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Characteristics of Australian isolates of Phytophthora infestans and planning to manage new and more aggressive strains of the fungus

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CRC for Tropical Plant Protection

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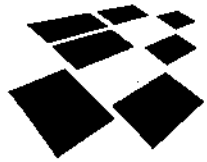

Horticulture Australia

Characterisation of Australian isolates of *Phytophthora infestans* and planning to manage new and more aggressive strains of the fungus

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Horticulture Australia

PT98009 Characterisation of Australian isolates of *Phytophthora infestans* and planning to manage new and more aggressive strains of the fungus

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Purpose of the report

New strains of the potato late blight fungus, *Phytophthora infestans*, are severely damaging potato crops overseas and have spread rapidly around the globe since the early 1980's. These new and more aggressive pathogen strains have forced affected countries to introduce intensive and expensive spray programs, which are only partially effective. Australia is currently believed to be among the few countries in the world still free of the new strains of this pathogen, and as a result, yield loss and costs of managing the disease are relatively low. The aim of this investigation was to characterise the Australian *P. infestans* population in detail according to mating type, metalaxyl sensitivity and DNA profiles. This would provide a reference against which to test populations in the future in light of the risk of introducing new strains, raise awareness of this disease in the industry and provide a basis for developing incursion management strategies for new strains.

Acknowledgements

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1 Media Summary

A study of Australian populations of the potato late blight fungus, *Phytophthora infestans*, has found none of the new strains that are wreaking havoc in potato crops across Europe, North and South America and parts of Asia. The Australian strains tested so far are like the old European and North American strains, belonging only to the A_1 mating type, and proved to be very sensitive to the *Phytophthora* specific fungicide metalaxyl (Ridomil®). Further evidence that we do not have the new strains is that late blight still only occurs sporadically in Australia, usually on the north-west coast of Tasmania and in the central highlands region of Victoria (Ballarat), and that serious outbreaks have been managed effectively with metalaxyl. However, in many other countries, late blight has become the single most destructive and costly disease of potatoes.

Late blight caused the Irish potato famine during the 1850's. The original populations of the fungus, which spread to Europe with the introduction of potatoes from South America, consisted of only one mating type of the fungus, the A_1 type. The fungus could only survive from one season to the next on volunteer potatoes and infected seed tubers.

A second wave of migration of the fungus from central Mexico, began in the mid 1980's, spreading new A_1 strains, as well as a second mating type (A_2) throughout the world. The new populations now consist of many different strains of the A_1 and A_2 mating types. These strains are much more aggressive and cause severe stem lesions, readily infect tubers and produce sexual spores (oospores) which can survive in a dormant state in soil for several years in the absence of a host. They are able to infect the potato crops under a much wider range of environmental conditions, causing major losses where the disease was easily managed in the past. They are also resistant to metalaxyl, with the result that crops in Europe must be sprayed continuously with protectant chemicals, at intervals as little as five days apart, to achieve control. Because of the presence of the two mating strains, the new populations are more genetically diverse and adaptable and have very quickly replaced the old, less aggressive strains in Europe, North and South America, Russia and parts of Asia.

The aim of this study was to characterise or 'fingerprint' the Australian late blight fungus against other strains from around the world. Samples were taken from several diseased crops and diseased volunteer potatoes in northwest Tasmania and Victoria during the 1998-99 and 1999-2000 seasons. The disease develops in these areas after several days of warm, sultry weather conditions in mid to late summer, which allow the formation of dew on leaves.

The Australian late blight fungus proved to be more difficult to isolate from potato tissue and to grow in culture than reference strains from Europe and North America. Nevertheless, 28 cultures were successfully obtained from the Tasmanian samples. Unfortunately, culturing from the Victorian material was not successful. In all likelihood, the Victorian strains will be similar to those from Tasmania but further sampling and testing will be necessary to confirm this.

Although the evidence suggests that we only have the old A_1 strains in this country, we cannot afford to be complacent. The inadvertent introduction of the new strains of *Phytophthora infestans* into Australia could be very costly. We must determine the risk of this happening and develop strategies to minimise the risks and deal with any incursion. The first signs of the new strains will be an increased frequency and severity of outbreaks of late blight and an inability to control the disease with metalaxyl.

2 Technical Summary

The aim of this project was to characterise Australian strains of *Phytophthora infestans* causing late blight of potatoes, in light of the development of new and more aggressive strains in North America and Europe, which are resistant to the fungicide metalaxyl and are causing major economic losses. Samples of diseased material were collected during the summers of 1998/99 and 1999/2000 from outbreaks of late blight in crops on the north coast of Tasmania and the Central Highlands region of Victoria where the disease traditionally occurs.

Twenty-eight isolates were successfully brought into culture from the Tasmanian late blight samples. However, the fungus was not successfully isolated and cultured from Victorian samples. Compared to reference isolates from overseas, the Tasmanian isolates grew rather poorly on different media and under different conditions which can account for difficulties in isolating and culturing the fungus. This observation is consistent with findings from overseas where isolates representing the old A1 mating type population are harder to culture than representative of the new *P. infestans* population which grow readily in culture.

None of the isolates tested were found to have characteristics that were consistent with the strains presently found in Europe and North America. Of 24 of the Tasmanian isolates paired against tester strains of the A_1 and A_2 mating types, all were found to be A_1 types. Of 21 isolates tested, all proved to be very sensitive to metalaxyl *in-vitro*. DNA fingerprinting with the RG-57 probe revealed that all of 20 isolates tested were identical and had a fingerprint pattern with a high level of homology to the old *P. infestans* populations which were found throughout the world prior to 1980. They proved to be closely related to the old US1.3 clonal line. The RG-57 fingerprint pattern for the 20 isolates varied slightly from patterns previously described for Australian isolates (AU-1 and AU-2) and were designated AU-3. The isozyme and DNA fingerprint pattern of these isolates provide evidence that the Australian isolates are somewhat different from the ones spread around the globe from Europe.

The characteristics of the Tasmanian strain is in line with field observations that late blight is a minor disease, occurring sporadically and locally in some districts, and readily controlled with foliar applications of metalaxyl. The new strains overseas, which include both the A_1 and A_2 mating types, are proving to be more aggressive and adaptable, resistant to metalaxyl, able to survive in soil without a host for several seasons as oospores and require very costly spray programs which are less effective than metalaxyl. Evidence of new strains in Australia would include a breakdown in control with metalaxyl, a greater incidence and severity of outbreaks, an earlier start to epidemics and a more widespread occurrence of the disease.

Australia is one of the few countries in the world not to be affected by a serious late blight problem. In order to maintain this status, we must be informed and vigilant. Recommendations for further work include an assessment of the risk of introducing new strains into Australia, determining the likely scenario's for the potato industry should new populations of *P. infestans* occur and to develop contingencies to deal with possible incursions of the new strains of the fungus.

3 Technical Report – *Phytophthora infestans* in Australia

3.1 Background

Late blight, caused by the fungus *Phytophthora infestans*, was the cause of the Irish potato famine of the 1850's but in modern times was effectively managed through the use of more resistant cultivars and fungicide spray programs.

Phytophthora infestans exits in two different forms known as different 'mating' types, A_1 and A_2. The *Phytophthora* fungi reproduce sexually through mating between male (antheridia) and female structures (oogonia) to produce thick-walled spores, the oospores. Some species of *Phytophthora* are self-fertile, producing both male and female mating structures. However, *Phytophthora infestans* only produces oospores when the two mating types (A_1 and A_2) come together. This means that genetic information (DNA) from two different parents is combined resulting in greater genetic diversity packaged in the hardy oospore.

Original populations of the late blight fungus, which spread from Europe with the introduction of potatoes from South America, consisted of only one mating type of the fungus, the A_1 type. This fungus could only 'overwinter' as mycelia in potato tubers or in other hosts.

A second wave of migration of the fungus from central Mexico, began in the mid 1980's, spreading new A_1 strains, as well as a second mating type (A_2) throughout the world. The new populations now consist of many different strains of the A_1 and A_2 mating types. These strains are much more aggressive and cause severe stem lesions, readily infect tubers and produce sexual spores (oospores) which can survive in a dormant state in soil for several years in the absence of a host. They are able to infect the potato crops under a much wider range of environmental conditions, causing major losses where the disease was easily managed in the past. They are also resistant to metalaxyl, with the result that crops in Europe must be sprayed continuously with protectant chemicals, at intervals as little as five days apart, to achieve control. Because of the presence of the two mating strains, the new populations are more genetically diverse and adaptable and have very quickly replaced the old, less aggressive strains in Europe, North and South America, Russia and parts of Asia. Late blight is now one of the most destructive diseases of potato around the world.

The following review describes the biology of late blight and highlights the differences between the old populations of the fungus and the new more aggressive strains.

3.2 Review of the literature

3.2.1 The original 1840's late blight migration

The first global migration of late blight occurred in the 1840's when a new disease was reported on potatoes in Philadelphia (Petersen *et al.*, 1992; Stevens, 1933). Over time, reports of this disease came from more distant locations and by 1845 the disease had spread through the northeastern USA, into the midwest states of the USA and into Canada. The first reports of the disease in Europe came from Belgium in June 1845 (Bourke, 1964). During that summer late blight spread over Europe and by mid October most of the potatoes in northwest Europe, from Poland to Ireland, were affected by the disease (Bourke, 1964). The resulting disease epidemics in the years to follow led to crop failure and rotting of potatoes in storage,

giving rise to food shortages in northern Europe followed by starvation and mass migration from Ireland where people relied on the potato as a staple food (Woodham-Smith, 1962).

From that time onward, the disease spread throughout the world, most likely in seed potatoes, to almost all potato producing parts of the world (reviewed in Cox and Large, 1960). Recent studies using mating type analysis, isozymes and DNA fingerprinting have revealed that there was very little genetic diversity among these strains and only one mating type was found of this heterothallic fungus. It appeared that a single strain had escaped from central Mexico and spread globally (Spielman *et al.*, 1991; Goodwin *et al.*, 1994ab; Goodwin and Drenth, 1997).

3.2.2 Late blight in Australia

Late blight was first reported on potatoes in Australia in 1907 (reviewed by Oldaker, 1947). The Victorian Government Pathologist, Daniel McAline, recorded the development of epidemics during 1909 to 1911 through all the major production districts around the state (McAlpine, 1911) and cited particularly high rainfall during the summer months as a cause of the epidemics. He cited infected seed potatoes were the more likely means of introduction into Australia.

Today, the disease is generally considered to be of little economic importance. This is most likely due to relatively unfavourable conditions of low humidity during the summer months in most production areas around the country. However, sporadic and localised outbreaks have caused significant damage in individual crops every five to seven years or so, most commonly in northern Tasmania and the Central Highlands (Ballarat) regions of Victoria. These outbreaks are usually associated with several days of warm, sultry weather during the summer months which favour periods of dew formation. The disease generally appears in crops after flowering. Early blight (*Alternaria solani*) spray programs probably slow the development of late blight during favourable weather conditions and sprays of the systemic fungicide metalaxyl have usually been effective in halting disease spread once outbreaks were detected.

3.2.3 The 1970's migration of *P. infestans* from Central America

A report indicating the presence of an A_2 mating type of *Phytophthora infestans* in Europe was the first evidence that something had changed in the pathogen population (Hohl and Iselin, 1984). This report stimulated plant pathologists all over the world to analyse local *P. infestans* populations since previously only the A_1 mating type had been detected outside of central Mexico. These studies indicated that changes were not restricted to Western Europe but rather were worldwide (Spielman *et al.*, 1991; Fry *et al.*, 1993).

The characterisation of these populations has relied on mating type analysis and a series of genetic markers. The first commonly available genetic markers were isozymes (Tooley *et al.* 1985). Different allozyme alleles of glucose-6-phosphate isomerase (*Gpi*) and peptidase (*Pep*) loci could differentiate the old A1 mating type population from the new population consisting of A_1 and A_2 mating type isolates (Spielman *et al.*, 1991). DNA fingerprinting using a multicopy probe (RG-57) (Goodwin *et al.*, 1992a) has enabled much greater resolution of population structure and could be used to accurately describe and identify clonal lines. This DNA fingerprinting technique also provided convincing evidence that the old A_1 mating type population consisted of only a single clonal line and that migration of new strains was responsible for the resurgence of late blight (Goodwin *et al.*, 1994ab; Goodwin and Drenth, 1997).

The use of isozymes and DNA fingerprinting in combination with mating type analysis revealed that the new population had already spread to all continents by the late 1980's, with the exception of Australia and Antarctica (Fry *et al*, 1993). The mechanism of the initial migration was probably via infected potato tubers imported from Mexico to Western Europe in the late 1970's (Niederhauser, 1991). Infected daughter tubers produced in crops grown from these imported tubers may have been replanted or discarded in a compost pile from which infected tubers would produce foliage able to support the growth of the fungus in spring. Sporangiospores produced on these plants may then have infected nearby potato or tomato fields. Through conducive weather conditions a number of serious disease outbreaks of late blight occurred in the late 1970's and early 1980's and this lead to the rapid spread of the new strains all over Europe. Migration to other continents probably occurred through the trading of seed potatoes from Europe.

Up until the late 1980's, only representatives of the old A_1 mating type population were found in the USA and Canada. However, this population also rapidly changed in the early 1990's as a new population of A_1 and A_2 mating type isolates was identified. Isozyme analysis and DNA fingerprinting revealed that these isolates were different from elsewhere in the world and more similar to strains found in northern Mexico. This provided support for the hypothesis that the fungus had spread with the increasing importation of tomatoes into the USA (Goodwin et al, 1994a).

3.2.4 Population displacement

New populations of *P. infestans* quickly displaced the old populations of *P. infestans* wherever new strains spread. This took place over only a few years and, in most cases, new populations completely replaced old ones. Intensive sampling in Europe showed that no representatives of the old populations could be found within a few years of the introduction of the new strains (Fry *et al.*, 1992,1993; Spielman *et al.*, 1991).

A number of factors may be involved in this rapid and complete population displacement.

1. **Higher level of fitness and aggressiveness.** The strains of the new population have a higher fitness level and are more aggressive, thus outcompeting the old strains (Kato *et al.*, 1997). The new strains are significantly more difficult and more expensive to control (Johnson *et al.*, 1997). Resistance to metalaxyl makes suppression of an initiated epidemic very difficult. The new strains also more aggressively infect and damage the stems and tubers of potatoes (Kato *et al.*, 1997). They can survive in the stems during periods of dry, hot weather, sporulating rapidly after a short period of wet and humid weather.

2. **Metalaxyl resistance.** No strains representing the old *P. infestans* populations were found to have any significant level of resistance to the fungicide metalaxyl. However, representatives of the new population very rapidly developed resistance to this fungicide (Goodwin *et al.*, 1996). The development of resistance to metalaxyl and the subsequent failure of disease control gave rise to major late blight outbreaks. Strains with metalaxyl resistance have, of course, a huge selective advantage.

3. **Effects of sexual reproduction.** Before the introduction of the new strains the old population of *P. infestans* consisted of only A_1 mating type isolates. Thus, *P. infestans* survived only as mycelia in seed or volunteer potato tubers. However the new

populations, consisting of both mating types, have the ability to reproduce sexually resulting in the formation of oospores. The presence of oospores of *P. infestans* in soil, together with overwintering mycelium in potato tubers, has many consequences for the control of late blight.

- Whenever weather conditions are favourable, germinating oospores can initiate a late blight epidemic where potatoes (planted or volunteer) grow in soil contaminated with oospores. This will lead to earlier and more massive breakouts of late blight epidemics.
- Oospores are most likely to result in infection of leaves in the lower, rather than the upper part of the leaf canopy, as happens with sporangia initiated late blight disease. Protectant fungicides, which are applied mainly to the top of the canopy, may fail to protect the lower canopy. (These factors may increase the chances for the development of initial foci and occurrence of epidemics). This means a higher risk of the development of initial disease foci and the subsequent development of epidemics.
- Sexual reproduction results in genetically more diverse populations of *P. infestans* with greater adaptability to environmental conditions and control strategies such as fungicides.
- The production of oospores in infected leaves and stems enables the fungus to survive unfavourable conditions during the season. Oospores can germinate rapidly and colonise the remaining healthy foliage as soon as conditions are once again favourable for the disease.

3.2.4.1 What are the consequences of population displacement in Australia?

The introduction of new strains of the two different mating types into Australia will have dramatic consequences for potato producers. Populations of *P. infestans* will change from ones that only reproduce asexually and overwinter only as mycelium in potato tubers to ones that reproduce both sexually and asexually and produce oospores that can survive for several seasons in soil without potatoes. These new populations will be more aggressive and adaptable and will be resistant to metalaxyl. Epidemics will start earlier in the season, provided weather conditions are favourable, and progress much more rapidly through the crop (Drenth *et al.*, 1993). Rather than starting in the upper petioles and progressing down the canopy, as is the case in Australia at present, the fungus will attack lower stems directly, resulting in the dramatic and rapid destruction of plants and crops. Expensive spray programs will be required to keep the disease in check because of metalaxyl resistance. Overseas experience suggests that the seasonal incidence of late blight will increase and that the disease will be more common within a district where once it occurred only sporadically in a few crops.

3.2.5 Disease cycle of *P. infestans*

3.2.5.1 Asexual life cycle

In spring, when potato tubers are planted, diseased sprouts may arise from those tubers which are infested with mycelium of *P. infestans* (Fig. 1). Under favourable conditions, sporangiophores emerge from the stomata and release numerous airborne sporangia causing a rapid spread of the disease. At temperatures above 12 - 15 °C sporangia may germinate directly.

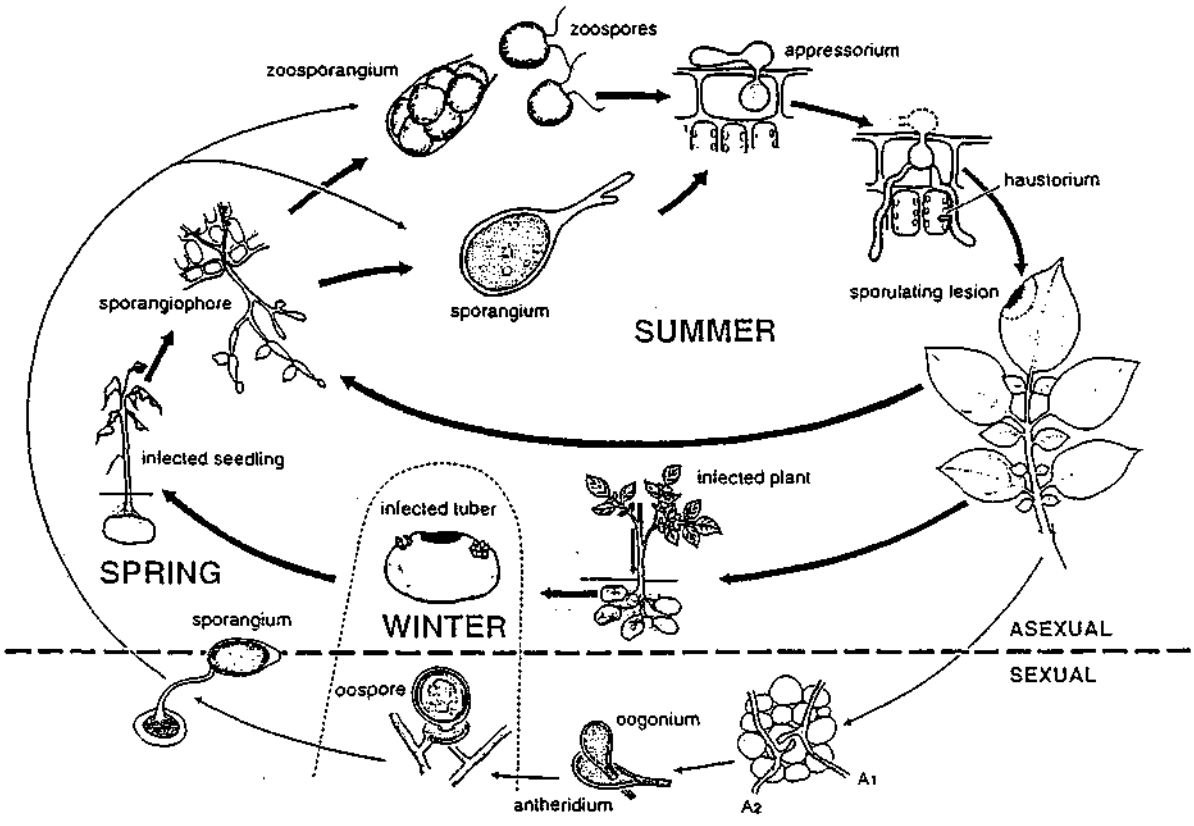

Figure 1. Disease cycle of potato late blight caused by *Phytophthora infestans* (reprinted with permission from Drenth, 1994)

Below 12 °C sporangia may differentiate into numerous motile zoospores which germinate after encystment. On leaf surfaces and stems, germinated sporangia and cysts form germtubes with appressoria from which penetration hyphae arise. Characteristically, a host cell next to stomatal guard cells is penetrated by the penetration hypha. Hyphal structures are formed in the epidermal cell from which the mycelium grows, initially intercellularly, while intracellular haustoria are formed in the mesophyll cell layer (Pristou & Gallegly, 1954; Gees & Hohl, 1988). In the first stages of lesion development, a water-soaked area appears where sporangiophores emerge predominantly from the stomata. Under favourable conditions, a rapidly expanding lesion carrying numerous sporangiophores with abundant sporangia is formed within four to five days after infection. Many asexual generations may be produced in one growing season, which explains the tremendous potential for spread and epidemic development of the disease. The airborne sporangia can spread over distances of up to several hundred kilometres (Aylor, 1986). In wet weather conditions, sporangia or zoospores are washed down from the leaves and carried into the soil. Here the spores germinate and the germtubes may penetrate the tubers through lenticels, wounds and eyes or at sites where the surface is not completely suberised. Most of the blighted tubers will rot in the soil or during storage. However, a few will survive and they may be planted in the following season.

Van der Zaag (1956) and Hirst and Stedman (1960) gathered conclusive evidence to support the hypothesis stated by de Bary (1876) that *P. infestans* can indeed overwinter as a mycelium within infected tubers. They showed that only a small proportion of infected tubers give rise to

infected sprouts. Nevertheless, infected sprouts will emerge and act as a source of inoculum. Van der Zaag (1956) estimated that one infected sprout per 20 to 600 square kilometre is sufficient to start the annual late blight epidemic. In this way, the disease is carried on from season to season. The hypothesis of Brefeld (1883) and de Bruyn (1926) that *P. infestans* can also overwinter as a saprophyte in the soil has not been substantiated. Zan (1962) and Lacey (1965) demonstrated that soil infectivity did not persist for more than 11 weeks in non-sterilised soils. This is by no means long enough to bridge the gap between successive potato crops in most potato growing areas and thus excludes a saprophytic phase in the disease cycle.

3.2.5.2 Sexual life cycle

P. infestans is a heterothallic species and when hyphae of opposite mating type make contact, antheridia and oogonia are formed. The oogonium grows through the antheridium in an amphigynous configuration in which the antheridium surrounds the oogonial stalk (Fig. 1). There is no exchange of cytoplasm between antheridium and oogonium (Hemmes, 1983). The oogonium expands rapidly due to the flow of cytoplasm through the oogonial stalk from its own thallus. After this expansion phase the oogonial stalk is plugged. All nuclei in the oogonium except one migrate to the periphery where they disintegrate. Meiosis occurs in the multinucleate gametangium (Shaw, 1983ab). A fertilisation tube grows from the antheridium through the oogonial wall to deposit an antheridial nucleus in the oogonium. Subsequently, lipid bodies and vacuoles are formed in the cytoplasm of the oogonium and they also migrate to the periphery. A thick oospore wall develops and the remaining cytoplasm is located in the centre of the ooplast. Blighted potato plants, containing oospores, remain in the field, decompose and oospores are liberated. Oospores can germinate and infect tubers and stolons of newly planted potatoes, as well as stems and leaves, which come into contact with the soil (Schöber & Turkensteen, 1992). Oospore germination involves consumption of the lipid bodies in the ooplast, dissolution of the oospore wall and the formation of one or more germtubes. These germtubes can either initiate mycelial growth directly or terminate in a sporangium which can germinate directly or produce zoospores. As oospore formation was unknown from most potato growing areas of the world, there have been relatively few studies of the conditions which favour sexual reproduction of *P. infestans* in nature.

3.2.5.3 Oospore formation and survival

In general, oospores of oomycetous fungi are highly persistent structures. For example, oospores from *P. cactorum* can survive for at least one year in soil (Malajczuk, 1983) and in mummified strawberries (Grove *et al.*, 1985). Oospores of *P. fragariae* can survive for at least three years in soil (Duncan, 1980). For the Oomycete *Aphanomyces euteiches*, survival in soil for up to four years was reported (Kotova, 1979). To our knowledge only one study on the survival of *P. infestans* oospores was published. Perches and Galindo (1969) collected soil from a Mexican field two years after a severely blighted potato crop had been grown there. In greenhouse experiments, potatoes planted in this soil were infected on the lower part of the stems and on the leaves which were close to or in contact with the soil. Moreover, *P. infestans* could be isolated from this soil using selective media. They suggested that *P. infestans* oospores were responsible for the infection.

In order to prove that pairings of *P. infestans* isolates produce functional oospores which are able to survive the winter in the soil and to cause infections in the next growing season, it is of the utmost importance to determine whether sexually derived progeny caused infections. Therefore, one must be able to distinguish sexual and asexual progeny. For that purpose Goodwin *et al.* (1992a) employed DNA fingerprint probe RG-57 which is used to characterise *P. infestans*

isolates. Two *P. infestans* isolates, collected in The Netherlands, were paired *in vitro* resulting in hybrid progeny as was shown after DNA fingerprint analyses (Goodwin *et al.*, 1992a). The same two isolates were used for the generation of oospores *in planta*. In floating potato leaf discs, inoculated with a mixture of sporangia from the parental isolates and incubated between 5 to 25 °C for several days, immense numbers of oospores were formed (Drenth *et al.*, 1994). Oospores extracted from the leaves germinated *in vitro* and the resulting isolates were found to be pathogenic. DNA fingerprinting revealed that these isolates were hybrids from the two parental strains. Drenth *et al.*, 1995 also showed that oospores can overwinter in soil and that after eight months, the soil still contains infectious material. DNA fingerprints of the isolates obtained revealed that the infections were caused by sexual progeny originating from oospores (Drenth *et al.*, 1994). Continuation of these same oospore survival experiments revealed that the soils stayed infectious up to 4 years (Turkensteen *et al.*, 2000).

3.2.5.4 *P. infestans* in the centre of origin, central Mexico

Several wild *Solanum* species native to Mexico have resistance against *P. infestans*. Using that information, Reddick and Crosier (1933) were among the first to postulate that central Mexico might be the centre of origin of *P. infestans*. Diversity observed in the host plant and in the fungus supported the hypothesis that the *Solanum-P. infestans* pathosystem had co-evolved there (Niederhauser & Mills, 1953). In 1956 the A_2 mating type of *P. infestans* was identified in central Mexico (Niederhauser, 1956; Smoot *et al.*, 1958; Gallegly & Galindo, 1958). Besides oospore formation *in vitro* on different agar media, oospores were found *in planta* in leaves and stems of potato plants (Gallegly & Galindo, 1958; Smoot *et al.*, 1958; Estrada, 1967). These oospores were able to germinate and to produce pathogenic progeny (Smoot *et al.*, 1958; Romero & Erwin, 1967).

In central Mexico, A_1 and A_2 mating type isolates appear at equal frequency in the fungal population (Gallegly & Galindo, 1958). The Mexican isolates showed more virulence factors than isolates from asexually propagated populations collected in the United States (Tooley *et al.*, 1986). Five of the six known glucose phosphate isomerase (*Gpi*) allozyme alleles (83, 86, 90, 100, and 122) and all known peptidase (*Pep*) alleles (83, 92, 100) were found in Mexican *P. infestans* isolates collected in the 1980s (Goodwin *et al.*, 1992b). The frequency of allozyme alleles for *Gpi* and *Pep* does not deviate significantly from the Hardy-Weinberg equilibrium indicating random mating (Tooley *et al.*, 1985). DNA fingerprinting of isolates collected in central Mexico revealed that almost every isolate has a unique RG-57 genotype and all RG-57 hybridising fragments known so far are represented (Goodwin *et al.*, 1992b). The high level of genetic diversity and the presence of allozyme alleles in Hardy-Weinberg equilibrium are consistent with the hypothesis that sexual reproduction occurs frequently in *P. infestans* populations in central Mexico (Goodwin *et al.*, 1992b).

3.2.5.5 Managing potato late blight

When *P. infestans* first appeared in Europe in 1845, most potato cultivars were extremely susceptible. By the end of the 19th century, moderately resistant potato lines, generated through selection for resistance, were available (Salaman, 1910). In the first half of 20th century, resistance governed by single resistance genes (R-genes) was regarded as a breakthrough. The R-genes were introduced by crossing *Solanum tuberosum* with species of *Solanum* that are highly resistant to late blight. In particular *Solanum demissum* (Lindl.) has been widely used as a source of R-genes against late blight. However, resistance based on R-genes was short-lived, as the fungus rapidly overcame resistance governed by the R-genes. This so-called breakdown of resistance is due to the appearance of virulent races which supposedly emerged either through

mutation (Peterson & Mills, 1953; Toxopeus, 1956; Gallegly, 1968), sexual reproduction (Pristou & Gallegly, 1956), parasexuality (Leach & Rich, 1969) or somatic variation (Caten & Jinks, 1968).

In large parts of the world, late blight control is heavily dependent on the frequent application of protectant fungicides which are applied every 5-14 days. The frequency of application depends on the weather conditions and the susceptibility of the potato cultivar. Fungicides used to control *P. infestans* are Bordeaux Mixture, copperoxychloride, dithiocarbamates, triphenyltin compounds, cymoxanil and phenylamides. With the exception of the latter two, all these compounds function by killing spores and their germlings on the plant surface before infection occurs. They have little curative activity and are only effective when a complete cover of the foliage is maintained. Therefore, frequent applications are required for effective control. Once *P. infestans* has penetrated the host tissue, these fungicides can no longer affect the fungus. Once late blight becomes established in a field it is difficult to control. The phenylamides, metalaxyl and cymoxanil, have systemic and eradicant properties. Metalaxyl has been used to control potato late blight since the late 1970s. However, metalaxyl resistant *P. infestans* isolates were found within one year after the introduction of this fungicide in Europe in 1979 (Davidse *et al.*, 1981) and the use of metalaxyl is now discouraged and rather limited in Europe.

During the last four decades frequent and large scale applications of fungicides have been used to control potato late blight adequately. Hence, breeding for late blight resistant potato cultivars had low priority. Nowadays the growing environmental awareness of the general public forces growers to reduce fungicide applications drastically. In addition, the use of some effective fungicides may be forbidden in the near future. Moreover, the appearance of the A_2 mating type and the possibility of sexual reproduction forming oospores, which act as additional inoculum, might initiate *P. infestans* epidemics earlier in the season, thus requiring a higher input of fungicides to control the disease. Therefore, late blight resistant cultivars are needed more than ever.

3.3 Project objectives

Based on current overseas experience, it can be hypothesised that the population of *P. infestans* found in Australia is made up of metalaxyl susceptible strains of the A_1 mating type which are similar to the strains that occurred in North America and Europe before the introduction of new A_1 and A_2 strains. This is based on the fact that late blight is not a significant disease of potatoes in Australia and those outbreaks can be controlled with metalaxyl.

The objectives of this project were to:

1. Characterise Australian populations of *P. infestans*;

2. Establish that the Australian *P. infestans* population consists only of strains that represent the old global A_1 mating type population;

3. Establish that the A_2 mating type does not occur in Australia;

4. Determine the sensitivity of the Australian *P. infestans* population to metalaxyl;

The project would also serve to raise awareness in the industry of the potential problems with late blight and provides the basis on which to build contingency plans for minimising the risk of introducing new strains of *P. infestans* into Australia and managing an incursion of the fungus.

3.4 Materials and Methods

The project involved a number of steps. These included:

- Informing the industry of the project, its objectives and the need to be alerted to any late blight outbreaks;
- Collection of specimens from late blight outbreaks;
- Isolation and culturing of *P. infestans*;
- Determining the mating type of isolates collected;
- Determining the DNA 'fingerprint' of each isolate and comparing them to international standards or 'type' specimens and;
- Determining the relative sensitivity of each isolate to the fungicide metalaxyl.

3.4.1 Isolation and culturing of *Phytophthora infestans*

Protocols developed for the collection, isolation and culturing *P. infestans* from diseased specimens are outlined in the following sections:

- Selection of freshly diseased plant tissue
- Transport of samples
- Surface preparation and sterilisation
- Rye media
- Selective media
- Incubation

Selection of freshly diseased plant tissue

Phytophthora infestans is hemi-biotroph and attacks only healthy plant material. Often when a plant has died it is difficult to isolate *Phytophthora* species as they have a rather limited saprophytic ability and dead plants tend to harbour many secondary pathogens. Infected tissue selected for isolation should ideally contain part diseased and part healthy tissue. Please remember that *Phytophthora* is especially hard to isolate from necrotic tissue. Therefore, it is best to obtain material from the edge of an actively growing lesion.

When potato leaves carrying lesions are placed in a petri dish on a wet piece of filter paper at 15°C they will sporulate profusely. Using a loop, which has been dipped into an agar plate and has some agar attached to it, touch the mycelia lightly to release the spores and then place them on a plate of Rye agar. Another method is to place infected leaf tissue under thin slices of potato tubers and incubate them at 15°C for 4-5 days and the mycelia will grow through the tubers and can be harvested from the top and placed on selective Rye media.

Transport of samples

If samples are exposed to drying or high temperatures (35 °C) they will lose their viability. If you leave your samples in an enclosed vehicle in summer *Phytophthora* will die fairly quickly. Place your samples in small plastic bags and blow them up before closure to prevent drying out and put them in an esky (icebox) to prevent overheating. Also, avoid low temperatures, as Phytophthora will not withstand freezing either. Hence, do not put the samples in direct contact with ice. Wrap a few ice blocks in newspapers and add to an icebox to keep the temperature of your leaves within normal range. In case the samples need to be stored, place them in a refrigerator at 5 °C and ensure that the samples are moist but not wet. It is best to isolate *Phytophthora* from leaf tissue the same day but samples will keep for a few days.

Surface preparation and sterilisation

Cut out small (0.5 x 1cm) sections of leaf tissue from the edge of an actively growing lesion. Surface sterilise leaf tissue by either one of the methods described below:

1. Dip pieces of leaf tissue in 70% ethanol for about one minute, wash for 10-20 seconds in sterile distilled water and blot dry onto sterile paper tissues before placing leaf tissue onto selective media.

2. Dip root tissue in a bleach solution (sodium hypochlorite 0.5%) for about 30 seconds. Then wash the bleach solution off using sterile distilled water and blot dry onto sterile paper tissues and place it onto selective media.

We routinely use the ethanol method, which tends to give fewer problems with bacterial contamination and gives good recovery. It is also important that the small tissue samples are blotted dry very well and pushed into the agar instead of just placing it on the top. This will ensure good contact between the bacteria in the tissue and the antibiotics in the media. *Phytophthora* species will quickly grow through the media, leaving bacterial infections behind.

Rye media

Rye media was prepared according to the methods of Judelson adapted from Caten and Jinks, 1968, Can. J. Bot. 46:329.

1. Soak 60 gm of rye seeds (preferably organic) in 500 mL of autoclaved tap water for 24-36 hours at room temperature.
2. Blend the seeds and water at high speed for 20 seconds (use a milkshake blender). Pour into a 2 L flask and rinse out the blender with another 200 mL of water. Now you will have 60 gm of rye seeds in 700 mL of water.
3. Autoclave for 30 min at 121 °C.
4. While still hot (about 60-70 °C); filter out the large chunks by passing it through a kitchen strainer (1mm mesh). Resuspend the rye seeds in 200 mL of water and refilter (this helps improve the yield by releasing more nutrients).
5. Bring the volume to 1 litre for every 60 gm of seeds. Make sure the media is evenly distributed over several beakers.
6. Add 20 gm of sucrose per litre. Mix well. Freeze in aliquots for later use, or use immediately by adding 15 gm of agar per litre and autoclave.

Selective media

There are a number of different selective media commonly used for isolation of Phytophthora species (see Erwin and Ribeiro, 1996). For *Phytophthora infestans* a good media is Rye agar plus **P_{10}ARP** which is an effective antibiotic cocktail which contains Pimaricin (10 µg/mL), Ampicillin (250 µg/mL), Rifampicin (10 µg/mL), and Pentachloronitrobenzene [PCNB] (100 µg/mL).

Table 1. Media used to culture *P. infestans*

Compound	Stock solution	In 100 mL	Final concentration
Pimaricin	2 mg/mL MeOH	500 µL	10 µg/mL
Ampicillin	250 mg/mL H2O	100 µL	250 µg/mL
Rifampicin	10 mg/mL DMSO	100 µL	10 µg/mL
PCNB	10 mg/mL DMSO	1000 µL	100 µg/mL

Note that most antibiotics cannot be autoclaved and need to be filter sterilised after being put in solution. The antibiotics should be added after the media has been autoclaved and cooled down to about 50 °C immediately prior to pouring the media. Plates used for isolations should not contain any free water or condensation on the lids. If that is the case, dry the plates with the lids half off in the laminar flow for 10-30 minutes. Many antibiotics are sensitive to light thus solutions and plates containing antibiotics should be kept in the dark.

Incubation

Phytophthora infestans grows between 5-25 °C and its optimal incubation temperature is between 15–20 °C. Slightly cooler temperatures than 15 °C tend to favour *Phytophthora infestans* and slow down bacteria and are therefore preferred. Place your plates in a separate incubator away from clean *Phytophthora* cultures, because of heavy sporulating fungi and mite infestations, which pose a serious contamination threat.

3.4.2 Mating type analysis

Mating type of isolates was determined by pairing isolates with unknown mating type with tester strains of known mating types. Small strips of the tester strains and the strain of unknown mating type were placed parallel to each other on clarified Rye agar and incubated in the dark at 25 °C. After 3-4 weeks plates were microscopically examined for the presence of oospores.

3.4.3 Sensitivity to metalaxyl

The relative sensitivity of each isolate to the *Phytophthora* specific fungicide metalaxyl (Ridomil®) was determined by measuring the radial growth of cultures on Rye media amended with different concentrations of the chemical (0, 0.02, 0.1, 0.5, 1 and 5 ppm). This range of concentrations is used to calculate the EC50 concentration which is the effective concentration at which the growth of the fungus is inhibited by 50%.

3.4.4 DNA fingerprint analysis

This DNA fingerprint method is described in detail in Drenth and Govers (1993). Mycelium for DNA extraction was grown in liquid Rye A medium (Caten & Jinks, 1968) in the dark at 18°C for about 14 days. Mycelium was ground to a fine powder in liquid nitrogen and mixed for 4 min at 55°C with 2.5 mL of extraction buffer containing three parts of Tris equilibrated phenol, two parts of triisopropylnaphthalene sulfonic acid (20 mg/mL), two parts of 4-aminosalicylic acid (120 mg/mL), and one part of 5x RNB (1 M Tris/HCL, 1.25 M NaCl, 0.25 M ethylene glycol-bis-(2-aminoethyl ether) N,N,N',N'-tetraacetic acid). The mixture was shaken vigorously for 2 min, and 0.25 Vol of chloroform-isoamyl alcohol (24:1, v/v) was added. The aqueous phase was then extracted once with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1, v/v/v) and twice with an equal volume of chloroform-isoamyl alcohol (24:1, v/v). Nucleic acids were precipitated with 0.6 Vol of 2-propanol, dissolved in $T_{10}E_1$ (10 mM Tris-HCl [pH 8], 1 mM EDTA), and treated for 30 min with RNase A (20 µg/mL) at 37°C. This method yields 250-500 µg of DNA per gram (wet weight) of mycelium. For Southern blot analysis, approximately 5 µg of genomic DNA was digested with the restriction enzyme EcoRI according to the manufacturer's instructions and size-fractionated on a 0.8% agarose gel for 14-16 hr at 40 V (600 Vh) before alkaline transfer to Hybond N^+ (Amersham) hybridization membrane.

Membranes were prehybridized for 2 hr at 65°C in 10 mL of hybridization solution (5x SSC, 5x Denhardt's solution, 0.5% sodium dodecyl sulfate [SDS], 100 µg of salmon sperm DNA per milliliter). Probes were labelled with [α-^{32}P]dATP according to the random-primer labelling method of Feinberg and Vogelstein (1983) and allowed to hybridize overnight. Filters were then washed three times for 20 min each at 65°C in 2x SSC, 0.5% SDS, and three times for 20 min each in 0.5x SSC, 0.1% SDS, and exposed for 1-3 days at 80°C to Kodak Xomat S film backed with an intensifier screen.

3.5 RESULTS

3.5.1 Late blight outbreaks and the collection of isolates

Collections of late blight infected leaves were made in Tasmania and Victoria in the summers of 1998/1999 and 1999/2000. Although weather conditions in the summer of 1998/1999 were conducive for development of the disease, only 18 samples from Tasmania and 3 from Victoria were obtained, and of these only a single sample was brought into pure culture. Treatment of diseased fields with metalaxyl may have significantly reduced the chances of obtaining pure cultures from the diseased leaf and petiole samples.

During February and March 2000, a total of 30 samples of late blight affected material was obtained from Tasmania from which 28 different cultures were obtained (Table 2). Only one late blight affected crop was found in the Central Highlands of Victoria during the summer of 2000. When it was found, this infection was in decline and too old for a successful isolation of *P. infestans*. Additional diseased material was collected from an outbreak in a glasshouse in April 2000 but the fungus could not be successfully isolated from diseased leaf samples.

The cultured isolates grew rather slowly on a specially prepared Rye Agar and generally grew very poorly on a range of different media and under different conditions compared to reference isolates from overseas. The slow growth of the Australian isolates may explain the poor recovery rate of the fungus from diseased material during the 1998/1999 season. Experiments with a range of different media (pea agar, carrot agar, artificial cornmeal agar, fresh cornmeal agar, oatmeal agar, 5%, 10% and 20% rye agar) and at different incubation temperatures only resulted in a slight improvement in growth rate. The maximum growth we could obtain was 2-3 cm radius in 7 days at 16 °C in the dark. The optimal media and growth conditions for *P. infestans* are described in the material and methods section.

3.5.2 Mating type analysis

Mating type analysis revealed that all the Tasmanian isolates tested are only of the A_1 mating type. No A_2 types were found.

3.5.3 Sensitivity to metalaxyl

Sensitivity to metalaxyl was assessed by growing the cultures on Rye media amended with increasing amounts (0. 0.02, 0.1, 0.5, 1 and 5 ppm) of metalaxyl. Most of the Tasmanian isolates proved to be extremely sensitive to metalaxyl and the growth of all isolates was completely inhibited at 5ppm metalaxyl. The isolates were so sensitive that we could not reliably calculate an EC50 value based on the range of concentrations used in the tests (EC50 is the effective concentration at which the growth of the fungus is inhibited by 50%).

3.5.4 DNA fingerprinting

DNA fingerprinting using probe RG57 revealed that all 20 isolates analysed were identical to each other (Table 2). Hence, no genetic variation was found among the isolates observed. Comparison of the DNA fingerprints with published records and a global database of isolates (Goodwin and Drenth, 1997; Forbes *et al.*, 1998) revealed that the Australian isolates are most closely related to clonal line US1.3. In fact, there is only a single band difference between the Australian isolate and this particular clonal genotype. Details of the nomenclature of the isolates is described in detail in Forbes *et al.* (1998).

DNA fingerprints of isolates representing the old US-1 clonal lineage show some minor variation. This variation is often expressed as a loss of a single DNA fingerprint fragment. These minor changes are due to mutation and mitotic recombination.

The RG57 DNA fingerprint patterns of 20 Tasmanian isolates also vary sightly from two patterns, designated AU-1 and AU-2, previously described for Australian isolates by Goodwin *et al.*, 1994b) (see also Forbes *et al.*, 1998). Therefore, the DNA fingerprint pattern observed in the current study has been designated AU-3. The isozyme and DNA fingerprint pattern of these isolates provided evidence that the Australian isolates are somewhat different from the ones spread around the globe from Europe.

Table 2. Collection details and results of mating type, metalaxyl sensitivity and DNA fingerprint analysis of 20 Australian isolates of *P. infestans*.

UQ accession number	Date collected	Host	State	Collector	Other codes	Mating type	Metalaxyl resistance	DNA fingerprint
UQ4861	26/04/1999	Potato	TAS	H. Pung		A_1		AU-3
UQ5222	24/02/2000	Potato	TAS	H. Pung	P1.4	A_1		
UQ5223	24/02/2000	Potato	TAS	H. Pung	P4.1	A_1	Very low	
UQ5224	24/02/2000	Potato	TAS	H. Pung	P5.4	A_1	Very low	
UQ5225	24/02/2000	Potato	TAS	H. Pung	P6.1	A_1	Very low	
UQ5226	24/02/2000	Potato	TAS	H. Pung	P6.2	A_1	Very low	
UQ5227	28/03/2000	Potato	TAS	H. Pung	P1.2	A_1	Very low	AU-3
UQ5228	28/03/2000	Potato	TAS	H. Pung	P1.3	A_1	Very low	
UQ5229	28/03/2000	Potato	TAS	H. Pung	P3.1			AU-3
UQ5230	28/03/2000	Potato	TAS	H. Pung	P3.2	A_1	Very low	AU-3
UQ5231	28/03/2000	Potato	TAS	H. Pung	P3.3			
UQ5232	28/03/2000	Potato	TAS	H. Pung	P4.2	A_1	Very low	AU-3
UQ5233	28/03/2000	Potato	TAS	H. Pung	P5.2	A_1	Very low	AU-3
UQ5234	28/03/2000	Potato	TAS	H. Pung	P5.3			
UQ5235	28/03/2000	Potato	TAS	H. Pung	P6.3	A_1	Very low	AU-3
UQ5236	28/03/2000	Potato	TAS	H. Pung	P6.4	A_1	Very low	AU-3
UQ5237	28/03/2000	Potato	TAS	H. Pung	P9.1	A_1	Very low	AU-3
UQ5238	28/03/2000	Potato	TAS	H. Pung	P9.4	A_1		AU-3
UQ5239	28/03/2000	Potato	TAS	H. Pung	P10.1	A_1	Very low	AU-3
UQ5240	28/03/2000	Potato	TAS	H. Pung	P10.2	A_1	Very low	AU-3
UQ5241	28/03/2000	Potato	TAS	H. Pung	P10.4	A_1	Very low	AU-3
UQ5242	28/03/2000	Potato	TAS	H. Pung	P10.5			AU-3
UQ5243	28/03/2000	Potato	TAS	H. Pung	P11.1	A_1	Very low	AU-3
UQ5244	28/03/2000	Potato	TAS	H. Pung	P11.2	A_1	Very low	AU-3
UQ5245	28/03/2000	Potato	TAS	H. Pung	P11.3	A_1	Very low	AU-3
UQ5246	28/03/2000	Potato	TAS	H. Pung	P11.4	A_1	Very low	AU-3
UQ5247	28/03/2000	Potato	TAS	H. Pung	P25	A_1	Very low	AU-3
UQ5248	28/03/2000	Potato	TAS	H. Pung	P25.2	A_1	Very low	AU-3

3.6 Discussion

The aim of this project was to characterise the Australian *P. infestans* population, using mating type tests and DNA fingerprinting, in order to establish the absence of the new and more aggressive, metalaxyl resistant strains of both A_1 and A_2 mating types which have spread to most other potato producing areas of the world since the late 1970's.

Twenty isolates of *P. infestans* were successfully cultured from diseased material collected from the north coast of Tasmania. They were characterised as belonging only to the A_1 mating type, proved to be very sensitive to metalaxyl and all belonged to the same clonal group designated AU-3, similar to US1.3. None of the isolates tested had characteristics that were consistent with the populations of new strains that have migrated to North America and Europe in the past two decades. The characteristics of late light in Australia, i.e. a generally low incidence, only sporadic and localised outbreaks and effective control with metalaxyl, are consistent with disease associated with the types of strains found in Australia.

Unfortunately, *P. infestans* was not successfully cultured from diseased material in Victoria and the Victorian population of the fungus could, therefore, not be characterised. At this stage, there is no evidence to indicate that the Victorian population is the same as Tasmanian population and further tests will need to be done over the coming seasons to characterise the Victorian population. However, based on the fact that late blight outbreaks in Victoria are sporadic and localised and can be controlled with metalaxyl, it is likely that the Victorian population is similar in make-up to the Tasmanian population. We attempt to make collections of the fungus when late blight outbreaks occur in the future.

There is no evidence to date of the presence of the A_2 mating type of *P infestans* in Australia. It should be noted however, that the new populations of the fungus in other countries belong to both the A_1 and A_2 mating types. The phenotype of mating type alone cannot be used as an indication of the presence of new strains in this country as new A_1 strains are far more aggressive than the old ones and likely to be resistant to metalaxyl. In addition, the introduction of A_2 mating type strains is likely to have a dramatic impact on the local populations because the mating between A_1 and A_2 strains will result in strains that are more ecologically adaptable than the present population.

All isolates tested proved to be very sensitive to metalaxyl and this is consistent with the field observations that the fungicide effectively controls late blight outbreaks in Australia. The new overseas populations are metalaxyl resistant. Sensitivity to metalaxyl provides further evidence that the new strains do not occur in Australia.

DNA fingerprinting was used to determine the genetic make-up of the Australian *P. infestans* isolates (at the RG-57 loci). All 20 isolates shared the same DNA fingerprint pattern indicating that they all belong to the same clone. The Australian clone, designated AU-3 was unique but very similar to US1.3 DNA fingerprint pattern (only one DNA fragment difference). Changes do accumulate in a clonal line with time. If we consider that the strains presently analysed are direct descendents of the strains first reported in Tasmania in 1907, a single fragment difference over a time span of almost a century is more an indication of the stability of the DNA fingerprint pattern. Variation may occur due to changes from heterozygosity to homozygozity at single allozyme and DNA fingerprint loci. Mitotic recombination could have generated the observed variants at the heterozygous loci. An RG-57

DNA fingerprint locus which is heterozygous for a particular band could easily become +/+ or -/- through mitotic recombination

The Australian *P. infestans* isolates grow rather poorly on artificial media compared to isolates from overseas. This was noted before by Goodwin (pers. comm.) and the characteristic helps explain why Australian isolates were difficult to isolate into pure culture, even with the help of antibiotics. This problem hampered work on the project because characterisation of this fungus depended largely on testing pure cultures on artificial media, and hence the lack of data on the Victorian strains which were not isolated into culture.

The slow growth of the fungus on artificial media *in vitro* is an interesting characteristic that has been observed in other parts of the world but not as severe as in Australia. A check of global culture collections also revealed the absence *P. infestans* isolates from Australia. In Australia, late blight outbreaks are sporadic and localised, probably because sufficiently long periods of cool and sultry weather conditions required for infection and disease development are rare. The use of protectant fungicides over the past four decades, usually for target spot control, also helps control the disease. Hence, we could assume that the *P. infestans* population in a particular district is rather small compared to those in other potato producing regions of the world where the disease is more prevalent. In terms of survival during unfavourable seasonal conditions, the Australian population faces severe bottlenecks between potato growing seasons. Dramatic fluctuations in the size of the *P. infestans* population may give rise to the accumulation of deleterious mutations in the late blight population. This mutational load in the fungal population may result in reduced fitness in the pathogen population as described by Muller *et al.* (1964) and Lynch and Gabriel (1990). However, it is difficult to test this hypothesis experimentally.

Goodwin *et al.* (1994b) speculated that the Australian isolates may have directly migrated from Mexico. However, this seems unlikely as the Australian DNA fingerprint pattern is nearly identical to the US1.3 clonal line which was once quite common in the US and Canada. Populations originating in Mexico show a tremendous amount of genetic variation for all markers, including the RG-57 DNA fingerprint marker. Hence, we propose that it is more likely that late blight was introduced into Australia from the US and or Canada on seed potatoes in the early part of this century. Certainly a number of potato cultivars were shared between both continents at the early part of this century.

3.6.1 Implications for the Australian Potato Industry

Generally, late blight outbreaks are only reported to occur in some Tasmanian and Victorian production areas. So far, only *P. infestans* isolates representing the Tasmania population have been characterised. Given that the characteristics of the disease and its management do not differ significantly between the two states it is likely that *P infestans* populations in Victoria may share similar characteristics to those in Tasmania. Our research indicates thus far that:

- Australia only has the A_1 mating type of *P. infestans*;
- Australia does not have representatives of the new and more aggressive populations of *P. infestans* found overseas; and
- There is no evidence of resistance to metalaxyl in Australian populations of *P. infestans*.

This helps explain why late blight is not a major disease in Australian potato production.

However, the danger lies in the accidental introduction of new aggressive strains from overseas. The most likely sources of new strains of the late blight fungus appear to be seed and other fresh potatoes and fresh tomatoes. Consignments of tomatoes shipped around the US appear to have been a major means of spreading the new *P. infestans* strains within that country. The consequences of introducing new strains of *P. infestans* to Australia could be costly. Table 3 compares some of the characteristics of the old and new strains of *P. infestans* and the disease they cause. Some of the implications of new strains for the Australian potato industry include:

- Populations of *P. infestans* will change from ones that only reproduce asexually and overwinter only as mycelia in potato tubers to ones that reproduce both sexually and asexually and produce oospores that can survive for several seasons in soil without potatoes;
- New populations will be more aggressive and adaptable and will be resistant to metalaxyl;
- Epidemics will start earlier in the season, provided weather conditions are favourable, and progress much more rapidly through the crop. Rather than starting in the upper petioles and progressing down the canopy, as is the case in Australia at present, the fungus will attack lower stems directly, resulting in the dramatic and rapid destruction of plants and crops;
- Expensive spray programs will be required to keep the disease in check because of metalaxyl resistance. These fungicides are not registered at present.

Overseas experience suggests that the seasonal incidence of late blight will increase and that the disease will be more common within a district where once it occurred only sporadically in a few crops. Such a situation would not be dissimilar to the introduction and subsequent economic losses experienced in the Pacific northwest in the USA (Johnson *et al.*, 1996). In the state of Wisconsin in the United States, for example, late blight was once considered to be a sporadic and localised problem much like that in Australia. However, since the introduction of new strains of the fungus the disease has become an annual problem requiring expensive fungicide spray programs to control (Walt Stephenson, pers. comm.).

Australia is in the enviable position of being free of strains of a pathogen that are creating havoc in many other countries. We must remain vigilant to maintain this position. It is imperative that the Australian potato industry is aware of this problem, develops the necessary strategies to ensure that there is minimal risk of introducing new strains of *P. infestans* and develops the contingencies to deal with the new late blight scenarios should these new strains be inadvertently introduced. Unfortunately, the first signs of new strains are likely to be an increased incidence and severity of the disease and the inability to control outbreaks with metalaxyl.

Table 3. A comparison of the different characteristics of late blight and *P. infestans* in Australia and overseas

Disease and strain characteristics	**Australian populations**	**Overseas populations**
Mating type	A_1	A_1 & A_2
Survival – over-wintering and long-term	Short term only, not without the host. As mycelia in potato tubers and other hosts	Long-term with and without the host - Oospores & mycelia.
Population	Low	Relatively high because of oospores.
Disease epidemic	Low incidence, sporadic and local – epidemics slow to start because of low population	Relatively high incidence, more widespread, more damage – earlier infection of crop, more disease foci, larger epidemics.
Control with fungicides	Sensitive to metalaxyl	Resistant to metalaxyl – control requires frequent applications of expensive protectant fungicides that will only prevent new infections.
Disease characteristics	Initial infection of petioles at top of plant progressing down to stems	More aggressive, direct infection of main stems – rapid destruction of plants.
Seed infection	Low risk	Very high risk – high inoculum potential on seed tubers
Fungus strain characteristics	Genetically stable	A_1 & A_2 mating results in genetic recombination and diversity, more adaptable populations – rapid and complete replacement of 'old', less aggressive and adaptable populations.
Economics	Generally low disease incidence, low levels of damage and yield loss, relatively low cost of management with fungicides	High incidence, high levels of damage, significant yield loss, reduced seed quality, expensive fungicide spray programs.

4 Technology Transfer

The objectives of this project were publicised through the distribution of a leaflet and articles in Eyes on Potatoes.

- Drenth *et al.* (1998). Potato Late Bight – Leaflet for distribution to the key growers and industry representatives.

- de Boer *et al.*(1998). Wanted – late blight! *Eyes on Potatoes, The Australian Potato Industry Council Newsletter, Volume 5, December 1998.* p 4.

- de Boer *et al.* (1999). The hunt for the potato late blight fungus continues. *Eyes on Potatoes, The Australian Potato Industry Council Newsletter, Volume 8, December 1999.* p 10.

The results of the project were publicised in the 2001 edition of Potato Australia.

- de Boer *et al.* (2001). No new late blight strains – yet! *Potato Australia,* **12,** 36.

4.1 Intellectual property

All methods used for the characterisation of the Australian *P. infestans* populations, such as DNA fingerprinting using the probe RG-57, mating type analysis, and metalaxyl sensitivity, are in the public domain. No intellectual property was created as part of this research project.

5 Recommendations

The key outcome of the project is that Australia is free of the strains of *P. infestans* that are causing significant economic damage in potato crops in many other countries. The following recommendations are aimed at ensuring that the Australian potato industry is in a high state of preparedness to prevent the introduction of new strains of *P. infestans,* and to deal with epidemics arising from the inadvertent introduction of new strains. Because we do not have the new strains, Australia has a trade advantage from the point of view of exporting seed potatoes to other countries.

- A continued flow of information to the industry on the threat and consequences of the introduction of new strains to increase awareness in the industry and to ensure early warning in case late blight management strategies fail.

- The further testing of both Victorian and Tasmanian populations of *P. infestans,* which is essential in developing good understanding of the fungal populations in Australia, as well as ensuring the necessary expertise in the diagnostic techniques. Limited surveys and analysis of the genetic make-up could be done between the collaborating laboratories in case late blight epidemics develop.

- An assessment of the risk of incursion of new strains of *P. infestans* into Australia. The most likely means of introduction is on fresh potato and tomato products. This has implications for the trade of potatoes and tomatoes into Australia.

- Determining the likely scenarios for late blight incidence and severity, management and economic costs should new strains of *P. infestans* take hold in this country in light of climate and production systems.

- The development of contingencies to minimise the risk of incursions and deal with incursions should they occur. Eradication of new strains has not been successful overseas. However, given our geographic isolation, climatic conditions and relatively low intensity of potato farming compared to Europe for example, containment of an outbreak attributed to a new strain may be possible if such an incursion is recognised early enough.

- A review of the current availability of alternative fungicides to metalaxyl should resistance develop and their registration status. Experience overseas shows that the introduction of new strains with resistance to metalaxyl rapidly leads to serious epidemics of late blight and the continuous use of metalaxyl ensures the establishment of these resistant strains within a very short time frame of 1-2 years based on overseas experience.

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