Improving the reliability and consistency of processing beetroot production

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This report summarises the results of a four-year study investigating yield and quality decline in Australian processing beetroot crops. It provides information on the identification and management of soil-borne fungal diseases of beetroot and identifies prospective beetroot varieties that may be used as alternatives to the varieties currently grown by the industry.

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Improving the Reliability and Consistency of Processing Beetroot Production

(Completion October 2004)

Final Report

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Media Summary

Soilborne fungal diseases threaten the viability of the Australian processing beetroot industry. The disease problems have been exacerbated in recent years because crops are now grown virtually year round, and under environmental conditions that favour disease outbreaks. The long beetroot growing-window has reduced the opportunity for farmers to rotate out of beetroot and if rotations are completed, they are generally only short, which further increases the disease problems.

This work was completed to help the Australian beetroot industry better manage its soilborne diseases and improve the quality and consistency of the product it grows. The problems faced by this industry are not unique to Australia, however nowhere in the world does there seem to be a single strategy that is completely efficacious in controlling soilborne diseases in beet crops and so we anticipated that an effective management strategy will almost certainly involve a combination of tactics. Our work indicates that the most prospective strategy will involve: 1) reducing the length of the growing window so that beets are no longer grown during the periods of highest disease risk, 2) utilising knowledge of disease species at particular sites to make more informed decisions about when blocks should be planted to minimise disease outbreaks, 3) Ensuring that all beet seed is treated with a Rizolex and Apron seed dressing combination prior to planting, and 4) Utilising crop species that are not hosts for the soilborne beet pathogens, as rotation crops (barley and dolichos seemed to be the best for sites with mixed disease infections).

In addition to increasing disease issues, the Australian beetroot industry relies on only a few standard beetroot varieties for its slicing product and only one baby beet variety. In recent years the industry has become concerned that these varieties are no longer providing the high quality, consistent product that the processor and consumer demands. We identified beet varieties that offer promise as alternatives to the current industry standards, and for more efficient production, suggest that the industry would benefit by switching to monogerm beet varieties.
Technical Summary

Soilborne fungal diseases are killing the Australian beetroot industry. Infections of young plants reduce crop stands, and later infections attack developing beets, reducing quality and increasing processing costs. Additionally, this industry currently relies on only 3 slicing type beetroot varieties and 1 baby beet variety and anecdotal evidence suggests that the varieties are no longer meeting the quality requirements demanded by the processor or the consumer. We completed a 4-year study to investigate methods by which the industry might better manage its soilborne diseases and improve the varietal options of the industry.

We completed surveys of beetroot soils in the Lockyer and Fassifern Valleys and found that *Rhizoctonia* and *Pythium* species are the most important soilborne diseases. Furthermore, we determined which pathogens predominated in specific blocks of beetroot using glasshouse soil-indexing bioassays. Additionally, Dr Paul Scott (UQ Gatton) characterised the pathogenic *Pythium* isolates using molecular methods and found three main species: *P. aphanidermatum*, *P. ultimum* and *P. dissotocum*. Pathogenicity studies with the *Pythium* species indicated that disease severity is influenced by both temperature and the age of plants at inoculation. Furthermore, each *Pythium* species produced disease at specific temperature and plant age combinations.

Field and glasshouse fungicide trials showed that a combination seed dressing of Rizolex and Apron reduced disease, and was the best treatment out of more than 30 fungicides that were assessed. For best results, the dressing should be applied to seed as a slurry.

Glasshouse assessments of 22 crop types as alternate hosts for *P. aphanidermatum* and *Rhizoctonia* revealed that barley and dolichos were the least susceptible to infection by either pathogen.

A prospective management strategy for these soilborne diseases might therefore involve a combination of tactics. First, manipulation of planting dates so that sites with high inoculum potential are sown when environmental conditions do not favour the development of disease epidemics. Second, seed dressings with a Rizolex and Apron fungicide combination. Third, planting crops in rotation with beetroot that are poor hosts for the beetroot soilborne pathogens eg. barley, dolichos.

In terms of varietal improvements, we screened more than 90 beet varieties in field trials conducted in all parts of the growing window. We identified some alternatives to the current slicing type varieties and some of these lines are now being grown by this industry. In addition our research suggests that the industry would benefit by growing monogerm beet varieties.
Introduction

The Lockyer and Fassifern valleys of south-east Queensland supply approximately 90% of Australian processed beetroot. In 2003, this region produced 28 700t of slicing beetroot and 2500t of baby beets. There are many factors that stand in the way of profitable, efficient production but an important one for this industry is crop establishment problems and quality reductions due to soil-borne diseases. Golden Circle P/L, the only company now processing beets in Australia, requires a consistent supply of quality raw product from May through December for processing to be completed efficiently and competitively. This has meant that the planting window has been extended so that beetroot are now being grown at times of the year when environmental conditions are not favourable for beetroot production. The extremities of the growing season, February-March and October –December, are typically extremely hot, and are periods of high risk for heavy rain, storm and hail events and losses due to soilborne fungal diseases.

As an additional complication, the number of growers supplying raw product to Golden Circle has been steadily declining in recent years, and now the cannery relies on fewer than 10 growers to supply the entire contract. Since there are fewer suppliers of raw product, each grower is required to plant more area to beets so that the total contract requirement can be met. Many of the growers have insufficient land to meet their contract requirements with the cannery, as well as plant other rotational crops. Consequently, on some farms a monoculture production system exists.

The specialisation of many farms to beetroot, often with short rotations, and the demands by the processor to extend the growing season, have been two critical factors that have led to the prevalence of soil-borne fungal disease issues in this industry. In the past 5 years, soilborne diseases have reduced crop yields and the quality of harvested product dramatically, particularly in crops planted in February-March or those harvesting October-December. Infections of young plants reduce crop stands and later infections attack developing beets, reducing quality and increasing processing costs.

In addition to an increased prevalence of soil-borne diseases, the industry relies on 3 slicing beet varieties (Detroit Dark Red, Garnet and Pablo) and only one baby beet type (New Globe). The beet growers have reported that the quality of Detroit Dark Red, Garnet and New Globe have declined in recent years and they no longer have confidence that these varieties can meet their processing needs. Despite this, no alternative replacement varieties have been identified.

Currently, the Australian beetroot industry is at a crossroad. It can either continue on its current path and risk failing completely, or it can implement changes to its production and processing practices so that its diseases can be better managed and the quality of its raw product improved. To change, members of the industry must have a clear understanding of the causes of the diseases and the management possibilities that are available and which are likely to reduce disease severity. They also must invest in a varietal assessment program so that alternative varieties to the current standard types may be identified. This project commenced in 2000 with the objective of identifying the diseases responsible for the losses and determining practical ways they should be managed, as well as improving varietal options for this industry.
CHAPTER 1: Literature Review

At the start of this research program the project team completed a review of the scientific literature relevant to beet soil-borne diseases and beet production. The purpose of this review was several-fold. First, it allowed the problems facing the Australian beetroot industry to be viewed in an international context. Second, it bought the Australian industry members and the research team up to date with relevant research being conducted in other parts of the world. Third, it provided the project team and the Australian industry with direction when they came to develop their research strategy for managing beet diseases and improving beet quality and production in Australia.

Each member of the Queensland beetroot industry was provided with a copy of the literature review and a similar version of this document has been published in The Australian Journal of Experimental Agriculture (www.publish.csiro.au/journals/ajea) (Martin, 2003).

Management Options for Soil-borne Diseases of Beetroot

1. Introduction

1.1 The Plant

Beetroot (Beta vulgaris var. vulgaris) or table beet, is a member of the Chenopodiaceae plant family, which encompasses a diverse range of both economically important species as well as numerous agricultural weeds. Beta vulgaris L. is the species of greatest economic importance within the genus Beta. This species is further divided into four important cultural types: sugar beet, fodder beet, Swiss chard and beetroot, each of which has been developed for a particular use. These plants are indigenous to the Mediterranean region and western and eastern Europe.

Beta vulgaris is either annual or biennial in its reproductive habit. Seed may be produced in the first year after planting or an extended period (50-120 days) of cool weather may be required to stimulate flowering. Seed is produced in regions with relatively low temperatures (0-10°C). Oregon, Utah and Arizona, in the United States, and France and Italy are important seed producing areas (Whitney and Duffas, 1986). Beet may be either self-sterile or self-fertile, and its seed may be either multigerm or monogerm. Monogerm seed can be planted at regular intervals in a row to produce an even stand.

Beet production, predominantly of sugar beet, is widespread. Beetroot is produced in The United States, The United Kingdom, northern and eastern Europe, Japan and Australia. From an economic standpoint, sugar beet is the most important cultural type and it accounts for the majority of beet production worldwide. For this reason, most of the available literature on beet relates to sugar beet, however much of this information also applies to beetroot as well as the other types.
1.2 The Beetroot Industry in Queensland

Most beetroot in Queensland is grown for processing, with only small areas planted for fresh market production. The Lockyer and Fassifern valleys of south-east Queensland supply approximately 90% of Australian processed beetroot. In 1995, this region produced 32000 t. Processing, carried out by Golden Circle Ltd., carries a high value-added component. The processed product is valued at about $33M pa.

For competitive processing and marketing, a consistent supply of quality beetroot is required from May until December. Efficient field production through high production per unit area and utilisation of the processing plant through an extended harvest period is required to maintain the viability of the Queensland industry.

Inconsistent quality of raw product, particularly at the extremities of the growing season is currently threatening the profitability of the Queensland industry. There are many factors that impede efficient production, but an important one for this industry is pre- and post-emergence losses and beet quality reductions caused by soil-borne diseases (O’Brien, et al., 1998).

2. Soil-borne Diseases of Beetroot

A complex of fungal pathogens is known to be capable of causing root rot disease of beet both in Queensland and in other parts of the world. Early in the season, disease reduces plant stands and later infections attack developing beets, reducing quality and increasing processing costs. Short crop rotations and demands by processors to extend the harvest period into times of high disease risk further exacerbate the problem.

2.1 The Pathogens

In Queensland, three genera of soil-borne fungi *Pythium* spp., *Aphanomyces* cochliloides Dresch. (Hutton and O’Brien, 1986) and *Rhizoctonia solani* Kuehn have been reported as the predominant soil-borne pathogens involved in the beetroot root rot complex. The same fungi have also been recognised as pathogens of beet for many years in other parts of the world. Coons et al. (1946) differentiated acute and chronic phases of this disease complex. In the acute stage, young plants are killed during germination or a week or two after emergence from the soil. The seedlings that survive the initial attack often show disease symptoms on their main or lateral roots that are characteristic of the primary pathogen involved. The symptoms in the later stages of growth are manifestations of the chronic phase.

2.1.1 *Pythium*

The genus *Pythium* contains saprophytic, facultatively parasitic, obligately plant pathogenic, and mycopathogenic taxa (Kato et al., 1990). Pathogenic *Pythium* species are important in both the acute and chronic phases of the disease, causing both pre- and post-emergence damping off problems in beet seedlings as well as root rots in older plants. Worldwide, twelve species of *Pythium* (including *P. ultimum* (Trow), *P. aphanidermatum* (Edson) Fitzp., *P. irregularare, P. debaryanum, P. salpingophorum, P. dissotocum, P. deliense Meurs, P. acanthicum and P. myriotylum Drechs.) are known to attack beets (Windels and Kuznia, 1993). *Pythium ultimum* Trow and *Pythium aphanidermatum* (Edson) Fitzp. are generally considered to be the
most important pathogenic species. Serious losses due to *Pythium* have been reported in Minnesota and North Dakota (Kuznia and Windels, 1993; Brantner and Windels, 1998), Michigan (Mumford, 1968; Johnson and Halloin, 2000), California (Hancock, 1977); Colorado (Ruppel et al., 1988); Arizona (Bretzel et al., 1988); Finland (Vestburg et al., 1982); England (Asher and Payne, 1989); Northern France and Yugoslavia (Asher and Payne, 1989).

In young seedlings *Pythium* spreads quickly from the roots to the stem, which becomes soft and watery before the seedling collapses. Root infection of mature plants causes death of sections of feeder roots. Excessive proliferation of secondary feeder roots may also be indicative of infection by *Pythium* (O’Brien, 1988). Aboveground symptoms also include wilting, yellowing and death of lower leaves (Williams and Duffas, 1986).

*Pythium* spp. are adapted to survive and proliferate in wet soils, particularly when moist conditions prevail for prolonged periods of time. The fungus spreads through the soil via motile zoospores. Thick-walled oospores enable long-term survival of the pathogen. In wet soils, seed treated with fungicides may not be able to withstand high disease pressure, resulting in poor stand establishment. Conditions that delay emergence, such as compacted soil or excessively deep planting, increase the time of exposure of germinating seed to possible infection by *Pythium*.

Relationships between soil temperature, soil moisture and organic matter are critical in the development of *Pythium* epidemics in the field (Hancock, 1977). Species-specific temperature requirements have been demonstrated in several studies (Hancock, 1977; Bretzel et al., 1988; Kuznia and Windels, 1993; Raftoyannis and Dick, 2002). For example, in a field survey in North Dakota and Minnesota, Kunzia and Windels (1993) found that *P. aphanidermatum* caused more seed rot and damping off when soil temperatures increased from 14°C to 31°C, whereas the reverse relationship was observed for *P. ultimum*. Similarly, high soil temperatures (30 to 37°C during July and August) were identified as contributing to the uniformly low levels of *P. ultimum* in field soils of the San Joaquin Valley in California (Hancock, 1977), whereas the onset of disease caused by *P. aphanidermatum* in central Arizona coincided with the occurrence of temperatures of 27°C or more for at least 12 hours per day provided adequate soil moisture was available (Bretzel et al., 1988). *P. aphanidermatum* caused significant damage of sugar beet roots at temperatures as high as 35°C in another recent *in vitro* study (Raftoyannis and Dick, 2002).

The importance of soil moisture for the onset of epidemics was demonstrated in California. Field populations of *P. ultimum* increased substantially at water potentials between –0.3 and –8 bars, whereas no population increases occurred under drier conditions. In this instance the disease increased rapidly in the autumn months (when temperatures were favourable) when moisture was available after irrigation (Hancock, 1977). In a similar study conducted in Arizona, the infection rate of mature sugar beet by *P. aphanidermatum* fell dramatically when the irrigation was cut off, even in the presence of soil temperatures conducive to infection (Bretzel et al., 1988).
2.1.2 *Aphanomyces cochlioides*

*Aphanomyces cochlioides* causes an acute seedling blight and a chronic root rot of beets. This fungus causes only minimal pre-emergence damping off, but infection immediately after emergence produces a red discoulouration of the cotyledons and young leaves. The fungus invades the hypocotyls of young seedlings, causing a brown discoulouration that may extend up to the base of the cotyledons. The hypocotyls are weakened by this attack and the seedlings may fall over and die. Those that do survive are usually stunted. Below ground, the taproot also becomes withered, blackened and “wiry” in appearance. In a survey of 27 farms in the Lockyer Valley of SE Queensland, *A. cochlioides* was the pathogen most commonly isolated from diseased beetroot seedlings during 1979-1981 (Hutton and O’Brien, 1979). In Texas, this pathogen is also considered the primary cause of seedling disease in sugar beet (Rush and Vaughn, 1993).

Mature plants may also be affected and show yellowing and wilting of foliage and unthrifty top growth as well as black lesions on roots and large (up to 5cm diam.) depressed black lesions on the beets themselves (Hutton and O’Brien, 1986; Whitney and Duffus, 1986). Overseas, abundant lateral root development has been reported as a symptom of this disease, however in Queensland this symptom is more commonly associated with infection by *Pythium* spp. (O’Brien, 1988). The chronic phase of the disease has been reported in the United States, Canada, England, Germany and Japan as well as Australia.

This pathogen is similar to *Pythium* in its environmental requirements. High soil temperatures (20-28°C) and wet conditions favour infection. Plant infections are initiated by swimming zoospores that are released during wet weather or following irrigation. In a similar way to *Pythium*, this fungus also produces thick-walled highly resistant spores that allow long-term survival of the pathogen, even when the soil dries out. In England, the disease has been reported to occur most frequently in acid soils (Byford, 1975), however both in England and in parts of the United States, *Aphanomyces* is not an important cause of seedling disease, presumably because most seed is sown while soil is too cold to favour the pathogen. Increasing pressure on Queensland producers to sow beet crops during the summer months under warm, wet conditions, increases the risk of root rots by *Aphanomyces* and may explain the prevalence of this fungus in previous surveys of the Lockyer Valley (Hutton and O’Brien, 1986; O’Brien *et al*., 1998).

2.1.3 *Rhizoctonia solani*

*Rhizoctonia solani* causes pre and post-emergence seedling death as well as a root, foliar and crown rot of beet plants. The fungus is found in agricultural soils throughout the world and it attacks many crop species, including beans, cabbage, lettuce, peas and potatoes. This pathogen is the most common and serious root disease of beet in the United States and it has been reported as an important disease in most other areas of the world where beet crops are grown (Whitney and Duffas, 1986). *Rhizoctonia* spp. have been classified by means of hyphal anastomosis reactions between isolates. In sugar beet anastomosis group (AG) 2-2 is the major AG world-wide causing crown and root rot.
As a seedling pathogen, *R. solani* causes some pre-emergence death but it inflicts most of its damage on seedlings that have already emerged. Infection is initiated below ground and extends up the hypocotyl, producing a dark pinched area near ground level (O’Brien *et al.*, 1998). The seedling often collapses at this point.

Later in the growing season, the same fungus may cause a crown rot on maturing beets. Initial diagnostic symptoms include brown to black cankers on petioles and crown tissues. Rotting proceeds toward the crown and roots of the plant and is accompanied by wilting and yellowing of the leaves (Abawi and Ludwig, 2000; Scholten *et al.*, 2001). Irregular, depressed dark lesions may develop on the surfaces of the beets. Fungal growth is often visible on the surfaces of these lesions (Whitney and Duffas, 1986).

*Rhizoctonia*, in the form of hyphae, survives in soil in colonised host tissues. Survival in the soil as sclerotia has also been reported in Japan (Hyakumachi and Ui, 1979) and in New York State (Abawi *et al.*, 1986). Under warm soil temperatures (25°C-33°C) the fungus grows through the soil and infects the plant through its leaves, petioles, crown and roots. The disease occurs in most types of soil, but is most severe in heavy poorly drained soils, especially in low areas where water collects (Whitney and Duffas, 1986). Since organic matter plays an important role in harboring the pathogen between crops, sowing too soon after plowing in a cover crop may lead to high levels of infection if the cover crop debris is not adequately broken down before the new crop is planted. This factor was identified as an important consideration for beetroot producers in the Lockyer Valley of SE Queensland (O’Brien *et al.*, 1998).

### 3. Control of the Soil-borne Pathogens of Beet

#### 3.1 Pre-plant Treatment Options

##### 3.1.1 Seed Treatments

**Fungicides**

The three major soil-borne fungal pathogens that cause root rot in beetroot all cause losses early in the growth of the beet crop if environmental conditions are favourable. For this reason, control or reduction of the diseases through application of fungicides to the beet seed seems a plausible control method. In the case of beetroot however, the simultaneous association of three fungi with this disease in the field complicates fungicide control options, since fungicides frequently vary in their activity against different taxonomic groups of fungi. A single chemical with broad-spectrum activity against all the soil-borne fungi of beetroot is currently unavailable and is not likely in the foreseeable future (Leach and MacDonald, 1978). Knowledge of the identity of the pathogens involved is therefore important. A combination of two or more fungicides is often necessary to protect against the spectrum of anticipated pathogens. As an additional complication, the simultaneous application of several different fungicides to the seed increases the likelihood of toxicity to the young germinating seedling.

Since the mid 1970’s trial work in several countries has been conducted to determine the best fungicide/s to use in the seed pellet to control these pathogens. Until 1981, most countries were relying on thiram to give control of both seed and soil-borne diseases. Thiram, a protectant fungicide, is relatively effective under low disease
pressure and is one of the least expensive fungicides available. Thiram is applied as a standard seed dressing to beetroot seed in Australia, however because of its mode of action, it only creates a localised zone of protection around the germinating seed. Thiram is also used in England to control deep-seated seed infection of *Phoma betae* in sugarbeet and beetroot seed. In this case, the fungicide (0.2%) is applied as a prolonged (24 hr) steep (Maude *et al.*, 1969). The steeping process not only provides good control of the pathogen, but also results in more rapid emergence (Durrant *et al.*, 1988).

Systemic fungicides, which can be absorbed during early seedling growth, should offer more extensive protection than thiram, particularly when infection takes place not through the root systems and the seed, but directly into the hypocotyl at some distance from the pellet, as is often the case with *Aphanomyces* infection. A range of systemic products have been examined for control of *Pythium* and *Aphanomyces* including captafol (Orthodifolotan), hymexazol (Tachigaren), metalaxyl (Apron, Ridomil) and propamocarb (Previcur) (Asher and Payne, 1989).

Metalaxyl has shown considerable promise as a beet seed dressing for control of *Pythium* spp both in Australia (O’Brien *et al.*, 1998) and overseas (Crosier *et al.*, 1986; Brantner and Windels, 1998). Since 1992, all sugarbeet seed sold to producers in the Red River Valley of Minnesota and North Dakota and in west-central Minnesota has been treated with metalaxyl (Brantner and Windels, 1998). Emergence of seedlings in this region is still often reduced by *Pythium* infections however, leading researchers to suggest and test the theory that variation in sensitivity of *Pythium* isolates to metalaxyl may be partly responsible (Brantner and Windels, 1998). They concluded, however, that the continued use of this fungicide as a seed treatment poses only limited risk for the development of metalaxyl–resistant isolates. This conclusion was based on the consideration that only a small amount of the fungicide is applied to the seed; it persists in the soil for only a short time and only a small proportion of the *Pythium* population in the soil is exposed to the treatment. However, they warned that if soil applications of metalaxyl are used in addition to seed treatments, the risk of selecting insensitive strains of the pathogen will increase and possibly reduce disease control.

Metalaxyl, although effective for *Pythium* control, shows no activity against either *Aphanomyces* or *Rhizoctonia*. For this reason, there has recently been a lot of interest in hymexazol as a seed treatment, since it has shown activity against both *Pythium* and *Aphanomyces* in trials in several countries (Byford and Payne, 1983; Payne and Williams, 1990; Heijbroek and Huijbregts, 1995).

In England and Finland, a rate of 10 g.a.i/kg of seed has been found to be optimal in field trials (Vestburg *et al.*, 1982; Byford and Payne, 1983). Since 1983, sugarbeet seed treated with hymexazol at 10g g.a.i./kg has been commercially available in England. Elsewhere in Europe the rates of hymexazol used for *Aphanomyces* and *Pythium* control vary widely, probably reflecting the different disease situations in the growing regions (Asher and Payne, 1989). In France, seed treated with 28 or 42 g.a.i/unit is available to growers in regions with serious problems. In The Netherlands, early in the season quantities of about 10 g.a.i/unit provide sufficient control, whereas by the end of the season rates of greater than 20 g.a.i/unit are necessary (Heijbroek and Huijbregts, 1995). Similarly, in England under conditions of severe disease pressure rates of 21 g.a.i/kg are required for maximum disease control (Payne and Williams, 1990).
Evidence from a large number of trials indicates that rates in excess of 20g.a.i/kg can slow emergence (Asher and Payne, 1989). If high rates are to be applied as single applications, as in England, any potential benefits must be balanced with the possible adverse effect of the chemical on seedling emergence. The alternative strategy, as adopted in France, is to provide seed treated with different rates for use where the disease is anticipated. This strategy may create logistic difficulties and would only be effective if sites prone to the disease and the environmental conditions favourable for its development each year can be predicted with some degree of accuracy (Asher and Payne, 1989).

Deviations between the actual rate of application and the target rate of application may occur and have been noted when hymexazol is incorporated in pelleted seed (Heijbroek and Huijbregts, 1995). These deviations have been attributed to the unstable nature of hymexazol and its tendency to degrade in the pellet. The composition of the pelleting mass and the addition of carbamate insecticides along with hymexazol may also influence the degradation. Heijbroek and Huijbregts suggest that an overdose of hymexazol should be applied to pelleted seed to compensate for the degradation and mention the importance of sowing the seed soon after pelleting to limit its extent (1995).

Several fungicides have been reported to show efficacy for control of Rhizoctonia solani when applied to beet seed. In the western United States, most beet seed is treated with pentanitrochlorobenzene (PCNB) (Quintozene) to protect against Rhizoctonia infection (Whitney and Duffas, 1986). Tolclophos methyl (Rizolex) has also been found to be effective against R. solani both in Australia (O’Brien et al., 1998) and overseas. In a glasshouse assessment of fungicidal seed dressings, tolclophos methyl gave complete protection against Rhizoctonia infection when it was applied as a slurry (2g/kg seed) to monogerm beetroot seed (O’Brien et al., 1998). This chemical is currently registered for use on potatoes in Australia.

Pencycuron (Monceren) is another chemical with specific activity against R. solani (Yamada, 1986). In experimental trials, dry seed dressings of Monceren at 0.5-1.5g a.i./kg seed were promising, particularly when combined with Euparen (Yamada, 1986).

Seed Priming Treatments

Seed priming is a pre-sowing treatment in which seed germination processes are initiated and stopped prior to radicle emergence. Seed priming typically increases the rate, uniformity, and percentage of seed germination, resulting in improvement of stand and often of yield. It is usually of greatest benefit under environmental conditions that are suboptimal for seed germination and emergence, such as cool, wet conditions (Osburn and Schroth, 1988). The duration of the priming treatment can range from less than 24 hours (Guedes and Cantliffe, 1980) to several weeks (Khan et al., 1980). Various priming techniques have been developed including osmopriming, in which seeds are allowed to imbibe in an aerated osmotic solution such as polyethylene glycol (PEG) or inorganic salts. The osmotic potential of the solution regulates the amount of water uptake by the seeds, thus enabling the completion of the early phases of germination under controlled conditions (Osburn and Schroth, 1988). Osmoprimed seed is available commercially in the United States for some small seeded vegetables and also has been produced experimentally for a number of crops including sugar beet and some grain crops (Osburn and Schroth, 1988). Solid matrix
priming is a relatively recent development and uses a solid carrier to regulate water availability to seeds. This type of priming has been shown to be as good or better than osmopriming with regard to speeding seedling germination (Harman et al., 1989; Rush, 1991).

Beetroot seeds are slow and often asynchronous in their germination. These characteristics interfere with the early establishment of a uniform, vigorous stand of seedlings, particularly in cold, wet soil (Khan et al., 1983). Several U.S. studies have indicated that osmopriming beetroot seed in PEG improves the emergence rate, the final stand and total yield of beetroot crops (Khan et al., 1983; Khan and Taylor, 1986).

In addition to improvements in germination rate and emergence, osmopriming with PEG or NaCl (Taylor et al., 1985; Osburn and Schrot, 1988; Osburn and Schrot, 1989; Rush, 1992) and solid matrix priming with water and hydrous silicate clay (Rush, 1991; Rush and Vaughn, 1993) have both been reported to significantly reduce pre-emergence damping off in beets caused by *Pythium* spp. *Pythium* spores germinate in response to nutrients diffusing from imbibing seeds (Osburn and Schrot, 1988). Priming treatments leach soluble exudates from the seed and as a consequence seed colonisation by the pathogen is reduced when the seed is rewetted. In addition, indigenous bacteria, present on the seed coat, that multiply in the osmotic solution during seed treatment may prevent colonisation of the seed by *Pythium* spp. (Taylor et al., 1985).

Studies on the effect of seed priming on post-emergence damping off caused by *Aphanomyces cochlioides* indicate that seed priming treatments are ineffective for control of this pathogen (Rush, 1992; Rush and Vaughn, 1993). In comparison, a Californian field study indicated that osmopriming sugarbeet seed in either PEG or NaCl gave comparable or better control of *Pythium* spp. than treating the seed with metalaxyl and when combined, the osmoprimed and fungicide seed treatments resulted in even greater disease reductions (Osburn and Schrot, 1989). In the same study, pre-emergence damping off caused by *Rhizoctonia solani* was also reduced by osmopriming. This pathogen is not controlled by metalaxyl and therefore planting osmoprimed seed in soils known to be infested with *R. solani* may offer additional protection against this pathogen when the standard fungicide seed dressings are inadequate.

Although the efficacy of osmopriming beet seed has been demonstrated in the numerous studies discussed above, several technical difficulties have been encountered with current methods. Osmotic solutions require continuous aeration and a large volume of priming solution is required per quantity of seed. As well as this, the use of high concentrations of PEG in solution has low oxygen solubility and diffusivity (Mexal et al., 1975). These complications will need to be adequately addressed if osmopriming of beetroot seed is to be commercially feasible.

**Biological Seed Treatments**

Most of the available literature on control of soil-borne beet pathogens with biological treatments relates to the biological control of *Pythium ultimum*. Only very limited consideration has been given to the possibility of biological control of either *Aphanomyces cochlioides* or *Rhizoctonia solani* in beet crops. Indeed, as for
fungicide seed treatments, the discovery of a single broadspectrum biological control agent with activity against the three major soil-borne pathogens of beets is unlikely.

*Pythium ultimum* was considered by Osburn *et al.*, to be an ideal candidate for biological control because the susceptible period for the host is relatively short and high populations of the biological control organism would not be required for extended periods of time (1989). They identified two bacterial strains: *Pseudomonas fluorescens-putida* (R20) and *Pseudomonas putida* (ML5), which, when inoculated onto sugarbeet seed resulted in marked reductions in colonisation by *P. ultimum* and gave comparable control to fungicides (metalaxyl or fenamino sulf) in suppressing damping off by *P. ultimum* in greenhouse experiments. Williams and Asher (1996) also assessed potential bacterial biological control agents for *P. ultimum* and *A. cochlioides*. In this case, however, the level of protection fell short of that achieved with standard fungicide seed treatments. In other studies, *Pythium oligandrum* was identified as a potential biological control agent of *P. ultimum, Aphanomyces cochlioides* and *Rhizoctonia solani* in sugar beet, however control levels provided by this organism were inferior to those achieved with fungicide drenches and failed to control any of the pathogens when the disease pressure was high (Whipps *et al.*, 1993). In addition, the control achieved with *P. oligandrum* was shown to vary with the type and amount of inoculum applied as well as the method of application, and only controlled damping off over a narrow pH range (pH 7.0-7.5) (Holmes *et al.*, 1998).

*Trichoderma hamatum* was also shown promise as a biological control agent for *Rhizoctonia solani* in beet seed (Lewis and Papavizas, 1987a; 1987b), reducing the survival of the pathogen by about 90% after one week. In this instance, however, the timing of application of the biological control agent proved to be important for good control of the pathogen. If the control agent was added to soil prior to the presence of the pathogen, the pathogen survival was not reduced. Also, the age of the inoculum greatly affected the ability of *T. hamatum* to limit survival and growth of the pathogen in soil. Young, actively growing inoculum was effective whereas inoculum consisting mainly of resting spores was not (Lewis and Papavizas, 1987a). An alginate pellet formulation of *T. hamatum* was developed to assist with application, however the storage of the pellets for more than 6 weeks at 5 or 25°C reduced their effectiveness against *R. solani* (Lewis and Papavizas, 1987b).

The literature would suggest that at this stage, seed applications of biological control agents for control of soil-borne beet pathogens is unlikely to represent a feasible commercial proposition since the control levels achieved are generally inferior to those currently provided by fungicides, and environmental variables markedly influence the efficacy of biological treatments.

**Fumigation**

Methyl bromide (CH₃Br) has for many years been the most widely used and effective fumigant worldwide. This compound will be phased out of use by 2005, under the Montreal Protocol, because it is an ozone depleting chemical (Ohr *et al.*, 1996). Assessment of potential replacements for methyl bromide has been the focus of much research effort. In beet production, aside from efficacy constraints, many fumigants are also not economically feasible (Harveson and Rush, 1994). It seems likely that a beneficial response to fumigation would need to be observed across several cropping
cycles and/or to crops grown in rotation with beet for these economic constraints to be overcome.

Metham sodium is currently the only commercially available alternative to methyl bromide and it is widely used in Australia and overseas for control of a range of soil-borne diseases in horticultural crops including *Pythium* spp. (Roberts et al., 1988; Stephens et al., 1999) and *Rhizoctonia solani* (Wicks et al., 1996; Stephens et al., 1999). Unfortunately, metham sodium is prone to biodegradation (Matthiessen, 1999). Biodegradation is a phenomenon whereby soil micro-organisms that metabolise a pesticide are stimulated to dominate the soil microbial population by repeated applications of the chemical. This is particularly a problem with modern pesticides because they are not halogenated and are therefore more prone to breakdown via microbial activity (Matthiesen, 1999).

Other potential fumigants have been trialed but as yet, remain uncommercial. Harveson and Rush evaluated the fumigant Telone II (1,3-dichloropropene) for control of soil-borne pathogens of sugar beet because of its cost efficiency and efficacy at low rates. Yields were significantly increased in fumigated plots in a field study (1994). Methyl iodide has also been evaluated as a potential methyl bromide replacement and was found to be as effective or more effective than methyl bromide as a fumigant for control of soil-borne fungi, including *Rhizoctonia solani* (Ohr, et al., 1996; Becker, et al., 1998). Unfortunately, the relative cost of methyl iodide compared to other fumigants was not discussed in these publications and would need to be investigated in any future consideration of this product. Benzaldehyde was also shown to reduce the viability of *R. solani* and reduce populations of *Pythium aphanidermatum* in a laboratory study (Wilson et al., 1999). This chemical is considered a desirable alternative to methyl bromide because it is inexpensive and its breakdown products (CO2 and H2O) are harmless to the environment.

**Biofumigation**

The problem of biodegradation of metham sodium and the relatively high cost of this chemical has prompted researchers to investigate other non-synthetic methods of fumigation. The term biofumigation has been coined and adopted to describe the concept of using *Brassica* plants to control soil-borne pests and diseases in other crops (Matthiessen, 1994).

The ability of brassica amendments to reduce some fungal and nematode populations has been attributed to their production of glucosinolates (Lewis and Papavizas, 1971b). These compounds are released when *Brassica* plants are physically broken up and during the breakdown of *Brassica* residues in soil. Glucosinolates are precursors of isothiocyanates. Metham sodium is an isothiocyanate (Matthiessen, 1994).

Biofumigation effects on many soil-borne pathogens have been reported in a range of cropping systems. For example, dried cabbage residue reduced soil populations of *Fusarium oxysporum* f.sp. *conglutinans* (Ramirez-Villapudua and Munnecke, 1988) and superior growth of wheat following *Brassica* crops was attributed to suppression of soil-borne fungal pathogens, including *Rhizoctonia solani* (Kirkegaard et al., 1996). Damping-off of sugarbeet caused by *Aphanomyces cochlioides* was reduced by soil amendments of eight *Brassica* species (mustard, brussel sprouts, kale, collards, cress, cabbage, turnip and kohlrabi), in a glasshouse study (Lewis and Papavizas,
1971a). Similarly, *Aphanomyces euteiches*, the causative agent of root rot of pea, was suppressed by volatiles, released during the breakdown of cabbage tissue in the laboratory (Lewis and Papavizas, 1971b). Field experiments in which white mustard (*Sinapis alba*) grown after peas significantly reduced root rot (*A. euteiches*) in pea plants grown the following year, further supported these early glasshouse experiments (Muehlchen *et al.*, 1990).

Although there are numerous reports in the literature highlighting the benefits of biofumigation, several studies investigating biofumigation for control of soil-borne *Pythium* diseases have not been promising (Kirkegaard *et al.*, 1996; Stephens *et al.*, 1999). Recently, *Pythium* spp. have been shown to be relatively insensitive to volatiles from mustard (Wong and Kirkegaard in Matthiessen and Kirkegaard, 1996).

The degree of fungal suppression by *Brassica* crops depends on the species, age and type of *Brassica* tissue, which influences the type and concentration of glucosinolates evolved; as well as the sensitivity of the pathogen. Recently in Australia, more than 100 different brassicas were assayed for glucosinolate production (Sarwar and Kirkegaard in Matthiessen and Kirkegaard, 1996) and seed of the most promising types is now being commercially produced for use in biofumigation.

**Crop Rotations**

The potential benefits of rotating beet crops with *Brassica* species have been already been discussed under the section on Biofumigation (section 3.1.1, pg 16).

When beet is grown on the same ground more than once in 3 years, it generally does not reach its full yield potential (Schauffle and Winner, 1979). Yield losses have been attributed to increases in soil-borne pathogen populations with increasing frequency of beet cultivation. For this reason, 3-5 year rotations out of beet is standard industry practice in beet cropping regions in the US (Herr, 1987; Rush and Winter, 1990).

Preceding cropping sequences have been shown to influence levels of disease caused by *Pythium* spp., *Aphanomyces cochlioides* and *Rhizoctonia solani* in beet crops in numerous pot and field studies.

In a Finnish glasshouse study, leguminous plants such as pea, field bean or red clover tended to raise the inoculum density of *Pythium* in soil and keep the level of damping-off unchanged or slightly elevated, when compared to continuously cultivated sugarbeet. In comparison, gramineous plants had the opposite effect, increasing emergence and the numbers of healthy plants and decreasing the inoculum density of *Pythium*. In this study, the influence of preceding crops on different soil types varied greatly, with sandy soils being more prone to increases in *Pythium* populations (Vestburg, 1987).

Losses due to *Aphanomyces* are most severe when beets are grown immediately following lucerne (Deems and Young, 1956; Mumford, 1968; Schneider and Robertson, 1975). Striking effects on disease incidence resulted from cropping soil thoroughly infested with *A. cochlioides* to lucerne, corn, oats and sugarbeet in an Ohio field trial. Sugarbeets maintained a 95-100% inoculum potential throughout the season. Cropping to corn and oats reduced disease. Corn was the most effective rotation in terms of *Aphanomyces* control, decreasing disease incidence to 10% after three months and maintaining disease at low levels. Oats decreased disease to 25%
during the first four months, however disease levels increased markedly once the oats were “ploughed down”. Lucerne did not decrease *Aphanomyces* incidence to levels below those in soils continuously cropped with sugarbeet (Deems and Young, 1956).

Crown rot caused by *Rhizoctonia solani* was also more severe where beets followed lucerne than where beets followed corn, soybeans or navybeans, in 5 year field rotations in Michigan (Schneider and Robertson, 1975). Similarly, cotton, fallow and sunflower were superior to lucerne as rotations to precede beet in *Rhizoctonia* infested soil in Texas (Rush and Winter, 1990).

In terms of *Rhizoctonia* control, tomato is also a poor crop to plant before beet, as is potato. High *R. solani* populations were shown to develop saprophytically on tomato crop residues in an Ohio field trial. Tomato is not considered to be a particularly good host for *R. solani*, however, after harvest the fruit and vines support the pathogen, allowing it to persist until the following spring (Herr, 1987). Two year rotations between beet and potato were also favourable for *R. solani* survival and development, presumably because the pathogen is able to survive between crops on undecomposed potato debris (Schuster and Harris, 1960).

### 3.2 Post-plant Treatment Options

**Fungicides**

The vast majority of research on fungicide treatments for the soil-borne pathogens of beetroot has been directed towards pre-plant seed applications. Since much of the damage by these pathogens is initiated during the early stages of plant development, this concentration of research effort is understandable since seed applications represent one of the best options for combating early infections. The main pathogens of beet do, however, cause appreciable losses and quality reductions of mature beets under appropriate environmental conditions, and hence fungicide applications following seedling emergence may offer some benefit.

In Australia, no systemic fungicides are currently registered for use on beetroot as post-emergence treatments. In the US, metalaxyl (Ridomil) is available for use as a supplemental in-furrow treatment applied at planting, however it is not commonly applied because early *Pythium* spp. infections are normally adequately controlled by seed dressings (Brantner and Windels, 1998).

A range of chemicals have been trialed experimentally for control of late *Rhizoctonia solani* infections. In three year field trials, single applications of triadimefon, triadamencol, the experimental protectant fungicide Bay NTN 19701 and the experimental systemic fungicide Bay HWG 1608 suppressed *Rhizoctonia* root rot of sugarbeets. Earlier applications, applied at the cotyledon to 4-6 leaf stage tended to be more effective and gave season-long protection (Ruppel and Hecker, 1987).

Triadimefon was also promising when applied to crowns and bases of plants in field plots artificially infected with *R. solani* (Schneider and Potter, 1983). In these experiments, chlorothalonil (2.49-2.64 kg/ha) and triphenyltin hydroxide (TPTH) (0.10-0.33 kg/ha) applications also suppressed *Rhizoctonia* infections. Preliminary assessments of PCNB and pencycuron were also promising, with pencycuron giving outstanding control of *Rhizoctonia* when applied as a crown application to sugarbeet. (Schneider and Potter, 1983).
More recently, the new strobularin chemical azoxystrobin was effective in reducing *Rhizoctonia solani* infections in a number of crops including sugarbeet (Zens, *et al.*, 1999) and celery (O’Neill *et al.*, 1999). This chemical warrants further assessment for control of other beet pathogens.

**Host Resistance**

In the U.S., beetroot breeding programs have been conducted at the University of Wisconsin-Madison and New York State Agricultural Experiment Station, Geneva. Unfortunately, only limited reference to variety releases from both programs could be found in the literature (Goldman, 1996; Marx, 1986). The inbred lines released from the Wisconsin breeding program have been used in the production of hybrid beet seed throughout the world. A list of releases from this program has been published (Goldman, 1996), however it gives no information on the relative susceptibility of each variety to soil-borne pathogens. A principle objective of the Geneva breeding program was to develop breeding material with tolerance or resistance to root rot. Some advanced breeding lines from this program have shown evidence of tolerance to *Pythium* root rot in field evaluations (Marx, 1986), however it is unclear at this stage, due to a paucity of information in the literature, whether any of these lines are now commercially available.

**Irrigation Management**

Wet soils favour the development of root rot epidemics in beets. There has been little consideration in the literature of the possibility of manipulating irrigation for the purpose of disease control in this crop. Piccinni and Rush found that sugar beets irrigated every 4 weeks had the lowest disease incidence and highest yield when grown in soils infested with Beet necrotic yellow vein virus (BNYVV) and Beet soilborne mosaic virus (BSBMV). In this study, sugarbeets irrigated every 2 or 3 weeks had significantly higher levels of disease than those irrigated every 4 or 5 weeks (2000).

Positive correlations between levels of *Aphanomyces* root rot and soil matric potential was also observed in a glasshouse study (Rush and Vaughn, 1993). In this instance, seedlings in pots irrigated only at pre-planting were significantly less diseased than those irrigated for emergence after planting.

In reality, manipulation of irrigation as a disease management tool in commercial cropping situations is unlikely to be a feasible option, however these studies have been mentioned as they help to highlight the importance of soil moisture in the development of soil-borne disease epidemics in beet.
CHAPTER 2: Identification and Characterisation of Soil-borne Pathogens

Introduction

In Australia, 3 genera of soil-borne fungi, *Pythium*, *Aphanomyces cochlioides* and *Rhizoctonia solani*, have been reported as the predominant soilborne pathogens involved in a beetroot root rot complex since the 1980s (Hutton and O’Brien, 1986; O’Brien et al., 1998; Tesoriero, 1993). The same 3 genera have also been recognised for many years as pathogens of beet in other parts of the world (Mumford, 1968; Vestbury et al., 1982; Whitney and Duffas, 1986; Asher and Payne, 1989). Since the mid-1990s, when soilborne diseases were last studied in detail in Australia, the severity of disease losses has increased substantially on Australian beet farms (Figure 1). In this project, we sought to understand the nature of this increase. We collected samples of diseased beets and soil samples from affected blocks, and isolated and characterised the organisms responsible for beet diseases.

Disease Indexing of Beetroot Soils

Materials and Methods

We used a modified version of the beetroot disease indexing test developed by O’Brien et al. (1998) to determine the disease potential of soils collected from beet farms throughout south-east Queensland. Fungi recovered from soils were identified to genus level by morphological features using light microscopy, and some were further characterised to species level by Dr Paul Scott (formerly of The University of Queensland, Gatton) via molecular analysis (refer to page xxx). The pathogenicity of the fungi (their ability to cause disease to beetroot) was determined in glasshouse bioassays with beetroot seedlings (refer to page xxx).

For the disease indexing tests, soil samples were collected from 39 beetroot blocks. For each sample, 20L of soil was collected by bulking together approximately 40-50 small sub-samples collected in a W pattern over each field from the top 10cm of soil. Each sample was thoroughly mixed and large clods removed. A sub-sample was autoclaved to provide a sterile control, which was used in 2 pots. The remaining soil was used to fill 20 pots.

In each pot a 30mL layer of medium grade (grade 3) vermiculite was covered with 100mL of soil. The soil was watered and then 12 beetroot seeds (cv. Detroit Dark Red) were sown in each pot. A layer of fine grade (grade 1) vermiculite was then added to cover the soil. An additional 2 pots of sterile UC mix were prepared for each test.

The test seed was left untreated, or was dressed with one of three fungicide treatments: a) Tachigaren 70 WP (7g/kg seed) b) Rizolex WP (8g/kg seed) c) Rizolex WP (8g/kg seed) + Apron (1mL/kg seed)
**Figure 1:** Symptoms of soilborne diseases of beetroot in south east Queensland

- Wilting of plants due to *Rhizoctonia*
- Plants collapse before harvest
- “Damping off” soon after emergence
- Poor taproots of infected seedlings (left), Healthy seedlings (right)
- Misshapen roots can be due to early disease infection or poor varietal characteristics
- “Hairy” roots may indicate infection by *Pythium*
- *Rhizoctonia* infection of mature beets
- *Sclerotium* causes secondary rot of mature beets
Five pots were sown with seed dressed with each fungicide and 5 with untreated seed. Untreated seed was sown into the pots of sterile soil and UC mix. After sowing, the pots were kept in a glasshouse, they were watered twice daily and fertilised with a general liquid fertiliser once per week.

After the seedlings started to emerge, the pots were assessed twice weekly. The total number of seedlings and the number of dead seedlings in each pot was recorded and the dead seedlings were removed. Isolations on non-selective culture media (1/2 strength potato dextrose agar) were completed for a selection of the seedlings removed from the pots, so that the organisms responsible for the seedling death could be identified. Information about sample sites is given in Table 1:
<table>
<thead>
<tr>
<th>Grower</th>
<th>Sample</th>
<th>Location Description</th>
<th>Collection Date</th>
<th>Time Planted to Beetroot</th>
<th>Time in Fallow</th>
<th>Time Planted to other Crops</th>
</tr>
</thead>
<tbody>
<tr>
<td>John Brent</td>
<td>A</td>
<td>Macs Farm Block 4, Northern side of block in line with middle of shed on southern side</td>
<td>18/06/03</td>
<td>2 x 16 weeks</td>
<td>20 weeks</td>
<td>-</td>
</tr>
<tr>
<td>John Brent</td>
<td>B</td>
<td>Home Farm Block 1. First block on right side of Brents Rd. Sampled in line with pump 10m into block</td>
<td>18/06/03</td>
<td>16 weeks</td>
<td>5 months</td>
<td>3 months (forage sorghum)</td>
</tr>
<tr>
<td>Moira Farms</td>
<td>A</td>
<td>Sample taken 20m in from headland at the end of the block directly behind sheds</td>
<td>30/04/03</td>
<td>6 months</td>
<td>6 months</td>
<td>-</td>
</tr>
<tr>
<td>Moira Farms</td>
<td>B</td>
<td>Sample taken 10m in from roadway and in line with the house on the hillside on the opposite side of the road</td>
<td>30/04/03</td>
<td>1 month</td>
<td>11 months</td>
<td>-</td>
</tr>
<tr>
<td>Glenn Lerch</td>
<td>A</td>
<td>Shed Block 4. First block past dam, sampled 10m in from road end</td>
<td>22/04/03</td>
<td>6 months</td>
<td>5 months</td>
<td>1 month (sorghum)</td>
</tr>
<tr>
<td>Glenn Lerch</td>
<td>B</td>
<td>Creek end of farm. Sampled 20m from NE corner of house</td>
<td>22/04/03</td>
<td>6 months</td>
<td>4 months</td>
<td>2 months (millet)</td>
</tr>
<tr>
<td>Peter Lerch</td>
<td>A</td>
<td>Sample taken in line with the two closely spaced power poles and 10m in from the headland</td>
<td>1/05/03</td>
<td>-</td>
<td>5 months</td>
<td>7 months (sorghum and sweetcorn)</td>
</tr>
<tr>
<td>Peter Lerch</td>
<td>B</td>
<td>Sample taken 20m into the block, directly in front of house</td>
<td>1/05/03</td>
<td>6 months</td>
<td>6 months</td>
<td>-</td>
</tr>
<tr>
<td>Litzow/Reddacliffe</td>
<td>A</td>
<td>Sampled 20m on western side of hydrant located in middle of the block</td>
<td>19/06/03</td>
<td>4 months</td>
<td>2 months</td>
<td>6 months (sorghum)</td>
</tr>
<tr>
<td>Litzow/Reddacliffe</td>
<td>B</td>
<td>Sampled 20m in from end of block &amp; 20m to the south of the pump in the field opposite that of Sample A</td>
<td>19/06/03</td>
<td>5 months</td>
<td>7 months</td>
<td>-</td>
</tr>
<tr>
<td>Neumann</td>
<td>A</td>
<td>Home farm in line with third hydrant past the end of the sheds and approx. 20m in from roadway</td>
<td>25/03/03</td>
<td>5 months</td>
<td>7 months</td>
<td>-</td>
</tr>
<tr>
<td>Neumann</td>
<td>B</td>
<td>Sample taken approx. 20m in from pump on the side of Qualischefski Road</td>
<td>25/03/03</td>
<td>2.5 months</td>
<td>3 months</td>
<td>6.5 months (onions)</td>
</tr>
<tr>
<td>Voight</td>
<td>A</td>
<td>Sampled from middle of the block in line with the pump shed</td>
<td>26/05/03</td>
<td>not determined</td>
<td>not determined</td>
<td>ND</td>
</tr>
<tr>
<td>Voight</td>
<td>B</td>
<td>Sampled 10-20m into the block in line with the first power pole from the creek end</td>
<td>26/05/03</td>
<td>not determined</td>
<td>not determined</td>
<td>ND</td>
</tr>
<tr>
<td>Zelinski</td>
<td>A</td>
<td>Sampled half-way between power pole and pump shed</td>
<td>27/02/03</td>
<td>32 weeks</td>
<td>5 weeks</td>
<td>sweetcorn</td>
</tr>
<tr>
<td>Zelinski</td>
<td>B</td>
<td>Block 29. 10m west of second hydrant from creek</td>
<td>27/02/03</td>
<td>-</td>
<td>6 months</td>
<td>potato</td>
</tr>
</tbody>
</table>
Results

A summary of disease severity information for the soil samples is given in Table 2:

Table 2: Severity of disease that developed in soil indexing tests

<table>
<thead>
<tr>
<th>Soil Sample</th>
<th>Location Description</th>
<th>No. dead seedlings removed</th>
<th>% of seedlings that died</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moira #4</td>
<td>Block at Laidley behind John Berlin's house</td>
<td>368</td>
<td>92</td>
</tr>
<tr>
<td>Neumann #2</td>
<td>Site of variety trial 2, 2001</td>
<td>400</td>
<td>89.89</td>
</tr>
<tr>
<td>Hauser</td>
<td>East side of house &amp; sheds, Gatton - Forest Hill Rd</td>
<td>351</td>
<td>84.58</td>
</tr>
<tr>
<td>Neumann #3</td>
<td>Qualischefski's block</td>
<td>369</td>
<td>81.1</td>
</tr>
<tr>
<td>Litzow #1</td>
<td>First field behind Voight's, Gatton – Forest Hill Rd</td>
<td>351</td>
<td>78</td>
</tr>
<tr>
<td>Voight #2</td>
<td>Off Gatton - Forest Hill Rd (opposite Litzow House)</td>
<td>297</td>
<td>67.5</td>
</tr>
<tr>
<td>Voight #3</td>
<td>Off Gatton - Forest Hill Rd (opposite Litzow House)</td>
<td>323</td>
<td>59.81</td>
</tr>
<tr>
<td>Voight #1</td>
<td>Site of fungicide trial 1, 2001</td>
<td>254</td>
<td>57.08</td>
</tr>
<tr>
<td>G. Lerch #4</td>
<td>Block on east side of Forest Hill - Blenheim Rd.</td>
<td>276</td>
<td>56.33</td>
</tr>
<tr>
<td>Neumann #1</td>
<td>Site of variety trial 1, 2001</td>
<td>295</td>
<td>56.19</td>
</tr>
<tr>
<td>G. Lerch #3</td>
<td>West side of Forest Hill - Blenheim Rd</td>
<td>216</td>
<td>46.45</td>
</tr>
<tr>
<td>Neumann</td>
<td>Sample A</td>
<td>231</td>
<td>45.74</td>
</tr>
<tr>
<td>Moira #2</td>
<td>Van de Weyer Rd (Forest Hill)</td>
<td>214</td>
<td>40.76</td>
</tr>
<tr>
<td>Zelinski</td>
<td>Site of commercial scale variety evaluation 2002</td>
<td>192</td>
<td>40.42</td>
</tr>
<tr>
<td>Litzow #2</td>
<td>Site of variety trial 2002</td>
<td>165</td>
<td>38.82</td>
</tr>
<tr>
<td>Litzow #3</td>
<td>First block on Hall Rd, Forest Hill.</td>
<td>180</td>
<td>38.71</td>
</tr>
<tr>
<td>Voight #4</td>
<td>Block Gatton - Forest Hill Rd, opposite Litzows house</td>
<td>150</td>
<td>37.5</td>
</tr>
<tr>
<td>Moira #1</td>
<td>Site of fungicide trial 2, 2001</td>
<td>178</td>
<td>37.08</td>
</tr>
<tr>
<td>Neumann</td>
<td>Sample B</td>
<td>151</td>
<td>35.95</td>
</tr>
<tr>
<td>Zelinski</td>
<td>Sample A</td>
<td>148</td>
<td>31.49</td>
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<tr>
<td>Zelinski</td>
<td>Sample B</td>
<td>134</td>
<td>27.63</td>
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<td>Sample B</td>
<td>114</td>
<td>25.33</td>
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<td>Sample A</td>
<td>109</td>
<td>23.7</td>
</tr>
<tr>
<td>Brent</td>
<td>Sample A</td>
<td>109</td>
<td>22.24</td>
</tr>
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<td>Corner of Forest Hill - Blenheim &amp; Woodland Rds</td>
<td>90</td>
<td>21.95</td>
</tr>
<tr>
<td>G. Lerch</td>
<td>Sample A</td>
<td>85</td>
<td>19.54</td>
</tr>
<tr>
<td>G. Lerch</td>
<td>Sample B</td>
<td>78</td>
<td>18.35</td>
</tr>
<tr>
<td>Moira Farms</td>
<td>Sample A</td>
<td>84</td>
<td>18.06</td>
</tr>
<tr>
<td>P. Lerch</td>
<td>Sample A</td>
<td>88</td>
<td>17.96</td>
</tr>
<tr>
<td>P. Lerch</td>
<td>Sample B</td>
<td>70</td>
<td>16.28</td>
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<tr>
<td>Moira Farms</td>
<td>Sample B</td>
<td>78</td>
<td>16.25</td>
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<td>G.Lerch #5</td>
<td>Lesters Lane (Laidley South)</td>
<td>53</td>
<td>12.62</td>
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<td>Voight</td>
<td>Sample B</td>
<td>52</td>
<td>11.3</td>
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<td>Moira #3</td>
<td>Site of commercial scale variety trial 2002</td>
<td>44</td>
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<td>G. Lerch #2</td>
<td>Site of fumigation trial 2002. After fumigation</td>
<td>27</td>
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<tr>
<td>Litzow</td>
<td>Sample B</td>
<td>23</td>
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<tr>
<td>Brent</td>
<td>Home farm block 5</td>
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<td>Sample A</td>
<td>13</td>
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<td>G. Lerch #1</td>
<td>Site of fumigation trial 2002. Before fumigation.</td>
<td>14</td>
<td>2.83</td>
</tr>
</tbody>
</table>
**Figure 2:** Fungi isolated from diseased seedlings grown in disease indexing soil samples

### Sites with Extreme Disease Potential

- Moira # 4
- Neumann # 2
- Hauser
- Newmann # 3
- Litzow # 1
- Voight # 2
- Voight # 3
- Voight # 1
- G. Lerch # 4
- Neumann # 1

### Sites with High Disease Potential

- G. Lerch # 3
- Neumann A
- Moira # 2
Sites with Moderate Disease Potential

Pythium aphanidermatum
Pythium ultimum
Pythium dissotocum
Pythium species (other)
Rhizoctonia
Aphanomyces
Fusarium
Sclerotium
Other

Pythium aphanidermatum
Pythium ultimum
Pythium dissotocum
Pythium species (other)
Rhizoctonia
Aphanomyces
Fusarium
Sclerotium
Other
The disease potentials of the soils varied widely, with up to 92% of seedlings dying in the most extreme instance (Moira #4) and losses as low as 2.83% in the best block (G.Lerch # 1). It is worth emphasising, that the indexing tests give an indication of the potential of a disease epidemic developing if environmental conditions are favourable. The soil indexing tests are completed under conditions highly conducive to disease infection (relatively high soil temperatures, and continual high soil moisture), so that disease development is maximised. A site with a high disease potential will not develop a disease epidemic unless the environmental conditions are conducive to
infection at the time a susceptible crop is planted. *Pythium* species and *Rhizoctonia* were the most commonly recovered organisms from the dead plants in the soil indexing tests (Figure 2).

**Figure 3:** The Apron + Rizolex seed dressing gives the greatest disease control in an indexing test on a soil containing pathogenic *Pythium* and *Rhizoctonia*

![Image of plants in different treatments](image)

**Untreated**  **Apron + Rizolex**  **Rizloex**  **Tachigaren**

**Identification of species of Pythium**

Traditionally, *Pythium* spp. have been identified by differences in morphological features. This method is time-consuming, cumbersome and sometimes open to subjective interpretation. More recently, DNA-based methods have been developed to identify the relevant *Pythium* spp. In contrast, DNA-based methods are rapid and unambiguous, and in many cases do not rely on the culture and propagation of the suspected pathogen. We provided 137 cultures of *Pythium* to Dr Paul Scott (University of Queensland, Gatton) for DNA analysis. The cultures selected were from 15 sites and were isolated from the soils in index tests as well as from ad hoc. samples of diseased beetroot. A more detailed account of this work is soon to be published in *Australasian Plant Pathology* (Scott et. al, in press.).

Briefly, 3 predominant *Pythium* species were identified: *Pythium aphanidermatum*, *P. ultimum* and *P. dissotocum*. The majority of the isolates (approx. 51%) were *P. ultimum*, 30.7% were *P. aphanidermatum* and 6.6% were *P. dissotocum*. 
Pathogenicity Testing

Materials and Methods

Not all fungi associated with diseased plants may be responsible for causing the disease symptoms. For this reason, we needed to test if the fungi we isolated from the dead seedlings in our index tests and from other plant samples, were pathogenic (able to cause disease) on beetroot plants. To do this we inoculated healthy beet plants in the glasshouse with fungal inoculum prepared from pure cultures, and assessed the plants for disease symptoms.

To prepare inoculum, 14-day-old cultures grown on half-strength PDA at 25°C were flooded with de-ionised water and scraped with a glass rod. A 200mL inoculum suspension was prepared from each culture. Beetroot seedlings (cv. Detroit Dark Red) were grown in 70 mm plastic pots filled with sterile UC mix. Four seeds were sown per pot and pots were watered twice daily and fertilised with Aquasol® liquid fertiliser twice per week. No fungicides were used on the seedlings during the tests.

To test the pathogenicity of each fungal isolate, 14-day-old and 28-day-old seedlings were drenched with inoculum (50mL per pot). Control plants were drenched with de-ionised water (50mL per pot). All pots were randomly arranged on benches in a glasshouse and the plants were assessed for disease symptoms two and four weeks after drenching. At each assessment, the total number of seedlings and number of sick and/or dead seedlings was counted in each pot. Sick and dead seedlings were removed after each assessment.

Root/hypocotyl tissue sections from a selection of symptomatic seedlings were surface sterilised in a 1% sodium hypochlorite solution and cultured on PDA. Resulting colonies were examined microscopically to confirm they were morphologically identical to the cultures used to produce the inoculum suspensions.

Results

Species of *Pythium* and *Rhizoctonia* were the most common pathogens identified. Of 275 *Pythium* isolates, 157 were pathogenic to beetroot seedlings, and for *Rhizoctonia*, 59 isolates of 78 were pathogenic (Figures 4, 5 & 6). *Pythium aphanidermatum* isolates were more pathogenic to younger seedlings. Only 3 of 34 *P. aphanidermatum* isolates killed greater than 50% of plants inoculated at four weeks of age compared to 13 of 34 that killed greater than 50% of plants that were inoculated at one week of age. The pathogenic *P. ultimum* isolates were equally pathogenic to plants regardless of the age at which the plants were inoculated.

Pathogenic *Fusarium* isolates were also recovered, but were far less frequent (18/71). *Fusarium* was more often a saprophyte of diseased tissue than the causal agent of the disease. *Sclerotium* was recovered occasionally from some sites. Usually it was associated with beets that were growing poorly because of attack by other pathogens or extreme growing conditions (lack of water/high temperatures). In pathogenicity tests only 1 of 9 *Sclerotium* isolates was pathogenic, and this isolate was only weakly pathogenic to young plants. A suite of other fungi belonging to the genera *Macrophomina, Bipolaris, Exserohilium, Phoma, Helminthosporium, Colletotrichum,*
*Rhizopus* and *Nigrospora* were also recovered from beet soils and symptomatic plants. None of these were pathogenic to beet seedlings in pathogenicity tests.

**Figure 4:** Seedlings infected by a pathogenic *Rhizoctonia* (right) have blackened, rotted roots compared to healthy seedlings (left)

![Figure 4: Seedlings infected by a pathogenic Rhizoctonia (right) have blackened, rotted roots compared to healthy seedlings (left)](image)

**Figure 5:** *Pythium aphanidermatum* is highly pathogenic to young beetroot seedlings

![Figure 5: Pythium aphanidermatum is highly pathogenic to young beetroot seedlings](image)
Figure 6: Not all *Pythium* isolates associated with beetroot are pathogenic. Plants inoculated with non-pathogenic *Pythium* remain healthy (left), while a pathogenic *Pythium* isolate kills young seedlings (centre). Uninoculated control (right)

A summary of the major pathogens associated with specific beetroot blocks is provided in Table 3.
Table 3: Pathogenic fungi associated with specific blocks of beetroot

<table>
<thead>
<tr>
<th>Sample</th>
<th>Location Description</th>
<th>Pathogenic Fungi Found</th>
<th>Pathogenicity¹</th>
<th>Main Pathogens at Site²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brent</td>
<td>Home farm block 5</td>
<td><em>Pythium ultimum</em></td>
<td>moderate-high on both young and older plants</td>
<td><em>Pythium ultimum</em></td>
</tr>
<tr>
<td>Moira #1</td>
<td>Site of Fungicide trial 2, 2001</td>
<td>1. <em>Rhizoctonia</em></td>
<td>1. moderate-high on both young and older plants</td>
<td><em>Rhizoctonia</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. <em>Pythium ultimum</em></td>
<td>2. high on young plants, weak on older plants</td>
<td><em>Pythium ultimum</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. <em>Rhizoctonia</em></td>
<td>3. moderate-high on young plants, non-pathogenic to older plants</td>
<td><em>Pythium ultimum (species not determined)</em></td>
</tr>
<tr>
<td>Moira #2</td>
<td>Van de Weyer Rd (Forest Hill)</td>
<td>1. <em>Rhizoctonia</em></td>
<td>1. high to young plants, weak-moderate to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. <em>Pythium (species not determined)</em></td>
<td>2. moderate-high on young, non-pathogenic to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
<tr>
<td>Hauser</td>
<td>East side of house &amp; sheds, Gatton -</td>
<td>1. <em>Pythium aphanidermatum</em></td>
<td>1. high to young plants, moderate to older plants</td>
<td><em>Pythium aphanidermatum</em></td>
</tr>
<tr>
<td></td>
<td>Forest Hill Rd</td>
<td>2. <em>Pythium ultimum</em></td>
<td>2. moderate to young plants, weak-moderate to older plants</td>
<td><em>Pythium ultimum</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. <em>Rhizoctonia</em></td>
<td>3. moderate to young plants, weak to older plants</td>
<td><em>Pythium ultimum</em></td>
</tr>
<tr>
<td>G. Lerch #2</td>
<td>Site of fumigation trial 2002. After</td>
<td>1. <em>Pythium ultimum</em></td>
<td>1. weak to young plants, moderate to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
<tr>
<td></td>
<td>fumigation</td>
<td></td>
<td>2. high to young plants, moderate to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
<tr>
<td>G. Lerch #3</td>
<td>West side of Forest Hill – Blenheim Rd</td>
<td>1. <em>Pythium (species not determined)</em></td>
<td>1. weak to young plants, moderate to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. <em>Rhizoctonia</em></td>
<td>2. high to young plants, non-pathogenic to older plants</td>
<td><em>Rhizoctonia</em></td>
</tr>
<tr>
<td>P. Lerch</td>
<td>Corner of Forest Hill - Blenheim &amp;</td>
<td>1. <em>Pythium ultimum</em></td>
<td>1. moderate-high to young plants, non-pathogenic to older plants</td>
<td><em>Pythium (species not determined)</em></td>
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<td></td>
<td>Woodland Rds</td>
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<td>2. high to young plants, moderate to older plants</td>
<td><em>Pythium (species not determined)</em></td>
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<td>Litzow #1</td>
<td>First field behind Voight’s, Gatton –</td>
<td>1. <em>Pythium aphanidermatum</em></td>
<td>1. high to young plants, weak-moderate to older plants</td>
<td><em>Pythium aphanidermatum</em></td>
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<td>Forest Hill Rd</td>
<td>2. <em>Pythium dissotocum</em></td>
<td>2. moderate to young plants, non-pathogenic to older plants</td>
<td><em>Pythium dissotocum</em></td>
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<td>3. moderate to young plants, non-pathogenic to older plants</td>
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<td>Site of variety trial 2002</td>
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<td>1. high to young plants, moderate-high to older plants</td>
<td><em>Rhizoctonia</em></td>
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<td>Neumann #1</td>
<td>Site of variety trial 1, 2001</td>
<td>1. <em>Pythium ultimum</em></td>
<td>1. moderate-high to young plants, high to older plants</td>
<td><em>Pythium ultimum</em></td>
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<td>2. <em>Pythium (species not determined)</em></td>
<td>2. high to young plants, moderate-high to older plants</td>
<td><em>Pythium (species not determined)</em></td>
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<td>3. <em>Fusarium</em></td>
<td>3. moderate-high to young plants, non-pathogenic to older plants</td>
<td><em>Fusarium</em></td>
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<td>4. <em>Rhizoctonia</em></td>
<td>4. high to young plants, moderate to older plants</td>
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<td>Neumann #2</td>
<td>Site of variety trial 2, 2001</td>
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<td>1. high to young plants, weak-moderate to older plants</td>
<td><em>Rhizoctonia</em></td>
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<td>Neumann #3</td>
<td>Qualischefski's block</td>
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<td><em>Pythium ultimum</em></td>
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<td>3. non-pathogenic to young plants, weak-moderate to older plants</td>
<td><em>Pythium dissotocum</em></td>
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<td></td>
<td>4. <em>Pythium (species not determined)</em></td>
<td>4. non-pathogenic to young plants, weak to older plants</td>
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</tr>
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<td>Voight #2</td>
<td>Off Gatton - Forest Hill Rd (opposite</td>
<td>1. <em>Rhizoctonia</em></td>
<td>1. high to young plants, moderate-high to older plants</td>
<td><em>Rhizoctonia</em></td>
</tr>
<tr>
<td></td>
<td>Litzow House)</td>
<td>2. <em>Fusarium</em></td>
<td>2. moderate to young plants, non-pathogenic to older plants</td>
<td><em>Fusarium</em></td>
</tr>
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<td>3. <em>Pythium aphanidermatum</em></td>
<td>3. moderate-high to young plants, non-pathogenic to older plants</td>
<td><em>Pythium aphanidermatum</em></td>
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<td></td>
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<td><em>Pythium ultimum</em></td>
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<tr>
<td>Voight #3</td>
<td>Off Gatton - Forest Hill Rd (opposite</td>
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<td>1. high to young plants, non-pathogenic to older plants</td>
<td><em>Pythium (species not determined)</em></td>
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<td><em>Pythium (species not determined)</em></td>
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<tr>
<td>Zelinski</td>
<td>Site of commercial scale variety trial</td>
<td>1. <em>Pythium (species not determined)</em></td>
<td>1. moderate-high to young plants, moderate-high to older plants</td>
<td><em>Pythium (species not determined)</em></td>
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<td>2. weak to young plants, weak to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
<tr>
<td>Moira #4</td>
<td>Block at Laidley behind John Berlin’s</td>
<td>1. <em>Pythium (species not determined)</em></td>
<td>1. moderate-high to young plants, non-pathogenic to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
<tr>
<td></td>
<td>house</td>
<td></td>
<td>2. weak-moderate to young plants, weak to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
<tr>
<td>Neumann</td>
<td>Sample A</td>
<td><em>Pythium (species not determined)</em></td>
<td>1. high to young plants, weak to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
<tr>
<td>P. Lerch</td>
<td>Sample A</td>
<td><em>Pythium (species not determined)</em></td>
<td>1. non-pathogenic to young plants, weak to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
<tr>
<td>Moira</td>
<td>Sample A</td>
<td><em>Pythium (species not determined)</em></td>
<td>1. non-pathogenic to young plants, weak to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
<tr>
<td>G. Lerch</td>
<td>Sample A</td>
<td><em>Pythium (species not determined)</em></td>
<td>1. moderate-high to young plants, non-pathogenic to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
<tr>
<td>G. Lerch</td>
<td>Sample B</td>
<td><em>Pythium (species not determined)</em></td>
<td>1. moderate-high to young plants, weak to older plants</td>
<td><em>Pythium (species not determined)</em></td>
</tr>
</tbody>
</table>

¹Pathogenicity rating scale (% plants dead): weak=1-15%, weak-moderate=16-30%, moderate=31-50%, moderate-high=51-75%, high=76-100%. Young plants inoc. 2 wk after sowing. Older plants inoc. 4 weeks after sowing

²Based on frequency of isolation from diseased plants in soil indexing assays

³Pathogenic *Fusarium* recovered from diseased plants in variety trial, not from soil indexing assay.
Variability in Pathogenicity of Pythium Species with Temperature and Plant Age

Introduction

Broadly, *Pythium* is often purported to be a more severe pathogen of young plants. Damping off symptoms are most often associated with *Pythium* infection in a range of hosts. In our pathogenicity testing in which we inoculated 2-week-old and 4-week-old beetroot plants however, some of the *Pythium* isolates were more pathogenic to the older seedlings and others were equally pathogenic to seedlings of both ages (Table 3). In addition to plant age, soil temperature is another factor that influences the severity of *Pythium* epidemics in the field (Hancock, 1977). Different pathogenic *Pythium* species are reported to require different temperatures for epidemic development in beetroot. For example, in a field survey in North Dakota and Minnesota, Kuznia and Windels (1993) found that *Pythium aphanidermatum* caused more seed rot and damping off when soil temperatures increased from 14 to 31ºC, whereas the reverse relationship was observed for *Pythium ultimum*.

In view of the fact that there are several different pathogenic species of *Pythium* on beetroot in Australia, species-specific temperature requirements may be an important consideration in a disease management strategy. In particular, there may be an opportunity to manipulate planting dates depending on which pathogens are present at particular sites. For example, sowing beets into soils in which species favoured by relatively high temperatures predominate, should be avoided during the hottest months of the growing season. We completed a controlled environment cabinet study to determine the influence of plant age and temperature on disease severity, for the three most common *Pythium* species in local beetroot soils: *Pythium aphanidermatum*, *Pythium ultimum* and *Pythium dissotocum*.

Materials and Methods

Pots of beetroot seedlings (cv. Detroit Dark Red) of 6 different ages (3, 10, 14, 21, 28 and 35 days after sowing) were drenched with inoculum of one of three *Pythium* species (*P. aphanidermatum*, *P. ultimum* or *P. dissotocum*) or water (control) and were maintained at one of six different temperatures (10, 15, 20, 25, 30, 35ºC) for four weeks. Each pot contained 10 plants. Twenty-four pots of seedlings of each age were drenched with each treatment. Four pots of each age/drench combination were incubated in each of 6 controlled environment cabinets at a different temperature, such that each incubator contained 24 pots of plants. Pots were watered twice daily and the humidity in each cabinet was set at approx 90%.

The *Pythium* isolates used were: #1339 (8) *P. aphanidermatum* ex. mature wilting beets (Peter Lerch), #1623 (10) *P. ultimum* ex. soil variety trial 1, 2001 (Merv Neumann) and #1688 (16) *P. dissotocum* ex. soil Qualischefski block (Merv Neumann). Inoculum was prepared by scraping 7-day-old *Pythium* cultures in sterile de-ionised water (1 plate/200mL water). Each pot was drenched with 50mL of inoculum and the pots were allowed to drain before they were transferred to the cabinets.

Pots were assessed weekly. Dead seedlings were counted and removed. At the final assessment time the shoots were cut from the plants and the shoots and roots were weighed separately.
Results

*Pythium aphanidermatum* was the most pathogenic of the three *Pythium* species. Infection by *P. aphanidermatum* developed only at temperatures of 15°C or above. Young plants were more susceptible to infection. At 15°C disease developed only in 3- and 10-day-old plants. Significant levels of disease developed in 10- and 14-day-old plants at temperatures ≥ 25°C, but temperatures of 30°C or greater were needed for the pathogen to infect 21-day-old-plants. For 28- and 35-day-old plants, significant levels of disease developed only when the temperature reached 35°C (Figure 7).

**Figure 7:** The severity of disease caused by *P. aphanidermatum* is strongly dependent on both plant age and temperature

% seedlings surviving 28 days after inoculation

*Bars with the same letters are not significantly different at the 5% level*

*Pythium ultimum* and *P. dissotocum* were less pathogenic than *P. aphanidermatum*. For *P. ultimum*, no clear relationship existed between disease severity and the plant age x temperature interaction (Figure 8).
**Figure 8:** No clear relationship exists between plant age x temperature and the severity of disease caused by *P. ultimum*

![Graph showing survival rate of seedlings at different temperatures and inoculation times.](attachment:graph.png)

*Pythium dissotocum* infected plants at temperatures of 30ºC or greater. Three-day-old plants were susceptible to infection at 30ºC, and plants of all ages were susceptible at 35ºC.

**Figure 9:** Beetroot plants were susceptible to infection by *Pythium dissotocum* at ≥ 30ºC

![Graph showing survival rate of beetroot plants at different temperatures and inoculation times.](attachment:graph2.png)

* Bars with the same letters are not significantly different at the 5% level

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33
For Queensland beetroot growers, it may be possible to limit disease epidemic development in blocks where pathogenic *Pythium aphanidermatum* occurs, by delaying planting until the winter months when soil temperatures are lower. Similarly, losses due to *P. dissotocum* may also be reduced on sites known to contain this pathogen by delaying sowing to the cooler months. Sites where this technique may be useful are Voight #1, Voight #2, Litzow #1, Neumann #3 and Hauser (Table 3).

**Pathogenicity of Pythium aphanidermatum and Rhizoctonia to crops other than beetroot**

**Introduction**

Cropping beets at high frequency increases soil-borne pathogen populations, resulting in yield losses. For this reason, 3-5 year rotations out of beet is standard industry practice in beet cropping regions in the United States (Herr, 1987; Rush and Winter, 1990). If an alternate crop is to be grown with the objective of reducing the levels of beet pathogens in the soil however, the alternate crop must not itself be a host for the beet pathogens, otherwise the pathogen inoculum level in the soil may be increased by planting the rotational crop. Published information identifies graminaceous crops such as corn or oats, or *Brassicas* as crops that will not increase pathogen inoculum density if grown in rotation with beetroot. We tested the susceptibility of 22 different crop types commonly grown in the Lockyer and Fassifern Valleys, to pathogenic *Rhizoctonia* and *Pythium aphanidermatum* isolates collected from local beetroot crops, in the glasshouse. Our objective was to identify non-susceptible crops that may be useful in helping to limit pathogen inoculum build-up when grown in rotation with beetroot.

**Materials and Methods**

Seeds of 22 types of crop species were planted in pots of UC mix in the glasshouse. Sowing dates were staggered so that all the plant types were at a similar stage of development when they were inoculated with either *Rhizoctonia* or *Pythium aphanidermatum* on 7 April 2003. Crop and cultivar information and sowing dates are provided in Table 4.

<table>
<thead>
<tr>
<th>Crop Type</th>
<th>Cultivar</th>
<th>Sowing Date</th>
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<tbody>
<tr>
<td>lettuce</td>
<td>Greenway</td>
<td>14 March 2003</td>
</tr>
<tr>
<td>capsicum</td>
<td>Giant Bell</td>
<td>14 March 2003</td>
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<tr>
<td>onion</td>
<td>Gladalan Brown</td>
<td>14 March 2003</td>
</tr>
<tr>
<td>beetroot</td>
<td>Detroit Dark Red</td>
<td>17 March 2003</td>
</tr>
<tr>
<td>carrot</td>
<td>Royal Chantenay</td>
<td>17 March 2003</td>
</tr>
<tr>
<td>silverbeet</td>
<td>Fordhook Giant</td>
<td>17 March 2003</td>
</tr>
<tr>
<td>watermelon</td>
<td>Country Sweet</td>
<td>17 March 2003</td>
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<tr>
<td>cotton</td>
<td>Sicala 40</td>
<td>21 March 2003</td>
</tr>
<tr>
<td>pea</td>
<td>Early Crop Massey</td>
<td>21 March 2003</td>
</tr>
<tr>
<td>tomato</td>
<td>Grosse Lisse</td>
<td>21 March 2003</td>
</tr>
<tr>
<td>oats</td>
<td>Moolah</td>
<td>21 March 2003</td>
</tr>
<tr>
<td>broccoli</td>
<td>Summer Green</td>
<td>21 March 2003</td>
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<tr>
<td>soybean</td>
<td>A6785</td>
<td>21 March 2003</td>
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<tr>
<td>pumpkin</td>
<td>Queensland Blue</td>
<td>25 March 2003</td>
</tr>
<tr>
<td>chinese cabbage</td>
<td>Wong Bok</td>
<td>25 March 2003</td>
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<tr>
<td>sweet corn</td>
<td>Pacific H3</td>
<td>25 March 2003</td>
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<tr>
<td>wheat</td>
<td>Giles</td>
<td>25 March 2003</td>
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<tr>
<td>French beans</td>
<td>Simba</td>
<td>25 March 2003</td>
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<tr>
<td>sunflower</td>
<td>-</td>
<td>28 March 2003</td>
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<tr>
<td>lucerne</td>
<td>Sequel</td>
<td>28 March 2003</td>
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<tr>
<td>sorghum</td>
<td>Jumbo</td>
<td>28 March 2003</td>
</tr>
<tr>
<td>Dolichos</td>
<td>Rongai</td>
<td>28 March 2003</td>
</tr>
</tbody>
</table>
Two pots of each type were planted and seedlings were thinned to 5 per pot before inoculation. We used *Pythium aphanidermatum* isolate #1339 (8) ex. mature wilting beets (Peter Lerch), and *Rhizoctonia* isolate #1669 (4) ex. soil Voight #2. Inoculum was prepared by scraping 7-day-old cultures in sterile de-ionised water (1 plate/200mL water). Each pot was drenched with 50mL of inoculum and the pots were arranged randomly on glasshouse benches. Pots were assessed weekly for 4 weeks. Dead seedlings were counted and removed.

**Results**

Barley was the crop that was the least susceptible to infection by either pathogen, followed by Dolichos (Figure 10). These two crop types would therefore, be the most suitable for planting as rotations with beetroot on sites that are known to be infested with both pathogen types (Voight #2, Neumann #3, Hauser). For sites where *Rhizoctonia* is the primary problem, barley, French beans, capsicum, soybean or tomatoes look to be the most prospective rotational crops (Moira #1, G.Lerch #3, Litzow #2, Neumann #1, Neumann #2). For sites where *P. aphanidermatum* is the primary pathogen (Litzow #1, Hauser), barley, millet, sorghum, sunflower, sweetcorn and wheat offer promise as rotational crops.

**Figure 10:** Relative susceptibility of a range of crop types to a *P. aphanidermatum* isolate and a *Rhizoctonia* isolate that are pathogenic to beetroot.
CHAPTER 3: Beetroot Variety Trials

Introduction

The Lockyer Valley district of south-east Queensland supplies approximately 90% of Australian processed beetroot. Beets are mechanically harvested and are processed by Golden Circle P/L. In order to ensure that the processed product is of a consistent high quality, Golden Circle imposes quality specifications on the raw material provided at the factory. Slicing beets are required to be globe shaped, 50-110mm in diameter, with a small crown and a small non-tapered taproot. Baby beets must fall within the 25-50mm diameter size range. Beets that are misshapen, cracked, or show evidence of disease lesions or other mechanical damage are rejected at the cannery or require additional manual processing, which substantially increases processing costs.

This industry now depends on only three slicing beet varieties, two open-pollinated varieties (Detroit Dark Red and Garnet) and one hybrid (Pablo), and one baby beet variety (New Globe). In recent years soil-borne diseases (Pythium, Rhizoctonia and Aphanomyces) have been reported to cause substantial yield and quality reductions, particularly in crops grown at the extremities of the growing season (i.e. those planted in February/March and those harvested October-December). These pathogens can result in poor stand establishment and may give rise to poor quality, misshapen beets that do not meet the processing specifications set by Golden Circle. Species of Pythium and Rhizoctonia are currently particularly problematic for this industry.

A) Field Assessments of Beetroot Varieties – 2001

Materials and Methods

Thirty-two beetroot varieties were compared in two field trials planted on-farm at Forest Hill at a site with a previous history of disease. The first trial was planted in an early planting window on 6th April 2001, and the second was planted in a later planting window on 6th June 2001. Seed lots were provided directly by seed companies (Table 5). The seeds provided had typically been treated with either thiram or a thiram/metalaxyl seed dressing combination. Prior to planting, seed lots that only had a thiram dressing were also treated with metalaxyl (Apron Liquid Formulation 35gai/100kg seed) to ensure that a standard treatment (thiram + metalaxyl) was applied to seed of each variety in the trials.
CHAPTER 6: Technology Transfer

- Workshops were held biannually throughout the project between the project team and key industry personnel (beetroot growers, Golden Circle employees, seed and chemical company representatives). The workshops have provided the industry with the opportunity to review progress and plan future research activities. At the first workshop (held in December 2000), each workshop attendee was provided with a copy of the literature review entitled “Management options for soil-borne diseases of beetroot”. At later workshops, attendees were provided with handouts detailing the results of latest research trials.

- In July 2001 and July 2002, as a component of the mid-year workshops, industry members and seed company representatives completed field walks of varietal trials at Merv Neumann’s farm (2001) and Moira Farms and Ashley Zelinski’s farm (2002) (Figure 58).

Figure 58: Field walks of varietal trials allowed the industry to assess the performance of beetroot varieties

- Several media releases have also highlighted the work completed by the industry and the project team. A story was filmed for “Landline” (ABC TV) on 10 September 2002 and was aired on 13 October 2002. A story was filmed for “Totally Wild” (Network 10) on 13 May 2003 and a newspaper article was published in “The Courier Mail” (Life liftout section) in June 2003.

- In July 2003, the project team compiled a survey that was sent to each beetroot grower from the Lockyer Valley and Bunny Bite Farms in the Fassifern Valley. The growers completed the survey anonymously. This survey was compiled to check the satisfaction of the industry with the work being done in the project. The survey was timed so that the project had been running for long enough for results to have been achieved, but in time for
improvements/changes to be made before the end of the project, if requested.  
A summary of the survey results are provided below:

**Beetroot Industry Questionnaire (no. of responses = 10)**

1. How would you rate the level of difficulty associated with producing consistent, high quality beetroot?

   - 1: no difficulty
   - 2: moderate
   - 3: extreme difficulty

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<td>0</td>
<td>1</td>
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2. On a scale of 1 (not important) – 5 (very important), how big a constraint are each of the following to your enterprise? If you are unsure, please mark with a U.

   a) soil-borne diseases  **Av. 4.44**  
   b) inconsistent availability of high quality seed  **Av. 4.00**  
   c) poor performance of existing varieties  **Av. 4.11**  
   d) lack of water  **Av. 4.11**  
   e) insufficient space to rotate out of beetroot  **Av. 3.22**  
   f) insect pests  **Av. 2.33**  
   g) weeds  **Av. 3.22**  
   h) lack of information about best practice for beetroot production  **Av. 3.00 (2U)**

3. On a scale of 1 (dissatisfied) to 5 (very satisfied), how satisfied are you with the research activities in the current beetroot project?

   - 1: dissatisfied
   - 2: moderately satisfied
   - 3: very satisfied

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<td>dissatisfied</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>1</td>
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4. Please list positive outcomes that you feel have arisen from the project.

   - organised and concerted approach to the whole issue
   - increased knowledge of disease types and presence (6)
   - rapid improvement in cooperation with seed companies
   - availability of wide selection of seed for trials
   - collation of trial results and information
   - trials of new chemicals
   - variety trials – new varieties (8)
   - moving towards creating a beet seed that suits our conditions (Seed improvement work) (2)
   - fungicide permits
   - identification of poor quality seed as a major issue
5. Please indicate areas that you think need improvement ie. what isn’t the project team doing that we should be doing, or what are we doing poorly?

- no suggestions (3)
- more work in rotational crops and soil indexing
- more work on plant nutrition for our soils. Ensuring plant is healthy so that it is better able to combat disease
- not enough is being done on disease problems. Disease is a bigger issue than seed quality/beet varieties
- more research is needed into finding/developing better seed
- faster response to problems needs to happen and attention to “hotspots”
- response to grower inquiry is very good, but follow-up takes a long time

6. Which of the following statements do you feel are true? Please mark with T=true, F=false, U=undecided.

1. The return on investment in the current beetroot project has been poor (F (5), T (1), U (2))
2. The project team are willing to listen to the needs of the industry (T (10))
3. I have little confidence in the ability of research to help achieve solutions that are meaningful on-farm (F (8), U (1))
4. The DPI staff should be doing more to help the industry (F (5), T (3), U (1))
5. Beetroot growers should be doing more to help the industry (F (3), T (4), U (2))
6. Golden Circle should be doing more to help the industry (T (6), F (1), U (2))
7. The DPI staff don’t understand the real issues facing the beetroot industry (F (8), U (2))
8. It is reasonable to expect that research should deliver solutions (T (9), F (1))
9. The research that is being done in the current project may be useful to some members of the group, but it is irrelevant to me (F (6), T (1))

7. How important is it for the beetroot industry to invest in another research project?

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<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>3</td>
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</table>

8. Briefly explain your response to Question 7.

- need results on hairy root
- I don’t want to go back to doing nothing
- We should continue with the work on soil-borne diseases. Seed production may have to be left to the seed companies because of the cost of our input plus theirs
- I would like to see some results first before investing in more projects
- I am very interested in the chemical and variety trials
- We need to continue the research, as long as it is targeted to where the growers see the greatest need
- There is still work to be done with disease and seed issues. If the day comes
when these issues are manageable and at an acceptable stage, other areas should be looked at for improvement. Whether it is at Golden Circle or DPI, research should never stop

9. Please list activities that should be the focus of future beetroot research,

- Diseases of beetroot (4)
- Variety trials (2)
- Variety trials – baby as well as slice (2)
- Rotational crops
- New herbicides for use in beetroot (3)
- Fungicide trials
- Residue testing for new chemicals
- Seed production (2)
- Hairy root problem
- Agronomy to improve yield. eg. Timing of fertiliser etc.
- Nutrition
- More efficient and effective irrigation

10. The following activities were suggested in the meeting held on 25/5/03 as areas where future research effort should be directed. Please indicate next to each activity how big a priority you feel each is (0 = forget it, 1 = minor importance, 2 = moderate priority, 3 = major priority, 4 = top priority).

a) Continuation of variety trials (on-farm assessments) (0(0), 1(1), 2(1), 3(5), 4(3))
b) Development of varieties with better disease tolerance (0(0), 1(2), 2(0), 3(3), 4(5))
c) Overseas study tour (0(0), 1(0), 2(4), 3(3), 4(3))
d) Continuation of varietal selection program with Henderson Seeds and at Stanthorpe (with the aim of ensuring genetic integrity and providing a consistent supply of high quality seed) (0(0), 1(0), 2(1), 3(4), 4(5))
e) Identification of the best rotational crops from the standpoint of disease management (0(0), 1(0), 2(1), 3(6), 4(3))
f) Identify and assess alternative herbicides (0(0), 1(0), 2(3), 3(5), 4(1))
g) Assess the influence of water quality on disease incidence and beetroot quality (0(1), 1(1), 2(2), 3(4), 4(2))
h) Best practice for fungicide spray application (0(1), 1(1), 2(3), 3(4), 4(1))
i) Identification of methods to improve soil health (0(0), 1(0), 2(1), 3(6), 4(3))

11. Other comments:

- problems identified need to be worked on
- The new project is a lot of money so we will need to see some positive outcomes. I know these type of trials can take time to see results
- I don’t mind more research as long as Golden Circle are happy to keep putting money forward and the money we have been giving to QFVG covers it with help from grants
- I think growers need to stay in touch and direct the DPI in the direction they wish DPI to go in these projects
- not enough research is going into disease
- need to focus on the issues at hand rather than taking on a great number of projects and doing them poorly. We are doing trials and all learning and this takes time – very frustrating. The growers and Golden Circle have always directed where they wanted the project to go – as it should be. This survey gives everyone a chance to say their piece – very good.
General Discussion

The soilborne fungal diseases currently jeopardising the Australian beetroot industry are not unique to Australia. The same diseases have been the focus of much research effort in sugarbeet and beetroot crops in many parts of the world. No single measure has been effective in controlling these diseases elsewhere, which, along with the research we have done in this project, supports the view that for Australian beetroot producers, a soil-borne disease management strategy comprising a combination of control tactics is likely to be required for effective disease control.

The fundamental issue that has lead to an increase in prevalence of soil-borne diseases in this industry has been the extension of the growing window into periods of high disease risk. As a consequence of this extended growing window, growers, particularly those with smaller farms, have less opportunity to rotate out of beetroot, or if they are able to rotate it is only for short periods. The result is a continual increase in the quantity of disease inoculum in the beet soils and heavy losses due to disease, particularly in crops planted during periods of high disease risk. Unless measures are taken to reduce pressure on growers to grow more beets over a longer window, the gravity of the soil-borne disease issues faced by this industry will only increase.

Aside from this fundamental change that needs to occur if this industry is to survive, this project has identified several tactics that will assist the beetroot industry to better manage its soilborne diseases and improve the quality of its product.

First, we have identified that *Rhizoctonia* and *Pythium* are the most important soilborne fungal pathogens responsible for disease outbreaks on farms throughout the beet-growing areas of south east Queensland and, with the assistance of Dr Paul Scott (UQ Gatton), have characterised the species of *Pythium* responsible. The three most common disease-causing *Pythium* species differ in their abilities to cause disease on plants of different ages and only cause disease at certain temperatures. Consequently, there is the opportunity to reduce disease losses by manipulating when blocks dominated by particular species are planted. For example, since *Pythium aphanidermatum* is highly pathogenic to very young plants at temperatures greater than 15°C, blocks in which this pathogen predominates should not be planted early in the season.

Second, we have identified fungicides that will help reduce disease losses. A combination of Apron and Rizolex WP will give significant disease control and for best results, it should be applied as a slurry to seed. Although promising in initial field trials, Tachigaren would appear to be of limited use in beetroot because it slows germination and may inhibit it completely if applied at high rates. We obtained a minor use permit for Rizolex as a seed dressing or in-furrow treatment for beetroot. Additional residue and efficacy trials must be completed for Rizolex for this permit to be extended beyond 2005.
Third, in glasshouse studies we identified prospective crops that if grown in rotation with beetroot do not promote further disease inoculum build-up. Barley and Dolichos were the poorest hosts of *Pythium aphanidermatum* and *Rhizoctonia* of 22 crop types assessed. Therefore, they may be useful as rotational crops at sites with mixed infections of both pathogen types. These glasshouse studies should be verified in field trials in a future research program.

Fourth, we have assessed more than 90 different beetroot varieties and have identified types that are prospective alternatives to the current standard lines. Detroit Dark Red, one of the industry standards was consistently a very poor performer in our trials, leading the project team and the industry to speculate that seed companies are no longer maintaining this open-pollinated variety. A seed production and improvement program for the open-pollinated lines has commenced with Henderson Seeds as a direct consequence of our research.

This industry would also benefit by switching to monogerm beet types. We demonstrated a clear inverse relationship between plant spacing and the quantity of misshapen material produced. The current standard varieties are all multigerm types. With multiple shoots arising from each seed cluster, the plants encroach on each other as they grow, increasing the quantity of misshapen material and reducing recovery at the cannery. With monogerm seed, the quantity of misshapen product is reduced, because plant spacing can be controlled. Monogerm beet seed is significantly more expensive than that of the standard lines, however the economic gains associated with improved quality should far outweigh the increased initial cost. If Golden Circle P/L are committed to improving efficiency in beet production, they should complete a cost/benefit analysis for monogerm varieties.
Acknowledgements

I would like to extend sincere thanks to the project team members, in particular, Scott Boreel, Vicki Hamilton, Peter Scholl, Eric Coleman and Bob Davis for their hard-work and dedication to this project. Assistance from Peter Case, Gerry Macmanus, Sandra Dennien, Carolyn Lee, Russell McCrystal, Belinda Bowe and Amanda Love in planting, harvesting and assessing field trials is also gratefully acknowledged. I also wish to thank Craig Henderson for developing the original project proposal for submission to HAL.

The work done by Dr Paul Scott (The University of Queensland) in identifying the species of pathogenic *Pythium* isolates added an additional dimension to the work that was not planned in the genesis of the project, but which contributed enormously to the project outcomes. We are extremely grateful to Paul for his efforts and his willingness to collaborate with us.

Considerable in-kind contributions were provided from seed and chemical companies. We are thankful to Barry Donahoe (Syngenta), Difang Chen (Alf Christianson Seed Co.), David Commens (SPS), Tim Lewis and Andrew Henderson (Henderson Seeds), Wayne Hoey (Le Froy Valley), Michael Sippel (Yates), Paul Hesseltine (Bejo), and Ole Johansen (Daehnfeldt), for their contributions. We also thank Rob Vitelli (Bayer), Chris Stuart (Sumitomo Australia Ltd), Patrick Press (Sumitomo Chemical Australia P/L), Tanya Middeldorp (Uniroyal Chemical), Natalie Rose (Syngenta Crop Protection P/L) and Matthew Gilmore (Barmac Industries) for supplying us with fungicides to trial.

John Hunt and Rob Stanic (Agrimm P/L), Steve Capeness (Vermitech), Alan Mudford (Wrightson’s Seeds), Richard Armstrong (Enviroganics P/L), Peter Thompson (Terra Firma Fertilisers) and Peter Stewart (Pacific Seeds) all provided in-kind contributions for the organic amendments and crop rotation trial, and their contributions are appreciated.

We thank Shane Litzow for taking the time to fix his metham applicator and fumigating the site for the organic amendments and crop rotation trial.

The beetroot growers of the Lockyer Valley contributed enormously to this work by providing us with sites on their farms to establish field trials, and by maintaining the trials from planting through until harvest. Specifically we wish to thank Merv Neumann, Peter Voight, Linton Brimblecombe, Tim Pocock, Glenn Lerch, Ashley Zelinski and Peter Lerch.

We thank HAL, Golden Circle P/L and Queensland Department of Primary Industries and Fisheries for funding this project.