**VG05029 (September 2009)**

# Fusarium wilt of snow peas.

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VG05029

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Report Completed-September 2009.

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## Media summary

Snow peas are a popular vegetable for consumers in Australia; however production has been declining. The main reasons for the decline have been the availability of cheaper imports from Zimbabwe, China and in the future Kenya and the high cost of domestic production especially the cost of hand harvesting. However Fusarium wilt has also had serious effects on snow pea production in Australia.

Fusarium wilt is caused by a fungus *F. oxysporum* f.sp. *pisi*. It is a soil borne pathogen that can rapidly build up in soil and can survive for many years. Symptoms caused by the disease include wilting and death of plants either early or after picking has been commenced. When the stems of plants are cut longitudinally a reddish discoloration is often observed.

A survey was conducted in three states to examine the spread of the disease and also to establish races of the pathogen in Australia. A total of 478 isolates of *F. oxysporum* f.sp. *pisi* were collected during this disease survey. Of these, 182 were collected from Queensland, 84 from New South Wales and 212 from Victoria. The results of this field survey indicate that the snow pea wilt pathogen, *F. oxysporum* f.sp. *pisi* is widespread across Australia's snow pea and pea growing regions.

There were no cultivars found to be resistant to Fusarium wilt in field and glasshouse trials. Current control options are limited to avoidance of infected fields. Fumigation with metham or chloropicrin is the preferred control option for some growers.

The best control option for this disease is resistant cultivars, but these are currently not available. Therefore farm hygiene is critical to reduce the spread of infected soil, plant debris and trellising infrastructure from diseased to disease free blocks. This should include cleaning farm equipment and footwear. Some seed dressings and plant activators may offer control early in the growing period, but infection may still occur later.

Managing this disease is critical to the snow pea industry and investigations should be carried out to determine the availability of resistant cultivars overseas or the potential of breeding resistant cultivars. Alternative options to fumigation should be investigated such as biofumigation crops and safe and sustainable chemical options.

# Technical summary

Snow peas are a popular vegetable for consumers in Australia, however production has been declining. The main reasons for the decline have been the availability of cheaper imports from Zimbabwe, China and in the future Kenya and the high cost of domestic production especially the cost of picking. However Fusarium wilt has also had serious effects on snow pea production in Australia.

Fusarium wilt is caused by a fungus *F. oxysporum* f.sp. *pisi* which is a soil borne pathogen that can rapidly build up in soil and can survive for many years. Symptoms caused by the disease include wilting and death of plants either early or after picking has been commenced. When the stems of plants are cut longitudinally a reddish discoloration is often observed.

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The results of this field survey indicate that the snow pea wilt pathogen, *F. oxysporum* f.sp. *pisi* is widespread across Australia's snow pea and pea growing regions. These findings were expected, as Fusarium wilt is the most significant disease in pea and snow pea crops worldwide after Aphanomyces root rot, and is present in all pea growing regions worldwide. The field survey data also showed that there is variation in the pattern of field symptoms across growing regions in Australia. These variations may be due to regional factors such as climate and soil, pathogenic race variation or genetic differences within the pathogen population.

A total of four races identified as 1, 2, 5 and 6 were found to exist in the main snow pea growing regions in Australia. These results were expected based on previous research which states that these races are the most common in pea growing regions worldwide and the only races of significant economic importance. No races unique to Australia were identified.

Race 1 was the predominant race found to exist within the population, comprising 52.14% of isolates studied. This finding was expected as Race 1 has been reported as the predominant race causing disease in pea growing regions worldwide, notably in the US where it was first reported. Race 5 is also significant within the population, with 26.42% of isolates belonging to this race. Races 2 and 6 are less significant within the population.

The genetic diversity and population structuring of *Fusarium oxysporum* f.sp. *pisi* obtained from the main growing regions in Australia was studied. A total of 140 isolates were analysed using molecular markers based on 6 primer sets. The results of the study showed that a total of 9 clonal haplotypes and 8 unique haplotypes exist within this population, indicating a moderate level of genetic

diversity. This level of genetic diversity has the potential to make disease control difficult. No geographic structure according to haplotype was found.

This is the first genetic survey of Fusarium wilt of peas in Australia and gives valuable information on the current population within snow pea growing systems within Australian growing regions but also valuable information for standard green peas.

There were no cultivars found to be resistant to Fusarium wilt in field and glasshouse trials. Current control options are limited to avoidance of infected fields. Fumigation with metham or chloropicrin is the preferred control option for some growers. Some seed dressings and plant activators may offer control early in the growing period, but infection may still occur later.

The best control option for this disease is resistant cultivars, but these are currently not available. Therefore farm hygiene is critical to reduce the spread of infected soil, infected plant debris and trellising infrastructure from diseased to disease free blocks. This should include cleaning farm equipment and footwear.

Managing this disease is critical to the snow pea industry and investigations should be carried out to determine the availability of resistant cultivars overseas or the potential of breeding resistant cultivars. Alternative options to fumigation should be investigated such as biofumigation crops and an evaluation of systemic acquired resistance products for example Bion Plant Activator®, Milsana and Actiguard. The value of soil amendments should be examined and the establishment of a routine disease screening protocol.

# 1. Introduction

## 1.1 Pea Agronomy

There are three main types of peas grown in Australia as vegetables for human consumption. The main one is the standard garden (green) pea (*Pisum sativum*), it is grown for processing i.e. for freezing or canning and for the fresh market. Green peas for the fresh market have been historically grown close to major cities, but as processing took more of the market, expansion away from cities commenced. Tasmania for example has become a large area of processing peas. Tasmania provides ideal soil conditions with high yielding areas on the north-west and north east of the state.

Green peas require cooler climates with winter rainfall. They are planted from June to September with harvesting from late October to mid February (Beckingham 2001).

The snow pea (*Pisum sativum* var. *macrocarpon*) is a distinct botanical cultivar of *Pisum sativum*. The pod is flatter and can be eaten before strings develop (Figure 1). Sugar snap peas are very similar to the green pea but they are eaten much like a green bean. They are thicker than snow peas but also similar to green peas in that they have strings.

Both sugar snap peas and snow peas prefer a cooler climate with temperatures above 30°C causing early maturity and lower yields. Conversely frosts may make the flowers sterile so no pods will form.

**Figure 1.** Snow peas are much flatter than the green pea. Oregon Giant in this photograph has large, long straight pods.

The most popular cultivar grown commercially is Oregon Giant, also marketed as Snowman. It has a high yield of long straight pods and has powdery mildew resistance. The market favours this cultivar because of its sweet tasting pods. It grows to 1 metre and therefore requires trellising (Figure 2). Trellising is usually constructed with posts and wires and must be taken out for sowing and if the block is to be used for another purpose after snow peas. Otherwise a mesh may be used as shown in Figure 3.

Snow peas must be hand picked and therefore a supply of labour nearby and available when needed can restrict the viability of large plantings of snow peas and also costly to the grower.

**Figure 2.** Post and wire trellis in Bundaberg. Snow peas being hand picked.

**Figure 3.** Mesh used for trellising of snow peas in Gympie Queensland.

## 1.2 Australian snow pea production information.

All green pea production has been declining from 47883 ha in 1983 to 37532 ha in 2007. Snow peas which are combined with sugar snap peas have also been declining (Table 1). The main reasons for the decline have been the availability of cheaper imports from Zimbabwe, China and in the future Kenya and the high cost of domestic production especially the cost of picking.

**Table 1.** The production in tonnes of peas and specifically snow peas and sugar snap peas.

| | **All peas Tonnes (Australia)[a]** | | **Tonnes of snow peas and sugar snap (Australia)[b]** | **Value of production. $M** |
|---|---|---|---|---|
| **1983** | 47883 | **2005/2006** | 5621 | 37.8 |
| **2007** | 37532 | **2006/2007** | 3490 | 28.3 |

[a] Data courtesy of the Australian Bureau of Statistics.
[b]Data courtesy of Ausveg.

## 1.3 Pea Diseases

Diseases of peas include ascochyta and mycosphaerella blight, foot rot, bacterial blight, downy mildew and powdery mildew. More recently Fusarium wilt has appeared as major issue in snow pea production areas.

*Fusarium* is a fungus that is commonly encountered in the environment especially associated with plant material. The fungus can be involved with plants without causing disease or by causing diseases such as root and cob rots, wilts and seedling blights. Fusarium wilts are often caused by types (also known as *formae speciales* or shortened to *f.sp*.) of *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen. Isolates that cause wilt diseases are usually host specific and over 100 *formae speciales* and races have been shown to exist.

## 1.4 *Fusarium oxysporum*

*Fusarium oxysporum* is a soil borne fungus that is quite commonly found in soil and often in plants. The survival and infection process of *F. oxysporum* is represented in Figure 4. In plants it may produce no symptoms at all, but occasionally a type of *F. oxysporum* can cause wilting. In the case of all pea types (green, snow, sugarsnap and field peas) the cause of wilt is *Fusarium oxysporum* f.sp. *pisi*.

There are a large number of plants affected by Fusarium wilts (Beckman 1987). Some examples of crop specific Fusarium wilts include Fusarium wilt of bananas cause by *F. oxysporum* f.sp. *cubense* Fusarium wilt of cantaloupe, *F. oxysporum*, f.sp. *melonis*, Fusarium wilt of cucumber caused by *F. oxysporum* f.sp. *cucumerinum*. The fungus *Fusarium oxysporum* f.sp. *tracheiphilum* (Fot) is the cause of Fusarium wilt of Snake Beans (*Vigna unguiculata* s.sp. *sesquipedalis*) and cowpea (*Vigna unguiculata* s.sp. *unguiculata*) world-wide.

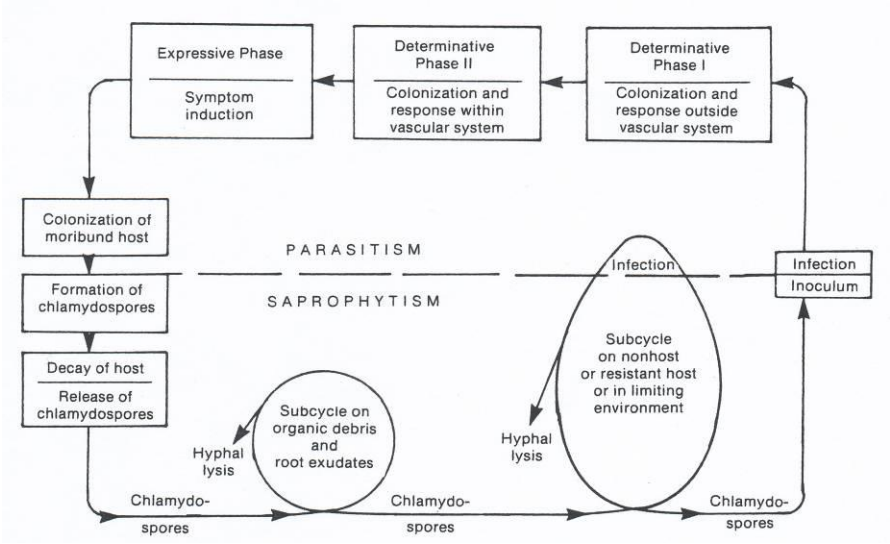


**Figure 4.** The survival and infection process of *F. oxysporum* in soil and plants. (Reproduced from "The Nature of Wilt Diseases of Plants" CH Beckman with permission from The American Phytopathological Society).

There are a number of races or types of *Fusarium oxysporum* f.sp. *pisi* that cause Fusarium Wilt of snow peas. These have been named races 1, 2, 5 and 6. The races 1, 5 and 6 have been described as true wilt, whereas race 2 has been given the name near-wilt, although symptoms of all the races are similar. Resistance was developed to race 1 for green peas many years ago. In Australia race 2 of Fusarium wilt was found in 1962 and race 1 and 6 are also present, but how widespread is not known (Kerr 1963; Armstrong and Armstrong 1974). Symptoms of Fusarium wilt include yellowing, curling down of leaves and stipules and eventual plant death (Figure 5). When infected plants have the lower stems cut the internal vascular tissue is coloured orange to red (Figure 6) (Hagedorn 1989).

**Figure 5.** The effects on plants may be variable across a block with a healthy plant next to one that has become infected (top) and more serious infection by Fusarium wilt (below).

**Figure 6.** Once the stem of an infected plant is cut open the internal vascular discoloration is clear**.**

## 1.5 Fusarium wilt disease cycle.

Fusarium wilt of snow peas is a soil inhabiting fungus. It survives in soils as chlamydospores which are tough survival spores. These may last for 10 years but the fungus itself may grow on weed roots as well, not causing any symptoms. The fungus can infect through spores produced on the plant, these include what are termed macroconidia and microconidia. Fusarium taxonomy, identification and images can be found in Leslie and Summerell (2006).

*Fusarium oxysporum* is common in cultivated soils of temperate and tropical areas of eastern Australia, and is common in soils from forested areas in tropical, subtropical and temperate forests (Summerell *et al.* 1993). It is also common in improved-pasture soils of the temperate areas but is not common in grassland soils of western Queensland (Burgess and Summerell 1992) and was not isolated from soil from the Simpson Desert.

Fusarium wilt invades roots of healthy pea plants and then gradually colonises the vascular tissue. The infection of the roots may be directly or associated with damage to the roots by other fungi or by nematodes. The growth of the fungus in the water conducting tissue contributes to the wilting of the plant. The plant is then readily invaded by the fungus and sporulates profusely on plant material (Figure 7). Fusarium wilt is extensive in wilt susceptible plants but is only limited to the basal part of resistant plants (Beckman 1987). Resistance to disease has been suggested to be associated with inhibitory root exudates, chemical defences in the host and physical barriers which prevent infection (Kraft 1994).


**Figure 7.** Pink coloured spore masses on plant material. These spores can move around the snow pea block in wind or water and carried on machinery and people. Soil and trash can be carried on machinery from an infected block to uninfected block quite easily.

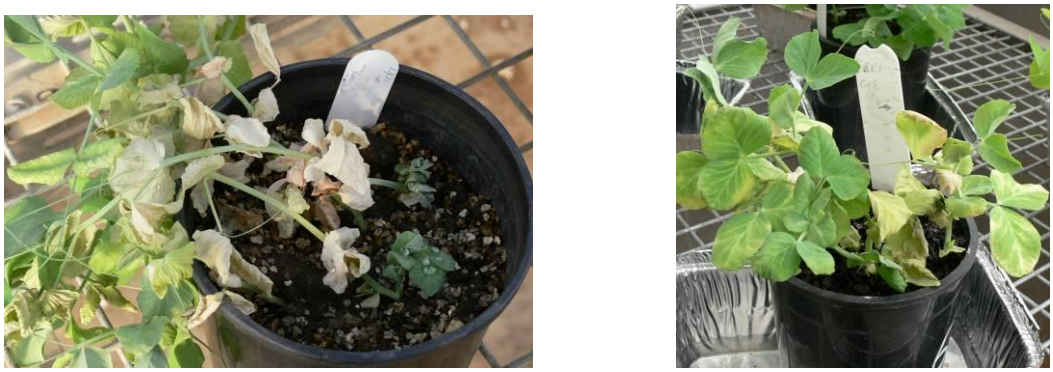

**Figure 8.** Infection in the greenhouse results in symptoms similar to the field. The peas planted into this soil (that contained Fusarium wilt) produced symptoms of Fusarium wilt. The pea plant on the right artificially infected with Fusarium wilt, by incorporating *Fusarium* into the potting mix.

## 1.6 Managing wilt diseases.

Developing disease resistant plants is by far the most reliable and environmentally friendly option for managing Fusarium wilt of peas. It has been carried out for many years in the green pea industry but it has not occurred in the development of snow peas. The information required for such a process is to know the races present in a growing area and some form of resistance available in a closely related plant. The problem with such plants as snow peas is maintaining the traits needed for the specific pod characteristics. It also requires a long process of breeding which is time consuming and costly. Other options for disease control

include fumigation, finding new ground or growing alternative non host crops. With some crops such as Fusarium wilt of snake bean, grafting susceptible plants onto plants with more resistance (but less desirable bean quality) has been trialled and shown to be successful. But this method is also laborious and expensive.

Fumigation with chloropicrin or other fumigants is another method of Fusarium wilt control as it is with other soil borne organisms. Soil sterilisation gives the best control available but may not be environmentally friendly and may in the long term select pathogenic fungi because of the loss of other beneficial microorganisms.

Fusarium wilt has been a problem in cotton in Australia for a number of years and the main control options have included more tolerant cultivars and rotations. In cotton rotation trials using alternatives to cotton crops and rotation sequences, more cotton plants were not affected when fallow preceded a cotton crop (Swan and Salmond 2005). The industry also has a seed dressing registered, Bion Plant Activator® (active ingredient Acibenzolar-S-Methyl 500 g/kg) for the suppression of Fusarium Wilt. Seed dressings were examined as potential control options.

The project within this report developed from grower concerns at the appearance of Fusarium wilt in various growing regions. The project commenced in mid 2005 and began with a disease survey. Over the duration of the project, snow pea production declined drastically, especially in the Sydney Basin, due to the importation of snow peas cheaper than what could be produced locally.

### 1.7 Fusarium wilt of snow peas.

Fusarium wilt is one of the most significant causes of crop losses in snow pea and pea worldwide, however; the extent to which this disease affects Australian production has never been quantified. This projects main aim was to establish a profile of the pathogen involved, this includes a genetic analysis of the pathogen involved. This was achieved through detailed surveys, isolate collection, race typing, population studies of the pathogen and cultivar evaluation. Disease management options based on current literature are limited and resistant cultivars are the main and best method for controlling wilt diseases. Bion Plant Activator® was examined for the suppression of Fusarium Wilt. A management plan was developed to assist growers with best options for controlling Fusarium wilt of snow peas with the current knowledge and experience of the disease gained through the course of the project.

VG05029 was a collaborative project carried out in three states and included four agencies; NSW Industry and Investment, the University of Sydney, Botanic Gardens Trust and Queensland Department of Employment, Economic Development and Innovation.

# 2. Disease Survey

## 2.1 Introduction

Fusarium wilt has been described as one of the most destructive limitations to pea production globally, and is second in importance next to *Aphanomyces* root rot in terms of crop losses. As global cultivation of this vegetable crop increases, so too does the incidence of disease. Snow pea crops are increasingly affected by a fungal disease, known as Fusarium Wilt, which has the potential to greatly reduce yield and economic returns. As production of green pea and snow pea cultivars has increased in Australia, the incidence of Fusarium wilt has subsequently increased. Pea growers in Australia are experiencing crop yield decline, resulting in direct financial loss. The recommended control strategies for Fusarium wilt do not appear to be effective in pea growing regions, as farmers are experiencing disease outbreaks in virgin soils. The occurrence of this disease in virgin soils indicates that crop rotation may not be a viable control strategy in Australian conditions, therefore in order to successfully cultivate pea and snow pea, novel management strategies must be developed.

Much of the research into snow pea wilt is conducted in the USA and India. Management strategies devised from the findings of such research are region specific and relate to the climatic features of the regions and the cultivar availability. Many Australian farmers are restricted to growing only one snow pea cultivar as a result of consumer preferences and seed availability; therefore the recommendations of international researchers are often irrelevant. In order to eradicate or control Fusarium wilt of snow pea in Australian soils, local populations of the fungus need to be studied to gain an in depth understanding of the pathogen biology and to determine population diversity. When developing control strategies, it is imperative to possess an in-depth understanding of the pathogen involved. An understanding of the genetic structure of the pathogen is required in order to determine the virulence genes responsible for disease, enabling the development of gene specific control programs and the development or location of resistant crop cultivars. In order to study the population structure of this pathogen, disease surveys were conducted and isolates of the pathogen were collected for analysis.

There have been no intensive disease surveys across Australia to identify this pathogen or describe its distribution. In order to confirm the presence of this pathogen in Australian growing regions and to determine its distribution, a disease survey was conducted in 2005/2006 across the major snow pea growing regions in Australia, Queensland, New South Wales and Victoria.

## 2.2 Fusarium Wilt of Snow Pea

The most common symptoms of disease include chlorosis of the leaves beginning from the lower regions of the plant, extending upwards as disease progresses, downward curling of the leaves and wilt (Figure 9). A red to brown discolouration of the vascular bundles is also a characteristic of infection. Infected pea plants are

often found to develop pods despite infection; however these pods are often dry, stunted and shrivelled, thus rendering them unsuitable for harvest and sale. Crop losses are devastating, particularly in snow pea crops, due to the curved shape of the pods. Few diseased plants will survive to anthesis and very few infected plants will set seed.

**Figure 9.** Wilted plant in field exhibiting symptoms of chlorosis and leaf curling

Wilt symptoms, apart from those previously discussed, include a bluish sheen, abrupt wilting, the expression of unilateral symptoms, vascular discolouration in upper part of plant, mycelium covered basal node and swelling of the basal internode. The below and above ground vascular system is usually coloured red to brown and the lower subterranean portion of the stem becomes enlarged during infection. Disease is often diagnosed in field conditions when large, chlorotic patches are observed within the crop (Figure 10). Symptoms of wilt affecting green pea crops are essentially identical to those observed in affected snow pea fields.

**Figure 10**. Chlorotic patches observed in a wilt affected snow pea field

## 2.3 Disease Observations

Fusarium Wilt of Snow Pea incidence was observed in the major growing regions of Queensland, New South Wales and Victoria. In Queensland, the survey was conducted in 2 separate areas; Bundaberg and Gympie. In New South Wales, the only area surveyed was Sydney and in Victoria the areas of Bairnsdale and Korumburra were surveyed (Figure 11).

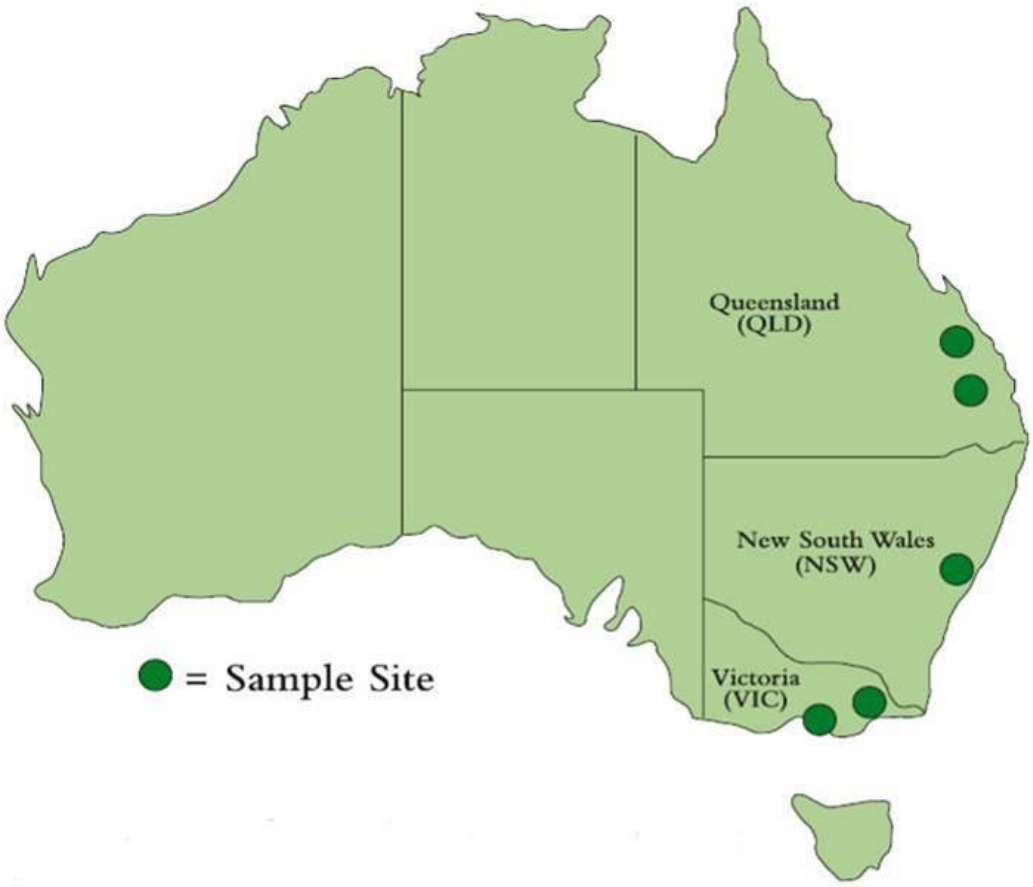


**Figure 11.** Snow pea growing areas surveyed for Fusarium wilt.

### *2.3.1 Queensland*

A total of 4 sites were surveyed in Queensland; 3 in Bundaberg and 1 in Gympie.

*Bundaberg*

Fusarium wilt was found in each of the three sample sites in Bundaberg. After field observations and discussion with growers, it was evident that wilt symptoms began to appear within crops late in the growing season. Most wilted plants had begun to develop pods prior to symptom development. Symptoms were typical of Fusarium wilt, with plants exhibiting yellowing of the lower leaves, chlorosis, leaf wilt and unilateral symptom expression. Disease was severe, and appeared to affect 80-100% of plants in the field (Figure 12 and Figure 13). It is important to note that in 2006, one grower did not harvest his snow pea crop due to the severity of wilt disease. Due to the late development of disease, yield loss was high which resulted in significant economic loss to growers.

**Figure 12**. Wilt symptoms developing in mature snow pea plants.

**Figure 13.** Wilted snow pea plants in Bundaberg.

Discussion with growers revealed that the land used for snow pea crops was often 'new' land which had been previously uncultivated or cultivated with other crops such as beans or sorghum. Growers revealed that they have begun to lease new land every growing season in an attempt to avoid Fusarium wilt in their new snow pea crops. Despite the virgin nature of the soils, snow pea wilt was present in the crops to a great extent. It is unlikely that the host specific pathogen existed within these soils prior to cultivation with snow pea, thus indicating that it was introduced into the crop. Transmission via the introduction of contaminated seed is possible, as all snow pea seed used in this growing area was sourced from the US, where Fusarium wilt was first described and continues to be a major disease in pea crops. An improvement in farm hygiene may be useful in reducing the severity of disease in this area. During the disease survey it was noted that growers continually move contaminated soil material between paddocks and farms by way of unwashed vehicles and footwear. Growers should ensure that all footwear and vehicle tyres are appropriately decontaminated prior to movement between farms in order to prevent the spread of this pathogen.

*Gympie*

Fusarium wilt was found on the sample site in Gympie. A high disease incidence was observed, with approximately 80% of the snow pea crop affected. As was observed in Bundaberg, wilt symptoms began to appear late in the growing season, just prior to pod set. Symptoms were typical of Fusarium wilt, with plants becoming increasingly chlorotic and wilted. Chlorotic patches of plants were observed within the field (Figure 14).

**Figure 14.** Chlorotic patches in snow pea crop.

Due to the late disease development in this growing area, yield loss was high resulting in significant economic loss to the grower. In 2008, the grower in Gympie ceased snow pea cultivation as a result of increasing disease incidence and economic loss.

The grower in Gympie had cultivated snow peas for a number of years and had experienced increasing disease incidence and severity over progressive growing seasons. The grower did not acquire new land each growing season, unlike the growers in Bundaberg. The grower practiced crop rotation on this land and rotated his snow pea crop with both maize and sorghum which were grazed by cattle. It is evident that crop rotation was not effective in reducing the snow pea wilt as disease increased in severity over time. It is also possible that the pathogen was introduced into Gympie via contaminated imported seed (sourced from the US) and via movement of vehicles between farms.

Farm hygiene may have been a contributing factor to the incidence of disease on this site. It was noted during the disease survey that the snow pea crop residues were often removed and placed in a pile adjacent to the snow pea fields. This practice provided a source of *F. oxysporum* f.sp. *pisi* inoculum and may have been responsible for disease occurrence in subsequent growing seasons.

### *2.3.2 New South Wales*

*Sydney*

A total of 4 sites were surveyed in Sydney. Fusarium wilt of snow pea was identified in all 4 sites. The snow pea growers in Sydney are of a Cambodian background and speak very little English. Communication with growers was aided by a translator.

Wilt symptoms in this growing area appear sporadically throughout the growing season. Symptoms appeared early in the growing season in some plants, and later in the growing season in others. This is in contrast to the observations in both Queensland and Victoria. Symptoms were typical of Fusarium wilt, with plants exhibiting yellowing of the lower leaves, chlorosis, leaf wilt and unilateral symptom expression (Figure 15). Fusarium wilt appeared to affect approximately 60% of the snow pea crop, which resulted in moderate yield reduction and economic loss to growers.

**Figure 15.** Wilted snow pea plants in Sydney, New South Wales.

Discussions with the growers in this area did not clarify the extent of crop rotation on these farms. Snow pea residue from previous seasons was evident in the field, indicating that crop rotation was not performed on these farms. The residue from previous seasons could provide a source of inoculum, resulting in disease in subsequent growing seasons. The snow pea crops were overrun with numerous weed species as seen in Figure 16. The weeds present in these crops could have the potential to act as asymptomatic hosts of the snow pea wilt pathogen, thus increasing inoculum levels within the soil. The increased level of inoculum as a result of both weed infestation and retention of crop residue will cause increase disease incidence and make disease control difficult for growers. An improvement in farm hygiene in this area may contribute to a reduction in disease severity, improving yield and income.

**Figure 16.** Weed infestation in Snow Pea crop in Sydney.

### *2.3.4 Victoria*

A total of 5 sites were surveyed in Victoria; 4 in Bairnsdale and 1 in Korumburra.

*Bairnsdale*

Fusarium wilt was found in each of the 4 sites in Bairnsdale. One site contained a green pea crop in addition to snow peas. This green pea crop was also affected by Fusarium wilt. After field observations and discussion with growers, it was evident that wilt symptoms began to appear within crops very early in the growing season. Most affected plants were very young seedlings (Figure 17). Symptoms in this growing area were typical Fusarium wilt symptoms, with plants becoming progressively chlorotic and wilted.

**Figure 17.** Young snow pea plants affected by Fusarium wilt in Bairnsdale.

Disease was very severe, with 80-100% of plants affected by wilt. This high disease severity resulted in extensive yield reduction and significant economic loss to growers. It is important to note that some growers in this region were unable to harvest their crops due to the severity of wilt in their fields. Figure 18 shows a typical snow pea crop in Bairnsdale with many affected seedlings.

**Figure 18.** Snow pea crop in Bairnsdale affected by wilt.

Growers in this area ensured that hygiene on their farms was maintained at a high standard. Growers were very aware of the implications of poor hygiene on disease incidence and severity. It does not appear that poor hygiene is a contributing factor to the high incidence of disease in this growing area.

Growers practiced crop rotation in this area and rotated with crops such as potatoes, capsicum and tomato. Weeds were controlled with regular spraying and manual removal. Despite efforts to reduce disease build-up in the soil via implementation of crop rotation and weed control, snow pea wilt was still prevalent in subsequent growing seasons. These findings suggest that the disease is introduced onto farms, possibly via contaminated seed. All snow pea seed used in Bairnsale, as in Queensland and New South Wales, is sourced from the US.

*Korumburra*

Fusarium wilt was found in the Korumburra sample site. A high disease incidence was observed on this site, with approximately 80% of plants affected by wilt. After field observations and discussion with growers, it was evident that wilt symptoms began to appear within crops very early in the growing season. Most affected plants were very young seedlings. Symptoms in this growing area were typical Fusarium wilt symptoms, with plants becoming progressively chlorotic

and wilted. Despite being affected early in the growing season, some plants did survive to anthesis and produce edible pods. Disease severity was moderate, with a yield loss of approximately 60%.

Hygiene on this farm was maintained to a very high standard. All crop residues were removed following harvest and weeds were controlled via chemical and mechanical means. These measures were performed in an attempt to reduce inoculum levels in the soil and reduce disease incidence and severity. Crop rotation was utilized on this site, with potatoes being the rotational crop. Despite efforts to reduce inoculum build-up in the soil, disease was prevalent in subsequent growing seasons. These findings suggest that the disease is introduced onto farms, possibly via contaminated seed. In contrast to Queensland, New South Wales and Bairnsdale, the snow pea seed sown in Korumburra is sourced from New Zealand, not the US.

## 2.4 Isolation of *Fusarium oxysporum* f.sp. *pisi*

### *2.4.1 Sample Collection*

During the disease survey in Australia's snow pea growing regions, samples of wilted plants and soils were collected in order to isolate the causal organism. Plants and soils were collected from each site. Plants were selected for sampling based on disease symptoms. Plants were only collected if they exhibited chlorosis of the leaves, wilting and vascular discolouration. Whole pants were removed from the soil, labeled and placed into plastic bags for transport back to the laboratory. Sites for soil sampling were randomly selected by use of a random number generator. A total of 20 soil samples were selected from each site. Approximately 2kg of soil was removed from each sampling site and was placed into a plastic bag with an appropriate label. Samples were transported back to the laboratory.

### *2.4.2 Isolation of Fusarium*

*Plant Material*

Once the plants arrived back at the laboratory, the plants were trimmed of excess soil and roots. Only 15cm of the lower stem was retained. The stem sections were washed in 70% ethanol solution and then rinsed with sterile deionised water. Stem sections were allowed to air dry (Figure 19).


**Figure 19.** Snow pea stem sections following ethanol wash.

The stem sections were cut into small cross-sectioned pieces, approximately 1cm long, and plated onto Peptone PCNB Agar (PPA) (Figure 20). Slightly fluffy colonies exhibiting a salmon to pink colour were retained as possible *F. oxysporum* cultures. Colonies were allowed to grow for 7 days and were then sub-cultured and plated onto Carnation Leaf-piece Agar (CLA). Media ingredients and preparation are in the Appendix.

**Figure 20**. Snow pea stem section on PPA

CLA cultures were single spored after 7 days growth and plated onto fresh CLA plates. Single spored cultures were examined under the microscope and all cultures identified as *F. oxysporum* were retained.

*F. oxysporum* isolates were stored in 15% Glycerol solution at -80$^{o}$C.

*Soil Material*

Soil samples were combined with commercial potting mix in a ratio of 1:1. This soil/potting mix mixture was placed into small plastic pots and labelled accordingly. Seeds of the snow pea cultivar 'Oregon Giant' were placed into the mixture and watered well. Following germination, seedlings were monitored for wilt disease symptoms. When seedlings appeared wilted, they were removed from their pot and washed with 70% ethanol solution. F. oxysporum was isolated from these seedlings using the same method described above.

### *2.4.3 Pathogen Population*

A total of 478 isolates of *F. oxysporum* f.sp. *pisi* were collected during this disease survey. Of these 478 isolates, 182 were collected from Queensland, 84 were collected from New South Wales and 212 were collected from Victoria.

## 2.5 Conclusion

The results of this field survey indicate that the snow pea wilt pathogen, *F. oxysporum* f.sp. *pisi* is widespread across Australia's snow pea and pea growing regions. These findings were expected, as Fusarium wilt is the most significant disease in pea and snow pea crops worldwide after Aphanomyces root rot, and is present in all pea growing regions worldwide. The field survey data also shows that there is variation in the pattern of field symptoms across growing regions in Australia. These variations may be due to regional factors such as climate and soil, pathogenic race variation or genetic differences within the pathogen population.

# 3. Pathogenic Races of the Snow Pea Wilt Pathogen in Australian Growing Regions

## 3.1 Introduction

The snow pea wilt pathogen, *Fusarium oxysporum* f.sp. *pisi*, exists in numerous forms across the globe. These physiologic forms of the fungus are known as races. Races of fungi are one of a group of forms alike in morphology but unlike in certain cultural, physiological, biochemical, pathological or other characters. Races of the pathogen are identified based on host response on differential cultivars. The resistance of pea plants to races of *F. oxysproum* f.sp. *pisi* is controlled by separate, single dominant genes in the host plant and the effectiveness of using resistant cultivars can be reduced by the occurrence of new pathogenic races.

The current criteria for the description of a race are 1) the isolate must occur in nature 2) the isolate must become established in nature and survive with time 3) the isolate must be of economic importance and 4) the race must be separated by other known Fusarium wilt races by utilising known gene difference of the host. Currently, 11 races of the pathogen have been identified worldwide. Despite this relatively high level of race diversity present within snow pea growing regions, it is only races 1, 2, 5 and 6 which cause significant disease symptoms resulting in large economic losses to growers. These races all incite disease in susceptible cultivars; however symptom expression varies among races. Previous reports suggest that races 1, 5 and 6 affect very young plants, resulting in rapid death, translating to high reduction in yield (Neumann and Xue 2003). Race 2 causes disease later in the growing season, usually at pod set (Kraft 1994). Previous findings suggest that Race 1 is the most common race causing disease in snow pea and pea growing regions worldwide and is the race of highest economic significance (Kraft 1994).

The race diversity within Australian snow pea growing regions has not previously been investigated, thus knowledge of race biology and diversity is very limited. It is important to determine the races present in a growing region in order to implement disease control strategies such as the use of race resistant cultivars. Knowledge of race diversity is also important as it can lead to the development of race-specific resistant cultivars, and the implementation of other race targeted control measures.

## 3.2 Materials and Methods

### *3.2.1 Isolate Selection*

A total of 140 isolates were selected as a representative sample population for inclusion in the race typing experiment. Ten isolates were randomly selected from each sample site for inclusion in the sample population. A total of 40 isolates were selected from both the New South Wales and Queensland populations, while 60 isolates were selected from the Victorian population.

### *3.2.2 Experimental Design*

The race typing experiment was conducted in several stages. A total of six *Pisum sativum* cultivars were selected as differential cultivars based on previous findings: Dark Gray Sugar, New Era, New Wales, Double One, Lincoln and WR Greenfeast.

The stages of the race typing experiment are illustrated below in Figure 21.

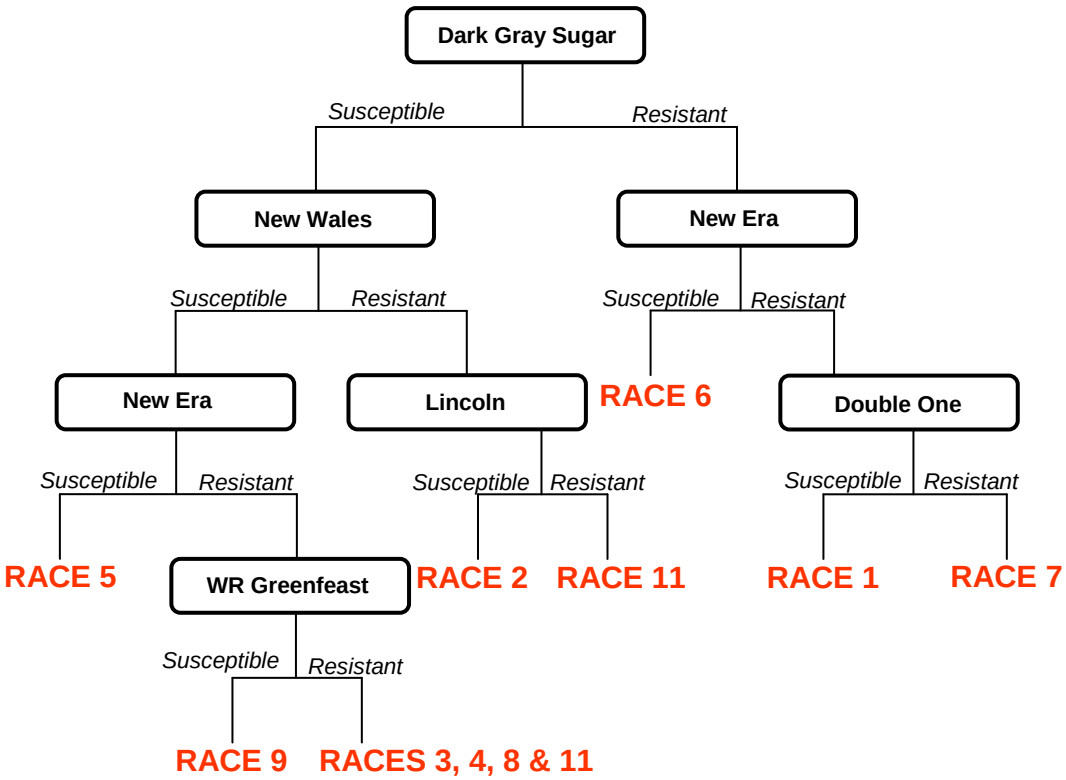


**Figure 21.** Stages of race typing experiment.

This experiment was a Completely Randomised Design. Each stage of the experiment included a positive control (Cultivar 'Oregon Giant' inoculated with *F. oxysporum* f.sp. *pisi*) and a negative control (uninoculated seedlings of differential cultivar). For each of the 140 isolated used in the experiment, 3 pots each containing 3 seedlings were inoculated.

### *3.2.3 Sowing*

Small pots each 10cm in diameter were filled with commercial potting mix. Five seeds of the cultivar being tested were sown 2cm deep into the potting mix and watered well. Once germination had occurred, extra seedlings were removed to ensure there were only 3 seedlings in each pot. The seedlings were watered daily and treated with fertiliser once every fortnight for the duration of the experiment.

### *3.2.4 Preparation of Inoculum*

Isolates were grown onto small petri dishes containing Carnation Leaf-Piece Agar (CLA). Each isolate was grown onto a total of 9 small (60mm) petri dishes. The isolates were grown under light for 10-14 days, until sporulation was evident.

### *3.2.5 Inoculation*

Seedlings were inoculated with *F. oxysporum* f.sp. *pisi* isolates 14 days after germination. For each isolate tested, the 9 small petri dishes were washed with sterile deionised water and the resulting spore suspension was placed into a beaker. The spore suspension was made to a total volume of 100mL using additional sterile deionised water. Three pots of seedlings were wounded around the root zone using a scalpel. A volume of 30mL of spore suspension was taken with a large plastic syringe and poured around the wounded seedlings in each pot. Extra potting mix was scattered over the seedlings. Pots were labelled with isolate number and pot number (1-3) and placed on metal racks over plastic trays to prevent cross contamination. The seedlings were allowed to grow in greenhouse conditions at 23$^{o}$C for a period of 4 weeks.

### *3.2.6 Disease Scoring*

The plants were observed weekly, over a period of four weeks, for disease development. Observations for each plant in each pot were recorded in a spreadsheet. Observations were recorded as W for plants exhibiting wilt symptoms and NW for plants not exhibiting wilt symptoms. After 4 weeks of observations, all results were analysed. Pots with at least 2 out of 3 seedlings showing wilt symptoms were recorded as ‘susceptible’. Pots with less than 2 wilted seedlings were recorded as ‘resistant’. The final results were analysed and race determinations were made based on cultivar response to each isolate over each stage of the experiment.

## 3.3 Results

The race typing experiment concluded that four races of *F. oxysporum* f.sp. *pisi* are present in Australian snow pea growing regions. These races and their significance within the pathogen are population are shown in Figure 22.

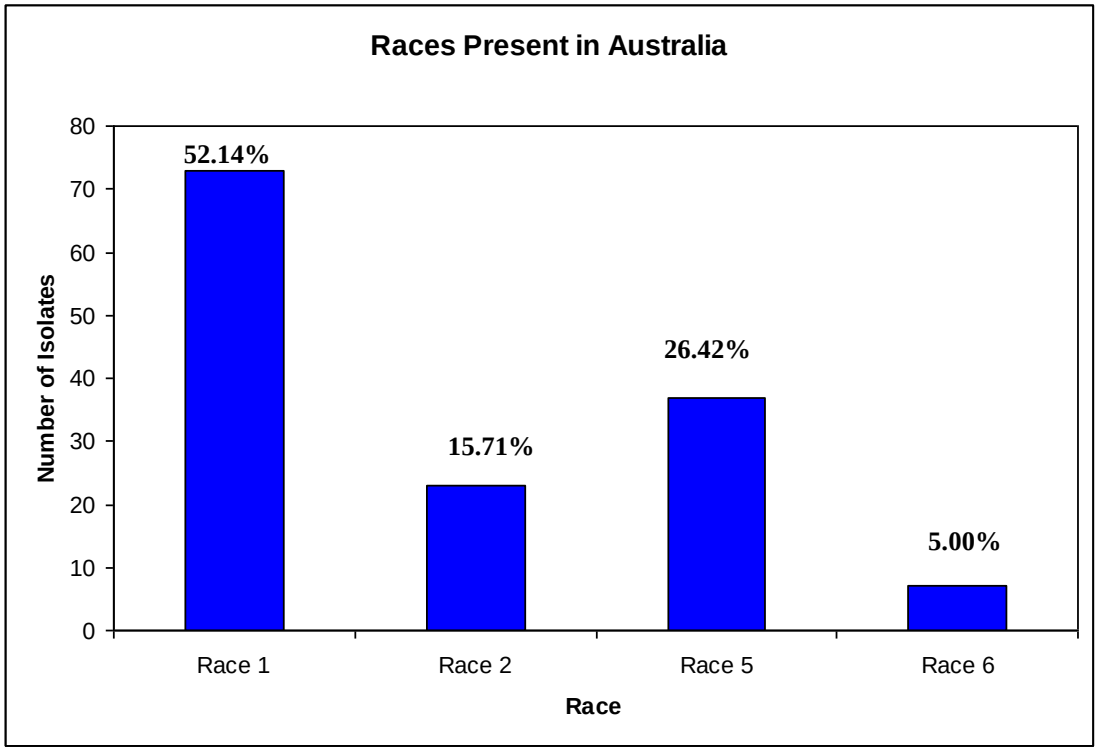


**Figure 22.** Races of *F. oxysporum* f.sp. *pisi* present in the Australian population.

The proportions of races within each growing region are illustrated below in Figure 23, Figure 24 and Figure 25.

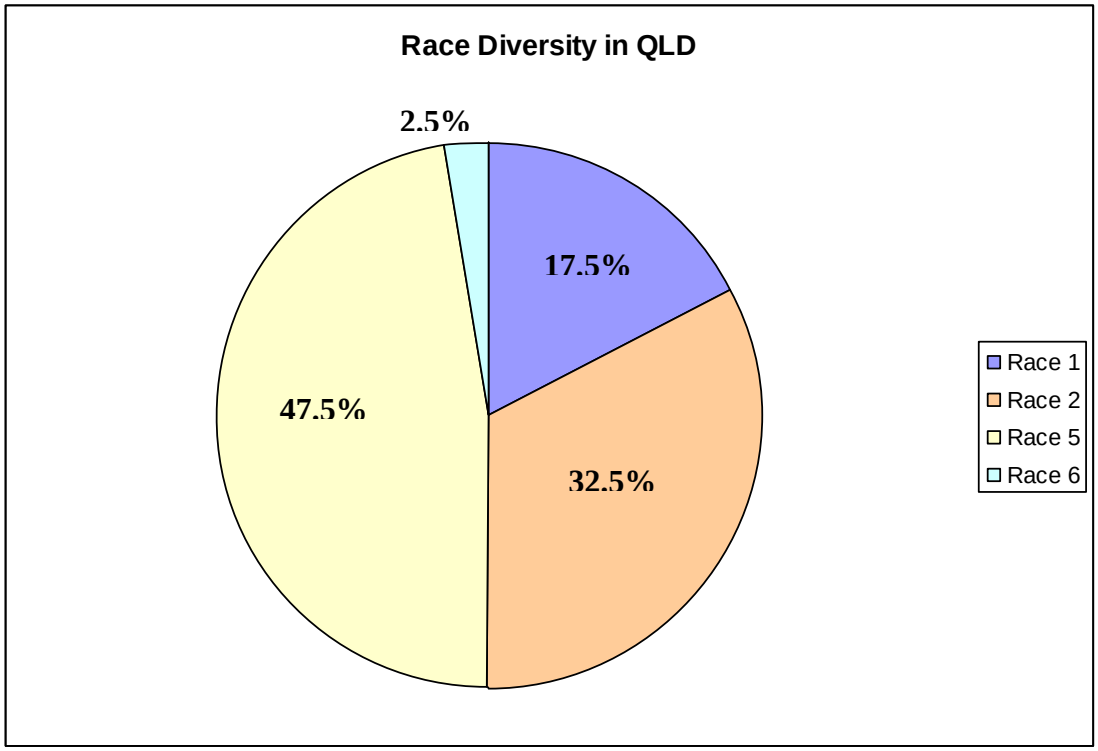


**Figure 23.** Race diversity in Queensland sub-population.

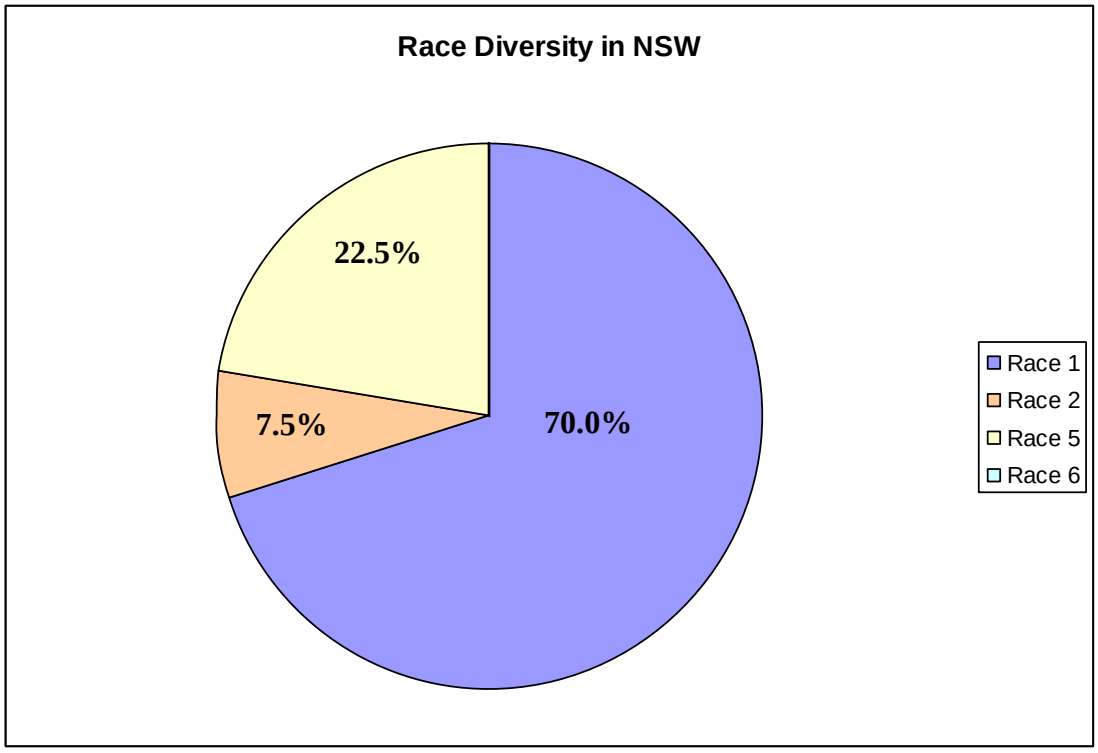


**Figure 24.** Race diversity in New South Wales sub-population.

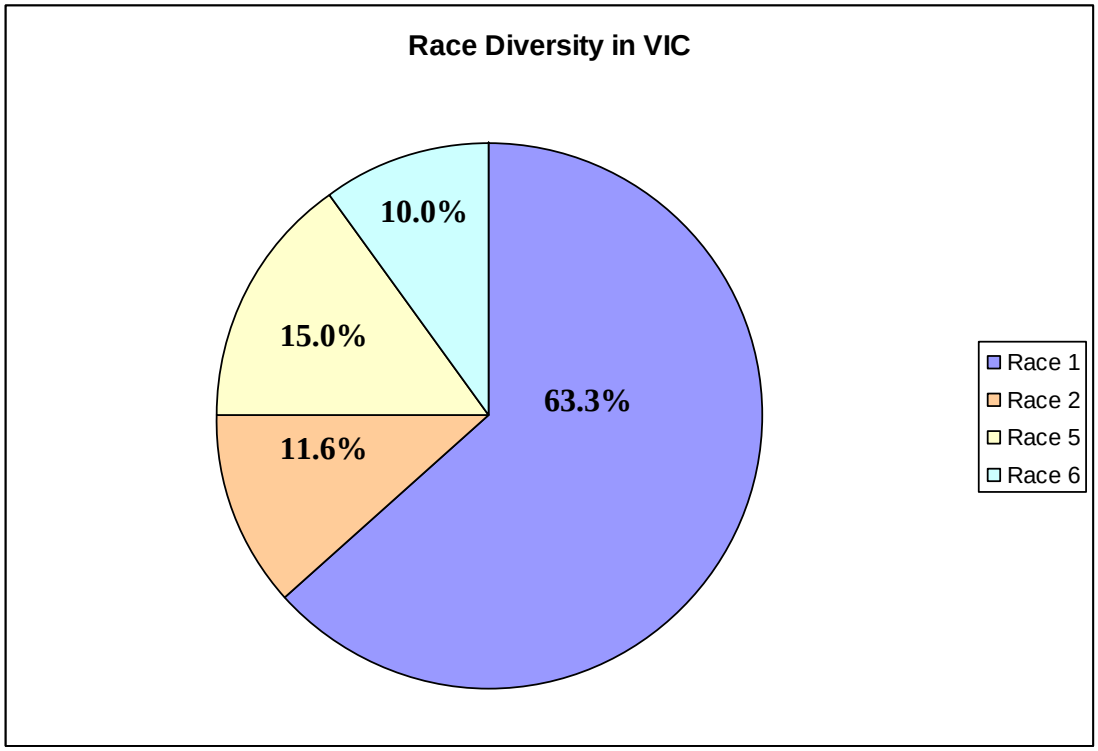


**Figure 25.** Race diversity in Victorian sub-population.

### 3.4 Discussion

A total of four races identified as 1, 2, 5 and 6 were found to exist in the main snow pea growing regions in Australia are races (Figure 22). These results were expected based on previous research which states that these races are the most common in pea growing regions worldwide and the only races of significant economic importance. No races unique to Australia were identified.

Race 1 was the predominant race found to exist within the population, comprising 52.14% of isolates studied. This finding was expected as Race 1 has been reported as the predominant race causing disease in pea growing regions worldwide, notably in the US where it was first reported. Race 5 is also significant within the population, with 26.42% of isolates belonging to this race (Figure 22). Races 2 and 6 are less significant within the population.

The race diversity differs among the three sub-populations from Queensland, New South Wales and Victoria. All 4 races are found within the Victorian sub-population (Figure 25). As predicted, Race 1 is the predominant race in this growing region. Race 1 is responsible for most wilt disease and has been reported to affect young seedlings and cause severe wilt symptoms. Races 2, 5 and 6 are found in similar, smaller numbers within this group. Field observations of this growing region indicate that pea and snow pea plants succumb to wilt early in the growing season, resulting in high disease severity and significant yield loss. This observation is congruent with the race findings in this region.

In contrast to the Victorian sub-population, only 3 races were found to exist within the New South Wales sub-population (Figure 24). As predicted, Race 1 was the predominant race, with 70% of isolates belonging to this group. Races 5 and 2 were also found within this group. Field observations in this reason indicate that disease symptoms appeared throughout the entire growing season. Disease incidence was not high in young snow pea plants, but was high during the middle and end of the growing season. Race 2 is the likely cause of late disease development. Race 5 was also found in significant numbers. Previous reports state that Race 5 causes disease and death in young pea plants; however these disease observations conflict with those findings. This disease pattern resulted in high yield loss as snow pea pods were absent or deformed and not suitable for sale.

All 4 races are also found within the Queensland sub-population (Figure 23). In contrast to the New South Wales and Victorian sub-population, Race 1 was not the predominant race. Race 5 was the most common race, with 47.5% of isolates belonging to this race group. Race 2 was also found in significant numbers, with 32.5% of isolates belonging to this group. Races 1 and 6 were found in minor quantities. Field observations in this growing region indicate that disease occurs very late in the growing season, usually beginning at the onset of pod development. Plants grow to maturity and appear symptom free, however succumb quickly to disease at pod set. Due to the prevalence of races 5 and 2, we can assume that these races are responsible for the late disease development. These findings are incongruent with previous findings which state that Race 5 causes disease in very young seedlings, resulting in rapid death. We do not see this pattern of disease in snow pea plants in Queensland. These findings suggest that Race 5 is similar in physiology to Race 2, which is known to cause 'Near Wilt' late in the growing season.

This race diversity may be the result of pathogen introduction. As the race diversity within the Australian population is similar to that of US populations and that most Australian snow pea seed is imported from the US, we can infer that races were introduced with imported seed. The race diversity may also be a result of parasexuality occurring between isolates in the field. Parasexuality has the potential to generate genetically unique strains of *F. oxysporum*. The creation of genetically unique strains may result in altered host response, and therefore new races of the pathogen. It is important to note that different cultivars of snow pea are grown in the different snow pea growing regions of Australia. In New South Wales and Victoria, the only cultivar grown is Oregon Giant. As previously mentioned, Race 1 was the predominant race in these growing regions. In Queensland, Oregon Giant is grown in addition to the cultivar Mammoth Melting. It is possible that the variation in cultivars grown may be significant in the detection of varying races in these regions.

As with most soil borne diseases, the use of resistant cultivars is often the most effective method of disease management. In order to effectively control snow pea wilt, resistant cultivars need to be developed. As race is not correlated to geography, we can state that control measures (namely resistant cultivars) can be implemented across all growing regions. As all growing regions are affected by Race 1, the use of a Race 1 resistant cultivar would be an effective method of controlling disease and reducing yield loss although disease would still occur due to the presence of races 2, 5 and 6. Due to the variation in race diversity across the

three growing regions, control measures will need to be targeted to race and region. Due to the predominance of Race 1 in both Victoria and New South Wales, it is recommended that a Race 1 resistant cultivar of snow pea is introduced to these regions. The introduction of this cultivar would significantly reduce disease in pea and snow pea crops, and increase yield and income. A Race 1 resistant cultivar would not be effective at controlling snow pea wilt in Queensland. It is recommended that a Race 5 resistant cultivar be introduced to this growing region.

Further studies are required to determine the genes which cause virulence in races of the snow pea wilt pathogen and genes which provide resistance in pea plants in order to develop race-specific resistant cultivars which are suitable for the Australian market.

## 3.5 Conclusion

Four races of *F. oxysporum* f.sp. *pisi* are present in the main snow pea growing regions in Australia. These races are the only races of economic significance worldwide. Race 1 is the predominant race in the population and is responsible for the majority of wilt disease in Victoria and New South Wales. Race 5 is responsible for the majority of wilt disease in Queensland. As the only viable control method for Fusarium wilt diseases is the use of resistant cultivars, race-specific resistant cultivars need to be developed and introduced into the major snow pea growing regions in order to reduce disease incidence. A Race 1 resistant cultivar would be most effective at reducing disease across Australian growing regions.

# 4. Population Diversity and Geographic Structuring of the Snow Pea Wilt Pathogen in Australia

## 4.1 Introduction

Fungal plant pathogens, such as *Fusarium oxysporum*, are extensively studied by plant pathologists in order to understand their population genetics. Population studies are an important aspect of plant pathology as plant pathogens are constantly moving and/or evolving. In agricultural systems, environmental changes including resistant plant cultivars, fungicide application, irrigation and crop rotation apply a selection pressure upon populations of fungi and influence their adaptation to suit their new environment. Recently, plant pathologists have begun to focus their studies on populations rather than individuals in order to understand pathogen dynamics and to develop effective, long term control strategies. DNA fingerprinting is a popular method of determining the genetic diversity present within a population of organisms. The techniques of Rep-PCR and RAMS-PCR are common molecular tools used in the study of population diversity. Rep-PCR has been described as a technique of genomic fingerprinting which makes use of DNA primers complimentary to naturally occurring, highly conserved, repetitive DNA sequences present in multiple copies in the genomes of many fungal species. Rep-PCR is the collective name for REP, BOX and ERIC sequences. Three families of repetitive sequences have been identified, including the 35-40 base pair repetitive extragenic palindromic (REP) sequence, the 124-127 bp enterobacterial repetitive intergenic consensus (ERIC) sequence and the 154 bp BOX element. The use of these three primers and PCR leads to the selective amplification of distinct genomic regions located between REP, ERIC or BOX elements. Random amplified microsatellite (RAMS) analysis involves PCR with primers containing repeat microsatellite sequences and degenerate anchors at one end of the sequence. This method has been found to be useful in describing variation in many groups of fungi. This study is unique in that the DNA fingerprints of fungal isolates were determined by a combination of six primers sets. The six primer sets were chosen for use in this study as a means if increasing sensitivity in determining the genetic variation between isolates in the population. The aim of this study is to test the null hypothesis that there is no genetic diversity within the *F. oxysporum* f.sp. *pisi* population in snow pea growing regions.

## 4.2 Materials and Methods

### *4.2.1 DNA Extraction*

Mycelia cultivated on plates of PDA were scraped off the agar surface using a scalpel and placed in a sterile 1.5ml tube containing 1ml of Extraction Buffer. The cells were lysed by rapid shaking in a homogeniser with the aid of quartz sand and ceramic beads (3 $ms^{-1}$ for 1 minute) in order to release nuclear material into solution. Cellular debris was pelleted by centrifugation at 12000 rpm for 5 minutes and removed. The resulting supernatant was transferred to a sterile 1.5ml tube containing 125 µL protein precipitation solution. The tubes were rotated for 10 minutes to bind and precipitate any protein remaining within the supernatant.

Subsequently, the solution was centrifuged at 14000 rpm for 5 minutes. The supernatant was transferred to 1.5ml sterile tubes to which 700 µL of binding matrix was added. Nucleic acid molecules present within the supernatant was bound to the binding matrix by rotating tubes for 20 minutes. This was followed by centrifugation at 14000 rpm for 1 minute. The resulting supernatant was discarded and the pellet (nucleic acids bound to matrix) was dried in a laminar flow cabinet for 15 minutes. Once dried, the pellet was re-suspended in 800 µL salt-ethanol wash solution. This step was followed by centrifugation at 14000 rpm for a further minute to separate the nucleic acids from the SEWS supernatant. The pellet was dried in a laminar flow cabinet prior to re-suspension with 120 µL of TE buffer + RNAse (10 µg/mL) solution. The nucleic acids were eluted into the TE buffer via a swirling action and placed into a 37$^{o}$C waterbath for 2 minutes to loosen matrix. The mixture was again centrifuged at 14000 rpm for 1 minute and the resulting supernatant was transferred to sterile Eppendorf tubes whilst the pellet was discarded. The tubes were placed into the waterbath at 37$^{o}$C for 1 hour in order to digest all RNA molecules and were stored at 4$^{o}$C.

### *4.2.2 Rep-PCR and RAMS-PCR*

PCRs were performed in 25 µL volumes containing the DNA template and 'master mix' which contains 0.2 µM of each primer (Table 2); 2.5 mM of dATP, dCTP, dTTP and dGTP; 2.5 mM $MgCl_2$; 1x PCR $NH_4$ Reaction Buffer; 1U of Taq Polymerase and sterile $H_2O$. A master mix only preparation was used as a negative control in every set of reactions. PCR was performed on each isolate six times, one reaction for each of the six genetic markers.

**Table 2.** Primer sequences of Rep and RAMS-PCR markers

| ***Primer Name*** | ***Sequence*** |
|---|---|
| BOX A1R | 5'-CTACGGCAAGGCGACGCTGACG-3' |
| ERIC 1R | 5'-ATGTAAGCTCCTGGGGATTCAC-3' |
| ERIC 2I | 5'-AAGTAAGTGACTGGGGTGAGCG-3' |
| REP 1R | 5'-IIIICGICGICATCIGGC-3' |
| REP 2I | 5'-ICGICTTATCIGGCCTAC-3' |
| ACA | 5'-BDBACAACAACAACAACA-3' |
| CCA | 5'-DDBCCACCACCACCACCA-3' |
| AG | 5'-HBHAGAGAGAGAGAGAGA-3' |

DNA amplifications were carried out in a thermal cycler with an initial denaturation followed by 36 cycles of denaturation annealing and extension with a final extension phase. Refer to

Table 3 and Table 4 for complete thermal cycling profiles. Following the completion of amplification, PCR products were cooled to 4$^{o}$C.

**Table 3.** Rep-PCR thermal cycling profiles.

| ***Process*** | ***Temperature $^{o}$C*** | ***Duration*** |
|---|---|---|
| Initial Denaturation | 94 | 3 min |
| Denaturation | 94 | 30 s |
| Annealing | | |
| ERIC | 56 | 30 s |
| REP | 45 | 30 s |
| BOX | 57 | 30 s |
| Extension | 72 | 2 min |
| Final Extension | 72 | 8 min |
| Storage | 4 | ∞ |

**Table 4.** RAMS-PCR thermal cycling profiles.

| ***Process*** | ***Temperature $^{o}$C*** | ***Duration*** |
|---|---|---|
| Initial Denaturation | 95 | 4 min |
| Denaturation | 95 | 30 s |
| Annealing | | |
| CCA | 57 | 45 s |
| AG | 54 | 45 s |
| ACA | 45 | 45 s |
| Extension | 72 | 2 min |
| Final Extension | 72 | 7 min |
| Storage | 4 | ∞ |

Aliquots (5 μL) of each PCR product were analysed by electrophoresis through a 2% (w/v) agarose gel in Tris-Boric EDTA (TBE) buffer. Gels were run at a voltage of 80V and stained with 100 μg $mL^{-1}$ ethidium bromide in TBE buffer. Gels were run for a period ranging from 90 min to 180 min. Photographs of gels were taken with a digital still camera over a UV transilluminator.

### *4.2.3 PCR Analysis*

DNA banding profiles obtained from gel electrophoresis were scored based on banding pattern similarity. Presence or absence of each band (locus) for each isolate was scored and transformed into binary code (1 or 0) and entered into a computer generated spreadsheet. The resulting data was imported into the statistical software package NTSYSpc®, similarity values based on the DICE coefficient of similarity were calculated and clustered using the Unweighted Pair Group Method with Arithmetic Means (UPGMA). The UPGMA clustering method was used to generate a dendrogram which illustrates the similarity between isolates and/or groups. Clonal lines observed in the dendrogram were assigned individual haplotype numbers.

## 4.3 Results

The haplotypes found to exist within the pathogen population are shown in Figure 26 and

Table 5. The geographic structure of the haplotypes is shown in Table 6.

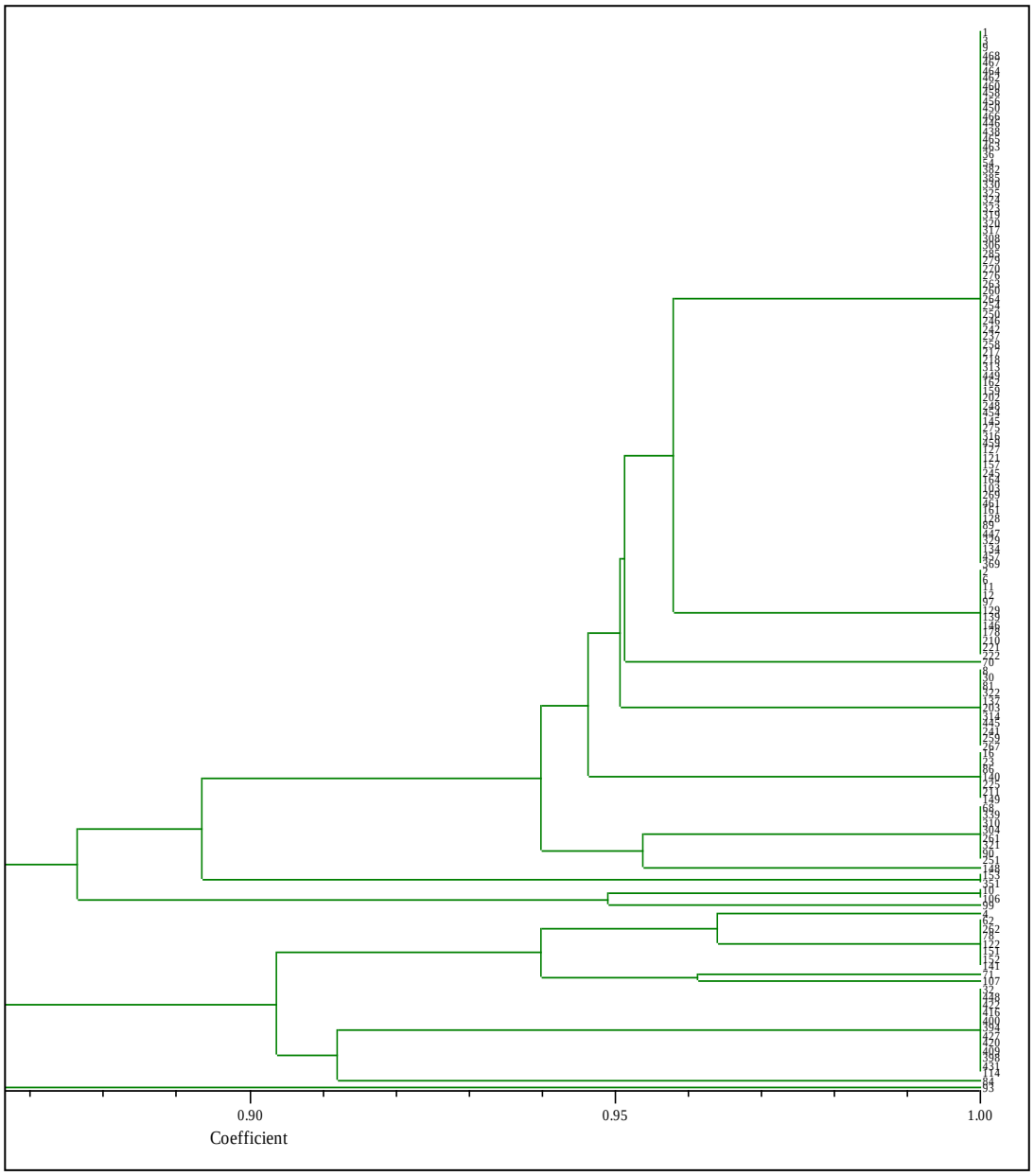


**Figure 26.** Dendrogram illustrating haplotypes present in the population.

**Table 5.** Genetic similarity within population.

| Clonal Haplotypes | Unique Haplotypes | % Similarity |
|---|---|---|
| 9 | 8 | 85 |

**Table 6**. Genetic similarity amongst sub-populations

| | Clonal Haplotypes | Unique Haplotypes | % Similarity |
|---|---|---|---|
| Queensland | 3 | 4 | 82 |
| New South Wales | 3 | 1 | 91 |
| Victoria | 8 | 9 | 79 |

## 4.4 Discussion

This study revealed a moderate level of diversity present within the population of this pathogen. A total diversity level of 15% was observed in the population (Table 5). Genetic analysis, based on Rep-PCR and RAMS-PCR fingerprinting markers determined that not all isolates of this asexual fungus were genetically identical. A large proportion of the population studied, however, was clonal in nature with a large clonal group present in the population. This clonal group comprised 51% of the population isolates. The remaining 49% of isolates belonged to one of 8 other clonal haplotypes or were genetically unique haplotypes (Figure 27). All of the haplotypes present within the population were not correlated to the geographic regions in which the isolates were collected. The large clonal group comprises isolates from all 3 growing regions (QLD, NSW and VIC). This indicates that genetic diversity of *F. oxysporum* f.sp *pisi* is widely and randomly distributed across the major growing regions in eastern Australia. The findings of this part of the diversity study dictate that the null hypothesis that there is no genetic diversity within the pathogen population be rejected and the alternate hypothesis accepted.

From the results of this study, we see that the Victorian sub-population is the most dissimilar or diverse, with a diversity level of 21%. A total of 17 haplotypes are present in the Victorian sub-population. The Queensland population is slightly less diverse, with a diversity level of 18%. Only 7 haplotypes exist within this population. The New South Wales sub-population exhibits the lowest level of diversity at only 9%, with a total of 4 haplotypes (Table 6).

The source of this genetic variation is not known or understood. One possible source of genetic variation within this fungal species is sexual reproduction occurring in field conditions. The sexual structures of the fungus have not been observed although it is probable, due to the high number of unique haplotypes in the population that various strains exist in the field which are of different mating types and are engaging in the sexual state of reproduction. The recombination of genes involved in sexual reproduction creates many new genotypes and allows new and existing virulence genes to be recombined into many different genetic

backgrounds with each sexual generation. This phenomenon may account for the substantial level of genetic diversity observed within the studied population. Although it is possible that the pathogen is reproducing sexually in field conditions growing regions, it is more probable that the parasexual cycle is conferring the high level of diversity upon the population. Parasexuality, or vegetative compatibility, occurs between isolates of fungal species, such as *Fusarium*, when the hyphae of compatible strains of the pathogen fuse and form a stable heterokaryon cell through which genetic material can be transmitted. The entity which emerges following the process of heterokaryosis may differ in its genetic constituents relating to virulence or host range. The extent to which vegetative compatibility occurs within this population is examined in the next chapter of this report.

The diversity within this population may also be the result of genetic introduction into snow pea growing regions in eastern Australia. As snow pea seed is imported (predominantly sourced from the US), it is possible that strains of the pathogen are being introduced via contaminated seed sources. The introduction of strains via importation of seed stock would explain the haplotype and race diversity observed within the pathogen population. It is interesting to note that some snow pea growers in Victoria import seed from New Zealand, as opposed to farmers in other regions who import all seed from the US. The Victorian sub-population exhibited the highest level of genetic diversity, and this may be attributed to the introduction of new strains via seed. Gene flow between growing regions may be the result of other factors such as transport of infected plant material, travel by farmers and labourers and shipment of seed from one region to another. It is suggested that a study commence to compare isolates of this pathogen obtained in Australia to isolates obtained from the United States to determine if the Australian population originated in the United States.

As no region specific genomes exist in the population, disease control strategies can be implemented across all of eastern Australia's snow pea growing regions. Control strategies, however, must be effective against a range of clonal lines and haplotypes in order to successfully control disease outbreak. Control of snow pea would be much less complex if clonal lines were located in specific production regions as single strategies could be implemented to prevent symptoms and spread of the pathogen. As is stands single control measures cannot be implemented in snow pea growing regions, as strategies need to be multi-faceted to ensure that the entire pathogen population will be targeted.

## 4.5 Conclusion

It is evident from the DNA fingerprinting results that there is a moderate level of genetic diversity within the population of *Fusarium oxysporum* f.sp. *pisi* in Australian snow pea growing regions. Despite this level of genetic diversity, a large proportion of the population is clonal. The high number of haplotypes within the population has the potential to make control of snow pea wilt difficult. The genetic variation between regions increases the difficulty in controlling this disease as control measures cannot be successfully implemented to target specific haplotypes. As the only viable control method for this disease is the use of wilt resistant cultivars, the pathogen haplotypes identified in this study can be screened in resistance breeding programs in order to select appropriate resistant snow pea

cultivars. Further work is needed in order to gain an increased knowledge of the population dynamics, as to allow for the development of effective, long term control strategies. It is essential to use the information generated from this population diversity study to develop resistance breeding programs. The implementation of Fusarium wilt resistant cultivars is essential to control this disease in Australia and worldwide.

# 5. Vegetative Compatibility Groups within the Pathogen Population in Australia

## 5.1 Introduction

During asexual reproduction of asexual fungi, hyphal strands may grow and meet in order to fuse and exchange genetic materials in a process known as anastomosis. The fusion of the two fungal hyphae during this process results in the formation of a fusion cell known as the heterokaryon. Heterokaryon formation is an important component of many fungal life cycles and may serve as the first step in the parasexual cycle and the transmission of genetic material. In plant pathogenic fungi, the entity which emerges after the formation of a heterokaryon may differ from its constituents in aggressiveness or host range. In effect, the process of heterokaryosis has the potential to generate genetic variability within a fungal population. Parasexuality or vegetative compatibility between isolates has the potential to render control of disease very difficult due to the creation of new, genetically unique fungal strains. It is difficult to initiate disease control programs in the field where the target pathogen is undergoing genetic variation and constantly changing.

The VCG technique has been used extensively as a tool to classify between pathogenic and non-pathogenic species, classify races and assess genetic homogeneity among fungal populations. VCG may also be used to assess whether isolates in particular VCGs are more associated with one site or geographic region. The method most commonly utilized in determining vegetative compatibility between isolates of *Fusarium* is the direct assessment of heterokaryon formation. Heterokaryon formation, and therefore vegetative compatibility, is indicated by the development of dense aerial growth where mycelia of the two thin nit mutant colonies anastomosed. Once vegetative compatibility between isolates has been established, the groups must be numbered. VCG's within a forma specialis are distinguished by a series of one or two digits which follow the 3-digit numerical code of the forma specialis.

## 5.2 Materials and Methods

### *5.2.1 Isolate Selection*

A total of 35 isolates were selected for VCG analysis. Each unique haplotype was included in the analysis as well as representative isolates from each large clonal haplotype. The isolates used in this study, along with their geographic origin and haplotype number are shown in Table 7.

**Table 7.** Isolates of *F. oxysporum* f.sp. *pisi* used in this study

| Isolate | Region | Haplotype |
|---|---|---|
| 1 | VIC | 1 |
| 3 | VIC | 1 |
| 4 | VIC | 11 |
| 6 | VIC | 2 |
| 10 | VIC | 9 |
| 23 | VIC | 5 |
| 32 | VIC | 15 |
| 70 | VIC | 3 |
| 71 | VIC | 13 |
| 81 | VIC | 4 |
| 84 | VIC | 16 |
| 93 | VIC | 17 |
| 99 | VIC | 10 |
| 107 | VIC | 14 |
| 127 | VIC | 1 |
| 134 | VIC | 1 |
| 148 | VIC | 7 |
| 152 | VIC | 2 |
| 153 | VIC | 8 |
| 178 | VIC | 2 |
| 225 | QLD | 5 |
| 259 | NSW | 4 |
| 262 | NSW | 12 |
| 269 | NSW | 1 |
| 306 | NSW | 1 |
| 317 | NSW | 1 |
| 321 | NSW | 6 |
| 339 | QLD | 6 |
| 398 | QLD | 15 |
| 445 | QLD | 4 |
| 448 | QLD | 15 |
| 457 | QLD | 1 |
| 461 | QLD | 1 |
| 466 | QLD | 1 |
| 468 | QLD | 1 |

### *5.2.2 Generation of Nitrate Non-Utilizing Mutants*

Each isolate was sub-cultured onto plates of complete media and allowed to grow under fluorescent light. Sections of colonies grown on complete media were sub-cultured into 1.5 ml tubes filled with minimal medium amended with potassium chlorate ranging in concentration from 2.0% to 2.5% in order to generate nitrate non-utilising (nit) mutants. Mutants were identified as thin, fast growing sectors on chlorate medium (Figure 27). A total of 10 mutants of each isolate were sub-cultured onto four ‘phenotyping media’; minimal media solutions amended with

1) Hypoxanthine (HX) 2) Ammonium tartrate ($NH_4$) 3) Sodium nitrite 4) Sodium nitrate (minimal media; negative control). The mutants were grown under fluorescent light for 4 days, after which, mutant types (Nit M, *nit* 1 or *nit* 3) were determined based on growth pattern (Table 8). Isolates which grew densely on all four phenotyping media were diagnosed as revertant mutants and were discarded.

**Figure 27.** Mutant of *F. oxysporum* f.sp. *pisi* growing on chlorate medium

**Table 8.** Determination of nit mutant type based on growth on phenotyping media.

| | *Nitrogen Source* | | | |
|---|---|---|---|---|
| *$NO_3$* | *$NH_4$* | *$NO_2$* | *HX* | *Mutant* |
| – | x | x | x | *nit* 1 |
| x | x | x | x | revertant |
| – | x | – | x | n*it* 3 |
| – | x | x | – | Nit M |

x = dense growth
– = sparse growth

### *5.2.3 Pairing of Mutants*

Once phenotyped, *nit* mutants were sub-cultured onto and stored in minimal media slants. After 4 days incubation, these mutants were subcultured onto 60mm petri dishes of minimal media and paired with complimentary mutants. Each isolate used in the VCG analysis was paired with a complimentary mutant of every other isolate used. A typical complimentary pairing is NitM and *nit1* mutants. When a *nit1* mutant is unavailable, a *nit3* is used. *Nit1* and *nit3* pairings often complement poorly and therefore do not give a clear indication of parasexuality between isolates. A total of 630 pairings were completed.

### *5.2.4 Determining Vegetative Compatibility*

Plates were incubated at 25$^{o}$C under fluorescent light for 5 days after which plates were scored for isolate compatibility. Vegetative compatibility was determined by the presence of dense mycelial growth at the junction between the two radial colonies, indicating that the mutants had undergone anastomosis and heterokaryon formation. An example of a compatible pairing, with heterokaryon formation is shown in Figure 28. Isolates were assigned to vegetative compatibility groups based on complementation with other isolates within the population.

**Figure 28.** Vegetatively compatible pairing of isolates. The central line of mycelial growth indicates anastomosis has occurred.

## 5.3 Results

The VCG analysis divided the 35 VCG isolates into 17 groups (Table 9) based on their vegetative compatibility alleles. Ten of the VCG groups contained only one isolate of the pathogen. VCGs 001 and 002 contained 2 isolates, VCGs 003, 004 and 005 contained 3 isolates, VCG 006 contained 4 isolates, and the largest groupVCG 007 contained 8 isolates. The overall VCG diversity was 48.57% as calculated by dividing the number of individual VCG groups by the total number of isolates.

**Table 9.** Classification of isolates according to haplotypes and vegetative compatibility grouping

| *VCG* | *Isolate* | *Haplotype* | *Region* |
|---|---|---|---|
| **OO1** | 1 | 1 | VIC |
| | 3 | 1 | VIC |
| **OO2** | 317 | 1 | NSW |
| | 321 | 6 | NSW |
| **OO3** | 152 | 2 | VIC |
| | 23 | 5 | VIC |
| | 32 | 15 | VIC |
| **OO4** | 178 | 2 | VIC |
| | 134 | 1 | VIC |
| | 153 | 8 | VIC |
| **OO5** | 269 | 1 | NSW |
| | 259 | 4 | NSW |
| | 262 | 12 | NSW |
| **OO6** | 10 | 9 | VIC |
| | 70 | 3 | VIC |
| | 71 | 13 | VIC |
| | 148 | 7 | VIC |
| **OO7** | 461 | 1 | QLD |
| | 93 | 17 | VIC |
| | 445 | 4 | QLD |
| | 225 | 5 | QLD |
| | 468 | 1 | QLD |
| | 448 | 15 | QLD |
| | 457 | 1 | QLD |
| | 466 | 1 | QLD |
| **OO8** | 4 | 11 | VIC |
| **OO9** | 6 | 2 | VIC |
| **O10** | 81 | 4 | VIC |
| **O11** | 84 | 16 | VIC |
| **O12** | 99 | 10 | VIC |
| **O13** | 107 | 14 | VIC |
| **O14** | 127 | 1 | VIC |
| **O15** | 306 | 1 | NSW |
| **O16** | 339 | 6 | QLD |
| **O17** | 398 | 15 | QLD |

As shown in Figure 29, a total of 3 VCGs were present in both QLD and NSW. Of the 3 groups found in QLD, 2 of these were composed of single isolates. Of the 3 groups found in NSW, only 1 was composed of a single isolate. As expected, based on both race typing and population diversity studies, VIC exhibited the highest level of VCG diversity with a total of 12 VCGs found to exist in the sub-population. Of the 12 groups found to exist in VIC, 7 of these are composed of a single isolate.

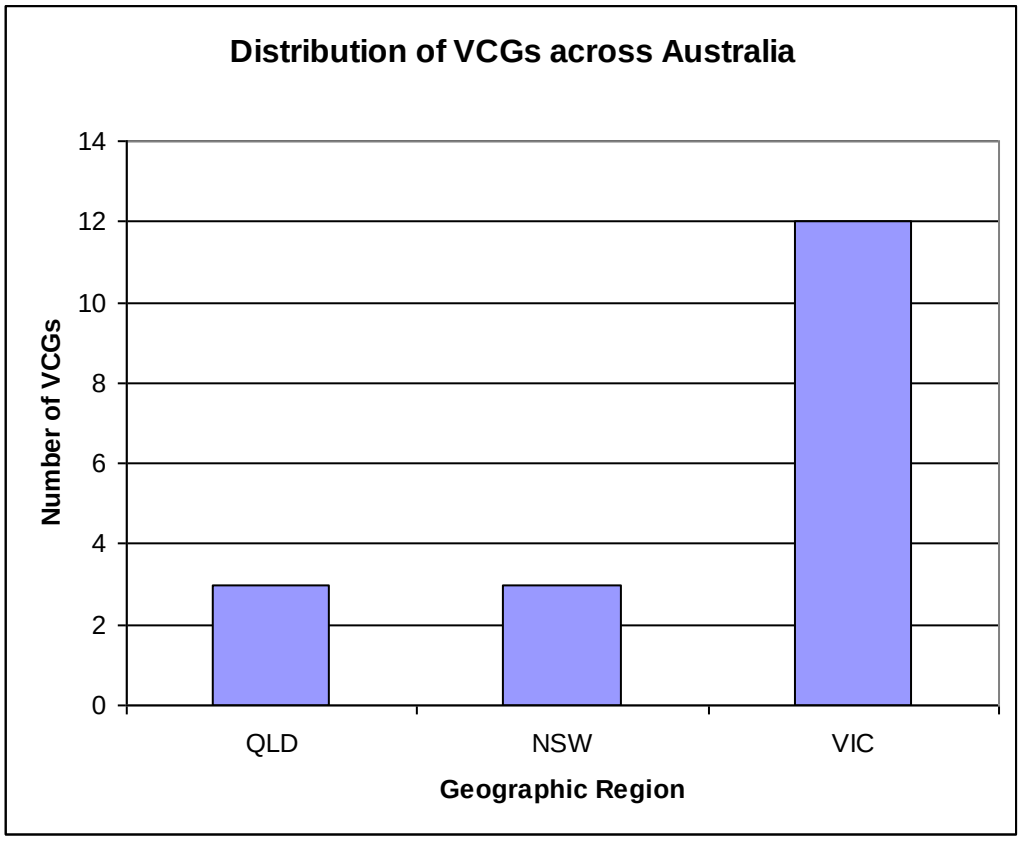


**Figure 29.** Distribution of VCGs across snow pea growing regions in Australia

VCGs of the *F. oxysporum* f.sp. *pisi* isolates did not correlate with genetic haplotypes, however; there does appear to be a correlation between VCG and geographic origin. Each of the VCGs which contain more than 1 isolate, besides 007, correlate to a specific growing region.

## 5,4 Discussion

Vegetative compatibility group analysis found that 17 distinct VCGs exist within the population. VCGs were not correlated with genetic clonality, however a correlation was observed between VCG and geographic region, indicating that vegetative compatibility amongst isolates is restricted to specific snow pea growing regions of Australia.

VCG analysis was chosen to study the diversity present within the population as it provides insight into the mode of reproduction and genetic transfer between pathogen isolates. It has been reported in previous studies that VCGs are often

correlated with clonal lineages, stating that each VCG observed is essentially a genetically isolated population. Although VCGs may be clonally derived, they may also be the chance products of genetic recombination and coincidentally received the same alleles at all of the vegetative incompatibility (vic) loci. A comprehensive vegetative compatibility study conducted by Croft and Jinks (1997) found that fungal strains within a VCG were genetically similar, whereas strains from different VCGs were genetically dissimilar. These researchers also showed that VCGs were not randomly distributed geographically. However, the current study showed that numerous haplotypes were present within VCGs which is similar to the findings of Bentley *et al*. (1998) which stated that forma specialis *cubense* isolates had a high degree of genetic variation within each VCG. It is possible that unique, new haplotypes are being generated within VCGs in the field.

Although it is possible that the pathogen is reproducing sexually in field conditions across snow pea growing regions in Australia, it is more probable that the parasexual cycle is conferring diversity upon the population. The entity which emerges following the process of heterokaryosis may differ in its genetic constituents relating to virulence or host range. Heterokaryosis acts as a means by which normally haploid fungi (such as *Fusarium*) may enjoy the benefits of functional diploidy (14), such as complementation or hybrid vigour. The transfer of genetic materials through this process may be responsible for increased virulence of the pathogen or an increase in resistance to many of the control methods currently employed in snow pea crops, increasing the level of difficulty involved in controlling this devastating disease. It is possible that the haplotype diversity observed in this population may be a result of heterokaryon formation between compatible isolates in the field.

The discovery of 17 VCG groups within Australian snow pea growing regions indicates that there is potential for isolates to undergo the parasexual cycle within field conditions. In both the New South Wales and Queensland sub-populations, there exists only 3 VCGs. The low number of VCGs present in these regions indicates that the potential for parasexuality between isolates is high. It is possible that the haplotype and race diversity within these sub-populations is influenced by the occurrence of parasexuality, and thus the transfer of genetic material, between isolates in these regions. It is interesting to note that the highest level of VCG diversity was found to exist in Victoria. As stated in previous chapters, the Victorian sub-population had the greatest diversity of both races and haplotypes. Typically, a high number of VCGs indicates that the potential for parasexuality is low, as most isolates are not compatible with others in the population.

This geographic isolation of VCGs may be beneficial in the implementation of control strategies, as specific groups of the pathogen can be targeted. The geographic isolation may also indicate that compatibility between isolates is influenced by factors other than clonality such as climatic or topographical factors unique to each region.

## 5.5 Conclusion

This study determined that a high number of VCGs exist within the Australian *F. oxysporum* f.sp. *pisi* population. The existence of these compatible groups within the population indicates that there is potential for the generation of new, genetically unique strains of the pathogen which have the ability to better adapt to environmental variation such as fungicide application and the cultivation of resistant cultivars. The generation of genetically unique strains of this pathogen has the potential to render many control strategies ineffective and can increase the difficulty in successfully managing this disease.

# 6. Grower survey and cultivar evaluation-Queensland

## 6.1 Introduction

Fusarium wilt is a consistent and severe production constraint for snow pea producers in certain growing regions of Queensland (particularly in the Bundaberg district). Snow pea crops suffered severe losses during the 2004 season with individual farms suffering losses of up to 25%. The disease is exacerbated when snow peas are grown frequently on the same site. Fusarium wilt affects the roots and lower stems of plants, plugging the water conducting tissues and causing the plants to yellow from the ground up and eventually to wilt and die prematurely. Four races of this disease have been described in snow peas worldwide (Races 1, 2, 5 & 6). The disease has the potential to be carried in infected seed and can be long lived in the soil making it a difficult task to develop an appropriate crop rotation and disease management program. Grower surveys will therefore help to give a better understanding of the problems faced by growers and how the growers go about trying to minimise the effect of Fusarium wilt on snow peas from year to year.

In terms of management, some cultivars of snow peas have putative resistance to Fusarium wilt, but these need to be assessed against the Australian races of the disease, in our growing environment, to see if they offer potential as a tool in a disease management program and also in terms of market requirements. Seven cultivars were subsequently looked at for their potential to resist Fusarium wilt under Queensland growing conditions.

## 6.2 Materials and Methods

### *6.2.1 Disease survey of growers in the Gympie and Bundaberg regions.*

A grower survey was undertaken in the Bundaberg region only, as the Gympie growers have stopped growing snow peas due to disease pressures and competition in the market place.

Two large snow pea growers were surveyed in the Bundaberg region. One grower grows 220 acres while the other grower will grow up to 200 acres of snow peas during the growing season.

### *6.2.2 Cultivar trials to assess current cultivars and their reaction to Fusarium Wilt.*

This work was carried out on the Gatton Research Station using an isolation area that was artificially inoculated with *Fusarium oxysporum* f.sp. *pisi* (*Fusarium wilt*).

The inoculum used in this trial was grown on sorghum seed that had been sterilised by autoclaving at 121ºC for 20 minutes. The seed was placed into three conical flasks. There was about a 2.5cm layer of seed in the bottom of each flask. *Fusarium wilt* was grown from cultures isolated from infected snow pea plants collected from the Bundaberg region and confirmed as Fusarium wilt by the research team at the University of Sydney. Three plugs of mycelium were placed

into each conical flask and placed in a 25ºC incubator. The flasks were shaken repeatedly to make sure all the seed were colonised with *Fusarium wilt* prior to infesting the soil.

The inoculated seed was placed into furrows prior to planting. The inoculum was covered over with soil and then the snow pea seeds were planted on top of this soil, before the furrow was completely covered over and irrigated using drip irrigation.

Seven snow pea cultivars and one sugar snap cultivar were grown using standard grower practices of irrigation and fertiliser with disease symptoms being recorded during the harvest period.

Two cultivar susceptibility trials were carried out over 2 growing seasons Figure 30. The first trial was planted August 2007 and continuing into November 2007. The second trial was planted late April 2008 and continued through to early September 2008 or until the plants succumbed to the Fusarium wilt.

## 6.3 Results

### *6.2.1 Disease survey of growers in the Gympie and Bundaberg regions.*

*Cultivars grown in this region*

Oregon Giant, Snow Manand Snow Crisp are three of the most common cultivars grown by these 2 growers. Sugar snaps are also grown to a lesser extent and do not seem to get Fusarium wilt as bad as the snow pea cultivars.

*Fusarium wilt incidence*

The growers surveyed observed the Fusarium wilt to be most prevalent during the warmer periods of the growing season. From planting to the end of May and again from September through until the end of October or final harvest were the worst times for Fusarium wilt. However the disease can attack almost any stage of the crop but more often than not from picking onwards.

If the plants are healthy and growing in disease free soil, the grower can pick the crop up to 12-15 times. However, if the plants are grown in a known infected block, the grower may only pick the crop 3-6 times. Once picking has commenced the plants very quickly exhibit disease symptoms and can die within 2 weeks.

Samples of infected plants were taken back to the laboratory for isolation and confirmation of Fusarium wilt. Vascular discolouration was evident in the plants collected in the field with *Fusarium oxysporum* being recovered from these plants when placed onto culture media.

*Fusarium wilt control-current methods.*

Fumigation is the preferred option for control, either Metham or C35 which is chloropicrin. These will also help manage root knot nematodes when there is an issue. Fumigation however only seems to last one season. Methyl bromide was the best option for growers but this product is no longer available. Fumigation

creates a biological vacuum within the soil so that when the Fusarium wilt is reintroduced into the area it colonises the ground quickly, which would account for the short term benefit of fumigation. One of the growers also found that flushing the trickle lines with Molasses had an effect on nematodes populations.

Agrifos/Ridomil was one grower's suggestion as he felt it did slow disease development down. This was not confirmed as both products are not known for their control of Fusarium. These would need to be looked at in a properly replicated trial with a number of other fungicide options in the field.

Rotations are used as part of the farm management program for Fusarium wilt, with snow peas generally following sugar cane.

Typical rotations by these growers may be the following:

snow peas - melons - sweet potato – snow peas

snow peas - tomatoes or capsicum – sorghum – snow peas

The rotation with sweet potato has had far fewer wireworm issues and sweet potato weevil issues than what other growers of sweet potatoes have.

The use of clean planting ground is becoming more and more difficult to find. When snow peas are planted into such ground, Fusarium wilt can be found attacking the snow peas within 2 years. This could be a result of direct movement of contaminate soil on the vehicles and/or equipment used by pickers. The growers did however have an understanding about cleaning down equipment between block but whether this is actually done is not clear. The fact that clean blocks show signs of Fusarium wilt within 2 years would indicate that this practice is not always carried out.

### *6.2.2 Cultivar trials to assess current cultivars and their reaction to Fusarium Wilt.*

Symptom expression was rated as shown in the Table 10. All cultivars exhibited some Fusarium wilt symptoms with a number of cultivars performing better than others. RS 08550820 performed poorly both times. HS 630 appeared as susceptible as RS 08550820 during the second planting. Oregon Giant and Oregon Sugar Pod exhibited only moderate symptom expression during the second planting as did HS 622. As mentioned by the growers, Sugar snap peas only showed mild symptoms of Fusarium wilt during both trial periods. Two HS lines also showed only mild symptoms during both planting periods, HS 618 and HS 623. It is possible that these results could be different if taken onto a grower property where more than one race of Fusarium may be present. The next step should therefore look at grower field sites that are know hot spots for Fusarium wilt with these cultivars and others if available.

**Figure 30.** Snow pea varietal trial at the Gatton research Station 2007**.**

**Table 10.** Disease symptom expression of snow pea lines to *Fusarium* wilt.

| **Cultivars** | **October 2007** | **August 2008** |
|---|---|---|
| | ***Fusarium oxysporum*** | |
| **HS 618** | + | + |
| **HS 622** | + | ++ |
| **HS 623** | + | + |
| **HS 630** | + | +++ |
| **RS 08550820** | +++ | +++ |
| **55505631 Oregon giant** | + | ++ |
| **35505626 Oregon sugar pod** | + | ++ |
| **Sugar Snap** | + | + |

+ = slight symptom expression; ++ = moderate symptom expression;
+++ = severe symptom expression

## 6.4 Discussion

Clearly all cultivars exhibit some susceptibility to Fusarium wilt, some more than others. Resistance breeding is one area that needs further work undertaken in any future research and should be undertaken in Australia under Australian conditions and with Australian races of Fusarium wilt. This would be the only reliable way of finding suitable cultivars that would exhibit tolerance or resistance to this devastating wilt disease.

# 7. Greenhouse trial to examine the reaction of the snow pea cultivar Oregon Giant with different isolates of Fusarium wilt.

## 7.1 Introduction

As races of Fusarium wilt became available after the survey and race typing, the effect on the most commonly grown snow pea cultivar Oregon Giant was examined in a greenhouse trial. The aim of the trial was to give an indication on the pathogenicity and variation in the pathogenicity across the different isolates and races on Oregon Giant. It also compared the isolates collected from different growing regions under the same controlled conditions.

## 7.2 Materials and Methods

Pots (100mm) were filled with vermiculite and seeds of Oregon Giant were sown (5 per pot). Each of seven isolates (Table 11) was grown on Carnation Leaf Agar (CLA) for two weeks. At the time of the trial no Race 1 isolates were available. Two weeks after planting the cultures were added to each pot. There were five replicate pots per isolate. One CLA plate was added to each pot by removing the seedlings, cutting 1cm sections from the end of the root mass, and then placing the culture underneath before replacing the plants back into the pot. Pots were placed in a greenhouse at 18/28°C. 30 days after sowing pots were examined for symptoms including the number of wilt affected plants. Results were analysed using Genstat 11$^{th}$ Edition Analysis of Variance.

## 7.3 Results

Plants were infected by the various isolates of Fusarium wilt, consisting of race 2 and race 5, displayed similar symptoms as compared to the non-inoculated controls (Table 11, Figure 31). Symptoms observed included yellowing and death of plants. The average number of affected plants ranged from two to four and were significantly different to the controls ($P<0.001$). Isolates 2, 309 and 32 had significantly less affected plants than 279. Overall those isolates belonging to race 5 had more symptoms than those of race 2.

**Table 11.** The number of affected plants and dry weights of Oregon Giant and Fusarium wilt isolates.

| Isolate | Race | Source | Affected (Plants)* |
|---|---|---|---|
| **279** | 5 | Vic | 4.2a |
| **313** | 5 | Vic | 3.4ab |
| **106** | 5 | Vic | 3.0abc |
| **138** | 2 | NSW | 3.0abc |
| **32** | 5 | NSW | 2.8bc |
| **309** | 2 | NSW | 2.4bc |
| **2** | 2 | NSW | 1.8bc |
| **Control** | N/A | N/A | 0.0c |

*Values with the same letter not significantly different at P<.001 using Genstat 12$^{th}$ Edition ANOVA

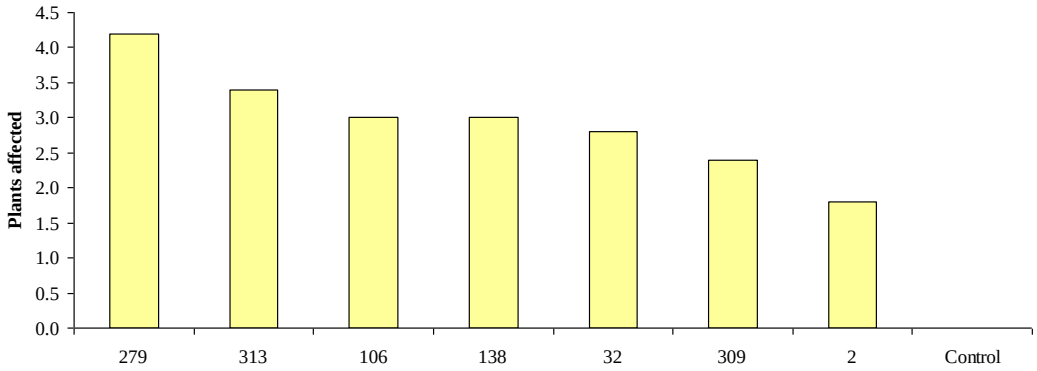


**Figure 31.** The affect of various isolates of Fusarium wilt on the snow pea cultivar Oregon Giant.

## 7.4 Discussion

Oregon Giant (Snow Man is the same cultivar but sold by a another seed company) is the most popular cultivar grown in Australia. Oregon Giant was affected similarly after inoculation with different races of Fusarium wilt. There was a tendency for those isolates from Victoria to be more severe than those from NSW and it appeared that race 5 isolates were more aggressive than the race 2 isolates. There is a high priority to develop a resistant cultivar of snow pea to reduce the impact of Fusarium wilt on the industry. As identified previously, with the predominance of race 1 in Victoria and New South Wales it is recommended that a race 1 resistant cultivar of snow pea is introduced to these regions. However as race 5 is dominant in Queensland, resistance to this is also desirable.

# 8. The effects of Fusarium wilt races on different cultivars of snow peas.

## 8.1 Introduction

As determined in the field trial in Queensland no cultivars were found to have any tolerance to one race of Fusarium wilt. As race typing had identified four races of the Fusarium wilt pathogen in Australia, a greenhouse trial was undertaken to establish if other races would similarly affect different cultivars. Seed companies were approached to supply any new cultivars that may show some tolerance or resistance to Fusarium wilt.

## 8.2 Materials and Methods.

Six cultivars of snow peas were examined for their reaction to combined inoculum of different races of Fusarium wilt. In this trial Race 1 (Isolate 68) and Race 6 (12) were combined and applied to each pot and similarly with Race 2 (97) and Race 5 (32). Three carnation leaf agar plates of each were grown under lights. Snow peas were sown (five per pot) into 100 mm pots containing potting mix. Four days after germination, potting mix was removed from around the plants and roots were slightly damaged with a scalpel. The combined cultures were mixed with sterile water (100ml) and 5 ml of this mixture placed around the damage roots. Pots where the roots were damaged but without any addition of Fusarium wilt were used for comparison, each treatment was replicated four times.

Plants were maintained in the greenhouse at 23/27°C for a further 5 weeks where plants were assessed for plant survival.

## 8.3 Results

All cultivars were affected by all the isolates (Figure 32). On statistical analysis those treated with the combined races of Fusarium wilt had a significantly higher number of affected plants (2.62 for 1/6 mixture and 2.58 for 2/5 mixture) compared to the control pots (0) at $P<.001$ (Genstat 11$^{th}$ Edition ANOVA). There was no significant difference between cultivars and races.

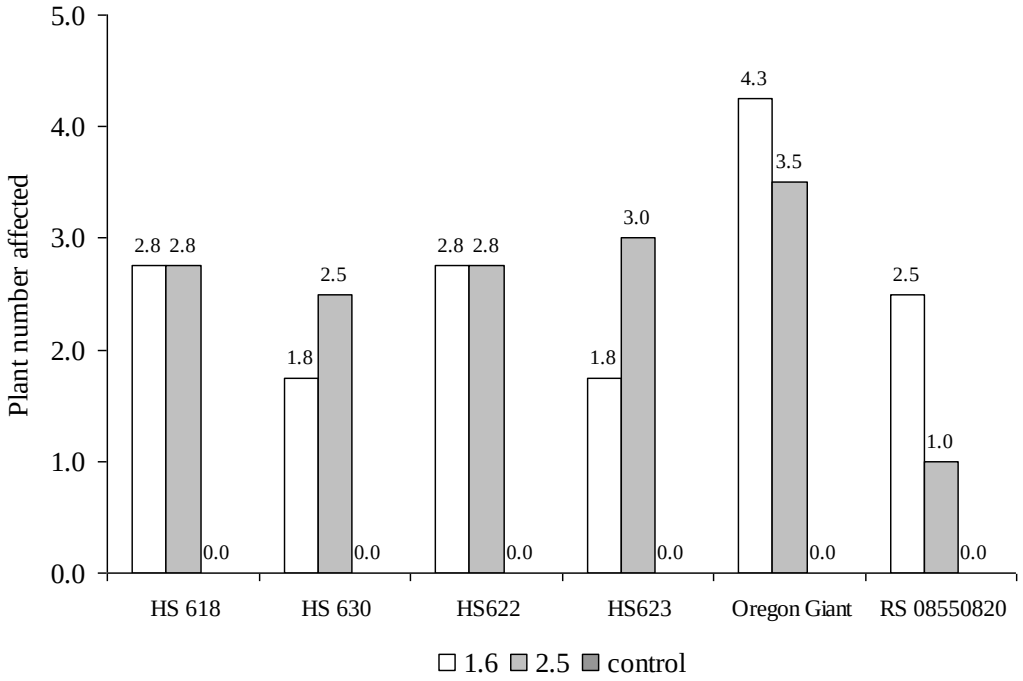


**Figure 32**. The effect of combined races on different cultivars of snow peas.

## 8.4 Discussion

There was no cultivar resistance to the races used in this trial. The cultivars used were supplied by seed companies, but their resistance to Fusarium wilt was not known. They were supplied with the possibility that they may have had some resistance. Where the different cultivars were grown to produce pods, the quality of pods of all the cultivars was less superior to Oregon Giant. The industry continues to favour Oregon Giant and therefore any breeding program must develop a cultivar equivalent in pod quality to Oregon Giant but with Fusarium wilt resistance. This assessment for tolerance to Fusarium wilt needs to be considered with any new cultivars that are released. When there is an introduction of new cultivars they should be screened for Fusarium resistance.

# 9. The effect of temperature on Fusarium wilt disease expression and progress in snow peas.

## 9.1 Introduction

Factors such as temperature, light, soil moisture and nutrition have been proposed as contributing to the expression of wilt symptoms (Beckman 1987). The success of resistant cultivars needs this information. For example a cultivar of chickpea was found to be moderately resistant to *F. oxysporum* f.sp. *ciceris* when inoculated plants were maintained at 21/24°C but were highly susceptible to the pathogen at 25/27°C (Landa *et al.* 2006). With Fusarium wilt of tomatoes it was found disease developed at temperatures from 20-34°C but was most rapid and severe at 27°C (Clayton 1923). *Fusarium oxysporum* f.sp. *pisi* has been found in a number of states in Australia, collecting information on the temperature preferences for Fusarium wilt would provide valuable information on the best planting times to reduce disease impact.

Eight Australian races of Fusarium wilt that were collected from the race study were used to compare different temperatures and the effect on disease expression.

## 9.2 Materials and Methods

Oregon giant seed was sown into single cells seedling trays filled with potting mix and maintained at 25°C till they were ready for transplanting and infecting. The method was similar to that carried out by Haglund (1989). Cultures of the isolates were grown on carnation leaf agar (CLA) (9 plates per isolate). After eight days the plants were carefully removed from the cells and transplanted into pots (5 per pot). At the time of transplanting 1cm was cut from the roots. Potting mix was placed in a 100mm pot onto which a layer of sphagnum moss was placed. 100mL of sterile water was added to the combined 9 CLA plates and 30mL (per pot) of this mixture was placed on the sphagnum moss. The seedlings (5) were then placed into the pots.

Pots were then placed in greenhouses maintained at 15°C, 20°C and 25°C. Pots were watered and symptoms were observed to identify the speed of development. After 28 days pots were removed from the greenhouses, plants were taken out of the pots and examined for vascular discoloration or total death. Plants stems were cut opened and examined for browning. The number of plants that were visibly dead was recorded. Data was analysed by Analysis of Variance using Genstat 11$^{th}$ Edition.

## 9.3 Results

Symptoms were first seen in the greenhouse whose temperature was 25°C, 11 days after inoculation (Isolates 217 and 225) and after 13 days in the 20°C greenhouse (Isolates 217 and 225). Symptoms were first seen in the 15°C at 16 days after inoculation where one isolate showed symptoms (Figure 33, Figure 34, Figure 35, Figure 36 and Figure 37).

No symptoms developed on any further isolates at any of the temperatures. The symptoms appeared as either dead plants or others with chlorosis and with

vascular discoloration (Table 12). Vascular discoloration was checked by cutting stems of plants longitudinally in half at and above the crown of the plant. Dead plants were significantly different across different temperatures for example between 15°C and 25°C but not between 15°C and 20°C and 20°C and 25°C. There was a temperature and isolate significant difference for vascular discoloration.

**Table 12.** The mean number of dead plants and those with vascular discoloration.

| Isolate | State | With Vascular Discoloration[1] | | | No. of Dead Plants[2] | | |
|---|---|---|---|---|---|---|---|
| | | 15°C | 20°C | 25°C | 15°C | 20°C | 25°C |
| **1** | Vic | 0a | 0a | 0a | 0 | 0 | 0 |
| **68** | Vic | 0a | 0a | 0a | 0 | 0 | 0 |
| **106** | Vic | 0a | 0a | 0a | 0 | 0 | 0 |
| **309** | NSW | 0a | 0a | 0a | 0 | 0 | 0 |
| **323** | NSW | 0a | 0a | 0a | 0 | 0 | 0 |
| **351** | NSW | 0a | 0a | 0a | 0 | 0 | 0 |
| **217** | Qld | 0.67b | 0.67b | 2.0c | 0 | 0.33 | 1 |
| **225** | Qld | 0a | 1.67c | 2.7d | 0 | 1.33 | 1.67 |
| **Control** | | 0a | 0a | 0a | 0 | 0 | 0 |

[1] Values with the same letter significant different at <0.01.

[2] Not significantly different, between isolates and temperature, but overall between temperatures.

**Figure 33** Isolate 225 at 25°C Plants in pots with variable symptoms i.e some dead others still green. Pattern similar to in the field.

**Figure 34.** Isolate 217 at 25°C. Symptoms similar to Race 225 at this temperature, with plants showing chlorotic leaves.

**Figure 35.** Isolate 225 at 20°C. Less severe symptoms than at 25°C.

**Figure 36.** Isolate 217 at 20°C.

**Figure 37.** Isolate 217 at 15°C. Minimal symptoms at this temperature.

## 9.4 Discussion

The higher temperature favoured Fusarium wilt disease expression with a small number of the isolates trialled. The symptoms were clearly similar to field symptoms with yellowing of leaves and plant death, vascular discoloration was also observed. This information although not conclusive for all races, identifies that planting in the warmer periods of the season, especially for race 2 and 6, will increase the rate of infection and severity of disease although of course the pot trials did not progress for the same period as in the field. This information confirms other studies with Fusarium wilt (Landa *et al.* 2006). Grower correspondence has indicated that they also have observed this effect in the field and delay planting later in the year (in Queensland).

Unfortunately many of the isolates tested did not produce symptoms on snow pea plants. This can partly be explained by the variability of conditions including inoculation procedures, timing of inoculation and environmental conditions may produce unsuccessful results (Kraft 1994). Also fungal cultures may lose pathogenicity in storage. All plants may have been colonised by the fungi but did not show any visible symptoms in the time period allowed using pots.

# 10. Evaluation of seed dressings to control Fusarium wilt.

## 10.1 Introduction

Seed dressings of various fungicides are commonly used across different crops to prevent pre-emergence and post-emergence damping off. Currently snow pea seed is treated with either of the fungicides Captan, Thiram or Apron XL®/Maxim® (which is metalaxyl-M and fludioxonil) or Alliette®. Alliette® is used as a seed dressing in peas to prevent downy mildew. Seed dressing selection appears to depend on the supplier of the seed. Damping off is the term used to describe the interruption of plant development at the seedling stage usually by soil borne fungi. The infection results in weakened or often dead plants. The fungi involved are usually those that have been referred to before such as *Pythium*, *Fusarium* or *Rhizoctonia*. This trial was established to evaluate seed dressings of various active ingredients on the effect of Fusarium wilt of snow pea.

## 10.2 Materials and method.

### *10.2.3 Trial 1*

Seed dressings (only codes used for some products) and active ingredients are listed in Table 13. These seed dressings are currently used in various crops however apart from Captan and Thiram, none have registration for vegetables. As Bion Plant Activator® has a registration in cotton for Fusarium wilt (suppression only) it was also trialled. Seed treatments (except for Bion Plant Activator®) were washed to remove seed dressing, dried and then the particular seed dressing was added to the seed and allowed to air dry. At assessment the survival of plants were examined. Oregon Giant seed as supplied by a seed company was used; this seed dressing was Apron XL/Maxim which includes two active ingredients (metalaxyl–m and fludioxonil). The Bion Plant Activator® treatment was added to the same seed.

A soil collected from a pea farm from Victoria known to have Fusarium wilt was used. Five seeds of each treatment were sown into each pot (three pots of each treatment) and after four weeks the number of plants remaining was counted and any disease symptoms noted. Roots were not damaged to induce disease as carried out in previous trials.

### *10.2.4 Trial 2*

The bean seed Oregon Giant that was used in trial 1 and treated similarly with Bion Plant Activator® were sown into seedling cells. A culture of the isolate 217 was grown on carnation leaf agar (CLA) (9 plates per isolate). After eight days the plants were carefully removed from the cells and transplanted into pots (5 per pot). At the time of transplanting 1cm was cut from the roots. Potting mix was placed in a 100mm pot onto which a layer of sphagnum moss was placed. 100mL of sterile water was added to the combined 9 CLA plates and 30mL (per pot) of this mixture was placed onto the sphagnum moss. The seedlings (5) were then placed into the pots. Treatments (4) included, Bion Plant Activator® treated seed plus isolate 217, Oregon Giant plus isolate 217, Bion Plant Activator® treated seed only and Oregon Giant seed only. There were five replicates per treatment.

After four weeks plants were rated for disease symptoms from 0-5 where zero was disease free and five was most affected. Data was analysed using Genstat 11th Edition.

**Table 13.** The details of fungicide seed dressings, active ingredients, rates and the target organisms.

| Seed treatment Code | Active ingredient(ai) | Concentration of ai | Product rate/100kg seed | Active ingredient concentration g/100 kg seed | Target Organisms |
|---|---|---|---|---|---|
| Captan | captan | 800 g/kg | 1 kg | 800 | *Pythium* and *Rhizoctonia* |
| Thiram | thiram | 800g/kg | 1 kg | 800 | *Pythium* and *Fusarium* |
| A | azoxystrobin | 100 g/L | 50 ml | 5 | Broad spectrum |
| F | fludioxonil | 100 g/L | 50 ml | 5 | *Fusarium*, *Penicillium* |
| FM | fludioxonil + metalaxyl-M | 25 g/L + 10 g/L | 150 ml | 3.75 + 1.5 | *Rhizoctonia*, *Fusarium* and *Pythium* |
| MF | metalaxyl-M + fludioxonil | 37.5 g/L + 25 g/L | 150 ml | 5.625 + 3.75 | *Pythium* and *Rhizoctonia* |
| DM | difenconazole + metalaxyl-M | 92 g/L + 23 g/L | 130 ml | 11.96 + 2.99 | *Rhizoctonia* and *Pythium* |
| AFM | azoxystrobin + fludioxonil + metalaxyl-M | 75 g/L + 12.5 g/L + 37.5 g/L | 100 ml | 7.5 + 1.25 + 3.75 | *Pythium* and *Rhizoctonia* |
| Bion Plant Activator® | Acibenzolar-s-methyl | 500g/L | 0.006g | 0.6g | *Fusarium* |
| Seed company | metalaxyl-m + fludioxonil | 350g/L +100g/L | ? | ? | *Pythium* and *Fusarium* |

## 10.3 Results

### *10.3.1 Trial 1*

Disease symptoms on snow peas were severe, they including severe wilting and death of seedlings (Figure 38). Not all plants were infected in the pots. Infected plants were examined and vascular browning was evident on infected plants. Therefore Fusarium wilt was considered to be the main cause of these symptoms. The symptoms were different compared to those seen when plants were artificially infected. Seed dressings were similar but those that had fewer affected plants included the AFM, seed company and seed company plus Bion Plant

Activator® (Table 13). There was however no significant difference with an analysis of variance.

### *10.3.2 Trial 2*

Bion Plant Activator® did reduce disease symptoms in the greenhouse trial in pots. Symptoms were typical, with yellowing and wilting (Figure 40). Bion Plant Activator® reduced the effects of Fusarium wilt by 50% as seen in Figure 41. The product had no effect on the germination and growth of snow peas as can happen if the rate used is incorrect.

**Figure 38.** Wilting of plants caused by severe symptoms of Fusarium wilt.

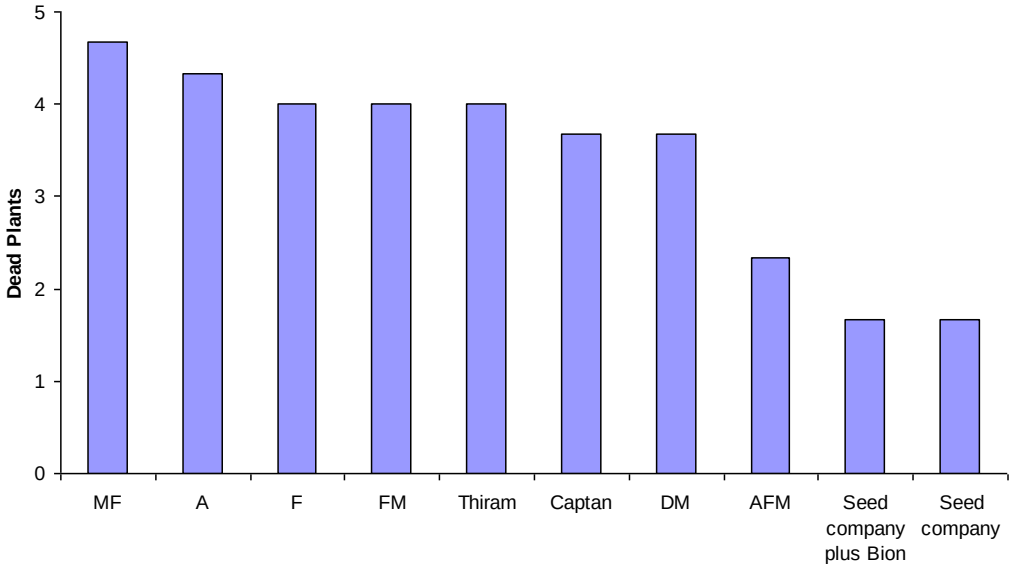


**Figure 39.** The effect of seed dressings on disease levels in soil known to contain Fusarium wilt.

**Figure 40.** Symptoms from left to right, Oregon giant +Bion Plant Activator® + Isolate 217, Oregon giant + Isolate 217, Oregon giant + Bion Plant Activator® and Oregon giant.

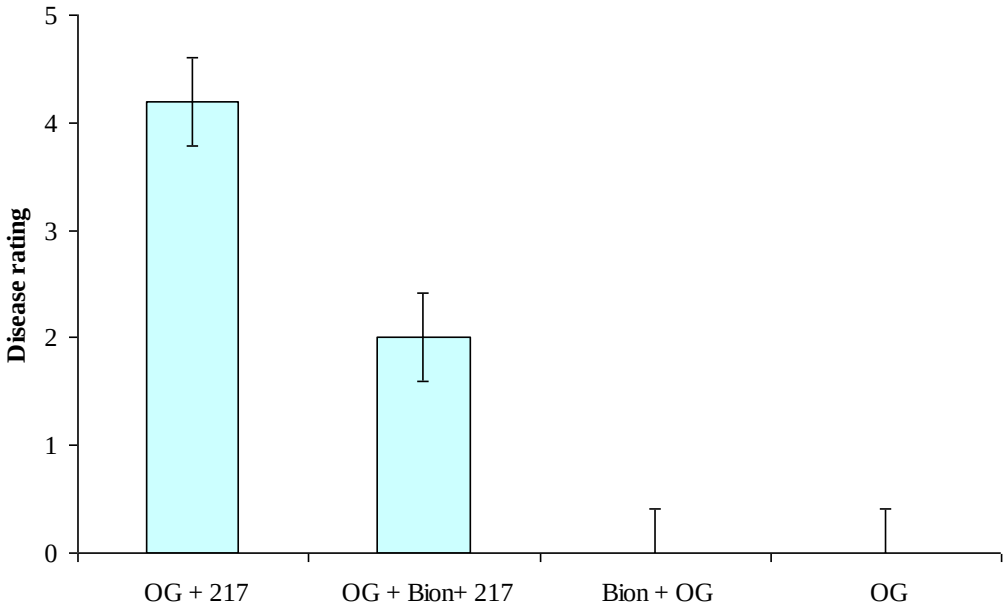


**Figure 41**. The effect of using Bion Plant Activator® on Fusarium wilt.

## 10.4 Discussion.

The seed dressings had some effect on Fusarium wilt in this study with the seed company seed dressing reducing the affects of this disease. In trial 1 the product F contained similar quantities of fludioxinil but did not have the same effect as the seed company seed treatment. Their product contained high rates of metalaxyl-M and fludioxinil, so these in combination were superior in controlling this wilt. Metalaxyl-M has activity towards *Pythium*, a fungus which can cause root rotting and damping off. Although no other products apart from the seed company seed dressing had any affect on disease levels it is possible that *Pythium* may have

contributed to disease symptoms. It is possible fungi such as *Pythium* and *Fusarium* may act in a disease complex.

There was no additional affect of Bion Plant Activator® after adding it to the already treated seed. This soil contained a severe form of Fusarium wilt which caused severe wilting in young plants, therefore seed dressings especially those that contain high rates of fludioxonil may assist in reducing disease levels.

In Trial 2 the there was a positive effect of Bion Plant Activator® on disease levels in artificially infected pots, even though the plants roots had been damaged. Various rates and formulations of this product should be examined further.

There are many seed dressings currently used in field crops that should be registered for use on vegetables in Australia. Seed examined during the project had various seed dressings depending on the seed company. Some seed was treated with Alliette® which will provide protection against Downy Mildew but will not have any affect on *Fusarium*.

As seed dressings only provide some disease control for a limited period early in the crops growth, their use would be part of a management strategy. These trials should be repeated in the greenhouse and in the field with known infected grower soil.

# 11. Integrated management options for Fusarium Wilt of Snow peas.

Control of Fusarium wilt is difficult once established in a field. The first stage of any disease control programme involves the examination of the disease and host interaction. This project has achieved this through identifying the causal organism and the spread of the disease within Australian snow pea growing regions.

Fusarium wilt must be detected in an infected crop especially when disease symptoms are not widespread. An infected crop with minimal symptoms will provide inoculum for rapid build up of disease in subsequent cops and decisions must be made on moving from an infected block to new ground. If any symptoms are noticed that include yellowing of leaves, loss of plant vigour and reduced survival of plants when pods have developed, then correct identification of the cause of these symptoms is critical. They could be related to water or nutritional stresses but they could be the first signs of Fusarium wilt. Check for vascular discoloration by slicing the stem of an infected plant longitudinally at ground level and examine for a reddening inside the stem. Send plants with symptoms to a plant disease diagnostic laboratory for confirmation.

Fusarium wilt is a soil borne fungus that can rapidly build up in soil and can survive for many years. It has been shown to exist in the Australian environment as different races and therefore this must be considered in future research and disease management planning.

The best control option for this disease is resistant cultivars, but these are currently not available. The main control options for growers in Australia include, soil fumigation, avoidance of infected fields, planting in cooler months of the year, adopting a strict hygiene plan and growing non susceptible crops.

Fumigation has been found to be successful but may not be economically viable in some situations. Fumigation is currently carried out by some growers where the main products used include metham or chloropicrin. Fumigation will also control weeds, insects and nematodes. However fumigation may only be effective for one season. Fumigation kills many beneficial antagonistic organisms in the soil and if Fusarium wilt is reintroduced into that soil it can colonise the substrate rapidly with limited competition from other soil borne organisms and can contribute to the short term benefit of fumigation.

Seed, although is suspected of carrying Fusarium wilt is not considered likely. Seed is selected by seed companies from crops grown in areas free of disease and monitored for disease during the crops growth. Seed quality is therefore assured. Seed transmission if possible, will only occur if growers keep seed from known infected plants.

It appears through work in this project that warmer temperatures favour the disease; therefore planting in cooler temperatures will delay the disease symptoms especially with races 2 and 5. Unfortunately whether this is the same for races 1 and 6 cannot be confirmed. In Queensland where races 2 and 5 are more common, this temperature effect needs to be considered in relation to planting time.

Management options include maintaining a farm hygiene policy, especially when moving from infected ground to a clean property or block. Key points of this plan would include restricting soil movement by any means from one property to the next. The fungus is easily transferred in soil.

Restrict the movement of machinery such as tractors and wash them before going from an infected block to a clean block. Snow peas are hand picked so even pickers will need to clean boots and wash them in a disinfectant before moving to a clean block.

Snow peas require a great deal of infrastructure for trellising such as posts and wires. This trellising is moved from block to block, but would be capable of moving the Fusarium wilt pathogen with it. Soil on posts, plant material and *Fusarium* spores on wires, all would provide inoculum when moved from an infected block to uninfected. Reducing this source of Fusarium wilt inoculum would be very difficult. The only method to "clean" this material would be to clean of soil and plant debris and/or dip in disinfectant type products which are available for such purposes. This however would be a huge task.

Valuable information is available to growers for assistance with planning farm hygiene with the publication "Farm hygiene for vegetable blocks" which is a note published by The Queensland Department of Primary Industries and is available at http://www2.dpi.qld.gov.au/horticulture/4753.html

Fusarium wilt of snow peas is specific to a host crop; therefore disease will not occur on a crop not belonging to the pea family. However the fungus will survive in the soil and if peas are reintroduced then wilt infection may occur. Growers that have Fusarium wilt may need to change to a different crop to break the disease build up. Reducing the frequency of snow peas grown in the same block will reduce disease levels.

During the course of this project a number of cases have been reported of rapid build up of Fusarium wilt in snow peas grown in land that had previously not recorded the disease. The disease can build up rapidly as inoculum is spread by the movement of infected soil. As seen in this report symptoms in plants may not be noticeable when the inoculum level is at low levels. However with the movement of soil, vehicles, machinery and people through an infected block Fusarium wilt propagules can build up in the soil to a level that will result in increased infection.

# 12. Discussion

Snow peas are an important and popular vegetable for Australian growers and consumers. However snow pea production within Australia has been declining because of cheaper imported product. Snow peas have to be hand picked and labour costs are high compared to other countries such Zimbabwe, China and Kenya.

The most popular cultivar grown commercially is Oregon Giant, also marketed as Snow Man. It has a high yield of long straight pods and has powdery mildew resistance. The market favours this cultivar because of its sweet tasting pods.

Fusarium wilt caused by *F. oxysporum* f.sp. *pisi* has also affected local production by causing disease outbreaks in all states where snow peas are grown. It is a soil borne pathogen that can rapidly build up and can survive for many years. Symptoms caused by the disease include leaf chlorosis (yellowing), wilting and death of plants either early or after picking has been commenced. When the stems of plants are cut longitudinally a reddish discoloration is often observed. Infected pea plants are often found to develop pods despite infection; however these pods are often dry, stunted and shrivelled, thus rendering them unsuitable for harvest and sale.

This projects main aim was to establish a profile through genetic analysis of the Fusarium wilt pathogen and its interaction with the snow pea plant. This was achieved through detailed surveys, isolate collection, race typing, population studies of the pathogen and cultivar evaluation. Disease management options based on current literature are limited and resistant cultivars are the main and best method for controlling wilt diseases. Bion Plant Activator® (active ingredient is Acibenzolar-S-Methyl) as well as seed dressings were examined for the suppression of Fusarium wilt. A management plan was developed to assist growers with best options for controlling Fusarium wilt of snow peas currently.

The results of the field survey indicated that the snow pea wilt pathogen, *F. oxysporum* f.sp. *pisi* is widespread across Australia's snow pea and pea growing regions. These findings were expected, as Fusarium wilt is the most significant disease in pea and snow pea crops worldwide after Aphanomyces root rot, and is present in all pea growing regions worldwide. The field survey data also showed that there is variation in the pattern of field symptoms across growing regions in Australia. These variations may be due to regional factors such as climate and soil, pathogenic race variation or genetic differences within the pathogen population.

The isolates collected in the survey were subsequently used to determine the races of the snow pea wilt pathogen, *Fusarium oxysporum* f.sp. *pisi*, present in the main snow pea growing regions in Australia. A population of 140 isolates was selected for inclusion in this study and 4 races were found to exist within the population. The 4 races found (Races 1, 2, 5 and 6) are considered to be the only races of economic significance worldwide. Race 1 is the predominant race in the population and is responsible for the majority of wilt disease in Victoria and New South Wales. Race 5 is responsible for the majority of wilt disease in Queensland. The level of race diversity found to exist within the population may have

implications for the development of disease control measures. As the only viable control method for Fusarium wilt diseases is the use of resistant cultivars, race-specific resistant cultivars need to be developed and introduced into the major snow pea growing regions in order to reduce disease incidence. A Race 1 resistant cultivar would be most effective at reducing disease across Australian growing regions.

The genetic diversity and population structuring of *Fusarium oxysporum* f.sp. *pisi* obtained from the main growing regions in Australia was studied. A total of 140 isolates were analysed using molecular markers based on 6 primer sets. The results of the study showed that a total of 9 clonal haplotypes and 8 unique haplotypes exist within this population, indicating a moderate level of genetic diversity. This level of genetic diversity has the potential to make disease control difficult.

It is evident from the DNA fingerprinting results, that there is a moderate level of genetic diversity within the population of *Fusarium oxysporum* f.sp. *pisi* in Australian snow pea growing regions. Despite this level of genetic diversity, a large proportion of the population is clonal. The high number of haplotypes within the population has the potential to make control of snow pea wilt difficult. The genetic variation between regions increases the difficulty in controlling this disease as control measures cannot be successfully implemented to target specific haplotypes. As the only viable control method for this disease is the use of wilt resistant cultivars, the pathogen haplotypes identified in this study can be screened in resistance breeding programs in order to select appropriate resistant snow pea cultivars. Further work is needed in order to gain an increased knowledge of the population dynamics, as to allow for the development of effective, long term control strategies. It is essential to use the information generated from this population diversity study to develop resistance breeding programs. The implementation of Fusarium wilt resistant cultivars is essential to control this disease in Australia and worldwide.

A study during the period of the project was conducted to determine the number of vegetative compatibility groups (VCGs) which exist within the *F. oxysporum* f.sp. *pisi* population in snow pea growing regions in Australia. It was found that 17 VCGs exist within the population, 12 of these consisting of single isolates. The existence of these VCGs within the population indicates the potential for isolates to undergo parasexuality within the field which may result in the generation of new, possibly more virulent strains of the pathogen.

There was no resistance in cultivars tested with races that were found from the disease survey and race typing, but there was some indication that fungicides as seed dressings may reduce symptoms. Bion Plant Activator® reduced symptoms on snow pea plants early and should be considered for further field trial work, both as a seed dressing and soil drench. Other types of plant activators should also be trialled and compared. From the research in this project it was identified that planting snow peas when soil temperatures are too warm favours disease development. Temperature had an influence on the progress of disease and this should be considered in future crop management. Soil and crop refuse management is critical to reduce the spread of the disease within and between

farms. Trellising is also considered a potential source of infection as it can transport infected soil (posts) and wires (spores and infected plant material).

A great deal of screening of disease was achieved in this project. It would be timely for this screening experience to be developed further into a routine assessment for wilt with any new cultivars that may be introduced to Australia.

## Recommendations.

- Breeding cultivars for resistance or tolerance to Fusarium wilt should be considered a priority.

- Overseas experience should be accessed through an examination of snow pea growing areas, the current overseas experience with Fusarium wilt and an assessment of potential resistant cultivars.

- Based on the experience with working with Fusarium wilt of snow peas in this project including the availability of a number of isolates and races, a routine screening protocol needs to be developed.

- Conduct further greenhouse and field trials on SAR (systemic acquired resistance) and GRAS (generally regarded as safe) eg. Bion Plant Activator®, Milsana, Actiguard and potassium silicate.

- Biofumigation and alternate crops should be examined to assist with disease control.

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# Primefact -Management options for Fusarium wilt of snow pea

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Fusarium wilt of snow peas (Figures 1 and 2) is a devastating disease that has appeared in all snow pea growing regions in the eastern states of Australia. The causal organism of the disease is *Fusarium oxysporum* f.sp. *pisi* (Fop). All pea types are affected by the disease.

It is a soil-borne pathogen that can rapidly build up in soil and can survive for many years. Symptoms caused by the disease include wilting and death of plants either early or after picking has commenced. When the stems of plants are cut longitudinally a reddish discoloration is often observed (Figure 3).

The disease can attack snow pea plants at any stage, but in Queensland growing regions it appears to be worse during hotter periods of the growing season, especially after harvesting has started. In Victoria it has been observed attacking plants at a very young stage.

Early infections may cause plant death; late infections reduce the number of harvests. One plant may be affected next to a plant that is not affected.

## The Fusarium wilt fungus

*Fusarium oxysporum* is a soil-borne pathogen that is commonly found in soil and plants. In relation to wilt diseases caused by this fungus only specific types of *Fusarium oxysporum* affect certain crops. Fop only affects peas. Other wilt fungi are also specific, for example Fusarium wilt of cucumber is caused by *F. oxysporum* f.sp. *cucumerinum* and
*F. oxysporum* f.sp. *cubense* causes Fusarium wilt of cantaloupe.

Fusarium wilt invades roots of healthy pea plants and then gradually colonises the vascular tissue. The infection of the roots may be associated with damage to the roots by other fungi or by nematodes.

The growth of the fungus in the water conducting tissue contributes to the wilting of the plant. The fungus then quickly invades the remainder of the plant and produces huge numbers of spores (reproductive structures) which infect other plant material.

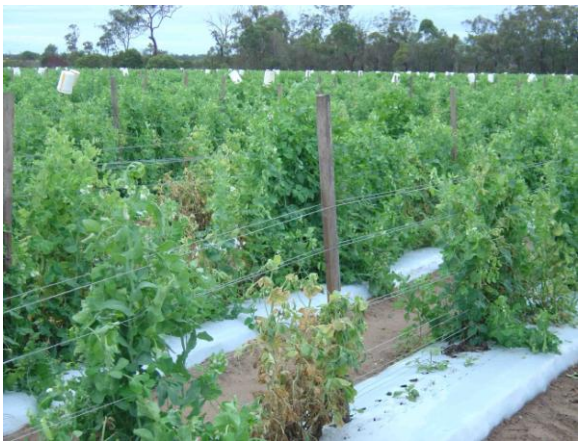

**Figure 1. Plant in foreground exhibiting wilt symptoms.**

**Figure 2. Close-up of infected plant.**

**Figure 3. Brown discoloration exhibited in affected plants.**

Different races of *Fusarium oxysporum* f.sp. *pisi* also exist that may show different reactions on different cultivars. The expression of disease may be different from one cultivar to the next.

## Races of Fop

During a survey of snow pea growing regions, 4 races of Fop have been identified in Australia – races 1, 2, 5 and 6. The number of each race found from isolates of the fungus collected from infected snow peas are shown in Figure 4.

Races 2 and 5 are more common in Queensland, whereas races 1 and 5 are more common in NSW and Victoria. Races of the fungus and the population types present in the environment need to be evaluated for disease control management.

Specifically this information assists when examining newly bred cultivars and for the development of new resistant cultivars.

## Management options

Control of Fusarium wilt is difficult once established in a field. The first stage of any

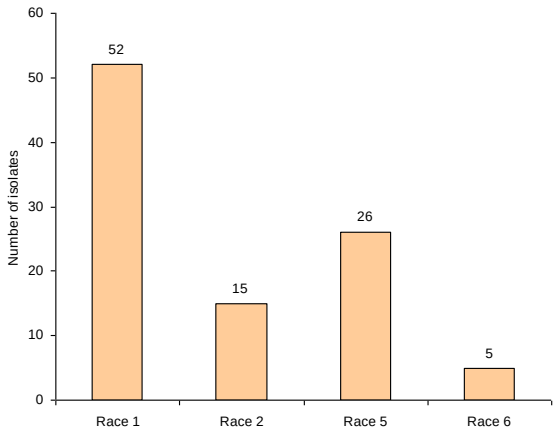


**Figure 4. Races of Fop and their frequency of isolation from growing regions surveyed.**

disease control program involves the examination of the disease and host interaction. Fusarium wilt must be detected in an infected crop especially when disease symptoms are not widespread. An infected crop with minimal symptoms will provide inoculum for rapid build-up of disease in subsequent crops and decisions must be made on moving from an infected block to new ground.

If any symptoms are noticed that include yellowing of leaves, loss of plant vigour and reduced survival of plants when pods have developed, then correct identification of the cause of these symptoms is critical. They could be related to water or nutritional stresses but they could be the first signs of Fusarium wilt. Check for vascular discoloration by slicing the stem of an infected plant longitudinally at ground level and examine for a reddening inside the stem. Send plants with symptoms to a plant disease diagnostic laboratory for confirmation.

The best control option for this disease is resistant cultivars, but these are currently not available. The main control options for growers in Australia include soil fumigation, avoidance of infected fields, planting in cooler months of the year, adopting a strict farm hygiene plan and growing non-susceptible crops.

Fumigation has been found to be successful but may not be economically viable in some situations. Fumigation is currently carried out by some growers where the main products used include metham or chloropicrin. Fumigation will also control weeds, insects and nematodes. However, fumigation may only be effective for short-term benefits. Fumigation kills many beneficial antagonistic organisms in the soil and if Fusarium wilt is re-introduced into that soil it can colonise the substrate rapidly with limited competition from other soil-borne organisms.

If beds only are treated with fumigants, infected soil from nearby can reintroduce Fop.

Although seed is suspected of carrying Fusarium wilt, it is not considered likely. Fop infects the lower parts of the plant and not parts near flowers; however, infected plant residue in with the seed may carry the disease.

Seed is selected by seed companies from crops grown in areas free of disease and monitored for disease during the crop's growth. Seed quality is therefore assured. Seed transmission if possible, will only occur if growers keep seed from known infected plants.

Management options for growers must include maintaining a farm hygiene policy, especially when moving from infected ground to a clean property or block. Key points of this plan would include restricting soil movement by any means from one property to the next. The fungus is easily transferred in soil.

Restrict the movement of machinery such as tractors and wash them before going from an infected block to a clean block. Snow peas are hand picked so even pickers will need to clean boots and wash them in a disinfectant before moving to a clean block.

Snow peas require a great deal of infrastructure for trellising such as posts and wires. This trellising is moved from block to block, but would be capable of moving the Fusarium wilt pathogen with it. Soil on posts, plant material and *Fusarium* spores on wires, all would provide inoculum when moved from an infected to uninfected block. Reducing this source of Fusarium wilt inoculum would be very difficult. The only method to 'clean' this material would be to clean off soil and plant debris and/or dip in disinfectant type products which are available for such purposes. This however would be a huge task.

Valuable information is available to growers for assistance with planning farm hygiene with the publication *Farm hygiene for vegetable blocks* which is published by Queensland Department of Employment, Economic Development and Innovation and is available at http://www2.dpi.qld.gov.au/horticulture/4753.html

Fusarium wilt of snow peas is specific to a host crop; therefore the disease will not occur on a crop not belonging to the pea family. However, the fungus will survive in the soil and if peas are reintroduced then wilt infection may occur. Growers that have Fusarium wilt may need to change to a different crop to break the disease build-up. Reducing the frequency of snow peas grown in the same block will reduce disease levels.

Cases have been reported of rapid build-up of Fusarium wilt in snow peas grown in land that had previously not recorded the disease. The disease can build up rapidly as inoculum is spread by the movement of infected soil. Plants with symptoms may not be noticeable when the inoculum level is at low levels. However, with the movement of soil, vehicles, machinery and people through an infected block the level Fusarium wilt in the soil increases to a level that results in increased infection especially in subsequent crops on the same land.

Information in this Primefact has been collected from a project, VG05029. VG05029 was a collaborative project that included four agencies; NSW Industry and Investment, the University of Sydney, Botanic Gardens Trust and Queensland Department of Employment, Economic Development and Innovation.

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# Appendix

***Media used for isolation, purification and identification of* Fusarium**

*Peptone PCNB Agar (PPA/Nash-Snyder Medium)*

This media contains antibiotics and a fungicide which inhibit most other fungi and bacteria but allows *Fusarium* species to grow. The basal medium includes 20g agar, 15g Difco peptone, 1g $KH_2PO_4$, 0.5g $MgSO_4.7H_2O$ and 0.1g Terrachlor® (contains Pentachloronitrobenzene (PCNB) 75% in 1L of distilled water. The medium was autoclaved for 20 min and cooled to 55$^oC$ before adding an antibiotic solution (1 g Streptomycin sulphate and 0.12 g Neomycin sulphate in 10 ml sterile water).

*Water Agar (WA)*

This medium contains 20g agar in 1l of distilled water and is autoclaved for 20 min.

*Carnation Leaf Agar (CLA)*

The medium is prepared by adding 4-5 pieces of sterilised carnation leaves on the surface of 2 % Water Agar in 6 mm Petri dish immediately after the pouring of agar.

*Potato Dextrose Agar (PDA)*

250 g white potatoes were washed, and diced (unpeeled) before boiling until just soft. The boiled potatoes were filtered through cheesecloth. The broth was added with 20 g Agar, 20 g Dextrose and water to make up 1 L.