DW/Area (mg/cm²)</th>
<th>Stem Diameter (mm)</th>
<th>Shoot length (mm)</th>
<th>Tree cross sectional area (area)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>3.75 a</td>
<td>2082 a</td>
<td>1.73 a</td>
<td>308 a</td>
<td>15.0 a</td>
<td>21.1 a</td>
<td>121.3 a</td>
<td>38.3 a</td>
</tr>
<tr>
<td>MeBr</td>
<td>9.38 e</td>
<td>2998 d</td>
<td>1.61 a</td>
<td>428 c</td>
<td>14.3 a</td>
<td>30.9 f</td>
<td>273.8 d</td>
<td>96.9 f</td>
</tr>
<tr>
<td>Dazomet</td>
<td>7.63 d</td>
<td>2702 cd</td>
<td>1.71 a</td>
<td>395 bc</td>
<td>14.7 a</td>
<td>26.4 cd</td>
<td>177.1 c</td>
<td>65.9 cd</td>
</tr>
<tr>
<td>MeBr+VAM</td>
<td>9 e</td>
<td>2938 d</td>
<td>1.64 a</td>
<td>432 c</td>
<td>14.7 a</td>
<td>28.4 de</td>
<td>244.2 d</td>
<td>75.2 de</td>
</tr>
<tr>
<td>MeBr+Antibiotic</td>
<td>8.88 e</td>
<td>2963 d</td>
<td>1.68 a</td>
<td>439 c</td>
<td>14.8 a</td>
<td>29.2 ef</td>
<td>244.1 d</td>
<td>84.3 e</td>
</tr>
<tr>
<td>Nematicide</td>
<td>5.55 be</td>
<td>2375 ab</td>
<td>1.65 a</td>
<td>322 a</td>
<td>13.7 a</td>
<td>24.8 bc</td>
<td>132.5 a</td>
<td>56.3 bc</td>
</tr>
<tr>
<td>Nem+Trichoderma</td>
<td>5.75 bc</td>
<td>2373 ab</td>
<td>1.66 a</td>
<td>335 a</td>
<td>14.1 a</td>
<td>23.2 ab</td>
<td>127.1 ab</td>
<td>48.8 ab</td>
</tr>
<tr>
<td>Nem+MAP</td>
<td>6.38 c</td>
<td>2546 bc</td>
<td>1.68 a</td>
<td>346 ab</td>
<td>13.6 a</td>
<td>24.9 bc</td>
<td>172.1 bc</td>
<td>65.9 cd</td>
</tr>
<tr>
<td>Nem+Ca(OH)₂</td>
<td>5.38 b</td>
<td>2349 ab</td>
<td>1.66 a</td>
<td>355 ab</td>
<td>15.3 a</td>
<td>24.5 bc</td>
<td>165.4 abc</td>
<td>55.7 bc</td>
</tr>
</tbody>
</table>

Means within a column different letters considered different (LSD p=0.05)

As this trial site was found to contain Pratylenchus penetrans nematodes which are known to be pathogenic to apple trees a small response to the treatments containing the nematicide was observed. After the nematicide treatment, the trees had a healthier appearance (visual score) and they were found to have significantly larger stem diameters and tree cross sectional areas when compared to the untreated control. With the exception of shoot length for the nematicide plus MAP treatment, there was no differences between the nematicide alone treatment and the other nematicide treatments that contained an additional treatment.

Of interest however, is the nematicide plus MAP treatment which tended to have a superior, although not statistically significant, growth performance for all the growth parameters measured when compared with the other nematicide treatments. The nematicide plus MAP treatment was not statistically different to the dazomet treatment for all growth parameters measured except visual appearance.

The results for the calcium hydroxide and Trichoderma treatments were disappointing and indicated that these cannot be recommended to industry. The reason why these treatments, which showed promise in the pot trials, failed in this field experiment could not be determined. The finding that root applied antibiotic and the mycorrhizae had no effects was discouraging, however, it is possible that these treatments will have significant effects on tree productivity in the second and third seasons.

Based on the final tree cross sectional area, the best indicator of overall tree performance during the first growing season, the treatments may be grouped into 4 groups as outlined in table 7.3. This identifies three groups of treatments, those containing the nematicide where non specific replant disease alone was controlled, MAP and dazomet where SARD was partially controlled and those treatments containing methyl bromide where SARD was well controlled. These results raise the possibility that the efficiency of MAP and dazomet may be improved to provide equivalent control of SARD to the methyl bromide treatment.
Table 7.3
Visual assessment treatment effects on performance of trees planted in an apple replant site

<table>
<thead>
<tr>
<th>Final tree cross sectional area range</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 +</td>
<td>All treatments with Methyl Bromide.</td>
</tr>
<tr>
<td>60 - 75</td>
<td>Dazomet, nematicide + MAP</td>
</tr>
<tr>
<td>45 - 60</td>
<td>All remaining nematicide treatments</td>
</tr>
<tr>
<td>less than 45</td>
<td>Untreated control</td>
</tr>
</tbody>
</table>

Conclusions

This field trial has identified that the addition of lime to the soil, or the placement of *Trichoderma spp* under the tree roots had no effect on SARD. However, it has also identified that MAP fertiliser and the soil sterilant dazomet, have potential to at least reduce the impact of SARD in apple replant sites. The mycorrhizae and root applied antibiotic treatment had no effect after the first growth season, may result in superior trees in later years by restricting or delaying the development of SARD in this orchard.

In this site there was a significant improvement in growth due to the use of the nematicide demonstrating that nematodes are present and do affect tree growth in southern Tasmania.
Project discussion and conclusions

Introduction

This project was conducted over three years. The first two years utilised orchard soils and tissue cultured ‘mm26’ apple rootstocks in glasshouse studies to characterise apple replant disease in Tasmania and to develop some potential control strategies that could be tested in the field. After the glasshouse studies were completed a field trial was established using potential treatment identified in the pot trials and the growth of the trees in the following season was monitored.

Non specific apple replant disease

The glasshouse studies confirmed that non specific apple replant disease, due to nematodes, was present in nearly half of the orchards studied. This is a new finding in Tasmania where it is commonly believed in the fruit industry that nematodes are not a problem. The practical ramifications of this are that growers have to be careful when replanting apple orchards to other crops such as cherries which will be affected by these nematodes. Further, some nematode studies of pasture paddocks to be planted to fruit crops have been analysed in a separate project and high numbers of Pratylenchus penetrans were found at one of these sites. Hence, it can be concluded that all sites to be planted to fruit crops in Tasmania should be assessed for their nematode populations prior to planting to ensure satisfactory tree performance.

Specific apple replant disease (SARD)

In addition to non specific apple replant disease the glasshouse studies also identified that specific apple replant disease (SARD) is far more common and severe than non specific apple replant disease. SARD was found to be present in all existing apple orchards tested, this resulted on average, in a 50 percent reduction in the growth rates of the trees. This growth reduction was however, variable between orchards and in one orchard the growth rate of affected trees was 30 percent of the trees growing in SARD free soil. Further, from overseas experience this variation in growth response is not constant across an affected orchard with SARD often being extremely severe in numerous isolated pockets within the orchard. This variation in tree growth in SARD affected orchards makes planning for replanting difficult and management of SARD affected orchards awkward. Affected orchards are not efficient and provide sub optimal returns to the growers. Hence SARD needs to be controlled when replanting apples.

The exact cause of SARD is desired in order to develop selective control strategies specific to the disease. Unfortunately, in overseas studies, examining the soil microbiota with traditional plant pathology techniques has never proved successful in the identifying of the cause of SARD. There are many potential reasons for this lack of success including the fact that only a small proportion of soil microbiota have been identified and the complex interactions that occur between different soil microbiota organisms is not well understood. As a result, in this study SARD was investigated by testing different soil responses to nematicides, fungicides and antibiotics to obtain a picture of the organism group responsible for SARD in Tasmania. The results for nematicides were outlined above and identified that non specific apple replant disease was present but that nematodes were not responsible for SARD. Three fungicides were also tested for their effectiveness against SARD.
Thiram (in trials not funded by this project) and matalaxyl are both broad spectrum fungicides with a history of controlling a wide range of soil borne fungi. Shirlan® is a fungicide active against basidiomycetes in the soil. None of these fungicides had any impact on SARD suggesting that fungi are not responsible for SARD in Tasmania. Finally the antibiotic streptomycin was added to the test soils and this material was found to be extremely effective at reducing SARD. Hence, it is concluded that the cause of SARD in Tasmania is sensitive to streptomycin, therefore it is probably a bacterium.

Unfortunately for industry antibiotics are not registered for use in any fruit crop and their registration at this stage, is unlikely. Hence, this method of controlling bacteria is not a commercial option. As a result other methods of controlling bacteria needed to be studied to attempt to find an economic control strategy for industry.

Control of SARD

Pesticide control

One potential option for the control of bacteria is the use of broad spectrum soil sterilant materials. Historically this has been the approach adopted around the world for the control of this disease, with the use of chemicals such as methyl bromide, chloropicrin, MIT (dazomet and metham sodium) and formaldehyde being used against this disease. In Australia chloropicrin is not registered for use on apples, however, it is a component of the existing methyl bromide fumigant and a new pesticide currently undergoing registration (Telone 35C®). The only current registration for MIT on apples is dazomet (Basamid®) allowing for the immediate use of this treatment by growers. Formaldehyde is not registered and has environmental issues that would limit its use. Hence, field trials were established to study the effect of chloropicrin and dazomet on SARD. Three of these field trials have been funded from other sources and have not been reported here however, in these trials these treatments have proved extremely effective against SARD. The only trial where control was not equal to methyl bromide was the results for dazomet for the field trial reported here. This unreliability is a global problem of MIT materials and is related to its activity in the soil water rather than the soil atmosphere. This different mode of activity results in the product being far more sensitive to application techniques such as method of incorporation and soil water status. Hence, of the two useful alternative soil sterilants both need further studies prior to their recommendation to industry. For Telone 35C® completed registration is necessary and the material still needs to be tested for its efficacy against SARD. For dazomet the application technology needs further research to develop a reliable system for its control of SARD.

Cultural control

The second potential method of controlling the bacterial agents of SARD are cultural methods. Bacteria are a problem for the cut flower industry where they grow and block the vascular system of the cut flower stem. In this situation bacteria have been traditionally controlled with the use of acid water and biocides such as ammonia and chlorine. In replanting orchards the soil pH is often modified with the addition of lime to the soil and ammonia based fertilisers such as mono ammonium phosphate (MAP) are often added for nutritional purposes. Hence, pot trials were conducted and confirmed that both these materials had an impact on the expression of SARD. On the basis of these pot trial results the treatments were included in the subsequent field trial. It was found that the addition of lime to the soil had no impact on SARD while the addition of MAP resulted in tree growth rates equivalent to the dazomet treatment. Further, soil life studies revealed that the MAP treatment dramatically reduced the soil microbial activity suggesting that this material had a direct controlling effect on SARD. More research is needed on application systems and rates to determine if equivalent control of SARD to that obtained using methyl bromide is possible with this treatment.
In addition to soil pH and ammonia based fertilizers the level of irrigation has been reported to have an impact on SARD (Utkhede, 1996). Pot experiments were conducted on Tasmanian orchard soils to investigate this possible control method. Unfortunately it was found that improved soil water status had no effect on tree growth in SARD affected soils in these pot trials.

**Biological control**

The final potential control method for bacteria investigated in this project were biological methods. In this part of the study mycorrhizae, which are reported to protect trees from root based disease attack, were tested as well as the soil pathogen antagonist *Trichoderma*. Of note is that many *Trichoderma* are also reported to produce antibiotics. In the pot trials there was no response to added mycorrhizae in the pot trials where the mycorrhizae and SARD were introduced to the plants simultaneously. It was concluded, however, that as mycorrhizae take several months to establish, this treatment would be better applied to trees prior to exposure to the disease. This method was undertaken in the field trial where the mycorrhizae were introduced to trees planted in sterile soil. While there was no effect of this treatment on tree performance after the first season of growth the effects of this treatment may become apparent in the second or third seasons. It is hoped that this treatment will reduce the rate of establishment of SARD on these trees.

It is unfortunate that while the *Trichoderma* demonstrated a control potential against SARD in the pot experiments this material failed to show significant activity in the field experiment such that its future inclusion in the project is unlikely.

**Conclusions**

This research project has clearly shown that non specific apple replant disease is present in about half of the Tasmanian soils tested. The effect of this disease, however, is minor when compared with SARD which was present in all orchard soils tested and resulted in, on average, a 50 percent reduction in tree growth and potential yield.

Nematodes and fungi were eliminated as the probable cause of SARD in Tasmania where a streptomycin-sensitive organism, such as a bacterium, appear to be responsible for the disease.

No alternative control strategies currently available to growers and as reliable as methyl bromide were identified. Chloropicrin and dazomet were the two treatments with the most potential for industry adoption. Chloropicrin is reliable, but not registered and dazomet is registered but not reliable. For chloropicrin a new product that contains 35 percent chloropicrin, Telone 35C® is currently undergoing registration and if successful will be available to growers. This material has however, not been tested for its effectiveness against SARD. Of the cultural control methods tested the fertiliser MAP showed the potential to be as effective as dazomet. The effectiveness of both MAP and dazomet is influenced by the method of application, which has not been fully determined or optimised. More research on application methods and rates are needed for these two treatments.

Other potential commercial treatments such as fungicides, nematicides, lime, irrigation, *Trichoderma*, and mycorrhiza appear to have no effect on SARD in the first year of growth of trees in the field. Nematicides were, however, effective against non specific replant disease where it was present.
Technology transfer

The results of this project have been communicated to industry in a number of ways. This process is dynamic and will continue to occur after the project has been terminated. In addition to informal presentations and discussions with growers at numerous meetings such as social events, at field days and conferences the project leader has also held informal discussions with various field officers from different State Departments. The following formal presentations have also been used to inform growers and scientists of the research results to date.


9. Milestone reports

10. Final report


