Combating apple replant disease in Australia

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Combating Apple Replant Disease in Australia

Final Report

April 2005

Martin Line, Stephen Wilson and Andrew Heath

Tasmanian Institute of Agricultural Research

Combating Apple Replant Disease in Australia
HAL Project AP00003
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The purpose of this report is to disseminate the results of a survey of the background literature on apple replant disease and the outcomes of research particularly aimed at potential alternatives to methyl bromide for the control of this disease.

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Media summary

Apple Replant Disease (ARD) is reported wherever apples are grown, and generally has a biological cause. It poses an acute dilemma for orchardists with the phasing out of the most effective treatment, methyl bromide (MeB), by 2005 under the Montreal Protocol because of its ozone-depleting properties. Horticulture Australia Limited (HAL) has provided funding to a group of Tasmanian researchers to assess alternatives.

The key components of this project comprised a comprehensive review of the literature, followed by glasshouse and field trials of a range of potential alternative treatments to MeB for apple-replant disease. Glasshouse trials using MeB as the standard indicated that monoammonium phosphate (MAP), Basamid®, Perlka® and various biological agents gave good protection against the disease. The beneficial effects of the first two agents support previous reports for these agents while Perlka® had not been previously recorded for this purpose. However since it is reported as a broad-spectrum biocide its efficacy in controlling apple replant disease is easily explicable. The benefits recorded for compost amendment were attributed to a nutrient effect on growth rather than inhibition of the disease.

A field trial undertaken at the Grove Research Centre included agents found to be effective in the glasshouse trials plus others being reported as promising alternatives. Telone C-35® was shown in the field trial to be at least as effective as methyl bromide, with Perlka® + MAP, Basamid® and compost additions resulting in only slight improvements in plant growth over the replant-disease controls. Other treatments including MAP, Trichopel®, Perlka®, and various biocontrol agents were ineffective in combating the disease in the field. It should be noted however that the poor result found in the field trial for Basamid® and MAP contrasts with previous results obtained by other workers and may possibly be the result of particular field conditions or method of application. In a second field trial, adding MAP or fresh soil to the planting hole were both found to substantially reduce the effects of ARD, in line with previously published results.

Recommendations

This study has confirmed Telone C-35® as a viable alternative to MeB for treating apple replant disease in replant orchards. Converting to Telone C-35® treatment has the advantage of growers being able to use the same equipment and similar field management methods as for MeB. For low impact (non-fumigant) control of ARD, continued use of MAP is recommended and for control without synthetic chemicals, addition of fresh (non-ARD) soil in the planting hole substantially reduces the impact on tree growth.

Technical summary

The nature of the problem

Apple Replant Disease (ARD) is a worldwide problem, arising when an orchard is planted in the same soil as a pre-existing orchard. The phasing out of the most effective treatment, methyl bromide (MeB), by 2005 under the Montreal Protocol poses an acute dilemma for orchardists. This project, supported by Horticulture Australia Limited (HAL) will assess alternative treatments of ARD.

Outcomes

Glasshouse trials using MeB as the standard indicated that monoammonium phosphate (MAP), Basamid®, Perlka® and various biological agents gave good protection against Apple Replant Disease. The beneficial effects of the first two agents support previous reports while Perlka® had not been previously recorded for this purpose. However since it is reported as a broad-spectrum biocide its efficacy in controlling ARD is easily explicable. The benefits recorded by a compost amendment were attributed to a nutrient effect on growth rather than inhibition of the disease.

The glasshouse trials were accompanied by field trials undertaken at the Grove Research Centre. The choice of treatments for a field trial was necessarily limited to those showing significant promise as potential alternatives to MeB from glasshouse trials, those previously reported in the literature as being promising alternatives and not easily assessable in a pot trial (particularly Telone C-35®), and those known to have broad-spectrum biocontrol activity (particularly Trichoflow®). In the primary trial, MeB was used as the standard against which all other treatments were compared, whilst the second used growth in untreated ARD soil as the control.

The difference between the MeB standard and the ARD-untreated control in the primary field trial was marked; shoot height of the untreated control at termination of the trial averaged 50% of the MeB standard, while cross-sectional area amounted to 67%. Both assays showed Telone C-35® to be at least as effective as methyl bromide in the field trial, with Perlka®/MAP and Basamid® treatments giving a significant (but slight) improvement over the control in cross-sectional area, but not in extension growth, and 20% compost amendment showing a significant (but slight) improvement over the controls in both measurements. All other treatments including MAP alone, Trichopel®, Perlka®, and various biocontrol agents were relatively ineffective, Addition of the *Bacillus* agent had a depressing effect on the growth of apples using both shoot height and cross-sectional area assays, the effect being significant using the former assay. This may possibly be attributed to the effect of the bacteria on root respiration, since a large bacterial inoculum coupled with water-saturated conditions at the time of planting could render the soil in the root-zone anoxic for a period of time. Although none of the potential biological control agents proved effective against apple replant disease, some showed broad-spectrum efficacy against a range of other fungal pathogens.
The poor result for Basamid® in this trial contrasts with previous results obtained by Brown & Schimanski (2002) where it was applied at least two to three months before planting and the present result may possibly result from poor application methods or inadequate attention to mixing within the soil profile. If so, Basamid® may be an attractive alternative from a practical viewpoint, since it does not presently require a licensed operator for application.

In a second field trial MAP, organic matter amendment (with various fertilizer additions) and replacement soil were compared with an untreated control in soil which had been planted to apples for 15 years prior to replanting with this trial. Organic matter incorporated into the top 20 cm of soil was ineffective, but MAP incorporated into the top 20 cm of soil or replacement soil

in the planting hole both resulted in near twofold increases in extension and radial growth measured after two seasons. Leaf analysis for macro and micronutrients showed most elements in all treatments to be marginal to adequate by accepted standards and there were was no evidence to indicate that the observed growth responses were associated with any nutritional effect. While the positive effect of MAP was supported by glasshouse experiments its benefits did not compare with those provided by MeB or Telone C-35® in the trial described above. However MAP does not have the major beneficial effect of broad-spectrum weed control exhibited by the fumigants. Indeed MAP appears to promote growth of competing weeds and this may account for the failure of the treatment in the first field trial.

Recommendations

This study has confirmed Telone C-35® as a viable alternative to MeB for treating apple replant disease in replacement orchards. No other treatment came close to MeB or Telone C-35® in effectiveness against the disease. Converting to Telone C-35® has the advantage of using the same equipment and similar field management as MeB, so contractors and growers are able to begin using it without any big change to current methods.

A REVIEW OF LITERATURE ON APPLE REPLANT DISEASE, ITS CAUSES AND REMEDIAL TREATMENTS
(N.B. At the time of submission of this Report to HAL, this review had not been published elsewhere)

1. General Introduction

Apple Replant Disease (ARD) is the description applied to the characteristic failure to thrive when apple trees are replanted into ground previously planted in apples. Symptoms usually become evident in the first year after planting (1) and include stunting, shortened internodes, rosetted leaves, 'witches broom' appearance (2), shortened orchard life, browning of infected roots (3, 4) and small root systems with many, poorly-functioning, fibrous roots (5). Such trees grow unevenly (5), remain stunted (5a) and bear low-quality and low-yield fruit 2-3 years later than normal (6). Young trees appear to be more vulnerable to damage than older trees and the above-ground symptoms are less obvious and more difficult to diagnose on established trees (2).

ARD is reported wherever apples are grown, and the causes appear to be both diverse and differing from region to region. The problem of ARD has become acute with the phasing out of perhaps the most effective treatment, methyl bromide (MeB), by 2005 under the Montreal Protocol because of its ozone-depleting properties. It has been claimed that a 25% loss in production efficiency across California's 2.2 million acres of tree and vine crops will occur should no viable replacement be found for MeB (1). Economic analysis in Washington State showed that ARD can turn potentially profitable projects into non-economic ones, and that loss-potentials were very significant, requiring the attention of orchard managers and professional advisors (7). In the case of apple varieties having average market value it was not economically viable to plant without first fixing the ARD problem (8).

Terms used for ARD include 'replant problem' and 'replant disorder' (2). Disorders of unknown cause were termed ***specific*** replant diseases by Pitcher *et al.* (1966) (9) and characterized 'by the severely reduced rate of shoot and root growth of the second planting of a given species following the same or closely related species on the same site'. The meaning of this terminology has since become muddied by e.g. Colbran, 1970 (10) who used ***'specific'*** ARD as a disease of heavy soils in Europe, the condition being to a marked degree specific to apples, in contrast to ***specific ARD in Australia*** meaning disease attributed to unknown organisms and controlled by fumigation. Incorrect use of fertilizers, poor soil structure, and poor drainage were identified among other (***non-specific***) reasons for replant disease. Campbell (2000) (11) added further confusion with '***special***' ARD, this being the primary cause of ARD in heavier basaltic soils of Orange and Batlow (NSW), and characterised in the negative by it not being caused by nematodes, fungi or bacteria. Brown et al. (2000) (6) concluded that ***non-specific*** ARD was usually associated with nematode attack and could be controlled by application of a nematicide prior to planting. Other causes of non-specific ARD identified included soil degradation, pH problems or the presence of toxic compounds such as herbicides, heavy metals and biological products. In contrast ***specific ARD*** was restricted to the disease resulting from replanting in soil where an apple orchard existed previously. Szczygiel & Zep (1998) (12) give a breakdown of ARD into ***specific, unspecific*** **(sic) or** ***mixed.*** If plant growth improved as well after drying as it did after steaming or formalin treatment, the disease was determined as ***unspecific***. '***Specific***' ARD was applied to cases where growth improved after steaming or formalin treatment only, and '***mixed***' was applied to growth improvement following both steaming and formalin treatments and also after drying, but to a lesser degree. Nematodes were strongly associated with the 'unspecific' or 'mixed' replant diseases. From this description it might be assumed the

'specific' relates to biological cause, but not nematodes. Physical factors of disease did not feature in this system (12).
Since the distinction particularly of specific, special, and non-specific is blurred by varied interpretations such terminology is best dropped, with the disease being restricted to the definition given in the first paragraph and characterized descriptively if the cause is known (e.g. 'ARD attributable to nematode attack').

2. Background Australian research leading to the present investigation

Colbran (1970) (10) noted that the reasons for many of the failures of apple-plantings in old-orchard soils in the Stanthorpe district (Queensland) were not clearly understood, although nematodes were implicated in many of the incidences. He reported that a nematicide gave similar regrowth responses to MeB in these soils, also reporting that growers ameliorated the problem by replacing soil at the planting site with virgin soil. Other agents stated to be effective were chloropicrin, methylisothiocyanate and Telone plus chloropicrin. A cover crop of sorghum-sudan grass hybrid was recommended when replanting orchards, (cowpeas, rye corn or lupins were not recommended as possibly increasing the severity of the problem), followed by deep ploughing, discing, and fumigation with nematicide which was to be left three weeks before tine-raking the soil deeply at least twice before planting. It was noted that some of the broad-spectrum biocides such as MeB and chloropicrin gave better results than the nematicide alone, but the cost of these was sometimes prohibitive. A bioassay of need for treatment was available at that time.

Campbell (2000) (11) in monitoring a trial at Orange of Delicious on MM 106 found (among other things noted elsewhere) that spelling the land for three years (rather than two) resulted in improved fruit production relative to other treatments (Basamid, high lime rates, high nitrogen rates).

Research on Tasmanian orchards undertaken by Brown et al. (2000) (6) resulted in the following conclusions:

- Pot trial studies confirmed that non-specific ARD (due to nematodes) was present in about half the orchards studied in Tasmania. Specific ARD (attributed to causes other than nematodes and restricted to ex-apple orchard soils) was found to be more common than non-specific ARD and was present in all Southern Tasmania (Huon Valley) soils examined that had a history of apple production. The disease resulted (on average) in a 50% reduction in growth rate of replanted apple rootstocks. Growers were also cautioned about replanting apple orchards to other crops such as cherries that may also be affected. The nematode *Pratylenchus penetrans* was found to be present in high numbers in a number of paddocks to be planted in fruit crops, indicating that such sites should be checked for nematode populations prior to tree planting.
- Sterilizing soil having no history of prior apple planting had no beneficial effect on subsequent apple replants.
- A limited response to nematicides was noted for 40% of the orchards examined, indicating nematodes to be a significant component of ARD.
- Effective fumigant treatments included chloropicrin and Dazomet [the active ingredient in Basamid which breaks down in soil to form methyl isothiocyanate (MITC)], with evidence that the fertilizer monoammonium phosphate (MAP) may also provide benefit.
- All soils from Southern Tasmanian apple-orchard sites tested responded to sterilization, indicating a biological cause. The fungicides Shirlan, Metalaxyl and Thiram were ineffective in pot trials, indicating the cause of ARD in these sites to be non-fungal. Introduction of the

anti-bacterial antibiotic streptomycin to pots was at least as effective as soil sterilization, indicating bacteria to be the primary cause of ARD in Southern Tasmania.
- Pot trials indicated that the biocontrol fungus, *Trichoderma*, or addition of calcium hydroxide, MAP or MAP plus nematicide had potential to reduce the impact of ARD. Streptomycin and mycorrhiza additions were also indicated to be beneficial for trees planted in sterile soil. Quantity of irrigation water was not a factor in the trials.

Brown & Schimanski (2002a) (13) reported that Dazomet was effective against ARD but not always reported as reliable. This was attributed to the liquid penetration of MITC, which is not as effective as the furmigant treatments and very dependent upon soil moisture conditions. It was contended that properly applied (under field capacity moisture conditions) Basamid should be effective in the control of ARD.
In other trials reported by the authors, chloropicrin was seen to be highly effective while Thiram was ineffective.

In a subsequent report on a field trial at two Tasmanian locations after two years trial Brown & Schimanski (2002b) (14) found that Basamid treatment of ARD soils led to apple yields almost as high as those from the MeB-treatment, and the quality of the fruit was reported to be as good as, or better than fruit from the MeB-treated soils.

3. Global Incidence of ARD

ARD is reported wherever apples are grown, and at least half the pome-fruit orchards are growing on soils with replant problems. ARD has been reported in 61% of 244 examined orchards in Poland (12), in 85% of replant orchards in Italy (15) or in 100% of apple-replant soils in Tasmania (7). Campbell (2000) (11) reported the incidence in Australian soils to be particularly common in a heavy basaltic soils, more frequent in light than heavy soils, and more frequent in neutral to slightly acidic soil than in acidic soils. The problem is particularly associated with soils that have carried apples for extended periods, but have been recorded in soils within three years of establishing an orchard on virgin soil (5).

Aerial multispectral imagery has become widely utilized for the detection of plant diseases including ARD in Washington State (16). It is claimed that the advent of the digital camera coupled with computer analysis should markedly increase the use of this methodology for scientific purposes.

4. Causative agents associated with ARD

The primary causes of ARD appear to be different in different regions, and different with different plants. Comparison of replant diseases of apples and roses found that the causative pathogenic agents were different (17), and remedial treatments which are effective against e.g. pear replant disease in Oregon (phosphorus addition) were different from those known to be dependable in California (1). In a review of the topic, McKenry (1999) (1) concluded that although nematodes, fungi, actinomycetes and other bacteria were possible contributors to ARD, none *per se* was the single cause; rather each was an important component of the disease.

Brown et al. (2000) (6) among others, considered the cause to be multi-faceted and never fully characterized. As examples of the multi-faceted nature of ARD, the causative agents in New York replant-orchards have been identified as including nematodes (*Pratylenchus penetrans*), parasitic fungi, bacteria and other soil-borne microorganisms. Complicating abiotic factors

included unbalanced or inadequate nutrient availability, impaired soil structure, loss of organic matter, herbicide residues, impervious soil layers and other, site-specific problems (2). A contrasting situation was apparent in Washington State soils, where *Pratylenchus penetrans* numbers were below the damage threshold level in eight of nine orchards surveyed and bacteria were not implicated in the disease, which was attributed to fungi, particularly of the genera *Cylindrocarpon destructans, Phythophthora cactorum, Pythium spp*. and *Rhizoctonia solani* (18). An interesting thought by Brown et al. (2000) (6) was that instead of being primary pathogens, pathogenic fungal genera commonly associated with ARD could possibly be secondary invaders rather than the causal agents.

It is not surprising that interactions between different causative agents have been reported, as in the case of ARD attributed to *Phytophthora cactorum* and *P. nicotianae* and/or the nematode *P. penetrans*, with enhanced pathogenicity observed where the fungi and nematodes were found together (19).

Summarised reports on the causative agents of ARD

4a. Physical factors
Low soil pH, poor irrigation practices, arsenic spray residues in the soil, soil compaction, nutrient deficiencies, and selection of an inappropriate orchard system (5a, 20)
Low soil fertility (ARD can be ameliorated by adding NPK) (4, 22)
Zinc deficiency (23)
Low temperatures (Cherry trees affected by ARD are reported to be more susceptible to cold injury) (2)
Soil type. ARD was more frequent in light sandy soil than heavy soils in Poland, while growth improvement following control measures was reported to be better in light rather than heavy soils (12). It was also more frequent in neutral or slightly acidic soils than in more strongly acid soils (12).

4b. Microbial agents
McKenry (1) noted that researchers in many countries have pointed to nematodes, actinomycetes, fungi and certain other bacteria as the greatest possible contributors to replant disease.
4b i. Fungi
Fungi have been strongly implicated as causal agents of ARD, these and nematodes being most frequently associated with this disease in reports.
The following fungi have been implicated by various authors as associated with ARD of apples, often in consortia with others:

Alternaria sp. (2)
Cylindrocarpon destructans (=Nectria radicicola) (4, 18, 26, 27), *C. lucidum* (2, 34a)
Cylindrocarpon spp. (2, 34a)
Dematophora necatrix (=*Rosellinia cecatrix*) (28, 29)
Fusarium oxysporum (28, 29), *F. tricinctum* (4, 26) *Fusarium spp.* (2)
Gilmaniella sp. (2)
Mortierella sp. (30) *Papulospora spp.* (2)
Penicillium spp. (2, 31)
Peniophora sacrata (*Phanerochaete sacrata*) (32)
Phytophthora cactorum (18, 19, 27)
Phytophthora cambivora (19)

P. cinnamomi (19)
Phytophthora spp. (2, 28, 29, 33a)
Pythium ultimum (19), *P. irregulare* (2, 34a), *Pythium spp.* (4, 18, 26, 27, 28, 33a, 34a)
Rhizoctonia solani (18, 24, 27), *Rhizoctonia* sp. (2, 33a)
Torulomyces lagena (30)
Trichoderma hamatum (30), *Trichoderma* sp. (2)

4b ii. Nematodes
Poor tree establishment coupled with extensive and possibly severe root damage is often associated with nematode attack (2). The species *Criconemella xenoplax*, *Meloidogyne chitwoodi*, *M. hapla*, *Xiphinema americanum*, *Pratylenchus jordanensis*, and *P. neglectus* have been intermittently reported, whereas *Pratylenchus penetrans* appears to be the species reported most frequently and globally in association with ARD (Nyczepir and Halbrendt 1993) (35). Initial population of 15/100 g soil are considered necessary for growth reduction, while 25 to 150/100 cc soil are considered damaging but can vary depending on soil texture, climate, and additional pathogens (35). The attribution of ARD to nematodes can be achieved either by a determination of high nematode populations in the vicinity of roots, or by comparison of reaction to nematicides relative to broad spectrum biocides and fungicides (2).

The geographic variability of nematodes as a primary cause of ARD has been noted previously. Nematodes (*Pratylenchus penetrans*) are reported to be the primary cause of 'non-specific' ARD in Polish soils (12), in the Beijing region, China (37) and an important component of the complex causing replant disease in cherry and apple orchards of New York (2). In a study of 244 apple orchards in Poland, Szczygiel & Zep (1998) (12) confirmed the root lesion nematode, *Pratylenchus penetrans* to be the cause of 'unspecific' ARD. This species was also higher in numbers in 'mixed' ARD infections.

In the Granite Belt of Queensland (4, 26) nematodes (*Pratylenchus penetrans* and *P. jordanensis*) were identified as being among the primary agents of ARD, and these nematodes were also reported from another two sites in Applethorpe, Queensland.
Campbell (1999) (33) also noted these species to be prevalent in NSW orchard soils. Brown et al. (2000) (6) reported nematodes in all Tasmanian apple orchards examined, but found that only 40% of replant soils responded in a limited way to nematicides and *Pratylenchus* sp. were considered to be of minor significance in Tasmanian orchard soils.

4b iii. Bacteria
Actinomycetes in particular have been positively associated with ARD by numerous workers (2, 34, 35a, 36, 38, 39, 41) and appear to damage rootlets, reducing the ability of plants to take up water and nutrients (35a). Symptoms appear early (within two weeks of planting) and are significant after six weeks, with infection of the plant rootlet epidermis and cortex leading to rapid decay (41). However the plants were reported to recover from the infection, a report contrasting with others of ARD in the field.

Otto, Winkler & Szabo (1993) (38) demonstrated a first peak of infection in young apple rootlets approximately five weeks after planting, to be followed by an apparent decrease in infestation that paralleled stagnation in growth. Pathogenic actinomycetes isolated from apple roots have been reported to inhibit a range of apple tree species (five were assessed) by 50% or more, regardless of species-type (17). Light & electron microscopy of seedling-tissue grown in ARD soil showed actinomycetes to dominate colonization of cortical cells, with fungal hyphae and

nematodes also being occasionally observed in these tissues (34). Actinomycete infection caused large-scale anomalies of the cell wall with demonstrated ability to penetrate to the cell lumen.

The only report of any other bacterial association with ARD (21) claims that the infection leads to a specialization of enzyme systems of the rhizosphere microbiota, with their conversion from being non-pathogens to parasitism. This may simply be a microbial attack on already damaged tissue, as occurs with other infection processes.

4c. Other biotic factors

Freedom from weeds has been reported to assist the ARD process, although this contrasts with other reports below (21)

5. Prevention and treatment of Apple Replant Disease

It is generally deemed important that prior to establishing an orchard a grower should undertake a soil analysis to rectify nutrient or pH imbalances (32, 43, 44) and determine counts of nematodes, especially in sandy soils, with nematicide treatment if numbers are high (32, 43). Assay for other causative agents (fungi, bacteria) are probably not likely to shed light on the problem (this reviewer's view).

Soil fertility is particularly important, with seedlings growing best in replant soils with appropriate N, P, K, Ca and Mg levels (26, 32, 43, 44). Also recommended is the removal of weeds using a broad-spectrum herbicide and the remediation of poor drainage (43, 44).

Removal of old root material from replant soils (ripping to 45cm) has been reported as essential (43). However deep ripping of soil as part of preparation of land must be used cautiously, since disturbed soil is prone to erosion and there may be a risk of bringing salt to the surface. Also newer apple varieties are relatively shallow-rooting. Use of an excavator with grab attachment to extract the tree and major root system may be a better option than either deep ripping or using a bulldozer to remove the existing trees.

The soil should ideally be tested for ARD in a pot trial (32), comparison being made with growth in similar soil having no history of apple planting. Since the 1960's the practice for controlling ARD in California include soil ripping, backhoeing of individual tree sites, soil trenching or slip ploughing coupled with soil fumigation. This two-punch treatment is reported to be effective in over 95% of instances (1). Excavation of ARD soil and replacement with fresh soil has been shown to be effective after three years trial (45).

Rootstock choice may be made for nematode-resistant varieties (32, 46). Addition of sawdust (old, not fresh) or straw mulch (1m wide, 7-10cm deep along the row of trees) has been recommended (11, 32), but growers need to be cautious about the creation of nutrient-imbalance inherent in addition of high-carbon, low-nitrogen materials in bulk. Planting of new trees into a potting soil in the field has been reported to be beneficial (47), as has planting two-year-old nursery trees, which were claimed to be more vigorous than grafted trees or trees from dormant buds (48). Interplanting of new trees between the rows of the previous trees was found to be effective after two years, but not significantly so after three years (45).

The problem of replant disease in trees and vines has been examined in detail by McKenry (2000) (1), with assessments of the field performance of over 150 potential alternatives to MeB. He predicts that following loss of MeB there will be a shift in California to Teldone, probably in combination with MITC-liberating compounds or chloropicrin. Without MeB McKenry claims

there will also be a shift to longer fallow periods and combinations of narrow-spectrum treatments to solve components of the replant problem, these requiring accurate knowledge of their limitations as well as accurate diagnosis of the specific problem. This will add great complexity to the task of replanting into old-orchard soil. 'Mistakes in proper assessment of the replant problem can greatly reduce production efficiency of the grower, frequently for the life of the new planting' (1). McKenry proposes management methodologies including trunk treatments with systemic 'herbicides' (sic; microbiocides?) and use of transported non-replant-problem soil. Depending on conditions and equipment availability, some low-volatility biocides were also delivered effectively to target pests using new techniques.

Review of treatments for ARD soils

5a. Fallowing, crop rotation, cover crops and mulches

Nematode (*Pratylenchus jordanensis*) populations have been reportedly reduced if apple-replant soil was left fallow for three months, but better results are likely to be obtained if the soil remained fallow for 12 months (49) or in fallow or rotation for 2-3 years (11). Often crop rotation or cover crops are used in combination with other treatments. For example, Stirling et al. (1995) (49) suggested that a combination of early removal of the previous orchard, incorporation of animal manure or a green manure crop with urea, choice of appropriate rootstock and maintenance of a layer of organic mulch around trees would provide protection against lesion nematodes without fumigants. These researchers found that after five years trees mulched with sawdust or sawdust and manure had the lowest nematode populations and produced apple yields as good as, or better than, MeB, although MeB was the only treatment to reduce nematode populations at planting to very low levels.

5b. Cover crops/green manure crops/mulches that have been reported as effective against ARD

5b i. Nematodes as the primary cause:

A wide variety of cover-crops have been found to be beneficial for inhibiting nematodes *viz.* …
Marigolds (*Tagetes patula* cv. Sparky) against *P. penetrans* in New York soils (2)
Forage sorghum (49), and oats (a wild type of *Avena sativa* cv. Saia) against unidentified nematodes (2). Oats were found to be the most effective of a large number of plants assessed; marigolds were as effective as steam pasteurization in the field, but other cover crops were incapable of preventing the rebound of nematode populations within six weeks of treatment (2).
Tagetes patula cv. Harmony against *P. penetrans* and fungal species of *Pythium* (50) *Festuca rubra* and *Agrostis alba*; against *P. penetrans* (50)
Brassica napus against *P. neglectus* (45, 49, 51) Brassicas are known to release isothiocyanates on decomposition producing high levels of glucosinolates. Although causing an *increase* in nematode populations while growing with sorghum, these populations were reduced substantially when the crop was ploughed in with urea (49).
Indian Mustard (*Brassica juncea*) against *Pratylenchus penetrans* and *P. projectus* (52).
Sudan grass, annual ryegrass or tall fescue (as green manure crops) against unidentified nematodes (43). Recommended use was for 1-2 seasons before replanting in apples. Cover crops (canola, Karoo, lupin, field pea, faba bean, canola) have been shown to be effective against nematode root-diseases of crops such as cereals (e.g. 51), hence would probably be effective against ARD where nematodes were the dominant causative agent. Between-tree herbicide strips was recommended to reduce weed competition and presence of alternate hosts for nematodes. Clovers and buckwheat also should be excluded from orchard covers, as these are excellent nematode hosts (52).

5b ii. Fungi as the primary cause:
Wheat (but only a winter variety, 'Eltan') was found to be effective against fungal ARD attack, planting in 3 x 3 week cycles (5). It was also noted that soil planted in Elan suppressed *Rhizoctonia solani* root rot of apples (5). Mulches in general are reported to be effective against fungal replant diseases.

5b iii. Unidentified cause:
Green manure crops of hemp, millet or cowpea (especially if covered with plastic), of sunflower & legumes (54) and of wheat and fodder beet (as well as other herbaceous species) (55) have been reported to be at least temporarily effective against ARD.
Likewise, **mulches** of decomposed bark (56), biohumus/compost (56, 57), straw, straw with dynamic lifter,straw with MAP or sawdust (old, not fresh) (11), composts inoculated with specific biocontrol agents (58), or addition of **peat** (59, 60) has been reported to be effective against ARD.

The experience gained from the treatment of root diseases of other crops (including break crops) is relevant to ARD. For example nematode-resistant crops are known to include field peas, triticale and lupin, though different responses are noted for *P. neglectus* and *P. thornei*. Lentils, medic and sub-clover are reported to be moderately resistant. *Allium* is of note as it produces diallyl disulfide, a trigger for germination of sclerotes (61, 62). The possible incorporation of 'biofumigation' crops to control fungi using *Brassica*, *Artemisia*, or canola spp. is also widely reported (e.g. 63, 64, 65, 66, 67, 68, 69). Thompson et al., (68) noted that poor growth of wheat can follow a canola crop, this being attributed to poor VAM colonisation in soils deficient in P and Zn. 'Tarping' soil after incorporating biofumigants such as *Brassica* (64) as part of an integrated pest management control is possibly beneficial.

5c. Selection of resistant tree species
Assessment of rootstock-resistance to ARD appears piecemeal (or confidential) since there is very little information available on controlled trials. The potential remains for the active selection of tree varieties for resistance to nematodes, since it is noted that resistance of wheat to the cereal-cyst nematode was achieved by selection (70). Campbell (1999) (33) has reviewed the situation in Australasia, claiming that MM.106 (a preferred rootstock for new land) had insufficient vigour for replant soils. She further reported (citing earlier studies) that Northern Spy rootstocks were sensitive to ARD and that M. 12 and M. 793 were reported to be resistant in some regions. The scions of Delicious and Golden Delicious were sensitive relative to Cox or Laxton Superb, although it is noted that sensitivities of scion varieties is also variable. Engel et al., (1994) (48) reported that two-year-old nursery trees growing on M9 rootstock were more vigorous in ARD soil than one-year old grafts or trees from dormant buds, and theses rootstocks were recommended for replant soil at a higher than normal density of 3.0 x 1.0 m. Merton 799 rootstock was reported by Stirling et al. (1995) (49) to be resistant to *Pratylenchus jordanensis* in field experiments at Applethorpe, Queensland.

In areas where *Phytophthora* was a problem, M.793 had more vigour than MM.106 and has been used successfully in NZ, with commercial application in India (Stephen Wilson, School of Agricultural Science, University of Tasmania *pers. comm*). Success has been reported locally with M.7, M.9 and M.26 rootstocks. Because rootstock 793 leads to a large tree cover, a dwarfing interstock variety such as M9 may be added to provide optimal tree size (Stephen Wilson, *pers. comm*).

In the case of ARD attributed to actinomycetes, Szabo (1999) (17) concluded that rootstock selection (five species of *Malus* were examined) had little effect on the severity of the disease, and that there was little chance of solving the problem of ARD by breeding. The possibility of inducing resistance in seedlings to fungi by application of e.g. β-aminobutyric acid, dichloroisonicotinic acid or salicylic acid (71) could be investigated.

5d. Chemical treatments

5d i. Specific Nematicides

Dichloropropene and 1,2-dichloropropane as separate treatments are reported to increase trunk circumference and fruit yield (72) in ARD soils. The mixed **nematicides** (2) (D-D) treatment of a N.Y. soil gave even better effectiveness than a broad-spectrum treatment with steam or chloropicrin, reducing *P. penetrans* populations in roots and soil over a two year period, or (in one study) maintaining good tree growth and yield response over the seven years of monitoring. Investigation of ARD in the Granite Belt of Australia indicated nematodes as an important component of the disease, since the nematicide fenamiphos gave consistently good results (4), as has ethylene dibromide and MeB in nematode-infested soils in Applethorpe, Queensland (49).

Vorlex® [1,3-dichloropropene (1,3-D) plus methyl-isothiocyanate], has been aimed specifically against nematodes. Treated trees in New York were 68-90% larger than untreated trees after two years, with the effectiveness being apparent for six years. Dichloropropene alone was less effective (2).

5d ii. Non-specific (broad-spectrum) chemical treatments or treatments known to be effective against fungal agents of ARD

Prior to 1990 Telone (1,3-D) (**dichloropropene**) was the preferred fumigant in the USA, but this use was suspended in 1990, leading to the takeover by MeB (1). MeB has since become the standard against which other treatments are compared and has invariably been among the most effective chemical control agents tested where these are compared.

The commercial agents **Dazomet®, Basamid®, Vapam® (= metham sodium)** are all agents of **methyl isothiocyanate** release. They are effective once incorporated into soil by drilling, ploughing (15) and wetting. Brown & Schimanski (2002) (13) noted that MITC is active in the aqueous phase and not in the soil air spaces, hence it is essential that the soil be near saturation capacity when the material is used. The occasional reports of failure of this agent may be attributed to insufficient wetting of the soil.

Chloropicrin® is reported to be very effective (2, 72) to outstanding (73) against ARD. The best treatment with this agent was found as a combination of MeB (70%) and chloropicrin 30% (72), a treatment that will not be permitted from 2005 due to the ban on MeB. In this regard, Porter & Mercado (74) have noted that with the phase-out of MeB, alternative fumigants such as **chloropicrin mixed with dichloropropene (Telone C35)**, methyl isothionate products (Basamid and Metham) applied with chloropicrin and new products such as methyl iodide, or propargyl bromide appear set to become important as soil fumigants.

Difenconozole® and **Metalaxyl**® were effective in inhibiting fungal ARD in nine orchards in Washington; **Fludioxinil**® was effective in two soils (5) and a combination of **Metalaxyl + Flutolanil** was effective in one soil (45). Growth improvement of up to 40% was noted for individual fungicide treatments. Although generally effective, resistance to **Metalaxyl**® by one *Pythium* population was observed (5).

Other agents such as calcium cyanimide (53) have been reported to be effective against pathogenic soil fungi and nematodes, but as yet unproven against ARD.

5d iii. Commercial chemicals reported as effective against ARD, either separately or in combination:

Captan® (56)
Carbendazim® (Bavistin®) (28)
Chloropicrin® (2, 72)
Dichloropropane-dichloropropene (2, 72, 73)
dichloropropene + methyl-isothiocyanate (Vorlex®) (2)
Difenconazole® (5, 27)
Ethylene dibromide (49)
Fenamiphos® (4)
Fludioxinil® (5, 27)
Formaldehyde (25, 28, 29, 56, 75)
Fosetyl® as Aliette® (56)
Metalaxyl® (5, 27)
Metalaxyl + Flutolanil (45)
Metham-sodium® (=Vapam®) (1, 15, 73, 76, 77) Reported to give inconsistent results; it is not a true fumigant and is a poor root-penetrant) (1).
MeB (45, 49, 57, 73, 76)
Methyl isothiocyanate (MITC) and related agents (Dazomet®, Basamid®) (13, 15, 72, 73, 77).
Streptomycin (specifically against bacterial infection) (6)
Telone (1,3-D) (dichloropropene) (1)
Telone C-35®, containing 65% 1,3 dichloropropene and 35% chloropicrin (6).

5d iv. Other chemical treatments reported to be effective

Addition of fertilizers: N & P (11, 78); NPK [where the soil was high (pH 8.7) giving a K deficiency] (79); Multiple N-applications as NH_4NO_3 (80); Slow release NPK fertilizers Dynamic Lifter®, Langley® tablets and Neuteboom® (33); MAP, (6, 11, 56, 57, 60, 81, 82, 83, 84, 85). Addition was primarily to soil, but foliar spray is also effective (84). Generally nutrient addition is effective by promoting the general health of the plant, but addition of N and P has also been reported to stimulate biocontrol bacteria in the rhizosphere (78).

Activated charcoal (reducing ethylene content) (57)

Lime in combination with fumigation (60)

Burning of old cherry trees with incorporation of **charcoal** into the soil resulted in 42% increased growth of new trees (trunk circumference) after 7 years relative to controls, or by 46% when nutrients addition was combined with burning/incorporation (2). Since ash has an alkaline pH, its addition may be equivalent to adding lime. Ash has the added advantage of the provision of potassium to a new crop. Temperatures of burning were insufficient to kill nematodes in the soil, leaving as a possible explanation the binding of toxic compounds by charcoal. Other treatments including addition of plant nutrients, nematicide treatment and fertilizer plus nematicide were much less effective.

5e. Physical treatments

Pasteurisation/steam sterilization has been widely used for treatment of ARD, both for field and glasshouse soils (3, 5, 6, 21, 25, 27, 33a, 56, 80, 81, 86). In the Granite Belt of Queensland pasteurisation of soil was more effective than treatment with fenamiphos (anti-nematode), indicating agents other than nematodes to be involved (26).

Air drying (only moderately successful) (25)
Irrigation (59)
Solarisation (45, 49, 72, 87). Although solarisation has been reported to be effective in protecting plants grown in ARD soils after one year (45, 87) it was found by Gur et al. (1991) (72) to be much less effective than double fumigation with chloropicrin followed by MeB, and by Stirling et al., (1995) (49) to be relatively ineffective against nematodes in the field.

5f. Biological control

The biological control of soilborne plant pathogens has received considerable attention in recent years. It has been the subject of a review by various authors in 'Principles and Practice of Managing Soilborne plant pathogens' (Hall, 1996) (88), and has been the topic of papers in plant pathology proceedings such as the Second Australasian Soilborne Disease Symposium in Lorne, Victoria (2001) and the 8th International Congress of Plant Pathology (2003) (89). The mechanisms of bacterial biological control of plant pathogens have also been reviewed by Glick et al. (1999) (90).

Although the biological control of ARD has not been specifically addressed in the literature, it would appear that there are reasonable grounds for future success of such control. Since the broader treatment is beyond this review, a restricted account will be given to demonstrate the potential of biological control of ARD. For example Sarathchandra, et al., (2001) (91), reported a variety of rhizosphere bacteria that control nematode populations; Stewart et al., (2001 (61), reported using *Trichoderma haziarnum* for protection of onions; Kurtboke (2001) (92) reported using actinomycetes against *Botrytis cinerea* and Wakelin, et al. (2001) (93) reported using *Paenobacillus polymyxa* against fungal root rot of peas. Kerry & Evans (1996) (94) in a review of management methods for plant parasitic nematodes give a more cautious appraisal for the biological control of nematodes, noting that although some fungi and bacteria have been commercialised, none has proved successful, with the control being inconsistent or requiring impracticable application rates.

Commercially available agents of biological control of diseases or damage of apples/pome fruits include AQ10® (*Ampelomyces quisqualis*) (USA EPA approved), for treatment of powdery mildew, Aspire® (*Candida oleophila*) and Bio-save 10LP® (*Pseudomonas syringae*) for treatment of *Botrytis* and *Penicillium* attack, BlightBan® (*Ps fluorescens*) for protection against frost damage, and Serenade® (*Bacillus subtilis*) for treatment of early blight, late blight, brown rot and fire blight, and Trichopel® (*Trichoderma hazianum* and *T. viride*) active against a broad range of fungal root pathogens. Essential to the commercial utilization of a biocontrol agent is its survival in storage for significant periods of time. While this aspect has not been examined in relation to agents active against ARD, considerable information is available on carrier formulations for these organisms. For example, Amer & Uthede (2000) (95) reported that *Pseudomonas putida* survived well in vermiculite, kaolin and bacterial broth, although only the first two had no detrimental effect on seedling germination. Storage of effective biocontrol agents that produce spores (especially *Bacillus* spp.) is not a problem, other organisms can be protected using freeze-drying in the presence of e.g. trehalose (5%) or sucrose (10%). Non-fat skimmed milk was found to be the best rehydrating medium, while commercial preparations of fungal hyphae can be made by spray drying in skimmed milk. The demonstration of methods allowing the high-biomass, low-cost growth of agents of biological control in compost holds considerable promise for the future (Ramona and Line, 2001) (96).

5f i. Fungal agents of biological control

Non-mycorrhizal agents of control

Central to this discussion are recent reviews in the book 'The Mycota. A comprehensive treatise on fungi as experimental systems for basic and applied research' Volume 4 (Wicklow & Soderstrom, 1997) (97), with relevant chapters by Jeffries on mycoparasitism (pp 149-164); by Chet, Inbar & Hadar on fungal antagonists and mycoparasites (pp165-184) and by by Kerry & Jaffee on fungi as agents for the control of plant parasitic nematodes (pp203-218). Though these reviews are general rather than specifically related to ARD, the pathogens and antagonists discussed are frequently the same as those relating to ARD. Jefferies reported that the use of mycoparasitism as a method for biological control is in its infancy, although it is suggested that it will become increasingly important in the development of integrated systems of plant disease control. Sometimes the result of mycoparasitism is simply a delayed onset of infection, but this can be sufficient to allow seedlings to overcome the short period of their susceptibility to damping-off diseases. Molecular engineering of various chitinases within a specific parasite has been mooted as having potential for improving plant protection, a procedure that would need to be considered very carefully given the potential for increasing the host range to include unintended fungi that are beneficial to the plant.

A number of nematophagous fungi have shown promise as biocontrol agents, although there is a problem that some countries have banned the release of non-indigenous organisms. The words of Kerry and Jaffee (1997) warrant repeating:

> 'There is still a need for a critical evaluation of the potential of biocontrol agents for plant parasitic nematodes in commercial cropping situations and their integration with existing production systems. Too often, agents have been released without knowledge of their biology and ecology, and their use has been limited because nematode control was often poor and inconsistent. Also, few attempts have been made to monitor the activity of agents after their release and so there is no understanding of the reasons for poor control. … If fungi are to be exploited for nematode control by growers, their selection and development must be based on sound science and their use carefully targeted to ensure commercial interest'. (97 p216).

Mycorrhizal agents of control

Vesicular arbuscular (VA) mycorrhizae are frequently associated with protection of the host plant against pathogen attack. VA mycorrhizae have been shown to have differing effects on the plant/rhizosphere/pathogen relationship, although the relationship is generally reported to be stimulatory to antagonists of plant pathogens and inhibitory to the pathogens. For example, conidial germination of *Trichoderma harzianum* and growth of *Pseudomonas chlororaphis* was stimulated by the presence of *Glomus intraradices* while the growth of the pathogen *Clavibacter michiganensis* and conidial germination of *Fusarium oxysporum* was reduced (98). Undoubtedly differing results reported on interactions between VA fungi and other microorganisms can be attributed to differing VA fungi and soil conditions (98). Apple rootstock generally spend one year in a stool bed and one year in a nursery bed prior to transplanting in the orchard, where plants are known to be generally mycorrhizal. Therefore Gamiet and Berch (1987) (99) examined the mycorrhizal status of rootstocks from stool or nursery beds, to find that between 3% (MM 106) and 34% (M 2) of plants from stool beds had been colonized by VAM, the colonization being dependent on type of rootstock (the lowest incidence being for MM 106, the highest for M 2 and M 7 rootstocks).

Assessment of mycorrhizae as agents of biological control of pathogens is confounded by their beneficial effects on apple seedlings resulting from the increased uptake of phosphorus, calcium, magnesium, zinc and copper (100). In a study on the effects of phosphate fertilization and inoculation of apple and plum rootstocks, Fortuna *et al.* (1996) (101) concluded that mycorrhizal

inoculation could overcome blocked apical growth and allowed for reduced chemical inputs, especially of P. Likewise Mei *et al.* (2001) (102) showed that VAM infection significantly increased apple seedling growth and P content, although curiously when P was applied, Fe content of seedlings decreased while Zn and Cu levels increased. Such benefit may be limited to P-deficient soils, since Gnekow & Marschner (1989) (103) reported that apple plants (one year old, M26) inoculated with *Glomus macrocarpon* had significantly increased growth only at the lowest rate of P application (20mg P/kg soil). However it was conceded that in high-P soils the plants may profit from the uptake of Zn and Cu. Likewise, *Glomus intraradices* alone or in combination with other agents was reported to be beneficial to apple growth and yield in British Columbian orchards for over six years (104). The optimal root length of apple rootstock M.25 for effective infection by *Glomus* strain A6 (*in vitro*) was reported to be 0.1-1.5cm, corresponding to the beginning of root elongation and such inoculation was claimed to enhancement of both the growth and survival of plants after transplantation to the field (105).

Despite the above reports of non-specific benefits of VA mycorrhizal inocula, there are a number of reports that these mycorrhizae will also directly antagonize pathogens associated with ARD. An inoculum of *G. fasciculatum* or *G. macrocarpum* has been suggested to be inhibitory to nematodes in apple replant soils (75), depending on soil type. Similarly *Glomus mosseae* has been reported to inhibit *Pratylenchus vulnus* attack of apple rootstock in glasshouse investigations. Root colonisation by *G. mosseae* was not affected by the presence of nematodes and addition of phosphate to non-mycorrhizal controls had little effect on plant growth, eliminating this element as a factor in the plant health (106). The abundance of nitrogen fixing *Azospirillum* (which likewise showed antagonism against fungal pathogens) also increased in the presence of the *Glomus* spp., leading to the suggestion that the colony-forming unit ratio of nitrogen-fixing bacteria to that of phytotoxic fungi could be used as an indicator of degree of ARD and that the use of some VAM fungi could replace chemical treatment of soil against ARD (39).

Glomus sp. D13 and *G. intraradices* were reported as effective in increasing the total shoot length and the number of shoots per rootstock planted in ARD-soil (107). It is also reported that *Glomus etunicatum* was successful in inhibiting ARD in the first six months of growth, but only when apple seedlings were grown for the first three weeks in a sterile substrate (sand-soil-peat) (108). Protection of plants inoculated with VA mycorrhizae and placed directly into ARD soil was negligible after a six-month period.

5f ii. Bacterial agents of biocontrol

There have been a number of promising reports of biological control of ARD using bacterial inocula, although some come from greenhouse trials (5, 30, 78). These however provide useful preliminary assistance for screening purposes prior to field trials of the most effective agents.

Glasshouse trials …

Pseudomonas putida and *Ps fluorescens* were found to be effective in protecting seedlings previously soaked in these bacterial inocula before planting in ARD-prone soil (109).
Bacillus subtilis (BACT-1 & EBW-4) applied as a soil drench increased apple (McIntosh seedlings) plant height in pasteurised, fertilized (esp. P) ARD soil (30).
Agrobacterium radiobacter was found to reduce plant death and improve growth compared with controls, it being suggested that this bacterium changes the composition of the rhizosphere microbiota by outcompeting 'micromycetes' (fungi imperfecti) (110). Catska and Hudska (1993b) (111) further reported that *A. radiobacter* inhibited pathogenic fungi and eliminated ARD both *in vitro* and *in vivo*.

Field trials …
A long-term trial of various treatments (including dip-treating seedlings in suspensions of various biological control agents) has been undertaken in British Columbia, using Gala trees on M. 9 rootstock, planted in ARD soil. *Enterobacter aerogenes* strain B-8 in combination with MAP was effective at one of two sites after one year (alone the bacterium was ineffective), and the *Bacillus subtilis* strains BACT-1, EBW-4 and B-10 were also reported as effective at one of the two sites (Utkhede and Li, 1989 (60). A follow-up of this trial after six years revealed that *Glomus intraradices* and the *combination* of *Enterobacter strain* B-8 (now identified as *E. agglomerans*) and EBW-4 had continued to significantly increase fruit yield, tree trunk growth and reduce infection by *Phytophthora cactorum* and *Pythium ultimum* (104).

In a further trial of McIntosh trees on M. 26 rootstock using various agents of biocontrol of ARD, 9/23 strains of *B. subtilis* isolates were reported to be effective after three years (81), but only *B. subtilis* strain EBW4 was consistently reported to be effective after three years (Utkhede (1993) (112) and five years (113) of trial. The combination of this bacterium with metham-sodium fumigation, peat or NPK fertilizers were also effective, but not as consistently as the application of the bacterium alone. After five years, none of the bacterial agents was found to be effective, while *B. subtilis* B-10 in combination with MAP actually reduced young tree growth and yield (Utkhede and Smith, 1993 (82). *B. subtilis* EBW4 alone was again found effective in increasing the growth of Jonagold on M. 9 rootstock planted in replant soil from an old cherry orchard after three years of field trial (114).

Field trials for the control of plant pathogenic nematodes (not necessarily those associated with ARD) have been proposed using the bacterium *Pasteuria penetrans*, with a focus on integrated control using *P. penetrans* in combination with crop rotation, cover cropping, specific soil amendments and selection for host resistance (115). However its integrated use with other means of control appears essential for success, and its commercial development will depend on the development of a simple method of mass production (94).

5f iii. Insect agents of biocontrol
The mite *Tyrophagus putrescentiae* will graze preferentially on some pathogenic fungi allowing increased growth of beneficial nitrogen-fixing *Azospirillum*. Inoculation of apple seedlings with mites under glasshouse conditions (together with *Penicillium claviforme*) prevented experimental induction of ARD (31).

5g. Integrated control
Unfortunately the term ‘integrated control’ means different things to different agencies; to those involved with chemical treatments, it means combining different chemicals, perhaps at different times to obtain optimal pathogen control, to biologists it generally means the combining of biological with selected or reduced chemical control. The use of integrated management of ARD in the latter is mentioned in a number of the above reports, and its use in other plant diseases is noted (61, 116).

***6. Ineffective* treatments or treatments that stimulate the disease agents**

Ineffective treatments could be attributable to target biota being different in different soils and that some treatments could have been applied at inappropriate rates or times. It is noted however that statistically it is not possible to demonstrate ‘no effect’ and any such conclusion based on

tree growth is limited by the conduct of the trial, with confounding factors being e.g. weed competition or crop management deficiencies.

Reports of ineffective treatments …
Ammonium nitrate (117)
Ammonium phosphate (87, 117)
Bacillus subtilis BACT-1 and B-10 biological control agents after two years in the field (82)
Basamid (33)
Brassica campestris (50) (populations of both *P. penetrans* nematodes and *Pythium* fungi were substantially *increased* following treatment)
Burning of trees on the plantation site (ineffective against nematodes) (2)
Chloramphenicol (17)
Composts (three types tested as 1:4 mixes in soil) (47)
Difenconazole (against fungal pathogens) (45)
Enterobacter aerogenes B-8 (60) (a potential biological control agent, after two years in the field)
Fallowing land for two years (11) (three years was effective)
Formalin (33)
High N levels (33)
Humic acid (45)
Magnesium limestone (56)
Mancozeb (112)
Metam-sodium (112)
Microjet and drip irrigation cf. sprinkler irrigation (117)
1-naphthylacetic acid and benzylaminopurine (42), both of which *promoted* infection by actinomycetes
Nematicides (6, 16)
NPK fertilizer (56, 112)
Peat (112)
Phosphite addition (5a)
*Pseudomonas putida*2C8 (45), a biological control agent against fungal pathogens, assessed after three years in the field
Shirlan, metalaxyl and thiram (6)
Trichoderma harzianum (Rootshield®) after three years in the field (45)
Trichoderma sp. in the field (6) (a strain showing promise in pot trials)
Inter-row planting of trees (72) (rather than planting on the site of uprooted trees).

7. Evolving research methods and prospects for further study

Mai et al., (1994) (2) noted that trends of research on ARD have been towards alternatives to chemical nematicides and broad-spectrum biocides, such as development of resistant rootstocks, biological control measures, nematode-resistant cover crops and the integration of ARD controls within comprehensive management strategies. Little has changed in these priorities to the present time, except that they are placed against a backdrop of increased environmental concerns relating to continued chemical use of biocides on agricultural crops.

Relatively new methodologies such as fluorescent antibody techniques, *in-situ* hybridization, Randomly Amplified Polymorphic DNA (RAPD) analysis, or targeted PCR fingerprinting (118) could readily be applied to the present topic, particularly allowing the detection of pathogens or the tracking of biological control agents released to the field (119). While not specifically

relating to ARD, Mukerji et al (1999) (120) discusses innovative concepts including microbial agents of weed and insect control, use of mycorrhizae in the control of plant pathogens, protoplast fusion and the role of tissue culture in disease control, genetic manipulation of antagonistic *Fusarium* spp. and application of lux-gene technology in disease control. It is noted that molecular- or fluorescent-tagged probes are commercially available against some of the fungi implicated in ARD as well as against *Pratylenchus* spp (121, 122, 123). These could be very useful for rapid disease-risk assessment, which is seen to be an important aspect in ARD. The substrate-induced respiration assay, mentioned by Herdina et al. (2001) (124) as a good indicator of disease incidence is simple, sensitive and rapid.

The possibility of producing VAM fungal inocula (*Glomus etunicatum, G. intraradices* and other *Glomus spp*) using an aeroponic system [living roots plus nutrients (125)] or on glass beads (126) also provides the potential for large-scale production of beneficial VAM. In this regard, germination of the VAM spores (*Glomus mosseae*) has been obtained on sphagnum peat, composted pine bark and composted olive pumice resulting in subsequent host colonization (127).

8. Educational packages

A package on the biological and physical factors associated with ARD in Washington State was developed as one of a variety of teaching methods for growers having different backgrounds (128). Resulting from the dissemination of these packages was an improved acceptance of soil fumigation as a management tool, easier introduction of fumigants other than MeB, increased recognition of soil physical, chemical and moisture problems, reduced reliance on seedling rootstocks with an increased use of dwarfing precocious rootstocks, better apple tree growth and production in ARD soils and a more optimistic view of sustainability in older orchard districts (128).

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Experimental Results and Discussion

1. GLASSHOUSE ASSESSMENT OF SELECTED TREATMENTS

Introduction

A review of the literature, including previous work undertaken in Tasmanian orchards, indicated several treatments of ARD deserving investigation in the glasshouse as a prelude to selection of treatments to assess in a field trial.

Methods in common to all pot trials

Topsoil (0 – 15 cm) used in all of the pot trials was collected from a recently grubbed orchard at the Grove Research Station in southern Tasmania. This area had been planted with a mixed variety orchard for the previous 15 years. The soil was a low fertility duplex, originally classified as Huon Loam and currently classified as a brown sodsol (Wilson, et al. 2004). Field trials were subsequently conducted at the same location. Plant material was removed from the soil using a coarse sieve and the soil was then mixed with perlite 70:30 v/v to provide an air filled porosity of approximately 13% (Australian Standard AS 3743, 1996). A sample of soil/perlite mix was pasteurised at 58 $^{\circ}$C (54 – 66 $^{\circ}$C range) for 40 minutes and then fan cooled for 20 minutes to provide a control non-ARD treatment. A further unpasteurised sample was included to provide a control ARD treatment.

The rootstocks used in these trails were MM102, MM 106 (a semi-dwarfing variety), or Bud 9 (a dwarfing variety very susceptible to apple replant). Rootstocks were ungrafted and had been lifted from commercial stoolbeds prior to cool-storage for 30 days prior to use. Unless stated otherwise rootstocks were planting one per 3.5L x 20 cm round pots and pruned to 40 cm high immediately after potting.

Irrigation was applied daily using porous mats under pots with a commercial fertilizer solution 'Peters Excel Hi N'. Pest and disease sprays were applied as required. Trunk circumferences were measured 15 cm above soil level at planting and at the end of the first growing season. At the latter time the following measurements were taken: trunk circumference, shoot number, extension growth, leaf number, leaf area and root volume. For the organic matter and Trichopel® trials root hydraulic conductivity was also measured using the method described by Nair (2003). Root and soil samples were collected at the termination of the trial to isolate potential biocontrol agents. Statistical analysis was carried out using the General Linear Models package in SPSS, following an arcsine square root transformation of data. Least significant differences were calculated using the method of Steel and Torrie (1981). Unless otherwise noted significant differences are at $P < 0.05$.

1.1 Fumigation Trial

A trial was undertaken of four commercial fumigants of interest and two control treatments, with five replicates of each. Treatments were:

Nil treatment control
Telone C35® at 50g/m^3 (8 ml/30L soil)
MeB at 500g/m^3 (7 ml/30L soil)
Chloropicrin at 50g/m^3 (8 ml/30L soil)
Metham® at 630 ml/1,000L (20 ml/30L soil)
Steam pasteurisation control.

Fumigation was conducted on the 19th October 2001; 30L quantities of soil were treated in plastic bags then sealed and stored at ambient air temperatures of 11°C – 27°C. The treatment with Telone C35® was kept sealed for one week, all other treatments were sealed for three days. All treatments were left open to the air for a minimum of one week before use. Treatments were arranged in a randomised block design. The trial was planted on the 1st November 2001 and harvested on the 19th April 2002.

Results and Discussion

The results of this trial are given in Table 1.1.1 and soil analyses after treatments but before planting are given in the Appendix (Table 1.1.1a). Fumigation with MeB, Telone C35® and chloropicrin all produced significant increases in extension growth relative to untreated controls, while Telone C35® and chloropicrin also produced significant ($P < 0.015$) increases in cross sectional area. MeB, Metham® and steam pasteurisation produced neither significant increases in extension growth or cross sectional area.

Table 1.1.1 Assessment of the relative effectiveness of fumigants against ARD. Root:shoot ratio figures are root volume to leaf area, TCSA is trunk cross sectional area.

Treatment	Extension Growth (mm)	Leaf Number	Leaf Area (cm^2)	Average Leaf Area (cm^2)	Root Dry Wt (g)	Root Volume (ml)	Root:shoot (cm)	TCSA % Increase
Control	775	58.2	597	10.29	17.4	36.4	0.06	17.6
Telone C35®	1,130	75	912	13.41	23.2	55.1	0.06	55.9
Methyl Bromide	1,118	86.2	863	10.65	28.8	58.8	0.07	32.7
Chloropicrin	1,168	78.4	813	10.7	30.1	60.6	0.07	59.9
Metham	670	47	543	11.79	19.4	38	0.07	34.2
Pasteurisation	912	61	646	11.19	24.5	46.2	0.07	35.9
LSD	320.5	21.9	ns	ns	5.61	15.3	ns	18.5
P level	0.05	0.039			0.01	0.024		0.015

ns – not significant, LSD – least significant difference at P=0.05, P level – probability level for treatment effects in the ANOVA..

Telone C35® and chloropicrin gave significant protection ($p < 0.05$) against ARD equivalent to MeB in extension growth, leaf number, root dry weight and root volume. Curiously, both the former agents gave significantly better ($p > 0.05$) performances than MeB in percentage increase in cross-sectional area. Metham and steam pasteurisation did not produce significant improvements under any parameter tested relative to the untreated control.

1.2 Organic Matter Trial

A trial was undertaken to assess the effect of different organic matter additions using MM 106 rootstock. Trees used in the trial were planted initially in sterile potting mix, or untreated (ARD) soil and then transplanted to the following treatments applied to ARD soil. The trial comprised seven treatments with five replicates arranged in a randomised block layout. Treatments were: Untreated control with planting initially into untreated ARD soil then transplanted to the same soil. For all other treatments, trees were grown initially in sterile potting mix before transplanting into ARD soil treated as follows: untreated soil, steam pasteurised ARD soil/perlite, ARD Soil/perlite with 10% (v/v) milled dry bark compost, ARD Soil/perlite with 20% (v/v) milled dry bark compost, ARD Soil/perlite with 10% (v/v) fish waste/wood fibre waste compost, ARD Soil/perlite with 20% (v/v) fish waste/wood fibre waste compost Peters Excel Hi N was applied weekly at half strength (0.5g/L), 160 ml per pot. The trial was planted on the 23rd October 2001, transplanted four weeks later and harvested on the 24th April 2002.

Results

The results of this trial are given in Table 1.2.1, soil analyses after treatments but before planting are given in the Appendix (Table 1.2.1a). The hydraulic conductivities and Huber values (Huber value is the ratio of trunk cross sectional area to leaf area and is a measure of the conductive efficiency of the above ground part of the tree) are given in Table 1.2.2. There was a significant increase in both extension growth and trunk cross sectional area increment in response to steam pasteurisation, compared with trees grown in non-pasteurised soil, The initial 4 weeks growth in soil-less conditions did not result in significantly improved growth for trees transplanted to ARD soil without amendment or to soil with 10% fish waste compost. Added organic matter as 10 and 20% peat and 20% fish waste compost, resulted in significantly ($P<0.05$) increased extension growth compared with trees planted direct to ARD soil, but there was no corresponding increase in radial growth. Pasteurisation produced significantly greater radial growth than all other treatments, but extension growth was not significantly greater than in 10 or 20% added peat or 20% added fish waste compost. There were no significant differences between the organic matter amendments.

Table 1.2.1 Effect of various organic amendments on apple growth in ARD soil mixes

Pretreatment	Treatment	Extn Growth	Root vol (ml)	Leaf No.	Leaf area (cm^2)	Shoot no.	% incr in CA	Root:Shoot	Av. Leaf area
Potting mix	**Untreated soil**	700	41	58	400	3.2	16	0.107	7.0
	Pasteurised soil	1000	47	67	681	2.8	51	0.076	11.2
	Soil+10% bark compost	875	40	61	463	2.8	30	0.093	7.6
	Soil+20% bark compost	842	38	63	446	2.4	23	0.096	7.2
	soil+10% fish compost	655	26	51	428	2.4	27	0.070	8.8
	soil+20% fish compost	798	26	58	471	2.8	22	0.059	8.3
Untreated soil	**Untreated soil**	538	43	41	303	2.6	20	0.142	7.5
	LSD	217	ns	ns	105	ns	12	0.043	ns
	P level	0.038			0.001		0.002	0.03	

ns – not significant, LSD – least significant difference, P level – probability level.

There was no change in either root hydraulic conductivity or root leaf specific conductivity but the two higher organic matter treatments and the pasteurised soil resulted in significantly higher Huber values compared with trees grown in ARD soil throughout.

Table 1.2.2 Assessment of the effects of organic matter addition on hydraulic conductivity of apple rootstock

Treatment	Root Conductivity (mmoles/ MPa/min)	Leaf-Specific Conductivity (mmoles mm^2/ MPa/min)	Huber value
Potting mix Untreated soil	8.88	0.02	0.229
Pasteurised soil	4.22	0.01	0.222
Soil+10% bark compost	5.76	0.02	0.197
Soil+20% bark compost	3.64	0.01	0.212
Soil+10% fish compost	7.04	0.02	0.179
Soil+20% fish compost			0.278
Untreated soil	8.79	0.02	0.153
LSD	ns	ns	0.048
P level			0.008

ns – not significant, LSD – least significant difference, P level – probability level.

Of all treatments in the trial, the direct to soil treatment is closest to a field planting into ARD soil, with some possible further setback with transplanting at week 4. Remaining treatments all allowed new growth to start under favourable conditions before transplanting to a mix containing ARD or steam pasteurised soil. All treatments except non-pasteurised soil alone and soil plus 10% fish waste compost, produced significantly longer extension growth, with no significant differences between effective treatments. The results suggest that an initial period of 4 weeks growth, without exposure to ARD, is not sufficient to overcome the influence of ARD on subsequent transplanting. However, significant differences between the direct to soil treatment and three of the organic matter treatments suggest some advantages for organic matter amendment as suggested in the review by Traquair (1984) and others. Failure of these treatments to give any advantage compared with the control (growth in potting mix then transferred to ARD soil) suggests limited value for organic matter amendment with this soil unless initial growth can be established without contact with the disease carrying soil. This observation agrees with field trials in which organic matter amendment seems to be most effective when added to the planting hole rather than incorporation into the topsoil. It is notable that there were no significant differences in extension growth between pasteurised soil and the best organic matter treatments, but radial growth was markedly greater in the pasteurised soil than in all other treatments. Further, radial growth in the direct to soil treatment did not differ significantly from any other treatment.

The water relations-results suggest that ARD does not influence water uptake, or alternatively by the end of the first season when these measurements were taken, the tree has adjusted to a reduced root hydraulic conductance. The significant change in Huber value probably reflects the changes in both leaf area and trunk diameter with stronger growing trees rather than any particular change in water uptake capacity.

1.3 Monoammonium Phosphate (MAP) Trial

The trial was factorial with five concentrations of MAP, two soil/perlite mixes and five replicates per treatment. Treatments were arranged in a randomised block design. MAP was added over the range of 0 – 6.0 g/L to pasteurised (non-replant) and non-pasteurised (replant) soil/perlite mix and incorporated by dry mixing into the soil/perlite mix prior to filling pots. The rootstock used was Budagovsky 9. Peters 'Excel Hi N' was applied once every three weeks at half strength (0.5g/L), 160 ml per pot. The trial was planted on the 22nd October 2001 and harvested on the 16th April 2002.

Soil salt levels associated with the highest MAP applications were high and all trees in the two highest MAP levels died before completion of the trial. For statistical analysis of growth data on the surviving trees, the number of MAP treatments was reduced to three.

In these two greenhouse trials, there was a significant increase in both extension growth and trunk cross sectional area increment in response to steam pasteurisation, compared with trees grown in non-pasteurised soil, as shown in Tables 1 and 2.
There was no interaction ($P>0.05$) between pasteurisation and MAP addition for either extension or radial growth. The two added MAP treatments resulted in significant ($P<0.01$) increases in trunk cross sectional area, but there was no difference between the two rates of MAP application. There was a similar ($P=0.02$) increase in extension growth at 1 g/l MAP, but 2 g/l was not effective and there was no significant difference between the two MAP treatments.

Death of trees in the two highest MAP application rate treatments appears to have been due to salt injury. Salt levels in these treatments were above the threshold electrical conductivity of 1.5dS/m for normal tree growth suggested by Noble and West (1989). Statistical analysis of the surviving treatments failed to show an interaction between MAP application rate and pasteurisation, confirming that newly planted trees respond to MAP in both replant and non-replant situations. The overall growth responses to MAP were an increase of around 35% in extension growth and an almost threefold increase in radial growth.

These results differ from those of Schupp and Moran (2002) who found no significant response to MAP in a field trial on an old orchard soil of unstated ARD status. However, Neilsen and Yorsten (1991) found a significant increase (about 40%) in trunk cross sectional area in response to MAP in the second year of a field trial, but extension growth was not recorded. While there is no obvious explanation for much greater responses in the current trial, clearly there would be a significant practical benefit if a similar response occurred in field plantings into both ARD and non-ARD soils.

1.4 Assessment of relative resistance of two rootstocks to ARD

Both rootstocks MM 106 and M 9 have been reported to be relatively susceptible to ARD in Australia and both are recommended rootstocks in some locations. The dwarfing rootstock M 9 is increasingly popular giving a small, manageable tree size whereas MM 106 is a medium dwarfing rootstock with a faster growth rate giving a larger tree size. Hence it was of interest to

compare these rootstocks in ARD soil in the presence of one of the effective agents (MAP) found to ameliorate ARD in the previous trial. The trial was a factorial design using two rootstocks, MM 106 and M 9, with seven replicates and three soil treatments:

Pasteurised soil with added nitrogen and phosphorous
non-pasteurised soil with added MAP
non-pasteurised soil with added nitrogen and phosphorous

Before planting into posts as described above, nitrogen was applied in the form of ammonium nitrate, phosphate was applied as single-superphosphate, both at rates equivalent to those of the MAP addition at 2.0 g/L of soil.. Pasteurisation was at 55°C for 20 minutes followed by 40 minutes fan cooling. Planting of rootstocks, watering and disease control were as previously described.

Results and Discussion

The results of the trial are given in Tables 1.4.1. There was a significant ($P<0.05$) interaction between rootstock and MAP treatment = the response to MAP addition relative to nil-MAP controls was significant for both rootstocks; in the case of MM 106 extension growth of the controls was 56% that of the MAP treatment, and of M 9, extension growth of the controls was 40% that of the MAP treatment. Hence the beneficial effect of MAP appears to be more pronounced with the very dwarfing M 9 compared with the more vigorous MM 106. The effect of the pasteurisation however was not as expected, with both rootstocks giving results that were not significantly different from their unpasteurised/non-MAP counterparts. This indicates that the pasteurisation treatment was only partially effective, with a significant level of ARD persisting following the steam treatment. Such partial treatment is common where bulky materials are treated, with steam not effectively penetrating the entire mass for sufficient time to eliminate pathogens. Alternatively, it may also indicate the apparent efficiency with which nitrogen and phosphorous are utilised when fertilizer is applied as MAP. There have been several reports in the literature that in non-ARD soils plant growth responses were greater than when the two elements were applied in different compounds.

Table 1.4.1. Extension Growth of different rootstocks with or without MAP addition[a]

Treatment	Extension growth-mm
MM 106 - pasteurised	1205.7
MM 106 - MAP	1847.9
MM 106 - No MAP	1040.1
M 9 - pasteurised	505.9
M 9 - MAP	718.6
M 9 - No MAP	288.7

LSD=360.2

1.5 Trial of Trichopel® and Trichoflow® as agents of protection against ARD

The trial used MM102 rootstock pruned to 25cm at planting. The treatment design was a factorial with six Trichopel® treatments by three concentrations of organic matter.

The Trichopel® treatments were:

(1) Untreated control
(2) Steam pasteurisation

(3) Trichopel® incorporated in into the soil/perlite mix at5g per pot at planting
(4) As for treatment (3) plus pre-planting drench at 5g in 1L per pot with Trichflow®
(5) As for (4) plus mid-season drench at 5g in 0.5L per pot 12 weeks, 5 days after planting.
(6) Plants that had been grown for four weeks in a composted pine bark:sand 70:30 mix, before transplanting into soil/perlite and peat mixes with Trichopel® incorporated at 5g per pot at planting only.

For the organic matter treatments, the perlite (see above) concentration was reduced and replaced with a corresponding volume of dried milled peat moss as follows:
(1) Soil/perlite mix with no peat moss added
(2) Soil/perlite mix with10% v/v moss added
(3) Soil/perlite mix with 20% v/v peat moss added.

Peters Excel Hi N was applied at half strength (0.5g/L), 160 ml per pot, treatments were arranged in a randomised block layout. The trial was planted on the 13th November 2001 and harvested on the 1st May 2002.

There was no interaction ($P<0.05$) between the organic matter and Trichopel® treatments and results for the two factors are given in Table 1.5.1, Results of hydraulic conductivity under varying conditions are given in Table 1.5.2. The treatments 'Trichopel® at planting + pre-drench', and 'Trichopel® at planting + pre-drench + mid season drench' both produced significant increases in extension growth over the control treatment. Trichopel® at planting + pre-drench also produced significant increase in growth as measured by increase in cross sectional area. Peatmoss addition did not produce significant changes in any of the major growth parameters. There was however a significant change in the percent increase in trunk cross-sectional area with the highest peat moss application resulting in an 80% increase in TCSA, compared with 50% for the control. Root hydraulic conductivity measurements were made on selected treatments, and there was a significant increase in root hydraulic conductivity from 3.25 mM/Mpa/min for the control to 4.9 for the steam pasteurised treatment and 4.3 for the two organic matter amendments. This change was not however reflected in the leaf specific conductivity which did not change with treatment. Consequently, the conductivity change almost certainly reflects the sum of several aspects of morphology, which individually have not been statistically significant, rather than any particular physiological effect. To conclude that there is any direct ARD effect on water relations of the tree, leaf specific conductivity would need to be reduced resulting in increased susceptibility to water stress. Differences in overall root conductivity are more indicative of changes in root volume or root to shoot ratio than any alteration in root function.

Table 1.5.1 Effect of various Trichopel®/ Trichoflow® amendments on apple growth in ARD soil mixes. See text for treatment details.

Treatment	Extension Growth (mm)	Shoot Number	Leaf Number	Leaf Area (cm^2)	Average Leaf Area (cm^2)	Root Dry Weight (g)	Root Volume (ml)	Root:shoot ratio	Initial Cross Sectional Area (mm^2)	Final Cross Sectional Area (mm^2)	Percentage Increase in Cross Sectional Area
Trichopel® effects											
Untreated control (1)	521	1.66	43.4	294	6.8	10.25	22.5	0.084	52.7	61.3	42.8
Steam pasteurised (2)	830	1.67	56.9	456	8.0	11.87	28.2	0.066	51.3	66.9	75.8
Trichopel® (3)	679	1.8	52.1	374	7.2	12.2	27	0.075	52.8	68.5	58.7
Trichopel® (4)	754	1.66	55.5	448	8.1	14.7	33.4	0.076	51.7	70.5	83.6
Trichopel® (5)	795	1.73	56	476	8.5	13.8	33.1	0.072	52.2	70.2	80.3
Pre-treatment + Trichopel® (6)	638	2.01	53.2	356	6.7	11.5	24.3	0.074	54.1	67.6	63.7
LSD	190.9	ns	ns	99.3	ns	3.4	10.3	ns	ns	9.3	39.0
P level	0.001			0.001		0.004	0.013			0.037	0.023
Organic matter effects											
Soil only (1)	710	1.77	53.4	422	7.9	12.5	25.9	0.064	53.4	65.4	51.6
Peatmoss 10 % (2)	681	1.84	53	378	7.1	12	27.5	0.08	51.1	67.3	69.6
Peatmoss 20 % (3)	717	1.66	52.1	403	7.7	12.7	30.9	0.079	52.9	69.8	81.2
LSD	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	5.16
P level											0.008

LSD – least significant difference at P=0.05, P level – probability level for treatment effects in the ANOVA.

1.6 Supplementary trials of Trichopel® and Trichoflow® against other commercial agents of potential ARD control

Two trials were conducted to evaluate a range of potential soil treatments on the growth of apple rootstocks. The soil used was the same as described previously.

Trial 1 had 11 treatments with 6 replicates arranged in a randomised block design. Treatments were:

(1) Trichopel® at 5g/pot and Trichoflow® at 5g/pot as a drench applied pre-planting
(2) Companion® a commercial strain of *Bacillus subtilis* applied as a pre-plant drench at 13ml/10L water equivalent to the commercial rate
(3) Mono-ammonium phosphate pre-mixed with the soil at 2g/L soil,
(4) Basamid® mixed into the soil at 7g/30L soil sealed in a plastic bag for 5 days, then aerated for 36 days prior to planting
(5) MeB applied at 7ml/30L soil equivalent to the commercial application rate, sealed in a plastic bag for 1 day then aerated for 35 days
(6) Perlka® applied at 12g/30L soil equivalent to the commercial application rate of 300 – 400 kg/ha, sealed in a plastic bag for 1 day then aerated for 37 days
(7) Untreated soil
(8) Compost applied at 20% by volume
(9) Trichopel® pre-cultured in a wood fibre waste:spent barley 80:20 mix, the spent grain provided by Cascade Brewery, Hobart, Tasmania; the mix was added to the soil at 20% by volume
(10) Locally-isolated biocontrol *Trichoderma* cultured as per treatment (9) and applied at 20% by volume
(11) Wood fibre:spent barley 80:20 mix applied alone at 20% by volume.

Apple rootstocks MM 106 were planted after soil treatments, one per 3.5L round 20 cm pot for all treatments, with rootstocks trimmed to 40 cm in height. Pots were placed on capillary matting and irrigation applied twice daily. Hoaglands solution (50% concentration) was applied on nine occasions over a 20-week growing season. Pest and disease control sprays were applied as required. At the completion of the trial, extension growth, root volume, root dry weight and shoot dry weight were measured and the results analysed using ANOVA General Linear Models package in SPSS.

Results

The results are given in Tables 1.6.1. Supplementation of 20% spent barley (and 20% spent barley in which was cultivated a locally-isolated *Trichoderma*) gave significantly better extension growth than Trichopel® provided under the same conditions, or any of the chemical or fumigant treatments. The Trichopel® plus Trichoflow® treatment and the Companion® treatments were the least effective against ARD in terms of shoot-extension, root volume, root dry weight and shoot dry weight, and the results obtained for these treatments were not significantly different from Perlka® or the untreated control treatments. MeB, Basamid® and MAP produced results comparable with organic matter addition but there was a notable change in morphology. In particular, MAP produced lower root volumes and hence root to shoot ratios than other treatments producing similar top growth.

Table 1.6.1 Relative effectiveness of fumigant and other potential treatments of ARD

Treatment	Extension growth (mm)	Root volume (ml)	Root Dry Weight (g)	Shoot Dry Weight (g)	Root:shoot
Trichopel® + Trichflow®	1,050.0	101.67	22.43	49.72	.35
Companion®	815.8	82.50	18.37	51.40	.31
MAP	1,658.3	155.00	32.35	91.00	.34
Basamid®	1,838.7	200.83	43.00	86.48	.43
Methyl Bromide	1,798.5	208.33	43.78	70.02	.49
Perlka®	1,680.5	163.33	39.02	58.75	.62
Untreated Control	1,135.0	120.00	29.30	51.40	.53
Compost – 20%	1,909.2	167.50	39.15	83.57	.39
Trichopel® in WFW/barley – 20%	1,727.5	190.83	40.05	82.07	.45
***Trichoderma* in WFW/barley – 20%**	2,415.0	202.65	42.85	98.87	.42
WFW/barley – 20%	1,975.0	187.50	40.32	89.58	.44
LSD, (P = 0.05%)	498	53.8	13.1	18.4	

LSD – least significant difference at P=0.05, P level – probability level for treatment effects in the ANOVA.

Discussion

The apparent failure of some of the commercial biocontrol treatments (Trichopel®/ Trichoflow® and Companion®) to overcome the negative effects of ARD was not surprising since these agents were not specifically intended for use against this complex disease profile. Other agents (MAP, Basamid® and Perlka® proved as effective (or better) in this trial as the standard, MeB. The beneficial effects seen for MAP, Basamid® and MeB support previous work (e.g. Brown et al.2000) for these agents, the results for Perlka® are however new. This agent comprises calcium cyanamide, which when wet releases hydrogen cyanamide and is reported to be effective against weeds and soil-borne pathogens including *Sclerotinia*, *Phytophthora* and *Fusarium*. The material is used by vegetable growers in all countries of the EU to overcome the problems of insufficient crop rotation. Since it is a broad-spectrum biocide its efficacy in controlling ARD is easily explicable.

Basamid® has previously been shown effective in countering apple replant providing a sufficient waiting period between treatment and planting is used. The manufacturer recommends a fallow period of between 10 and 30 days depending on temperature, or for early spring planting autumn treatment being recommended (BASF Corporation, 1998). Dr Gordon Brown (pers comm) has suggested an extended fallow period of 2-3 months in the case of ARD treatment. Basamid® has an advantage that it can be applied by growers without the specialised equipment required for

MeB or Telone C35® applications, and could be well suited to treatment of small areas where the use of conventional fumigation equipment may be impractical.

The significant results recorded for treatments containing compost or wood-fibre waste/barley supplements can be clearly attributed to a nutrient effect on growth, the effect of adding *Trichoderma* agents not having a significant effect over the control treatment.

From a perspective of causative agent, the improved growth of trees over controls shown by MAP, Basamid, MeB, Perlka® strongly indicates a biological cause of the disease, although the nature of the causative agent(s) remains unresolved.

2. ASSESSMENT OF SOIL SAMPLE FROM ORCHARDS IN GEOGRAPHICALLY SEPARATE LOCALITIES FOR PATHOGENS AGAINST APPLE SEEDLINGS AND FOR ANTAGONISTS TO THESE PATHOGENS

Introduction

Nematodes have been widely reported to be either a contributory or causative agent of ARD. Investigation of ARD in the Granite Belt of Australia indicated nematodes as an important component of the disease, since the nematicide fenamiphos gave consistently good results. Likewise in a study of Tasmanian orchards, Brown et al. (2000) reported a limited response to nematicides for 40% of the orchards examined, indicating nematodes to be a significant component of ARD. In the Huon Valley region however it was shown that the introduction of the anti-bacterial antibiotic streptomycin to pots was at least as effective as soil sterilization, indicating bacteria to be the primary cause of ARD in Southern Tasmania.

This trial was undertaken in an attempt to isolate root-associated pathogens (fungi, nematodes, bacteria) as well as potential antagonists to these pathogens (bacteria and fungi) from a range of orchards throughout Tasmania.

Methods

Soils

Soil samples were collected from a total of 31 sites of ten orchards in the Huon, Tamar and Spreyton regions of Tasmania and returned to the laboratory for investigation. Soils were stored at 3°C in sealed plastic bags until assessed, normally within one week of their collection.

Estimation of nematode numbers

The Whitehead and Hemming method as described by Hay (pers. comm.) was used. A tray with ornamesh was covered with four layers of tissue paper overlapping at the edges (these edges are sprayed with water to facilitate their binding). Soil (400mL) was then gently crumbled into a beaker and spread thinly over the tissues that were then folded over the soil. The tray was then half filled with water until the surface of the soil becomes slightly wet (with water top-up if necessary to give free water in the tray). The tray was then left at room temperature for two days before removing the soil, tissue and coarse mesh and passing the water + nematodes twice through a 25μ mesh with retrieval of nematodes after each pass. An aliquot of the combined filter-rinse water was used to assess nematode numbers.

Identification of nematodes

Selected (on morphology) nematodes were sent to TIAR NW Centre (Burnie) where identification was kindly performed by Dr Frank Hay.

Results and Discussion

The results of the trial are given in Table 2.1

Table 2.1 The incidence of nematodes in a number of Tasmanian orchards[a]
A total of 31Tasmanian localities representing 12 orchards were assessed for pathogenic nematodes.

Soil	# Nematodes in 2 ml	Bottle Vol (ml)	Nematodes /100 ml Soil	Comments
Kocsis Jonagold planted old orchard	17	23.5	998.75	
Kocsis Jonagold old orchard	14	24	840	10 years replanted
Kocsis orchard site-1, old orchard	0	25.5	0	
Kocsis orchard	15	25	937.5	Small sized nematodes
Kocsis New Jonagold tree	7	25	437.5	
Grove Research Station stoolbed	14	27	945	
Grove research station G 33	10	26	650	
Grove research station Plot G 33	9	26	585	
Grove research station G-14*	19	20.5	973.75	
Grove research station G-14	17	26	1105	
Grove research station	19	10	475	Replanted orchard
Grove research station	5	20	250	Replanted orchard
Grove research station Cherries- 33	3	27.5	206.25	
Grove research station G-12	16	23.5	940	MeB fumigated
Grove research station B-12	2	20	100	Non fumigated
Grove research station BG-12	21	26	1365	Telone fumigated
Grove research station old orchards	7	22.5	393.75	
Squibb Spreyton Cob	3	27.5	206.25	Rootstocks MeB fumigated 5 years prior to planting in 2000
Squibb Spreyton sundowner	1	25	62.5	
Hanson's orchard Hounville	20	27.5	1375	
Chris Steenholt's organic orchard	4	23.5	235	Planted 1year, Pletchey's bay
Chris Steenholt's middle orchard	12	24.5	735	
Chris Steenholt (trees planted)	8	24	480	Pletchen's Bay 2000
Shane Week's Spreyton	3	27.5	206.25	fumigated MeB, replanted 2000
Chris Burn's old orchard, Spreyton	10	25	625	Orchard had been cut down
Chris Burns control	7	26.5	463.75	Adjacent land with no history of apples
Shield's orchard Hounville	14	26	910	
Shield's orchard Huonville	11	25	687.5	Adjacent land with no history of apples
Shield's orchard Huonville	4	25	250	Trees removed 1 year prior to assay
Royal Gala Spreyton	23	15	862.5	
Collin's M-10 rootstock	5	27	337.5	

[a] The orchards were assessed in 2000. None of the nematodes observed were classified as pathogenic. The authors are grateful to Dr Frank Hay, Tasmanian Institute of Agricultural Research, Burnie for the identification of the nematodes reported in this study.

It is reported that as few as 25 to 150 *pathogenic* nematodes/100 cc soil can be damaging to apple trees, depending on soil texture, climate, and additional pathogens (Nyczepir and Halbrendt 1993). Since none of the orchards in this study showed detrimental signs of nematode

attack it was concluded that either the nematodes were non-pathogenic or the trees had developed resistance to the pathogens. *Pratylenchus* sp. were identified from most of the locations given in Fig. 1, and from a pot trial using soil from the Huon region, indicating potential pathogenicity for new orchards, especially in view of the population densities relative to those known to cause disease (no data on density of pathogenic spp). A plant parasitic nematode of the genus *Coslenchus* was also isolated from orchard samples from one orchard in the Huon Valley at levels that might be expected to cause disease. This is not a well-studied genus, but is not known to be a major parasite of plants.

3. ISOLATION OF BACTERIA OR FUNGI SHOWING INHIBITION OF ARD OR PATHOGENIC ACTIVITY AGAINST APPLE SEEDLINGS

Methods
Dominant cultivable isolates from washed apple root-surfaces or from ARD soil obtained as above were isolated by dilution plating onto TSA agar (bacteria) or Sabouraud-Dextrose Agar (fungi):

TSA Agar medium
0.3% Tryptone Soya Broth Powder (Oxoid)
0.1% Yeast Extract (Oxoid)
1.5% Davis Agar

Sabouraud-Dextrose Agar medium
3.0% Sabouraud liquid medium powder (Oxoid)
1.5% Davis Agar

Isolates were obtained from the dilution-plate cultures after incubating for up to 1 week at 22°C, and screened for:

a) Pathogenicity to apple plant seedlings. Seeds of apples obtained from the University of Tasmania Horticulture Centre were germinated, sub-cultured onto moistened blotting paper in large glass petri dishes and heavily inoculated with the isolates under test (five seedlings per microbial isolate). The seeds were maintained under saturated moisture conditions with appropriate controls for three weeks at 22°C before scoring.
b) Protection of radish seedlings against pathogen attack. Radishes were used rather than apple seedlings because no effective apple/pathogen system was found in the course of the investigation that resulted in significant mortality rates. In contrast radish seedling grown either on blotting paper or in standard potting mix were rapidly killed (up to 100% mortality) by *Pythium* inocula following sub-culture from the School of Agricultural Science culture collection. In either case the ability of a bacterial inoculum of between $10^7 – 10^8$ cells/mL (in a saline suspension) to protect the seeds (ten replicates/isolate) after soaking and placing on moist filter paper or in standard potting mix was scored on a zero to five scoring system, zero being given for nil protection, five being given for 100% protection against the pathogen after three weeks in the glasshouse. Seedlings inoculated with pathogen alone or with pathogen plus heat-killed bacterial cultures were used as controls.

Isolates showing antagonism to pathogens were assessed for ferric siderophores as follows: TSA was amended by the inclusion of iron as $FeCl_3$ at concentrations of iron at 100µM, 370µM, 1mM and 10mM before pouring and inoculating with bacterial isolates. Cultures producing ferric siderophores lost their ability to chelate the iron, eliminating the inhibition. Non-chelating cultures were not affected by the iron concentration.

Results
1. Isolation of potential biological control agents
No isolate (fungal or bacterial) obtained from the root surface of apple trees or ARD soil from orchards in any of the localities studied was found to be pathogenic to apple seedlings.
Radish seeds growing in potting mix were more readily protected against pathogen attack than the filter paper assay, the former method therefore being one of choice. Of the 138 cultures of bacteria showing antagonistic activity against at least one of the fungal pathogens were reduced

to 63 after secondary screening for identical colony morphology and other cultural characteristics. As before, none was found to be pathogenic to apple seedlings.

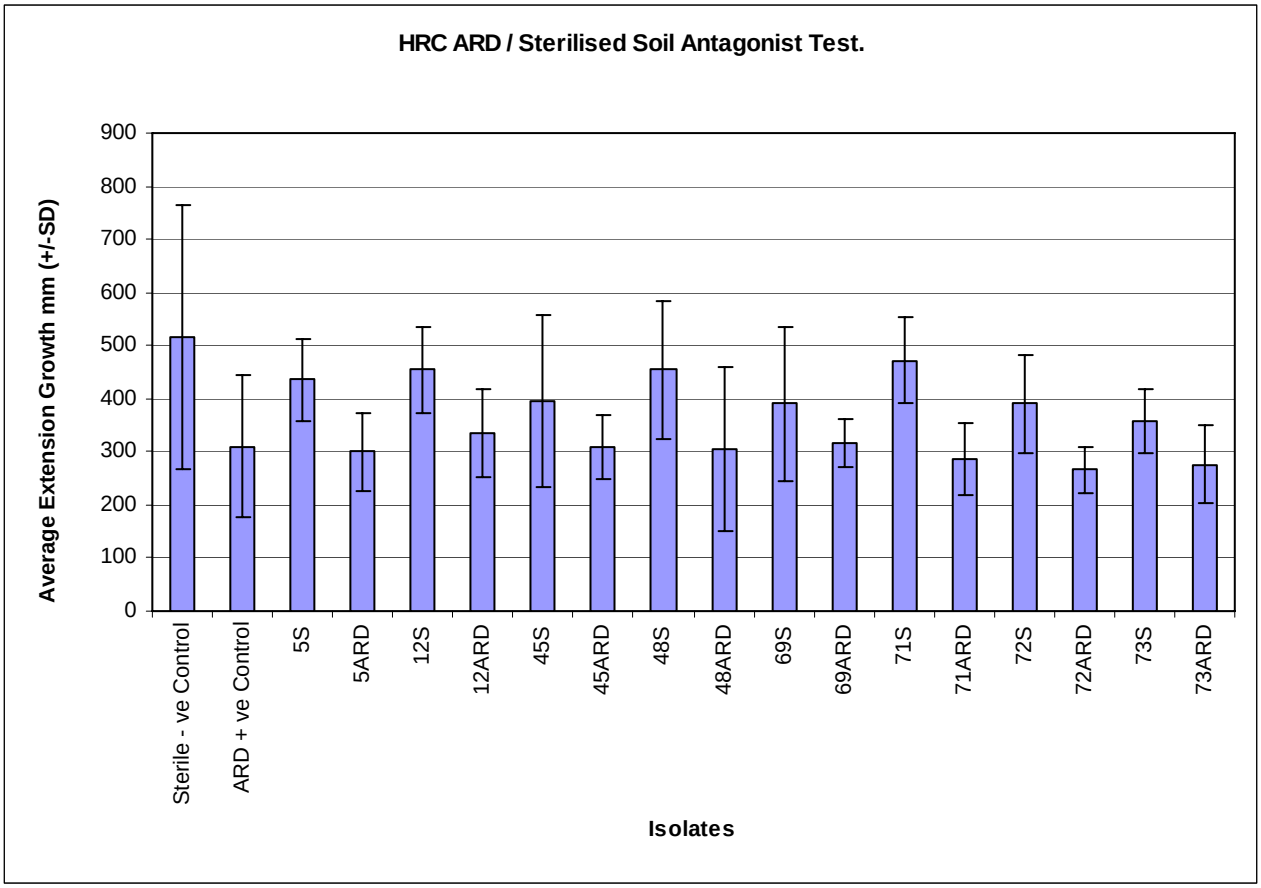
Of the 63 isolates, none gave 100% protection against pathogen challenge, but 12 scored 4/5 (80% protection) and 13 scored 3/5. The best of these were assessed in a pot trial as described below. As well as being inhibitory to the target *Pythium*, many were also inhibitory to other fungal pathogens when assessed on TSA. Active against *Sclerotinia minor*: 45/70 (64%), active against a *Rhizopus* sp.: 5/21 (24%), active against a *Phytophthora* sp.: 15/24 (63%), active against a *Fusarium* sp.; 4/38 (10.5%).

As for the mechanism of antagonism, all showed inhibition of the pathogen on TSA around colonies of the antagonist cultures indicating the production of a water-soluble antimicrobial compound. A total of 14 of 66 isolates tested (21%) showed production of ferric siderophores (an extinction of the inhibitory effect was evident with increasing iron addition to the medium, co-production of antibiotics not being excluded by these bacteria). The remainder presumably were antibiotic-producers but not ferric siderophore producers.

2. Pot trials of potential biocontrol agents against pathogenic attack

From a preliminary screening a number of isolates were found to protect radish seedlings from attack by a range isolates from apple orchards plus a number of known plant pathogens. Pot trials were then undertaken to screen promising isolates (following the *in vitro* assay) for ability to counter replant disease, or to establish other characteristics such as pathogenicity or the production of growth promoters. Shoots of M 9 rootstock were cut to the same length (40 cm) before soaking in washed cell-suspensions of potential biocontrol agents for 24 hours, before potting into ARD soil from the Grove research station. Controls were treated likewise but using heat-killed cultures of the microbial agents.
The results are shown in Figure 1.

Figure 1. Pot trial showing the relative extension growth of apple plants in apple replant disease soil[1].



[1]The sterile control was ARD soil autoclaved prior to the test. The ARD soil was soil from the Grove Research station as described previously. Apple trees (MM 106) were grown as described previously for nine weeks in the glasshouse. Treatments included sterile and untreated ARD controls as well as controls for each of the isolates tested (heat-killed cultures of the isolates assessed and marked 5ARD, 12ARD, 45ARD, 46ARD, 69ARD, 71ARD, 72ARD and 73ARD). Bars show standard errors.

Discussion

The mean heights of plants grown in sterile-soil (525mm) and in ARD-soil from the Grove Research Station (312mm) allowed for little chance of identifying isolates showing significant beneficial effect against ARD, especially given the large error bands of both controls. As a result, only one treatment (71S) was significantly better than its control, (although not significantly better than the ARD control) at $p < 0.05$.

4. GROVE FIELD TRIAL OF POTENTIAL AGENTS OF PROTECTION AGAINST ARD

Introduction

The choice of treatments for a field trial was necessarily limited to those showing significant promise as potential alternatives to MeB from glasshouse trials reported above (MAP, Basamid® (= Dazomet®) Perlka®, 20% compost, those previously reported in the literature as being promising alternatives and not easily assessable in a pot trial (Telone C35®), and potential biological control agents reported in the literature as having promise against ARD (Trichoflow® plus a local *Trichoderma* isolate cultivated in wood-fibre/barley waste). MeB was used as the standard against which all other treatments were compared.

Materials and Methods

The trial was undertaken on a near level, site, which had been planted to a mixed variety orchard and grubbed in winter 2002, at Grove Research Station over the 2002-2003 growing season. The design was a randomised complete blocks with 5 blocks x 14 treatments. There were 8 ungrafted rootstocks (MM 106) lifted from a stoolbed (ie not transplanted after a year in the nursery) per plot. Prior to conducting the trial the location was rotary hoed and graded to form raised beds for each row, with an inter-row spacing of 2 m . Trees were trimmed to 50 cm in length at the time of planting.

Treatments were:

(1) Telone C35® at 50g/m^3
(2) MeB at 500g/m^3
(3) Untreated control
(4) Mono-ammonium phosphate at 2g/L
(5) Trichopel® incorporated in the soil at 5g/L
(6) Perlka incorporated in the soil at *g/L
(7) Basamid® (Dazomet) incorporated in the soil at 40 g/m^2, rotary hoed into the soil and covered with plastic sheeting for 7 days after which the sheeting was removed, soil rotary hoed and left fallow for 32 days,
(8) Perlka® as per treatment (6) and mono-ammonium phosphate at 2g/L
(9) *Bacillus* sp. (GR05) grown in Tryptone Soy broth for 72 hours and added to the soil as a 20% v/v broth/perlite suspension at 100mL/L
(10) A locally-isolated *Trichoderma* cultured in wood fibre:spent barley waste (80:20), the spent grain provided by Cascade Brewery, Hobart, Tasmania added to the soil at 100mL/L
(11) A locally-isolated *Trichoderma* cultured as per treatment (10) and added to the soil at 200mL/L
(12) Wood fibre:spent barley waste as per treatment (10) with no *Trichoderma* amendment and added to the soil at 200mL/L.

Fumigation with Telone C35® and MeB was conducted by a local contractor on 11th November 2002 to allow time for agents used to dissipate prior to planting. The fumigation equipment laid a 2 m wide sheet of plastic sheeting over fumigated soil, covering the edges with soil to contain fumigants. Application of Basamid® was carried out on the 13th November 2002, granules were incorporated into the soil using a rotary hoe, plots were heavily watered, (this followed by a moderate rain) after which the plots were covered with 2 m wide plastic sheeting which was sealed with soil around the edges. The plastic sheeting was removed from all treated plots on the

21st November 2002 (after 8 days) and the plots rotary hoed to increase aeration and dissipation of remaining chemical agents. Soil assays for residual fumigant at weekly intervals (Australian Standards AS3743-1993) showed that planting could take place two weeks after removal of the sealing plastic.

Perlka®, MAP, cultured Trichoderma or Trichoflow® and compost additions were thoroughly mixed with soil, to a depth and diameter of approximately 40 cm around each tree. In the case of the biological control agent *Bacillus subtilis*, plant roots was soaked for 30 minutes in a washed cell-suspension of approximately 10^9 cells/mL prior to planting. Watering was provided by drip irrigation to all plants in the trial.

The trial was planted with MM 106 rootstocks, 90 cm apart, on the 13th December 2002 (22 days after plastic-cover removal). Initial extension and girth measurements were taken on the 19th December 2002. Extension and girth were measured on the 10th June 2003.

Results and Discussion
Results are given in Table 4.1.

Table 4.1 Extension Growth and Increase in Cross Sectional Area of plants in the Grove Field Trial. Figures with the same letter are not significantly different at P=0.05 based on the LSD calculated for arsine square root transformed data

Treatment	Mean extension growth (mm)	Percentage increase in cross sectional area
Telone C35	1,731.0	98.2a
Methyl bromide	1,692.2	80.7a
Control	835.2	26.0b
MAP	787.2	25.6b
Trichopel	881.8	28.0b
Perlka	746.6	31.2b
Basamid	1,012.6	34.8b
Perlka/MAP	788.0	40.5b
Bacillus (GR05)	560.8	17.3b
***Trichoderma* sp.**	878.6	30.7b
Trichopel®	924.6	28.3b
Compost	1,161.2	34.5b
LSD	429.4	

LSD – least significant difference at P=0.05, P level – probability level for treatment effects in the ANOVA

Telone C-35® and MeB treatments resulted in marked improvement in both extension and radial growth compared with the control and all other treatments. There were no significant differences between the control and any other treatments for either measure of tree size.
This study has therefore confirmed Telone C-35® as a viable alternative to MeB (MeBr) for treating apple replant disease in replant orchards. All other treatments including Basamid®, MAP, Trichopel®, Perlka®, Dazomet®, and various biocontrol agents including compost were relatively ineffective, although the poor result for Basamid® contrasts with previous results obtained by Brown & Schimanski (2002) and may possibly be the result of poor application methods or inadequate attention to mixing within the soil profile. It is also noted that MAP was found to be effective in a number of other studies (and was effective in our pot trial and the field trial reported below), and hence it is emphasised that the poor results obtained in the present study could simply be the result of factors other than ARD, such as competition with weeds.

Recommendations

1. The present trial clearly show the equivalence of MeB and Telone C-35® for the field treatment of ARD in both glasshouse and field trials. Converting to Telone C-35® has the advantage of using the same equipment and similar field management as MeB, so contractors and growers are able to begin using it without any big change to current methods. Although Basamid® is an attractive alternative from a practical viewpoint, since it does not presently require a licensed operator for application, it failed to give the consistent control of ARD in the field trial reported here. However Basamid® has previously been found to be effective in field trials where it was applied at least two to three months before planting.

2. The study has not ruled out the potential effectiveness of other treatments which have been found to be effective in other studies, but which gave mixed results in this study (generally showing promise in glasshouse trials but not being competitive in the Grove field trial). These particularly include Basamid® and MAP.

5. FIELD ASSESSMENT OF MONOAMMONIUM PHOSPHATE (MAP), ORGANIC MATTER OR REPLACEMENT SOIL IN EX-APPLE ORCHARD

Materials and methods

The trial was planted at Grove Research Station in southern Tasmania, Australia, on a low fertility duplex soil classified as Huon Loam by Taylor and Stephens (1935), and currently classified as a brown sodosol by Isbell (1996). The area had been planted with a mixed variety orchard for 15 years, prior to the present trial. Soil for the above greenhouse trials was from the same soil classification with a similar planting history.

The old orchard was removed using a grab to lift trees with minimal soil disturbance, and any remaining roots were carefully collected before the tree lines were rotavated. New trees (two local spur bearing red Delicious selections on M 9 rootstocks) were planted at 2.2 m spacing on the old tree lines. This spacing placed each replant tree 1.1 m from an old tree position. Rows were 4.3 m apart.

Treatments were as follows:

(1) Trees planted directly into rotavated soil with superphosphate added at 500kg/ha and ammonium nitrate at 125g/tree as a surface dressing in spring and late summer,
(2) Organic matter as composted pine bark added at a rate of 17 T (dry matter)/ha rotavated into the 0.8 m wide tree line, with ammonium nitrate at 125g/tree as a surface dressing in spring and late summer,
(3) Superphosphate at 500 kg/ha applied to soil with added organic matter
(4) Added organic matter with ammonium nitrate and superphosphate at above rates,
(5) MAP applied at 3.9 t/ha rotavated into the soil,
(6) Replacement of 50 l of soil in the planting hole with similar soil taken from a site which had never been planted to orchard. N and P were also added as in treatment 4. The trial was a randomised complete block of five replicates with six trees of each variety per sub-plot in a split plot design. Measurements were taken on four trees in each sub-plot with the remaining two acting as buffers.

The MAP treatment was approximately 2 g/l of soil and is based on the rate used by Slykhuis and Thomas (1986) assuming a treated area 0.8 m wide and a rotavation depth of 0.2 m. All plots received a base dressing of 200kg/ha of potassium chloride before planting. Rates based on unit area refer to actual soil area to which the treatment was applied, not area of orchard.

Irrigation was from individual drippers and the 0.8 m tree line was maintained essentially weed free using an appropriate herbicide program. Trees were marked for trunk circumference measurement and measured immediately after planting. Extension and radial growth were then measured on unpruned trees at the end of the second growing season after planting. Leaf samples for nutrient analysis were taken in mid summer of the second growing season. Pest and disease control was applied as required and followed normal commercial practice for the region.

Chemical analysis

Soil salinity levels and pH were determined before planting, were determined in a 1:5 soil: water extract. Sodium bicarbonate extractable P and K were estimated using the methods of Colwell (1963). Organic carbon was recorded as loss on ignition. Leaf nitrogen was determined, in a commercial laboratory, using a Kjeldahl digest and colorimetry. K, P Ca, Mg, Na, Mn, B and Fe

were determined on a nitric acid digest using inductively coupled plasma spectrometry in the same laboratory.

Calculations and Statistical analysis
Radial growth was calculated as the increase in trunk cross sectional area expressed as a proportion of initial cross sectional area. An arcsine square root transformation was applied to these data for statistical analysis. Statistical analysis of results was carried out using the General Linear Models package of SPSS. Least significant differences were calculated after the method of Steel and Torrie (1981). Unless noted otherwise, all differences referred to as significant are at $P < 0.05$

Results
There were no significant differences ($P>0.05$) between the two scion varieties (results not shown) and no interactions ($P>0.05$) between variety and soil treatment. MAP and soil replacement increased extension growth significantly ($P<0.05$) compared with all other treatments including the unmodified soil with added N, P and K (Table 1):

Table 1. Nutrient amendment/MAP trial

Treatment	Shoot Growth (mm)	Increase in TCA (% of initial)
NPK (control)	486	22a
Organic matter	536	26ab
Organic matter + N	491	19a
Organic Matter + NPK	544	25ab
MAP + K	885	36bc
Soil + NPK	1073	44c
LSD	313	
P level	0.003	

There was also a significant ($P=0.004$) effect of treatment on radial growth with MAP and replacement soil both resulting in increases compared with the control. Radial growth for these two treatments was not however significantly greater than either organic matter alone or with NPK. There were no significant differences in radial growth between the three organic matter treatments.

Prior to planting, soil pH, electrical conductivity, organic carbon and extractable P and K were similar across all treatments (Table 2). The effects of these treatments on the elemental uptake by trees are shown in Table 3.

Table 2. Physical characteristics of the soils assessed

Treatment	Soil pH	Conductivity	P(mg/Kg)	K (mg/kg)	Carbon (mg/kg)
Orchard soil+N+P	6.5	0.11	117	199	2.5
Organic matter+P	6.6	0.13	125	203	2.5
Organic matter+N	6.6	0.12	111	183	2.3
Organic Matter+N+P	6.6	0.13	100	199	2.4
MAP	6.5	0.12	129	220	2.5
Added soil+N+P	6.7	0.13	107	232	2.4
LSD	ns	ns	ns	ns	ns

Table 3. Effect of different organic/nutrient soil amendments on apple tree elemental uptake

Treatment	Leaf nutrient levels								
	N (weight %)	P (weight %)	K (weight %)	Ca (weight %)	Mg (weight %)	S (weight %)	Mn (ppm)	Fe (ppm)	B (ppm)
Orchard soil+N+P	3.09	0.16	11.2	1.13	0.25	0.18	45	106	37.3
Organic matter+P	2.36	0.26	13.4	0.86	0.21	0.16	31	105	39.9
Organic matter+N	3.08	0.17	12.4	1.08	0.26	0.171	43	105	38.2
Organic Matter+N+P	3.08	0.18	12.9	1.02	0.24	0.182	43	114	40.4
MAP	2.52	0.29	10.7	0.92	0.26	0.163	57	97	33.1
Added soil+N+P	3.08	0.17	13	0.67	0.3	0.178	76	94	29.7
LSD	0.16	0.055	ns	0.148	0.033	0.0125	16.5	10.45	2.94
P level	<.001	<.001		0.005	0.001	0.008	0.001	0.01	<0.001

As shown in Table 3, there were significant ($P<0.005$) effects of treatment on leaf N, P, Ca, Mg, S, B, Fe and Mn. There were no treatment effects on leaf K or the other micronutrients (micronutrient results not shown). Both leaf N and S were significantly reduced by the organic matter alone and MAP treatments, but there were no other treatment differences for either element. Leaf P was significantly higher in the same two treatments, again with no other differences.

Leaf Ca was significantly lower than the control in the organic matter alone and imported soil treatments but there were no other differences. Imported soil also significantly increased leaf Mg and organic matter alone caused a significant reduction compared with all other treatments except organic matter plus NPK. Compared with all other treatments, Fe and B levels were both significantly reduced in the imported soil. MAP treatment also resulted in a significant reduction in leaf B compared with the control and all of the organic matter amendments.

Discussion

Organic matter, fertilizers and MAP were incorporated in about 20 cm depth of soil along the planting line, so that trees in these treatments would have been exposed to ARD from planting. In contrast, imported soil was used to fill a planting hole so that trees in this treatment were not immediately exposed to ARD. Consequently, results generally confirm the results of the two greenhouse trials, with a useful response to added MAP, but no effect of organic matter mixed with ARD soil. Planting into imported soil produced stronger growth, further supporting the view that initial growth in a soil medium free of ARD will result in a major improvement in total growth. Added MAP produced a similar growth response to soil replacement, but there were no other significant treatment responses.

Soil treatment failed to produce significant changes in organic carbon or phosphorous levels in spite of added organic matter and the higher extractable phosphorous level in the MAP treatment. High variability in the phosphorous levels may account for lack of a significant response to MAP, but organic matter levels were similar across all treatments. The lack of a significant change in organic matter, in spite of the added composted pine bark, may explain the failure of the trees to respond.

The range of adequate leaf % N, P and K levels suggested by Reuter and Robinson (1986) were 2.0 to 2.4, 0.21 to 0.30 and 1.6 to 3.0 respectively. Consequently leaf K was excessive in all treatments, with leaf P marginally deficient in all except the MAP and high organic matter plus P and K treatments. Leaf N was adequate or excessive in all treatments but both of the treatments with high P levels also had significantly lower leaf N levels than all other treatments. Low N levels in the organic matter with no added N at planting was probably due to some nitrogen fixation with organic matter breakdown. The MAP treatment had no supplementary N and when samples were taken in the second growing season the trees had probably depleted the available soil N. It is important to note that levels in both of these treatments were still in the “high” range according to Reuter and Robinson (1986), but N added as a surface dressing of ammonium nitrate during the two growing seasons may have resulted in an even greater growth advantage for the MAP treatment.

Thus, while application of MAP with high available P, promoted growth and elevated leaf P to a level described as “high” by Reuter and Robinson (1986), the elevation was above a leaf P level already regarded as only marginal. Further, in the soil replacement treatment which produced strongest growth, leaf P did not differ significantly from the control.

It is notable that in both treatments with a significant increase in leaf P, there was an accompanying reduction in leaf N. Growth responses in these two treatments were significantly different when compared with each other, but only in the MAP treatment was there a significant increase in growth compared with the control. Organic matter, with supplementary P but no N, resulted in increased leaf P and reduced leaf N, but growth was similar to other organic matter treatments and the control. The results suggest an interrelationship between N and P uptake, but in this situation, with no evidence of either element being in a deficiency range, it is not possible to relate this suggestion to tree growth.

Mn levels were in the marginal to adequate range and highest in the imported soil, probably reflecting a higher Mn status for this soil. The higher Mn level in the MAP treatment accords with a similar observation by Neilsen and Yorsten (1991), who attributed the effect to acidification by MAP. In the present trial, pH did not change in response to treatment and there was no correlation between pH and leaf Mn. However, soil pH was only recorded immediately after MAP treatment and a longer-term change may have occurred as suggested by Raese (1998). The lack of any indication of N or P deficiency, failure of the low N with organic matter treatment to suppress growth lower than other higher N treatments, and lack of correlation between N or P levels and growth suggests that neither N nor P nutrition mediate responses to ARD.

The trials confirm the effectiveness of MAP in ameliorating the symptoms of ARD as reported by Neilsen (1994) and others. Myers (1991) reported a direct effect of MAP on soil organic matter, and while the present results do not include a determination of changes in organic matter with time after treatment, the nutrient analysis results do tend to support the view that the effectiveness of MAP may be related more to soil biology than to a direct effect on N or P nutrition of young trees.

The poor response to added organic matter in both the field and greenhouse trials was not encouraging for this soil type. However, results for the greenhouse trial indicated that an initial growth period without exposure to ARD has a marked effect on growth even with subsequent ARD exposure. Similarly, in the field trial, replacement soil in the planting hole resulted in a marked improvement in growth. Thus addition of non-ARD soil in the planting hole may emulate the pre-planting treatment of the greenhouse trial. Failure of trees to consistently respond to incorporated organic matter suggests that reported success with organic matter amendment may simply reflect an isolation of the tree from surrounding soil with organic matter placed in the planting hole. That is, the response may not be associated with any induced change is soil biota, but is more likely to be a physical effect of separating ARD soil from the initial root growth.

Combined the results of these trials suggest that the impact of ARD is in the very early stages of tree growth, possibly just weeks after bud burst. This initial impact appears to promote a shift in tree physiology which then stabilises and continues throughout the life of the orchard. Further work is needed to verify and elucidate this effect, but if it can be confirmed it suggest that in future, management of the physiology of the tree, rather than an ill-defined ‘disease’ complex may be a low impact control method. Options include carefully designed root/inter stock combinations, perhaps involving an idea proposed by Dr Brown to self-prune vigorous rootstocks leaving the preferred growth. Other options may include growth-promoting chemicals including growth substances or further development of the promotive effect of MAP.

Small scale local research is likely to continue on these options, but in the short to medium term, the research has shown that there are effective alternatives to MeB and that non fumigant control using MAP or soil replacement remain effective.

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APPENDIX

Table 1.1.1a Fumigation trial; soil analysis after treatment and before planting

Treatment	pH	Conductivity (dS/m)	Air filled porosity (%)	Water holding capacity (%)
Nil treatment	5.5	0.12	8.4	45
Telone C35	5.5	0.19	8.4	45
Methyl bromide	5.6	0.12	8.4	45
Chloropicrin	5.5	0.24	8.4	45
Metham	5.9	0.15	8.4	45
Steam pasteurisation	5.7	0.11	8.4	45

Table 1.2.1a Organic matter trial; soil analysis after soil treatment but before planting

Treatment	pH	Conductivity (dS/m)	Air filled porosity (%)	Water holding capacity (%)
Untreated control	6.2	0.07	18.9	27.5
Steam pasteurised	6.1	0.09	18.9	27.5
Sand and soil	5.5	0.12	13.2	45.5
Soil + 10% peatmoss	4.6	0.07	18.5	31.9
Soil + 20% peatmoss	3.9	0.12	18.4	36.0
Soil + 10% AFWC	7.2	0.22	21.2	31.3
Soil + 20% AFWC	7.1	0.50	25.1	31.1
Soil + 10% FWC	5.2	0.08	21.8	30.4
Soil + 20% FWC	5.0	0.18	21.9	32.4

OM - Organic matter (milled dry peat), AFWC – Fish waste compost amended with a biocontrol agent *Lysobacter antibiotcus*, FWC – Fish waste compost.

Table 1.3.1a MAP trial; soil analysis after soil treatment before planting

Treatment	pH	Conductivity (dS/m)	Air filled porosity (%)	Water holding capacity (%)
Nil MAP – non pasteurised	5.8	0.34	12.5	47.4
1.0 g/L MAP – non pasteurised	5.2	0.73	12.5	47.4
2.0 g/L MAP – non pasteurised	5.2	1.12	12.5	47.4
4.0 g/L MAP – non pasteurised	5.3	2.00	12.5	47.4
6.0 g/L MAP – non pasteurised	5.2	2.44	12.5	47.4
Nil MAP – pasteurised	5.9	0.030	12.5	47.4
1.0 g/L MAP – pasteurised	5.8	0.39	12.5	47.4
2.0 g/L MAP – pasteurised	5.5	0.93	12.5	47.4
4.0 g/L MAP – pasteurised	5.4	1.54	12.5	47.4
6.0 g/L MAP – pasteurised	5.2	3.01	12.5	47.4

Table 1.5.1a Trichopel® trial soil analysis for organic matter additions before planting

#	Treatment	pH	Conductivity (dS/m)	Air filled Porosity (%)	Water holding capacity (%)
1	Nil peat	6.1	0.09	12.5	47.4
2	10% peat	5.6	0.09	12.6	42.7
3	20% peat	5.3	0.09	13.2	45.6