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Genetic improvement of passionfruit to achieve improved disease resistance

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Department of Primary Industries & Fisheries, Queensland

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Purpose of report: The purpose of this report is to present the final results of all activities conducted under HAL Project PF04001 'Genetic improvement of passionfruit to achieve improved disease resistance'. The report provides a summary of project findings, a description of technology transfer activities, and recommendations arising from the outcomes of the project. The overall objective of this project was to develop improved disease management strategies for the Australian passionfruit industry through breeding and improvements in our understanding of fungal and viral diseases of passionfruit. This project targeted three areas; 1) breeding of rootstocks and scions with improved virus and cold tolerance, 2) the investigation of defence enzymes in passionfruit and the use of defence activators and 3) understanding the role of infection by single and multiple viruses and examining the use of PWV mild-strain cross-protection.

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Media summary

This project has made substantial progress in the goal to tackle passionfruit diseases. New rootstock material which is resistant to disease and tolerates cold and viruses was developed. The project also gained valuable knowledge about the workings of the passionfruit's natural defence system against disease.

Diseases caused by fungal and viral pathogens are a major limiting factor to profitable passionfruit production in Australia. Australian passionfruit also have very little cold tolerance which can limit their production and make them more susceptible to disease. The aims of this project were to develop new passionfruit genotypes with improved virus and cold tolerance, undertake investigations of virus diseases of passionfruit and understand more about production of defence related compounds by passionfruit which allow the plants to protect themselves from disease.

We used traditional breeding methods to incorporate good cold and virus tolerance into rootstocks and scions. We tested these new crosses under field conditions to determine their agronomic performance and resistance to other diseases. One of our rootstock selections, 'Heuston' x *P. incarnata* shows excellent virus and cold tolerance in the field. Unfortunately, we have not been able to get significant seed set for rootstock seed production. We have crossed 'Heuston' x *P. incarnata* with 'DPI' in order to improve seed set. The seed lines we have developed will be used in future breeding programs.

In order to develop new ways to control passionfruit viruses we need to understand more about them. We examined viruses either as single or mixed infections to determine their effect on virus symptoms and plant health. We recovered the original *passionfruit woodiness virus* mild strain and examined the use of it for cross-protection of passionfruit. Unfortunately the original mild-strain cross protection used for the control of woodiness virus from the 1950's is not effective.

Passionfruit produce compounds in response to infection by a pathogen to restrict disease development. We developed a method to measure these biochemical defence related compounds produced in response to treatment with defence activators or infection by a pathogen. This technique will be used in future work to better understand passionfruit's defence responses to diseases and to develop better options for field control of these diseases.

Technical summary

Passionfruit production in Australia is greatly limited by viral and fungal diseases. Australian passionfruit also have very little cold and virus tolerance which can limit their production and predispose them to increased disease susceptibility. The aims of this project were to develop new passionfruit genotypes with improved virus and cold tolerance, undertake investigations of virus diseases of passionfruit and understand more about production of defence related compounds by passionfruit.

We used traditional breeding methods to incorporate good cold and virus tolerance from *P. incarnata* and its derived crosses into rootstocks and scions. These new crosses were evaluated under field conditions to determine their agronomic performance and disease resistance. We have developed a cross of 'Heuston' x *P. incarnata* with potential use as a rootstock. Although the cross does not readily set seed we have produced seed from a cross between it and *P e.* f. *flavicarpa* 'DPI'. It is hoped that this cross will have high levels of virus and cold tolerance and also higher levels of seed set. This new cross will be assessed in future breeding programs.

We have also developed a system for producing cuttings from mature passionfruit vines. This system could be used to determine the usefulness of a clonally propagated rootstock of 'Heuston' x *P. incarnata* grafted to commercial varieties.

There were two parts to our scion breeding program. The first part was to introduce novel genes from the cold and virus tolerant *P. incarnata,* and we have produced a cross of 'Selection 1' x 'Misty Gem'. Initial screening of this cross showed good virus and cold tolerance as well as good fruit characteristics. We will plant more vines of this cross under field conditions for further assessment. The second part of the scion breeding program was to provide pathology support for industry development of varieties such as 'Sweetheart', 'Jumbo Gem' and 'Pandora' which have now been released to industry.

In order to develop new ways to control passionfruit viruses we need to understand more about disease development and virus interactions. We examined Passiflora latent virus (PLV), Passionfruit woodiness virus (PWV), Passiflora virus Y (PaVY) and Clover yellow vein virus (CYVV), either as single or mixed infections, to determine their effect on yields and quality.

We recovered the original *Passionfruit Woodiness Virus* mild strain and examined the use of it for cross-protection of passionfruit in a field trial. We found inoculation of seedling vines with the mild-strain prior to planting under field conditions did not protect against the development of virus symptoms.

We have developed a system to quantify the production of the defence related compounds β-1,3-glucanse, chitinase and total phenolics by passionfruit in response to treatment with defence activators or inoculation with pathogens or by different varieties of passionfruit. This technique will be used in future work to better understand the defence responses of passionfruit to diseases and to develop more options for field control of passionfruit diseases

Introduction

Since 1958 (Grozmann & Purss, 1958) *Passiflora edulis* f. *flavicarpa* has provided a rootstock for the industry in terms of overall production and resistance to Fusarium wilt, nematodes and *Phytophthora nicotianae* root and stem rot. However, its performance during the winter in sub-tropical production areas is unsatisfactory due to its lack of cold tolerance (*P. e.* f. *flavicarpa* is the least cold tolerant of *P. edulis* forms), which results in cessation of growth, poor production and severe virus infections. The *P. e.* f. *flavicarpa* rootstock itself is highly susceptible to virus diseases, and succumbs to base rot (caused by *Fusarium solani*) when it is below optimum health. To enhance the disease resistance and cold tolerance of current rootstocks we have been undertaking a rootstock breeding program which makes use of the cold and virus tolerance of *Passiflora incarnata* and its derivatives.

Virus diseases remain a major concern to the passionfruit industry. Due to the clonal propagation of new varieties, virus diseases can spread rapidly and impact severely on yield. In the 1950's passionfruit mild-strain cross protection was developed by Simmonds (1959) to protect *P. edulis* seedlings from infection by passionfruit woodiness virus. Plants were inoculated with a mild strain of the virus which prevented them from becoming infected with more severe strains. The use of mild strain cross protection provided good control of viruses until around the late 1960's/70's when the process seems to have been abandoned with the development of the more virus tolerant hybrids.

The successful revival of mild-strain cross protection would be most useful to the passionfruit industry. However, some fundamental questions need to be answered about the viruses infecting passionfruit and their cumulative effects. Virus surveys conducted in PF01001 showed that in most plantations surveyed, nearly all vines were equally infected with Passiflora latent virus (PLV), Passionfruit woodiness virus (PWV), Passiflora virus Y (PaVY) and Clover yellow vein virus (CYVV). Vines from one farm in northern NSW were also infected with Cucumber mosaic virus (CMV). Seedling Panama vines from central to north Queensland were generally only infected with one virus, PWV (Anderson *et al*, 2005). It is not known which of these viruses is/ are responsible for severe virus symptoms and declines in yield. This project aimed to answer some fundamental questions by examining the effect on passionfruit following infection by single and multiple viruses.

Current commercial passionfruit production in Australia is based on hybrids between *P. edulis* f. *edulis* and *P. e.* f. *flavicarpa* and selections within *P. e.* f. *flavicarpa* for more tropical production areas (Rigden and Newett, 2006). Previous scion breeding work has concentrated on the inter-crossing of commercially accepted hybrids to recombine the genetic variability that exists in the genotypes. Progeny has been selected for good agronomic performance and resistance to fruit and foliar diseases, with particular emphasis on resistance to alternata spot (caused by *Alternaria alternata*). However, this program did not provide genetic resistance to virus diseases or superior cold tolerance. For these traits it is necessary to investigate novel material. In this project we attempted crosses with novel material to introduce cold and virus tolerance which can be used for breeding commercially acceptable varieties

Plants have the ability to react to invasion by pathogens with defence responses. When a pathogen comes into contact with a host plant, the host plant is able to sense the pathogen through molecular signalling and respond in an attempt to prevent infection (Guest & Brown, 1997). Some artificially applied compounds (called defence activators) have been found to set off the cascade of responses without the pathogen being present, whilst other compounds have been found which 'prime' the plant so that it is able to respond faster when it senses a pathogen (Guest & Brown, 1997). Many of these compounds are used at very low doses and are environmentally friendly. They can also reduce the need for the use of some fungicides.

There have been successful trials using defence activators on passionfruit. Willingham *et al.* (2002) successfully controlled scab of passionfruit (caused by *Cladosporium oxysporum*) using combinations of a strobilurin fungicide and a defence activator (Bion) in field trials. In growth cabinet trials Bion alone successfully controlled scab (Willingham *et al.*, 2002).

However, considerable work needs to be done in understanding the mechanisms by which these activators work in passionfruit. In conjunction with understanding more about the mechanisms of defence activators is the study of the production of defence enzymes by passionfruit. Some plants have been shown to increase their production of defence enzymes after treatment with a defence activator or inoculation with a pathogen (Dann & Deverall, 2000). There may also be varietal effects where some cultivars produce higher levels of pathogenesis proteins in response to various stimuli. In this project we aimed to develop protocols for measuring the production of defence related compounds and examine the effect of treatment of plants with defence activators in growth cabinet studies.

The areas addressed in this project and final report are:

- review of breeding program conducted by Mr Peter Beal
- field evaluation of new rootstocks for disease resistance
- screening rootstocks and varieties for defence compounds and use of plant defence activators
- use of mild-strain cross protection to protect against passionfruit woodiness virus
- pathogenicity testing of individual viruses
- long term strategies to incorporate virus tolerance into hybrids and rootstocks
- mass screening of new hybrids and rootstocks for disease resistance
- investigate feasibility of using tissue culture to obtain virus-free hybrids
- other issues which were research during the course of this project.

Experiments

Review of breeding program by Peter Beal

Prior to the commencement of PF04001 Mr Peter Beal was engaged to undertake a review of the breeding program. In his report he made 14 recommendations. In summary he recommended; 1) maintaining a seed collection of known traits and explicitly list the traits for which we are breeding 2) increase the size of the germplasm source (including linking with overseas researchers) 3) retaining the good aspects of the previous crossing program including the comprehensive evaluation system 4) increasing the number of vines evaluated as this will improve selection efficiency and 5) strategic use of specialist support (e.g. horticultural and food technology). In July 2004 Beal updated this review and it is provided a table of passionfruit species and forms and their attributes of values for use in the genetic improvement of passionfruit (Appendix 1).

Experiment 1: Field evaluation of new rootstocks for disease resistance

The lack of cold and virus resistance in rootstocks is an on-going problem for the passionfruit industry. In PF01001 we showed that 'Selection 1' had excellent cold tolerance. We wanted to test if seedling 'Selection 1' vines would be a suitable rootstock for industry.

The aim of this experiment was to compare the field performance of two rootstocks 'Heuston' (selection of *P. edulis* f' *flavicarpa*) and 'Selection 1' (*P. edulis* f. *edulis* crossed with *P. incarnata* backcrossed to *P. edulis* f. *flavicarpa*) with the current industry standard 'DPI' rootstock when grafted to two commercial varieties with different characteristics, namely 'Misty Gem' and '152'.

Materials and methods

Tiny Tag data loggers were placed at each site to monitor temperature to be able to compare the two sites.

Trial site 1 – Duranbah, NSW

The rootstock evaluation trial was planted on the 2nd December 2003. It consisted of two scions ('Misty Gem' and '152') grafted to three rootstocks ('Selection 1', 'DPI' and 'Heuston'). Each combination was replicated 10 times. To avoid differences due to changes across the orchard, plants were grouped into 10 blocks (1 replicated of each rootstock/scion combination).

Yields and fruit and foliar disease assessment
Yields were obtained by harvesting the fruit from the autumn/winter crop. Vines were assessed for crop load and virus and disease symptoms at strategic time points throughout the growing season. On the 1st June 2004 the vines were visually rated for assessed for crop load, symptoms of virus in vine and symptoms of virus in fruit. On the 12th May 2005 fruit from each of the vines was assessed for the severity of anthracnose, Alternata spot and Septoria spot. The number of fruit fly stings per fruit was also recorded. On the 7th June 2005 vines and fruit were assessed for fungal infections on leaves and virus symptoms on leaves and fruit.

Photo-oxidation
A chlorophyll meter was used to determine how much chlorophyll was in the leaves and hence which vines were suffering from photo-oxidation. On the 17th September 2004 a handheld chlorophyll meter was used to assess photo-oxidation. Three measurements of chlorophyll content index (CCI) were made on each vine and an average value taken for statistical analysis

Stock-scion measurements
Stock-scion physiological compatibility may be indicated by the ratio of the girth of the stock to the scion. Overgrowths (scion much wider than stock) and benching (stock much wider than scion) indicate a physiological incompatibility. To examine the physiological compatibility of the different rootstock/scion combinations on the 17th September 2004 the girth of the stock and scion was measured on each vine and the resultant ratios compared using analysis of variance.

Trial site 2 – Nambour, Queensland

The trial at Nambour used the same stock/scion combinations as the trial at Duranbah namely 'Misty Gem' and '152' grafted to 'Selection 1', 'DPI' and 'Heuston'. Each combination was replicated 4 times. Due to labour limitations green counts were used as an indication of yield. Green counts were made 19th May 2004 and 8th December 2004.

Results:

The two sites were very similar in terms of temperatures. The trial site at Duranbah was only slightly cooler than the trial site at Nambour with average temperatures of 20.1°C and 20.5°C respectively. The minimum temperature at Nambour was 1.9°C whilst the minimum at Duranbah was 3.9°C. Both sites reached a maximum of 45.5°C.

Trial site 1- Duranbah, NSW

There was no significant effect of rootstock on yield (Table 1). There was, however, a significant effect of scion on yield; 'Misty Gem' yielded significantly more fruit than '152'.

Table 1. The effect of rootstock and scion on the average yields of vines planted at Duranbah for the autumn/ winter crop 2004 (In the same column, values followed by the same letter are not significantly different at P=0.05).

Treatments	**Average yield (number of fruit per vine)**
Rootstock	
'DPI'	51.6
'Heuston'	53.8
'Selection 1'	45.9
P	0.544
lsd	-
Scion	
'152'	27.7 a
'Misty Gem'	73.2 b
P	<0.001
lsd	12.08
Rootstock/Scion	
'152'/'DPI'	28.1
'152'/'Heuston'	32.6
'152'/'Selection 1'	22.4
'Misty Gem'/'DPI'	75.1
'Misty Gem'/'Heuston'	75.0
'Misty Gem'/'Selection 1'	69.4
P	0.936
lsd	-

There were no significant differences between rootstocks in the crop load, virus symptoms development on vines or fruit between the three rootstocks (Table 2). There was however, a significant difference between crop and fruit virus symptoms between 'Misty Gem' and '152'. 'Misty Gem' had a higher crop load and less severe virus symptoms on fruit. There was no interaction between crop load or virus symptoms for any of the rootstock/ scion combinations (data not shown).

When fruit were assessed for disease development in May 2005 there were no significant differences between the severity of anthracnose or Septoria spot or the average number of fruit fly stings per fruit for any of the treatments (Table 3). There was however a significant effect of scion on Alternata spot. 'Misty Gem' had significantly lower levels of Alternata spot than '152', although these levels were quite low. There was no interaction between rootstock and scion combinations for the parameters measured (data not shown).

There were no significant differences between the rootstocks, scions (Table 4) or rootstock scion combinations (data not shown) for the chlorophyll content as measured in September 2004.

There were no significant differences between rootstock-scion ratios for any of the tested rootstock/scion combinations (data not shown). The average scion to rootstock ratio was 0.821.

When vines were assessed for yield, foliar fungal and viral diseases and virus symptoms in fruit in June 2005, there were no significant differences between treatments for foliar fungal and viral disease symptoms (Table 5). There were significant differences between 'Misty Gem' and '152' for yield and fruit virus symptoms. 'Misty Gem' yielded more fruit and fruit were less distorted with virus than for '152'.

Table 2. The effect of rootstock on virus symptoms in vine and fruit and on crop load in '152' and 'Misty Gem'. Assessments were made on the 1st June 2004(In the same column, values followed by the same letter are not significantly different at P=0.05).

Treatments	Crop load on vine [a] (1-5)	Virus symptoms on vines [b] (1-5)	Virus symptoms on fruit [c] (1-5)
Rootstock			
'DPI'	2.80	3.11	3.17
'Heuston'	2.95	3.10	3.50
'Selection 1'	2.40	3.10	3.25
P	0.075	1.000	0.586
lsd	-	-	-
Scion			
'Misty Gem'	3.50 b	3.03	3.97 b
'152'	1.94 a	3.17	2.65 a
P	<0.001	0.495	<0.001
lsd	0.4154	-	0.548

* Overall transformation did not improve residuals hence untransformed data is presented.
[a]Crop load (1-only 1 or 2 fruit, 5-high crop load), [b]Vine - symptoms of virus, vigour, colour (1-poor heath, 5-good), [c]Fruit - Symptoms of virus in fruit (1- high no. of badly deformed fruit, 5-no deformed fruit)

Table 3. Disease assessments and number of fruit fly stings per fruit of rootstock scion combinations at Duranbah on 12th May 2005(In the same column, values followed by the same letter are not significantly different at P=0.05).

Cultivar	Severity (%) Alternaria	Anthracnose	Septoria	Average no. fruit fly stings/fruit
Rootstock				
'DPI'	1.25	0.38	0.48	0.346
'Heuston'	1.31	0.28	0.46	0.168
'Selection 1'	1.04	0.97	0.42	0.295
P	0.716	0.364	0.985	0.403
lsd	-	-	-	-
Scion				
'152'	1.61 b	0.84	0.410	0.224
'Misty Gem'	0.79 a	0.24	0.49	0.315
P	0.006	0.163	0.749	0.411
lsd	0.5722	-	-	-

Table 4. The effect of rootstock and scion on the chlorophyll reading. Three readings were taken from each vine on the 17th September 2004.

Rootstock	Average chlorophyll reading (CCI)
'DPI'	38.96
'Heuston'	41.79
'Selection 1'	39.75
P	0.469
lsd	-
Scion	
'152'	40.51
'Misty Gem'	39.83
P	0.727
lsd	-

Table 5. The effect of rootstock and scion on yield (as number of green fruit), fungal diseases on foliage, foliar virus symptoms and fruit virus symptoms (vines and fruit were assessed 7th June 2005) (In the same column, values followed by the same letter are not significantly different at P=0.05).

Cultivar	No. green fruit on vine	Foliar fungal symptoms [A]	Foliar virus symptoms [B]	Fruit virus symptoms [C]
Rootstock				
'DPI'	69.9	2.27	2.422	2.222
'Heuston'	69.2	2.05	2.350	2.05
'Selection 1'	76.7	2.00	2.222	2.056
P	0.415	0.524	0.484	0.098
lsd	-	-	-	-
Scion				
'152'	61.1 a	2.29	2.356	2.219 b
'Misty Gem'	82.8 b	1.93	2.307	2.000 a
P	<0.001	0.085	0.726	0.004
lsd	-	-	-	0.1454
Interaction				
'152'				
'DPI'	58.6	2.44	2.44	2.44
'Heuston'	54.6	2.20	2.40	2.10
'Selection 1	70.1	2.22	2.22	2.11
'Misty Gem'				
'DPI'	81.2	2.10	2.40	2.00
'Heuston'	83.9	1.90	2.30	2.00
'Selection 1'	83.2	1.78	2.22	2.00
P	0.422	0.958	0.956	0.098
lsd	-	-	-	-

[^]Transformation of data did not improve residuals. [A]Fungal disease rated on a 0-4 scale. [B]Fruit virus rated on a 1-3 scale: 1 – unaffected, 2 - moderate distortion and 3 - severe distortion. [C]Vine virus rated on a 1-5 scale; 1 – healthy, 2 - slight mottle, 3 - moderate mottle, 4 - distortion & vein clearing, 5 - tip blight

Trial site 2 – Nambour, Queensland

There were no significant differences in yields of fruit from different rootstocks for counts of green fruit done in both May and December 2004 (Table 6). 'Misty Gem' yielded significantly more fruit than '152'. There was no interaction effect between rootstock and scion on yield.

Table 6. The effect of rootstock on the yield (as measured by the number of green fruit on the vine) of '152' and 'Misty Gem' in field trial at Nambour (In the same column, values followed by the same letter are not significantly different at P=0.05)

Rootstock	Yield May 04	Yield Dec 04
'DPI'	40.0	130.2
'Heuston'	49.0	120.6
'Selection 1'	50.0	131.0
P	0.479	0.474
lsd	-	-
Scion		
'152'	19.1 a	109.0 a
'Misty Gem'	73.6 b	145.6 b
P	<0.001	<0.001
lsd	15.26	15.41
Interaction		
'152'		
'DPI'	17.8	114.8
'Heuston'	18.8	97.0
'Selection 1'	20.7	115.1
'Misty Gem'		
'DPI'	62.2	145.5
'Heuston'	79.2	144.2
'Selection 1'	79.2	147.0
P	0.626	0.623
lsd	-	-

Discussion/conclusions:

Although this trial was designed to examine the differences between three rootstocks it in fact showed the differences between the two scions being used in the trial. 'Misty Gem' produced more fruit than '152' under field conditions at Duranbah. At Nambour 'Misty Gem' vines also produced significantly more fruit in both May and December. At the time of this trial 'Misty Gem' was the most planted hybrid variety and this trial demonstrated one of the reasons why it is so favoured with growers.

There were no significant differences between the ratios of rootstock to scion girth measurements indicating that generally there is no stock scion incompatibility between 'Misty Gem' or '152' and 'Selection 1', 'DPI' or 'Heuston'.

For the parameters measured there were no differences between 'DPI', 'Heuston' and 'Selection 1'. Whilst the performance of 'DPI' and 'Heuston' was fairly uniform, the performance of the 'Selection 1' rootstocks was very variable and at this stage they are not suitable to release as a rootstock to industry. Further work would need to be undertaken to get a more uniform seed line of 'Selection 1' before release to industry as a rootstock.

Experiment 2: Screening of varieties and rootstocks for defence compounds

Development of assays for screening rootstocks and varieties for the production of defence related compounds

We have developed two different assays specifically in order to measure the production of defence related compounds of passionfruit. The first two assays are modifications of the methods of Dann and Deverall (2000) for measuring the defence related enzymes β-1,3-glucanase and chitinase. In our experiments we found we needed to greatly dilute (10x) the crude extract for measurement of chitinase. In this project we also optimised the use of the Qiagen Tissue Lyser for faster more precise preparation of crude extracts.

The total phenolics assay is based on the method used by Payet *et al.* (2006). For this assay we also used a 96-well assay plate which increased the speed at which samples were processed. We found that compared to other horticultural crops such as mango and avocado, passionfruit extracts require very little dilution as they do not contain the same level of phenolic compounds.

Increased chitinase, β-1,3-glucanase and total phenolic production has been correlated with an increase is resistance to pathogens. These assays will be used to examine the defence responses of different varieties of passionfruit. There will also be used in laboratory studies to screen putative defence activators for passionfruit.

Enzyme assays

Preparation of extracts from leaf material

This method is based on an extraction method form Dann and Deverall (2000).

To take a representative sample, leaves were harvested, placed in a stack and a cork borer used to punch out 5mm diameter disks. Tissue (0.2g fresh weight) was placed in a microcentrifuge tube with 0.02g PVPP and 1mL of sodium acetate buffer (50mM sodium acetate buffer pH 5.0 plus 1mM EDTA, 5mM glutathione). A Qiagen Tissue Lyser was used to macerate tissue, tube were then centrifuged at 13,000 rpm for 5 min. The supernatant was pippetted off and frozen at -70°C until enzyme analyses were performed.

β-1,3-glucanase assay

This method is a modified version of the one from Dann and Deverall (2000).

In a microfuge tube, 0.1mL of a suitably diluted crude leaf extract and 0.4mL of sodium acetate buffer (50mM, pH5.0) were equilibrated at 30°C for 3min. To initiate reaction 0.1mL of azurine-crosslinked pacyman (1 tablet suspended in 1mL double-deionized water AZCL-Pachyman, Megazyme Australia Pty Ltd) was added and tubes incubated at 30°C for 10 min. To terminate reaction 0.7mL 20%w/v Tris was added to each tube. Tubes were vortexed, incubated at room temperature for 5 min, vortexed again and centrifuged at 13,000rpm for 2min. Activity of β-1,3-glucanase was determined by measuring absorbance at 595nm using a 96-well assay plate reader.

Chitinase assay

This method is a modified version of the one from Dann and Deverall (2000).

In a microfuge tube, 0.1mL of a suitably diluted crude leaf extract (at least 10 fold) and 0.2mL of sodium acetate buffer (50mM, pH5.0) were equilibrated at 37°C for 3min. To initiate reaction 0.1mL of aqueous CM-Chitin-RBV (Loewe Biochemica, Germany) was added and tubes incubated at 37°C for 10 min. To terminate reaction 0.1mL 2NHCl was added to each

tube. Tubes were cooled on ice for 10min, and then centrifuged at 13,000rpm for 5min. Activity of chitinase was determined by measuring absorbance at 595nm using a 96-well assay plate reader.

Total phenolics assays

Based on the method of Payet, B., A. Shum Cheong Sing, and J. Smadja (2006)

Leaf material (1g fresh weight) was ground in liquid nitrogen, placed in 15mL Falcon tubes, 10mL methanol added, tubes vortexed and place in the refrigerator for 4hrs. Tubes were gently shaken each hour. Extract was filtered through Miracloth, centrifuged at 13,000rpm for 10mins and the supernatant transferred to 1.5mL tubes to prepare dilution series for phenolics assay. Gallic acid standard solutions (0.05 - 0.2mg/mL) were prepared fresh on the day of assay. To determine phenolic concentration a 96-well plate was used. To each cell 30 µl of sample or standard, 150 µl Folin-Ciocalteau reagent (10% v/v) and 120 µl of Na_2CO_3 (7.5% w/v) were added and plate incubated at room temperature in the dark for an hour. Absorbances were read at 690 nm and concentrations of total phenolics determined using absorbance curve developed from gallic acid standards.

Effect of defence activators on the development of diseases of passionfruit

A number of trials were conducted to examine the efficacy defence activators on development of diseases of passionfruit.

Effectiveness of silicon compounds against winter anthracnose of passionfruit

Aim To determine if treating passionfruit plants with Stand SKH (potassium silicate mixed with humic acid) or acidulated Olivine (Silvine) is effective in controlling winter anthracnose of passionfruit.

Materials and methods

On the 17th November 2005 30 vines of '152' (the cultivar most susceptible to winter anthracnose) were planted at the trial block at Duranbah. Vines were planted one per panel in two adjacent rows.

After the vines had established (16th December 2005) they were blocked according to position on the hill. Within each block, treatments were randomly assigned. Vines received either; no treatment, Silvine (50g mixed with 50g of sand) or Stand SKH (15mL in 4L of water). There were 10 replicates of each treatment.

On the 16th December 2005 the vines were rated for general health. Vines received a rating of 1=poor health, 2=moderately healthy or 3=good health. '152' often have 'blind' terminals. Unfortunately some of the rootstocks had grown past the scion so the ratings were not a true indication of the plants health. The rootstocks were pruned back and the vines were again rated for health on the 6th of January.

On the 9th February 2006 the vines were treated again with either Silvine (50g mixed with 50g of sand) or Stand SKH (30mL in 8L of water) and rated for height.

On the 15th March 2006 Stand SKH was reapplied. On the 11th April 2006 Stand SKH or the Silvine were reapplied. On the 6th June 2006 the trial was abandoned due to poor vines.

Results and discussion

There was no effect of treatment on vine vigour (Table 7). A high number of vines used in this trial had blind terminals (a common feature of '152') and never established properly hence the trial was not able to be followed through to completion.

Table 7. Average vigour of '152' vines treated with silicon products. Statistical analyses were not conducted on this data due to poor establishment of the vines meaning the data would not be reflective of the effects of silicon.

	Date of assessment		
Treatment	**16.12.05**	**6.1.06**	**9.2.06**
Untreated control	2.3	1.9	2.1
Stand SKH	2.0	1.8	2.1
Silvine	2.5	2.1	2.2

Fusarium oxysporum f.sp. *passiflorae* and potassium silicate growth cabinet trial

The aim of this experiment is to examine the effect of a putative defence activator (potassium silicate) against *Fusarium oxysporum* f.sp. *passiflorae* in passionfruit.

Materials and Methods

Three passionfruit lines were used in this experiment (Table 8). Seed were planted on the 28th January 2005 and grown in the growth cabinet set at 27°C. On the 23rd February 2005 plants were potted into individual beer can pots. On the 2nd March 2005 half of each of the lines were treated with 750ppm potassium silicate (Kasil, PQ Australia Pty Ltd). On the 7th March 2005 the same pots were treated again.

Table 8. Lines of passionfruit used in *F. o.* f.sp. *passiflorae* and potassium silicate trial, their origin and relative susceptibility to *F. o.* f.sp. *passiflorae*.

Variety	**Relative susceptibility to *F.o.* f.sp. *passiflorae***
P. edulis	Very susceptible
'DPI'	Resistant
'Selection 1' seed from open pollinated fruit from vine in breeding block	Susceptible

On the 10th of March 2005 all but one of the untreated plants and all of the treated plants were inoculated with *F. o.* f.sp. *passiflorae*. Plants at the 2nd or 3rd true leaf stage were dipped into a 1 x 10^6 conidia/mL solution and then potted into clean potting mix.
On the 1st April 2005 the vines were assessed for the development of wilt symptoms. On the 6th April plants were assessed for wilt symptoms and were destructively sampled to determine root health rating. Data was analysed using analysis of variance (Genstat 6th Edition).

Results and discussion

As expected there was a significant effect of cultivar on the development of wilting and root decay, *P. edulis* and 'Selection 1' showed significantly greater signs of wilting and root decay than 'DPI' plants (Table 9). Treatment with Kasil had no effect on disease development (Table 9) and there was no interaction between cultivar and treatment with Kasil (data not shown). Treatment with potassium silicate appears to not have an effect on the development of Fusarium wilt of passionfruit.

Table 9. Effect of cultivar and treatment with Kasil on the development of wilt and root rotting in plants inoculated with *Fusarium oxysporum* f.sp. *passiflorae*. (In the same column, values followed by the same letter are not significantly different at P=0.05)

Treatment		Wilt rating [A] 1st April	6th April	Root rot rating [B] 6th April
Cultivar				
'DPI'		1.00 a	1.00 a	1.00 a
'Selection 1'		3.15 b	3.20 b	2.95 b
P. edulis		3.46 b	3.85 b	3.54 b
	P	<0.001	<0.001	<0.001
	lsd	1.082	1.096	1.061
+Kasil		2.33	2.49	2.34
-Kasil		2.69	2.76	2.55
	P	0.414	0.551	0.614
	lsd	-	-	-

*No significant interaction (data not shown). *Transformation did not improve residuals hence untransformed data presented. [A] Wilt - above ground symptoms: yellowing, leaf fall and wilted leaves, rated from 1 (healthy) to 5 (wilted). [B] Root - root symptoms: discolouration of vascular strands and rotting of roots, rated from 1 (healthy) to 5 (decayed).

Phytophthora nicotianae and potassium silicate trial

The aim of this experiment was to examine the effect of a putative defence activator (potassium silicate) against *Phytophthora nicotianae* in passionfruit.

Materials and methods

Three passionfruit lines were used in this experiment (Table 10).

Table 10. Lines of passionfruit used in *Phytophthora nicotianae* and potassium silicate trial, their origin and relative susceptibility to *P. nicotianae*.

Variety	Relative susceptibility to *P. nicotianae*
P. edulis	Very susceptible
'DPI'	Resistant
'Selection 1' seed from open pollinated fruit from vine in breeding block	Susceptible

Seed were planted on the 20th January 2005 and grown in the growth cabinet set at 27°C. On the 23rd February 2005 plants were potted into individual beer can pots. On the 2nd March 2005 half of each of the lines were treated with 750ppm potassium silicate (PQ Australia Pty Ltd), each pot received 30mL of solution. On the 7th March 2005 the same pots were treated again. On the 10th of March 2005 all but one of the untreated plants and all of the treated plants were inoculated with *P. nicotianae*. *P. nicotianae* (BRIP 41880) had been used to inoculate wheat media which was incorporated into sterile potting mix so that the wheat media was 3% of the final volume of the potting mix. The plants were inoculated by removing plants from their pots, gently shaking off excess potting media and then potting the plants into the *P. nicotianae* amended potting mix. The seedlings were then placed into water up to a depth of 2cm above the base of the pots. Uninoculated plants were placed into a separate container but received the same treatment. The plants were placed into a growth cabinet set at 30°C. After four days the pots were removed from the water and allowed to drain freely for three days. This wetting/drying cycle was continued until the plants were assessed on the 23rd March, 1st April and 6th April 2005. At each time plants were assessed for wilt symptoms at the final assessment date the plants were destructively sampled to determine root health rating.

Results and discussion

There were significant effects of potassium silicate on the development of wilting and root rot symptoms. For the wilt ratings done 1st and 6th April the Kasil treated plants showed

significantly higher levels of wilting than the plants which did not received Kasil (Table 11). There was a variety effect on the development of root symptoms but not wilt symptoms. As would be expected *P. edulis* plants had more severe root rot symptoms than the 'DPI' and 'Selection 1' plants. For the wilt ratings done 1st and 6th April there was a significant interaction effect where Kasil treated *P. edulis* wilted more than the other plants.

The treatment of passionfruit with potassium silicate in the form of Kasil did not prevent the development of Phytophthora as measured by severity of wilting and root decay. Interestingly, the *P. edulis* plants that were treated with Kasil had significantly more severe wilt symptoms. The amount of Kasil applied to each plant may have been toxic for the slower growing *P. edulis.*

Table 11. Effect of cultivar and treatment with Kasil on the development of wilt and root rotting in plants inoculated with *Phytophthora nicotianae*. (In the same column, values followed by the same letter are not significantly different at P=0.05)

Treatment		Wilt rating [A]			Root rating [B]
		24th March	1st April	6th April	6th April
Cultivar					
'DPI'		1.16	1.26	1.32	2.05 b
'Selection 1'		1.24	1.41	1.47	2.24 b
P. edulis		*1.62*	2.08	2.23	3.85 a
	P	0.331	0.119	0.085	<0.001
	lsd	-	-	-	0.824
+ Kasil		1.46	1.84 a	1.96 a	2.65
-Kasil		1.13	1.18 b	1.22 b	2.53
	P	0.199	0.042	0.031	0.724
	lsd	-	0.6389	0.6672	-
+ Kasil					
'DPI'		1.00	1.10 b	1.10 b	1.8
'Selection 1'		1.44	1.78 b	1.89 b	4.29
P. edulis		2.14	3.00 a	3.29 a	2.33
-Kasil					
'DPI'		1.33	1.44 b	1.56 b	2.33
'Selection 1'		1.00	1.00 b	1.00 b	3.33
P. edulis		1.00	1.00 b	1.00 b	2.13
	P	0.073	0.019	0.008	0.211
	lsd	-	1.119	1.169	-

[A] Wilt - above ground symptoms: yellowing, leaf fall and wilted leaves, rated from 1 (healthy) to 5 (wilted). [B] Root - root symptoms: discolouration of vascular strands and rotting of roots, rated from 1 (healthy) to 5 (decayed).

Effectiveness of defence activators against *Alternaria alternata*, *Cladosporium oxysporum* and *Septoria passifloricola*

The aim of this experiment was to determine the effect of the defence activators Bion, potassium silicate, phosphorous acid and jasmonic acid against the fungal pathogens *Alternaria alternata*, *Cladosporium oxysporum* and *Septoria passifloricola* in growth cabinet studies.

Materials and methods

'DPI' plants were treated with the defence activators (Table 12), all treatments were applied as a foliar spray using an airbrush except for the potassium silicate treatment which was applied as a soil drench. Three days later the plants were divided up and inoculated with spore suspensions of *Alternaria alternata*, *Cladosporium oxysporum* or *Septoria passifloricola* each at a concentration of 1 x 10^6 spores/mL. Plants were placed in growth cabinets set to 27°C and 85% relative humidity.

The plants treated with *A. alternata* showed symptoms of infection within 10 days and were assessed for lesion development. The *C. oxysporum* and *S. passifloricola* plants were re-inoculated with their respective pathogens at spore concentrations of 5 x 10^6 spores /m, covered with plastic bags and placed back into growth cabinets at 27°C and 85% relative humidity. After 10 days the plants were assessed again for disease development.

Results and discussion

The *C. oxysporum* and *S. passifloricola* treated plants did not develop disease at all and hence no assessment of the effect of defence activators could be made. The reason why disease did not develop is unknown. Prior to the commencement of this experiment tests were carried out with each one of these isolates to confirm their pathogenicity.

The *A. alternata* inoculated plants developed a range of symptoms from the development of small lesions on leaves and stems to the loss of leaves and death. There was no effect of defence activator on the development of Alternata spot (Table 12). Some researchers have reported poor results from defence activators trials in growth cabinets where plants are subjected to constant light and temperature regimes. When the similar trials were undertaken under glasshouse and field conditions the treatment with defence activators significantly decreased disease (Reglinski *et al.* 2007)

The simplest explanation is that the chemicals either do not work in pathways that are involved in *A. alternata* infection or they were applied in insufficient quantities. Unlike for Bion, concentrations required of phosphorus acid, jasmonic acid or potassium silicate to elicit a response in passionfruit have not been determined.

There are possibly other defence activators which may elicit a defence response which would protect passionfruit from infection by *A. alternata* and testing of other putative defence activators should continue.

Table 12. The effect of treatment with defence activators on the development of disease on 'DPI' plants inoculated with *A. alternata*.

Treatment	Mean health rating [A]
Untreated control	1.50
Bion (0.1g/L)	1.67
Phosphorus acid (1% foliar spray)	1.83
Jasmonic acid (0.5mM 0.5mL of acetone /L of water)	2.50
Potassium silicate (750ppm)	3.17
P	0.063
lsd	-

[A] Health rating was a 1-5 scale where 1= healthy plant, 2- a few lesions on leaves or stems, 3=minor leaf loss, 4=major leaf loss and 5=all leaves lost, plant dead.

Experiment 3: Virus cross-protection

Plants can be protected from infection with severe strains of a virus by pre-inoculating them with very mild-strains, which cause little or no economic damage. This strategy is called "mild strain cross-protection" and was used in Queensland for the control of passionfruit woodiness disease in 1950s to 1960s in *P. edulis*. This practice ceased when *P. edulis* f. *flavicarpa* x *P. edulis* f. *edulis* hybrids were bred that had more tolerance of PWV infection than *P. edulis*. Very mild strains of PWV are still likely to be present in the field, following the extensive use of cross-protection in past years.

A cross protection field trial was set up to examine whether preinoculation (before planting) with a mild strain of passionfruit woodiness virus (PWV) would protect plants from subsequent infection with a typical severe strain of the virus.

Materials and methods

The two strains of PWV (mild isolate 1W-N and severe isolate 386) used in this work can be distinguished by the type of local lesion (necrotic or chlorotic, respectively) they cause on French bean.

DPI flavicarpa rootstock seedlings (*P. edulis* f sp *flavicarpa*) were used for this experiment, and four treatments were established while the seedlings were in the glasshouse:

1. uninfected (not inoculated)
2. infected with mild strain only (cross-protected)
3. infected with severe strain only
4. infected with mild strain, then 3 weeks later inoculated with the severe strain (challenged cross-protected)

Prior to planting, all plants were tested by ELISA for the presence of PWV, and by inoculation to French bean to determine the strain of PWV. All treatments gave the results expected; (1) uninfected), (2) infected with mild strain only, (3)infected with severe strain only, (4) infected with mild strain only or predominantly with the mild strain and also with low levels of the severe strain.

Seedlings were planted at the Duranbah field site 0n 29 November 2006. The four treatments were each replicated 15 times and planted in a randomised layout.

Results

The trial was rated on three occasions; 8 February 2007, 12 April 2007, 27 June 2007, i.e. 10, 19 and 30 weeks after planting. Plants were assessed for severity of virus symptoms, plant vigour, and fruit set/crop load.

At the first rating, virus symptoms were generally mild, and there were no significant differences between any of the virus treatments in plant vigour or symptom severity. All uninoculated plants appeared symptomless and were significantly more vigorous than the virus treatments.

At the second rating, results were variable. There was no significant difference between the plant vigour of any treatment. All treatments displayed more severe virus symptoms and many of the uninoculated vines had virus symptoms presumably due to natural infection at the field site. Surprisingly, the severe strain treatment plants averaged slightly less severe symptoms than the other virus treatments. Initial fruit yields were extremely variable between individual vines of all treatments, but cross-protected vines had the lowest fruit yields.

At the third rating, there were no significant differences in virus symptom severity or plant vigour between any treatments. Symptoms were more severe for all treatments that at the second rating, in line with the onset of cooler weather conditions. Fruit yield for the cross

protected treatment had improved relative to other treatments, but was still the lowest. No woody fruit were evident in any treatment, though many fruit had thickened pericarps.

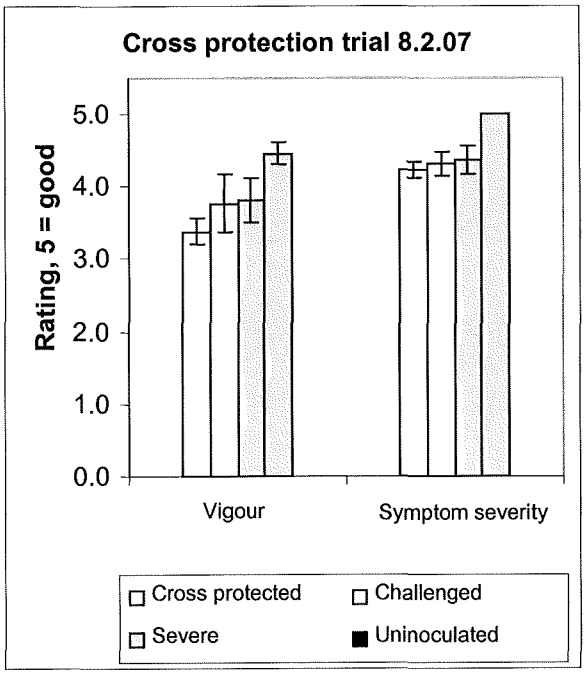

Figure 1. The effect of preinoculation with PWV mild-strain on vigour and virus symptom severity of passionfruit in block C at Duranbah in February 2007.

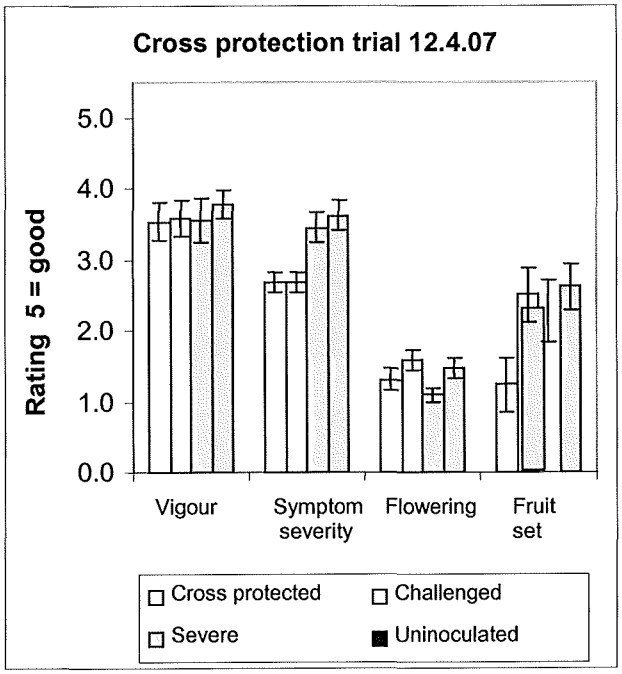

Figure 2. The effect of preinoculation with PWV mild-strain on vigour virus symptom severity, flowering and fruit set of passionfruit in block C at Duranbah in April 2007.

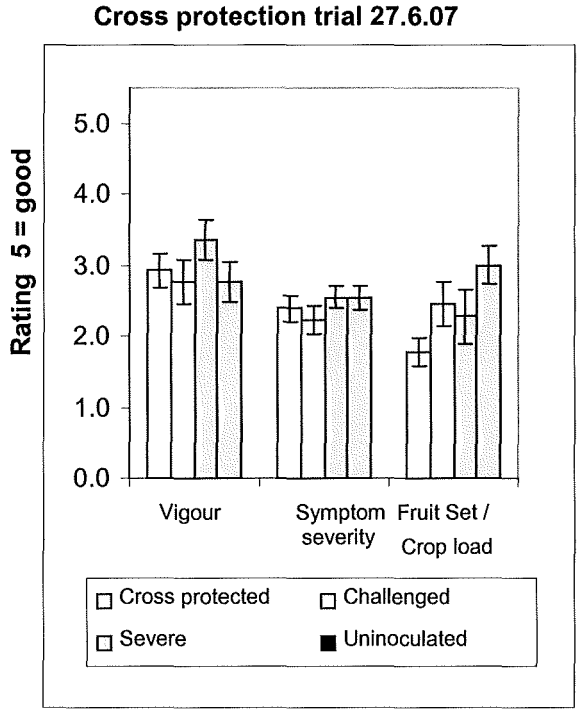

Figure 3. The effect of preinoculation with PWV mild-strain on vigour virus symptom severity, and fruit set/crop load of passionfruit in block C at Duranbah in June 2007.

Discussion

There was no positive, demonstrable effect of cross-protection on any of the rated parameters. The reason for its failure, in comparison to its success use in the past, is unclear. However, there is no evidence for the presence of viruses other than PWV in passionfruit in the older cross protection work, and the other viruses have infected the crops in more recent years. It is possible that one or a combination of the recently detected viruses (CYVV, PaVY, PLV) is responsible for overcoming the cross-protection with PWV or producing significant symptoms themselves. It is unlikely that cross protection can easily be achieved in passionfruit in the near future.

Experiment 4: Virus pathogenicity studies

Passionfruit woodiness virus (PWV), Passiflora virus Y, Passiflora latent virus and Cucumber mosaic virus have all been isolated from passionfruit as pure cultures. We have a potentially pure isolate of Clover yellow vein virus, which has only to be confirmed as being free of PWV. Single infections of PWV cause strong symptoms in passionfruit, and we are now in a position to test the other viruses individually.

Passionfruit badnavirus

Severe disease symptoms of leaf and fruit distortion and have been observed in *P. edulis* f sp. *flavicarpa* cv. Panama Gold from a range of locations including Mareeba and Proserpine. A diseased cutting has been established in the glasshouse at DPI&F Indooroopilly. Electron microscopic examination of the sap of diseased plants has revealed the presence of very low numbers of badnavirus-like particles but not other types of virus particles commonly associated with passionfruit.

Virus transmission

Nine *P. edulis* f sp. *flavicarpa* DPI rootstock seedlings were top grafted with scions of Panama Gold containing the badnavirus (isolate 1916). Four ungrafted plants were used as negative controls. Leaf tissue was taken from the stock plants before grafting, and stored at -20°C, to enable later checking for absence of badnavirus. Shoot growth from the stock was monitored for virus symptoms.

Three grafted plants showed suspect symptoms of leaf distortion, but PWV was subsequently detected in the mother plant. This invalidated the test, as the plants were not inoculated with the badnavirus alone, as so symptoms could not be ascribed solely to it.

Badnavirus integration

Initial testing of *P. edulis* f sp *flavicarpa* seedlings for badnavirus by PCR unexpectedly gave uniformly strong positive reactions. This raised the possibility that the badnavirus sequence was integrated into the genome of the passionfruit, as is known to occur with Banana streak badnaviruses (BSV) and several related viruses. In banana, the integrated form can lie dormant (latent) and later be activated to form virus infections under the stresses imposed by hybridisation or tissue culturing.

Materials and methods

Total nucleic extracts, containing genomic DNA, were prepared from the following *Passiflora* species, using the Qiagen biosprint system:
P. edulis f sp *edulis*
P. edulis f sp *flavicarpa*
P. incarnata
P. alata
P. vitifolia
P. subpetata
P. suberosa
P. foetida
P. aurantia
P. herbertiana

Extracts from these species were subjected to PCR using the badnavirus genus specific primer pair Badna FP/RP (Yang et al. 2003). The PCR products from three separate *P. edulis f sp flavicarpa* plants were cloned and the cloned DNA used for restriction enzyme analysis with enzymes EcoRI and Dde I. Representative clones of all unique restriction groups were then sequenced.

Results

A PCR product of about the expected size (550 bp) was obtained from all species using primer pair Badna FP/RP. Forty two clones were sequenced, 33 of which were badnavirus sequences, and nine were retrotransposon sequences. The badnavirus sequences separated into four distinct clades, each of which probably represents sequence from a different badnvirus species.

Discussion

These experiments established that badnavirus sequences are integrated into the genome of all *Passiflora* species tested, including both exotic and Australian native species. This complicates diagnostic assays for the virus, as any PCR method which has host DNA in the sample will give a positive reaction.

These integrated sequences are an historical record of infection of *Passiflora* by badnaviruses. However, we have no evidence whether or not complete badnavirus genomes are integrated (as occurs for BSV) and if so, whether or not these sequences could be activated for form virus infections.

Search for Badnavirus particles

Very low numbers of badnavirus particles were detected by electron microscopy of the original field sample. Several attempts were made to confirm these observations.

Materials and methods

Two separate experiments were conducted to confirm the presence of badnavirus particles in the symptomatic *P. edulis* f sp *flavicarpa* plants.

1. A partially purified viral minipreps was made from a plant established from a cutting of one of the original field samples (Isolate 1916), and examined in the electron microscope.
2. The partially purified minipreps from 1 was subjected to PCR amplification with and without prior DNase treatment. The DNase digests genomic (i.e integrated) DNA, but does not affect the DNA encapsidated within the virus particles

Results

No particles were observed by electron microscopy in experiment 1.

Badnavirus PCR products were obtained only without DNase treatment, implying that only genomic DNA was being amplified, and hence virus particles, if present, were at a concentration below the limit of detection.

Discussion

Badnavirus particles were observed in the initial field sample of *P. edulis* f sp *flavicarpa.* The detection of integrated badnavirus sequences supports the possibility that badnavirus infections occur in *Passiflora* species, but it appears that virus particle concentrations are very low and unpredictable.

Experiment 5: Long-term strategies to incorporate virus resistance into hybrids and rootstocks

Passiflora incarnata has been previously identified as an excellent source of cold tolerance in passionfruit. Farlow *et al.* (1984) investigated the use of crosses of *P. incarnata* with *P. edulis* f. *edulis* and *P. edulis* f. *flavicarpa* for use as rootstocks. With the decline of the Redlands breeding program the seed from the program was placed into storage, the only selection we could recover from the original batch was 'Selection 1' which we assessed in PF01001 and have used in subsequent breeding work. In the work of Farlow *et al.* (1984) 'Selection 1' was not the best performing cross and under guidance from Mr Peter Beal we attempted crosses using *P. incarnata* to try to obtain seed lines with superior performance.

In this series of experiments we further investigated *P. incarnata* and it's derived crosses in this project as potential sources of cold and virus tolerance for rootstocks and scions as part of a longer term breeding program.

Rootstocks

In PF01001 we found 'Selection 1' had had excellent cold and virus tolerance and root vigour however it is susceptible to Fusarium wilt (Anderson *et al.*, 2005). At the conclusion of project we had successfully produced a cross between 'Selection 1' and *Passiflora edulis* f. *flavicarpa* 'Heuston' to introduce Fusarium Wilt resistance. We had also produced seed from a cross between *Passiflora edulis* f. *flavicarpa* 'Heuston' x *Passiflora incarnata.*

'Selection 1' x *Passiflora edulis* f. *flavicarpa* 'Heuston'

Aim

To examine the usefulness of the cross between 'Selection 1' x *Passiflora edulis* f. *flavicarpa* 'Heuston' as a rootstock with increased cold tolerance.

Materials and methods

A cross between 'Selection 1' and 'Heuston' made in January 2005 was made and the seed recovered. Seedlings were raised in the glasshouse and 69 vines were planted at block C at Duranbah in November 2005.

In February 2006 the vines were assessed for vigour, flowering, fruit set, foliar fungal diseases, virus symptoms, suckering and, where suckering was present, nematode damage. In July 2006 the vines were rated for virus tolerance any vines which rated a 3 or below were selected for removal.

Results

In February 2006 the 'Selection 1' x 'Heuston' vines rated very well for vigour, foliar disease resistance and virus tolerance (Table 13). They however did not set many flowers and even less fruit.

By winter the vines were showing some loss of vigour and virus infection. When the vines were rated for virus symptoms in July 2006, 38 vines (out of 67) rated 4 or above for virus symptoms. The poorly rating vines were cut out whilst the vines which performed well were retained for further evaluation.

Table 13. The percentage of vines that rated particular scores for vigour, flowering, fruit set, foliar fungal disease and virus ratings for 'Selection 1' x *Passiflora edulis* f. *flavicarpa* 'Heuston' in block C at Duranbah. Vines were assessed in February 2006.

Rating	Vigour	Flowering	Fruit set	Foliar disease	Virus
1	0.0	77.9	98.5	0.0	0.0
2	0.0	19.1	1.5	0.0	0.0
3	1.5	2.9	0.0	0.0	0.0
4	23.5	0.0	0.0	4.4	0.0
5	75.0	0.0	0.0	95.6	100.0

[A] Vines were assessed on a 1-5 scale where 1 was an undesirable characteristic and 5 was a desirable characteristic

Discussion

During the course of PF04001 we found that the cross between 'Heuston' and *P. incarnata* was more promising and hence the research focus moved to that cross. The 'Selection 1' x 'Heuston' vines have been retained in the breeding block at Duranbah for future use if needed.

Passiflora edulis f. *flavicarpa* 'Heuston' x *Passiflora incarnata* cross

The aim of this experiment was to determine the usefulness of the cross between *Passiflora edulis* f. *flavicarpa* 'Heuston' x *Passiflora incarnata* as a rootstock with increased cold tolerance.

Materials and methods

A cross between *Passiflora edulis* f. *flavicarpa* 'Heuston' x *Passiflora incarnata* was made in January 2005. Seventy-two resultant seedlings were planted in block C at Duranbah in November 2005.

In February 2006 the vines were assessed for vigour, flowering, fruit set, foliar fungal diseases, virus symptoms, suckering and, where suckering was present, nematode damage. In July 2006 the vines were rated for virus tolerance.

In March 2007 all vines were checked for seed set. All fruit was collected from the zone 0.5m inwards from the post. Fruit were counted, cut open and the number of seed set counted. Any seeds produced were reserved for future use.

In an attempt to set seed for further trial use a cross between *Passiflora edulis* f. *flavicarpa* 'Heuston' x *Passiflora incarnata* was made.

Results

When vines were assessed in February 2006 they showed excellent vigour, excellent foliar disease resistance and excellent virus tolerance (Table 14). They had moderate levels of flowering but poor levels of fruit set. There were moderate levels of suckering and vines which produced suckers were assessed for nematode damage, most vines showed no nematode damage.

In winter 2006 the vines still showed excellent virus tolerance and vigour, in July 2006 all of the 'Heuston' x *P. incarnata* vines rated 5 for virus (no virus symptoms).

Of the 69 vines assessed in March 07 for fruit set only 8 vines set fruit with seed (Table 15.) many vines set a high number of fruit but they were all empty or had very poorly developed seeds. Interestingly the vines which set seed tended to have low levels of suckering.

Table 14. The percentage of vines that rated particular scores for vigour, flowering, fruit set, foliar fungal disease and virus ratings for *Passiflora edulis* f. *flavicarpa* 'Heuston' x *Passiflora incarnata* in block C at Duranbah in February 2006.

Rating	Vigour	Flowering	Fruit set	Foliar	Virus	Suckering	Nematode
1	1.4	4.2	81.9	0.0	0.0	0.0	0.0
2	0.0	13.9	12.5	0.0	0.0	8.3	0.0
3	5.6	38.9	4.2	0.0	0.0	70.8	0.0
4	5.6	19.4	1.4	0.0	0.0	8.3	1.6
5	87.5	23.6	0.0	100.0	100.0	12.5	98.4

[A] Vines were assessed on a 1-5 scale where 1 was an undesirable characteristic and 5 was a desirable characteristic.

Table 15. The seed set and suckering ratings of the cross between *Passiflora edulis* f. *flavicarpa* 'Heuston' x *Passiflora incarnata* planted in block C at Duranbah. Ratings were made in March 2007.

Vine [A]	No. of fruit	No. of fruit with seed	No. of seed	Suckering [B]
1	10	1	2	Low
2	37	1	14	Low
3	1	1	6	Low
4	343	6	6	Low
5	105	1	1	Low
6	61	1	1	Heavy
7	185	1	2	Moderate
8	13	2	2	Low

[A] Vine numbers have been changed as vines are still planted in trial block and are actively used in the breeding program. [B] Suckering ratings: Nil= no suckers, low= 1-5 suckers, moderate= 5-15 suckers, heavy=15-20 suckers, very heavy= more than 20 suckers.

The cross between a selected vine of 'Heuston' x *P. incarnata* and *P. edulis* f. *flavicarpa* 'DPI' was successful and seed has been put in storage at Indooroopilly Research Centre.

Discussion

The cross between *Passiflora edulis* f. *flavicarpa* 'Heuston' x *Passiflora incarnata* showed excellent cold tolerance, virus tolerance and resistance to foliar fungal diseases. This cross has excellent potential as a rootstock except for the inability to set seed. The next step should focus on the successful cross between seedlings of the selected vine of 'Heuston' x *P. incarnata* and *P. edulis* f. *flavicarpa* 'DPI'. Despite the suckering of the selections of *Passiflora edulis* f. *flavicarpa* 'Heuston' x *Passiflora incarnata*, they should also be investigated as a possible method for propagating cold and virus resistant rootstocks. Should this work be undertaken, the compatibility between the *Passiflora edulis* f. *flavicarpa* 'Heuston' x *Passiflora incarnata* rootstock selections and scion varieties will need to be investigated.

Scions

A single vine selection of a cross between 'Selection 1' and 'Misty Gem' was identified in this project as having promise in scion breeding work, showing good virus and fungal disease resistance. Tips were taken from this vine and 17 grafted plants were planted for further evaluation in block C. When assessed early in 2006 these vines had good vigour and disease resistance but had poor fruit set. When the vines were assessed in July 2006 about 1/3 of the vines had good fruit set and were still healthy despite the cooler weather.

Under instruction from Peter Beal the cross was made again as well as some other crosses for scion breeding, these include 'Misty Gem' crossed with *P. coccinea*, *P. incarnata* and

'Selection 1'. Of these crosses only 'Misty Gem' x 'Selection 1' was successful. Seed of these crosses is in cold storage at Indooroopilly, to be assessed under field conditions in the future.

Experiment 6: Mass screening of new hybrids and rootstocks for disease resistance

Mass screening methods were developed in PF01001 to screen large numbers of seedling populations for their resistance to fungal foliar and root diseases. In this process resistant progeny would be identified and selected for further field evaluation. In practice we found it difficult to get the seed numbers required for mass screening to foliar diseases. So for most of the crosses assessed they were planted directly in the field and evaluations for fungal foliar diseases were made there.

The mass screening methodologies were most useful for screening crosses for resistance to root pathogens. Here we report on the screening of 'Heuston' x *P. incarnata* and 'Pandora' for resistance to root pathogens.

Screening of 'Heuston' X *P. incarnata* cross for resistance to *Fusarium oxysporum* f.sp. *passiflorae*

In order to improve the cold and virus tolerance of currently available rootstocks, 'Heuston' has been crossed with *Passiflora incarnata. P. incarnata* has shown good cold tolerance and excellent virus tolerance, it does however, have poor resistance to *F. o.* f.sp. *passiflorae* and nematodes. 'Heuston' has good resistance to *F. o.* f. sp. *passiflorae*, nematodes and *Phytophthora nicotianae.*

The aim of this experiment was to examine the resistance of the 'Heuston' x *P. incarnata* cross to *F. o.* f.sp. *passiflorae* in a growth cabinet trial.

Materials and methods

On the 5th December 2005 the plants were inoculated by planting into a 5% by volume inoculated wheat media inoculated with *F. o.* f.sp. *passiflorae* (BRIP28044) mixed into Searles premium potting mix.

Highly susceptible to *F. o.* f.sp. *passiflorae, Passiflora edulis* ('2002') plants were used as a susceptible check. Only 2 *P. edulis* plants were inoculated and only 2 *P. edulis* plants were uninoculated as *P. edulis* plants were in limited supply. Four 'Heuston' x *P. incarnata* plants were left as untreated controls whilst 56 'Heuston' x *P. incarnata* plants were inoculated.

The plants were maintained in growth cabinets (25°C 12h day / 12h night) until the 1st February 2006 when they were assessed for vascular discolouration.

Results and discussion

Only one plant wilted in the trial, a '2002' *P. edulis*. *P. edulis* does have some resistance to *F. o.* f.sp. *passiflorae* so it is not unsurprising that the other plant did not wilt. Isolations from the wilted plant were made onto streptomycin amended potato dextrose agar (SPDA) with *Fusarium oxysporum* being recovered. None of the 'Heuston' x *P. incarnata* plants had any vascular discolouration indicating that there is a high level of resistance to *F. o.* f.sp. *passiflorae* in the population of plants tested.

This experiment has shown that there is resistance to *F. o.* f.sp. *passiflorae* in this particular cross of Heuston' x *P. incarnata*. Provided that the cross also has the characteristics of resistance to nematodes and *P. nicotianae* and line which does not sucker is selected, it will be very useful for future work and may itself be a useful as a rootstock.

Resistance of 'Pandora' to two soil-borne diseases

The *P. edulis* f. *flavicarpa* variety 'Pandora' was identified as having good fruit characteristics and being able to be propagated from seed and being true to type. It was released to industry in 2006. Two experiments were conducted to determine if 'Pandora' is resistant to Fusarium wilt and Phytophthora.

Experiment 1 – Resistance of 'Pandora' to *Fusarium oxysporum* f. sp. *passiflorae* and *Phytophthora nicotianae*

The aim of this experiment was to determine the resistance of the selection to *F. o.* f.sp. *passiflorae* and *Phytophthora nicotianae* in a growth cabinet trial and determine if 'Pandora' vines needed to be grafted to Fusarium wilt and Phytophthora resistant rootstocks.

Materials & Methods

Three passionfruit varieties were used in the trial: 'Pandora', *P. edulis* as a susceptible check and 'DPI' as a resistant commercial rootstock. Three treatments were used: untreated control, inoculation with *F. o.* f.sp. *passiflorae* and inoculation with *P. nicotianae.* Ten plants of each variety were used per treatment.

Seeds of 'Pandora', *P. edulis* and 'DPI' were germinated in a growth cabinet at day/night conditions of 27°C and 70% RH and 23°C and 70% RH. Seedlings were potted on to beer can pots 7/11/2006 using Searles premium potting mix.

On 17/11/2006 seedlings were inoculated by planting into a 5% by volume inoculated wheat media inoculated with either *F. o* f.sp. *passiflorae* (BRIP 28044) and *P. nicotianae* (BRIP 40949) mixed into Searles premium potting mix. The plants were placed into a growth cabinet on 12 hour alternating day/night cycle at 25°C and 70% relative humidity. The plants for the *P. nicotianae* experiment were placed on a wetting and drying cycle where pots were subjected to three days immersion in water and then three days free-draining conditions.

On the 15th December 2006 the plants were assessed for overall plant health (scale of 1-5) and percentage of healthy root tips. For the first five replicated plants inoculated with *F.o.* f.sp. *passiflorae* isolations were made onto SPDA. The same process was repeated with plants in the *P. nicotianae* trial except isolations were made onto 10PVP. The roots of each plant were then harvested and dried to determine the root dry weight of each plant in grams.

Results and discussion

There was no difference between overall plant health and root dry weight for the inoculated and the uninoculated plants in the *P. nicotianae* experiment (Table 16). There were, however, significant differences between percentage healthy roots. Inoculated 'Pandora' and 'DPI' plants were not significantly different to the uninoculated controls, but inoculated *P. edulis* plants had significantly fewer healthy roots than the untreated controls.

For the plants in the *F.o.* f. sp. *passiflorae* experiment, there were no significant differences between the percentage healthy roots and root dry weight (Table 17). There were significant differences in overall plant health. Inoculated *P. edulis* plants were significantly less healthy than plants from other treatments. 'Pandora' inoculated and *P. edulis* uninoculated both had poorer overall plant health than 'Pandora' uninoculated, 'DPI' uninoculated, and 'DPI' inoculated.

P. nicotianae was recovered from plants inoculated with *P. nicotianae*, and *F. o.* f.sp. *passiflorae* was recovered from plants inoculated with *F. o.* f.sp. *passiflorae*.
These results show that 'Pandora' is resistant to *P. nicotianae* in a growth cabinet situation. From the results of this trial the resistance of 'Pandora' to *F. o.* f.sp. *passiflorae* was not proven due to overall plant health not being as good as in the resistant check 'DPI'. Further screening of 'Pandora' using a more aggressive inoculation method is required.

Table 16. The effect of inoculation with *P. nicotianae* on % healthy roots, overall plant health and root dry weight of 'Pandora', *P. edulis* and 'DPI' (In the same column, values followed by the same letter are not significantly different at P=0.05).

Treatment	% Healthy roots	Overall plant health[a]	Root dry wgt (g)
'Pandora' uninoculated	99.5 a	4.7	0.194
P. edulis uninoculated	100.0 a	4.4	0.058
'DPI' uninoculated	100.0 a	4.9	0.112
'Pandora' inoculated	96.5 a	4.8	0.143
P. edulis inoculated	90.0 b	4.5	0.064
'DPI' inoculated	98.5 a	4.8	0.146
P	<0.001	0.122	0.096
lsd	3.61	-	-

^ Transformation did not improve residuals
[a] Overall plant health measured on a 1-5 scale; 1= Nearly dead, 2= Leaf loss and yellowing, 3= 1-2 leaves lost, 4= Stunted but healthy and 5= Vigorous

Table 17. The effect of inoculation with *F. o.* f.sp. *passiflorae* on % healthy roots, overall plant health and root dry weight of 'Pandora', *P. edulis* and 'DPI' (In the same column, values followed by the same letter are not significantly different at P=0.05).

Treatment	% Healthy roots	Overall plant health[a]	Root dry wgt (g)
'Pandora' uninoculated	99.5	4.7 a	0.194
P. edulis uninoculated	100.0	4.4 ab	0.058
'DPI' uninoculated	100.0	4.9 a	0.112
'Pandora' inoculated	91.0	4.0 b	0.108
P. edulis inoculated	99.0	3.3 c	0.059
'DPI' inoculated	100.0	4.8 a	0.159
P	0.464	<0.001	0.076
lsd	-	0.5605	-

^ Transformation did not improve residuals
[a] Overall plant health measured on a 1-5 scale; 1= Nearly dead, 2= Leaf loss and yellowing, 3= 1-2 leaves lost, 4= Stunted but healthy and 5= Vigorous

Experiment 2 - Screening of 'Pandora' for resistance to *Fusarium oxysporum* f.sp. *passiflorae* using the root dip inoculation method

The previous experiment to determine the resistance of 'Pandora' to *F.o.* f.sp. *passiflorae* using the wheat media inoculation method provided inconclusive results. This trial was conducted to determine the resistance of 'Pandora' to *F.o.* f.sp. *passiflorae* using the root dip inoculation method.

Materials and methods

Three passionfruit varieties were used in the trial: 'Pandora', *P. edulis* as a susceptible check and 'DPI' as a resistant commercial rootstock. Seeds of 'Pandora', *P. edulis* and 'DPI' were planted into Searles premium potting mix on 6/3/07 and germinated in the glass house.

F.o. f.sp. *passiflorae* (BRIP 28045) cultures were plated onto carnation leaf agar on the 30/3/07 and a spore suspension of 1 x 10^6 spores per mL produced using sterile distilled water on the 11/4/07.

On 11/4/07 when seedlings were at third true leaf stage they were separated and soil washed from their roots. Seedlings roots were then dipped into the spore suspension and the seedlings potted into seedling flats filled with Searles premium potting mix. Inoculated

seedlings were maintained in growth cabinets set at 27°C and 70% humidity to allow seedlings to grow. Seedlings were observed regularly for signs of disease development. On the 30/4/07 plants were assessed, and the number of healthy plants and the number of plants with vascular discolouration determined. Isolations were made from both inoculated and uninoculated plants onto SPDA.

Results and discussion

The 'Pandora' plants showed a very high level of resistance to *F. o.* f.sp. *passiflorae*. Of the 40 plants inoculated, all 40 plants survived (Table 18).

Table 18. The number of survivors and the number of 'DPI', *P. edulis*, and 'Pandora' vines with vascular discolouration after inoculation with *F.o.* f.sp. *passiflorae*.

Variety	Survivors	No of vines with vascular discolouration
'DPI'	40/40	0/40
P. edulis	16/40	0/16
'Pandora'	40/40	1/40

F. o. f.sp. *passiflorae* was not isolated from healthy vines. *F. o.* f.sp. *passiflorae* was consistently isolated from unhealthy *P. edulis* plants and from the one 'Pandora' showing vascular discolouration.

These results show that 'Pandora' could be planted in the field without first grafting provided the breeding lines of 'Pandora' are maintained in isolation and the high level of resistance to Fusarium wilt is maintained by preventing cross pollination. There is however a chance that a small number of plants will develop Fusarium wilt.

Pathogenicity Testing of *Phytophthora cinnamomi*

Phytophthora cinnamomi has not previously been suspected of infecting passionfruit and causing root death in Australian passionfruit, however there have been reports from overseas that it has been responsible for plant decline. Following initial testing of the pathogenicity of *P. c.* in three varieties of passionfruit, 'Heuston', 'DPI', and *P. edulis*, which showed possible infection and death of roots, further investigation was suggested to determine other possible causes of root decay.

The aim of this experiment was to determine whether the root decay observed in the initial pathogenicity testing of *P. cinnamomi* could be attributed to other factors such as anaerobic soil conditions brought on by water logging, toxic effect from wheat used in the wheat media, or a pre-existing pathogen in the potting mix.

Materials and methods

Eight plants of 'Heuston', 'DPI' and *P. edulis* were used in each of the treatments which were as follows:

1) Sterile potting mix - 'Searles premium potting mix' autoclaved for twenty minutes at 121°C
2) Standard potting mix - 'Searles premium potting mix' as taken from the bag
3) Standard potting mix with wheat media - 'Searles premium potting mix' mixed with triple autoclaved wheat media at a 5% concentration
4) Standard potting mix with *P. cinnamomi* - 'Searles premium potting mix' inoculated with *P. cinnamomi* wheat media at a 5% concentration

Plants used were raised from seed planted 10th March 2006 and potted on 7th April 2006. Plants were re-potted into their treatments 14th June 2006 and watered and allowed to settle in growth cabinets set at 27°C and 70% humidity. Pots were put into a waterlogged cycle from the 16th-19th June and watered as required from then onwards.

No symptoms were present by the 4th July 2006 so plants were placed on another wet cycle from the 4th-7th July 2006.

On the 31st July vines were removed from their pots, soils gently washed from roots and assessed for percentage of healthy root tips. Roots were cut from vine and dried at 60°C for 5 days and weighed to determine mass of dried roots. Isolations were made from diseased root tips onto SPDA and 10PVP.

Results and discussion

There were significant effects of potting media, variety and an interaction between potting media and variety for the percentage healthy root tips (Table 19). There was also a significant effect of potting media on dried root weight (Table 19).

Table 19. Effect of potting media and inoculation on percentage healthy root tips of 'DPI', 'Heuston' and *P. edulis* plants in growth cabinet trials June - July 2006 (In the same column, values followed by the same letter are not significantly different at P=0.05)

Treatment		Healthy Root Tips (%)	Root Weight (g)
Potting media			
Sterile potting mix		100.0 a	0.722 a
Potting mix		98.7 b	0.525 b
Potting mix with wheat media		99.0 ab	0.419 b
Potting mix with *P. cinnamomi*		97.62 c	0.751 a
	P	<0.001	<0.001
	lsd	1.002	0.1667
Variety			
'DPI'		99.7 a	0.590
P. edulis		98.84 a	0.608
'Heuston'		97.9 b	0.615
	P	<0.001	0.937
	lsd	0.8681	-
Interaction			
Sterile potting mix			
'DPI'		100.0 a	0.871
P. edulis		100.0 a	0.640
'Heuston'		100.0 a	0.656
Potting mix			
'DPI'		100.0 a	0.427
P. edulis		96.0 c	0.535
'Heuston'		100.0 a	0.613
Potting mix with wheat media			
'DPI'		100.0 a	0.432
P. edulis		99.4 a	0.385
'Heuston'		97.6 bc	0.441
Potting mix with *P. cinnamomi*			
'DPI'		98.8 ab	0.63
P. edulis		100.0 a	0.873
'Heuston'		94.1 d	0.752
	P	<0.001	0.278
	lsd	1.736	-

The plants with the highest percentage of healthy root tips were in the sterile potting mix. The plants in the media inoculated with *P. cinnamomi* had a significantly lower percentage of healthy root tips than the sterile potting mix, standard potting mix and potting mix with wheat media. Interestingly the dried root weight of plants grown in sterile potting mix and potting mix

inoculated with *P. cinnamomi* were not significantly different. 'Heuston' plants in potting mix inoculated with *P. cinnamomi* had the lowest percentage of healthy root tips, whilst *P. edulis* plants were the healthiest.

This experiment shows that *P. cinnamomi* is able to invade roots of Australian passionfruit to a limited extent. It is, however, unlikely that it will cause disease on passionfruit in the field.

The experiment also shows that the potting media has an effect on the percentage of healthy root tips and this factor must be considered in the future when conducting growth cabinet trials using commercial potting mix.

Experiment 7: Field evaluations of new hybrids from the variety evaluation program

The aim of this experiment was to assess the field performance of five new varieties grafted to two rootstocks.

Materials and methods

The trial was conducted in block B at Duranbah. 'Sweetheart', 'Jumbo Gem', 'Dawes', 'Tropic' and 'M2' were grafted to 'Selection 1' and 'Heuston' seedling rootstocks. Each combination was replicated three times. The vines were planted in December 2003. Yields were obtained by harvesting the mature fruit which had dropped to the ground. On the 1st June 2004 the vines were visually rated for assessed for crop load, symptoms of virus in vine and symptoms of virus in fruit.

On the 6th January 2005 fruit were harvested for internal quality tests by David Peasley. Fruit weight, pulp recovery and Brix were measured for 'Sweetheart', 'Jumbo Gem', 'Tropic' and 'M2'.

On the 12th May 2005, each fruit was harvested and assessed for the severity of Septoria, Alternata spot, and anthracnose. On the 7th June 2005 vines and fruit were assessed for fungal infections on leaves and virus symptoms on leaves and fruit.

Results

There were significant differences between yields for the scions tested. 'Sweetheart' yielded significantly more fruit than the other varieties during the autumn/winter crop (Table 20).

In the June 2004 assessments 'Jumbo Gem', 'Sweetheart', and 'M2' all had significantly less virus symptoms than 'Dawes' and 'Tropic' (Table 21). There was no rootstock effect on vine virus symptoms nor was there an interaction between scion and rootstock.

There was an interaction effect between scion and rootstock for the development of virus symptoms on fruit. 'M2' grafted to 'Heuston' developed significantly less severe virus symptoms on fruit than 'Jumbo Gem' and 'Dawes' grafted to 'Heuston' and 'Dawes' and 'M2' grafted to 'Selection 1'. However it was not significantly different to the majority of the rootstock scion combinations.

The internal quality tests of the fruit showed 'Sweetheart' to be significantly sweeter than the other varieties tested (Table 22). The highest pulp recovery was from 'Tropic'.

When fruit were assessed on the 12th May 2005 the disease levels were low and the severity of disease on most fruit was below a level which would affect marketability (data not shown). Stock and scion did not have any effect on the severity of Alternata spot, anthracnose or Septoria.

Discussion

The consistent cropping pattern, good autumn production, high sugar levels (Brix) and other agronomic characteristics identified by David Peasley and members of APIA meant 'Sweetheart' was deemed acceptable for release to industry and was released in 2005. Since it was released this variety has become very popular with growers. 'Jumbo Gem' has also found favour with a few growers but is not widely grown.

Table 20. Average yields for cultivar trial in block C at Duranbah for the autumn/winter crop 2004 (In the same column, values followed by the same letter are not significantly different at P=0.05).

Treatment	**Yield (fruit per vine)**
Scion	
'Sweetheart'	72.83 c
'Jumbo Gem'	46.33 b
'Dawes'	29.04 ab
'Tropic'	18.83 a
'M2'	13.51 a
P	<0.001
lsd	21.46
Stock	
'Heuston'	36.4
'Selection 1'	35.8
P	0.929
lsd	-
Interaction	
'Heuston'	
'Sweetheart'	83.67 e
'Jumbo Gem'	32.33 abcd
'Dawes'	42.67 bcd
'Tropic'	7.67 a
'M2'	15.67 ab
'Selection 1'	
'Sweetheart'	62.0 de
'Jumbo Gem'	60.33 cde
'Dawes'	15.41 ab
'Tropic'	30.0 abc
'M2'	11.35 a
P	0.047
lsd	30.34

Table 21. Effect of cultivar and rootstock on fruit and vine virus symptoms and crop load for trial in block B at Duranbah in 2004 (In the same column, values followed by the same letter are not significantly different at P=0.05).

Treatment	Virus symptoms on vines [a] (1-5)	Virus symptoms on fruit [b] (1-5)	Crop load on vine [c] (1-5)
Scion			
'Sweetheart'	2.54 b	3.93	2.79 b
'Jumbo Gem'	2.67 b	3.00	2.5 0 b
'Dawes'	1.54 a	2.84	3.62 c
'Tropic'	1.66 a	3.59	2.83 b
'M2'	2.78 b	3.60	1.62 a
P	0.014	0.167	<0.001
lsd	0.8254	-	0.6397
Stock			
'Heuston'	2.15	3.44	2.65
'Selection 1'	2.33	3.34	2.70
P	0.46	0.749	0.803
lsd	-	-	-
Interaction			
'Heuston'			
'Sweetheart'	2.67	3.67 abc	3.00
'Jumbo Gem'	2.33	2.33 a	2.33
'Dawes'	1.67	3.00 ab	3.67
'Tropic'	1.65	3.51 abc	2.66
'M2'	2.42	4.69 c	1.57
'Selection 1'			
'Sweetheart'	2.42	4.19 bc	2.57
'Jumbo Gem'	3.00	3.67 abc	2.67
'Dawes'	1.42	2.69 a	3.57
'Tropic'	1.67	3.67 abc	3.00
'M2'	3.15	2.51 a	1.66
P	0.547	0.024	0.676
lsd	-	1.413	-

* Overall transformation did not improve residuals hence untransformed data is presented.
[a]Vine - symptoms of virus, vigour, colour (1-poor heath, 5-good), [b]Fruit - Symptoms of virus in fruit (1- high no. of badly deformed fruit, 5-no deformed fruit), [C]Crop load (1-only 1 or 2 fruit, 5-high crop load),

Table 22. The internal quality of 'Sweetheart', 'Jumbo Gem', 'Tropic' and 'M2' of fruit harvested 6th January 2005.

Variety	Fruit weight (g)	Pulp recovery (%)	Brix (°)
'Sweetheart'	66.6	41.5	15.7
'Jumbo Gem'	93.2	40.5	13.1
'Tropic'	96.3	45.4	13.7
'M2'	83.3	36.6	13.8

Table 23. The effect of rootstock and scion on yield (as number of green fruit), fungal diseases on foliage, foliar virus symptoms and fruit virus symptoms (vines and fruit were assessed 7th June 2005) (In the same column, values followed by the same letter are not significantly different at P=0.05).

Cultivar	No of fruit	Foliar fungal symptoms [A]	Foliar virus symptoms [B]	Fruit virus symptoms [C]
Scion				
'Sweetheart'	83.8 ab	1.67 ab	2.67	2.00 a
'Dawes'	58.6 ab	1.95 b	2.96	2.98 c
'Tropic'	45.7 a	1.83 b	3.17	2.00 a
'Jumbo Gem'	169.8 c	2.50 b	2.42	2.15 b
'M2'	129.8 bc	0.75 a	2.75	2.00 a
P	0.013	0.02	0.11	<0.001
lsd	72.8	0.94	-	0.364
Rootstock				
'Heuston'	91.0	1.79	2.83	2.28
'Selection 1'	107.0	1.67	2.74	2.23
P	0.475	0.653	0.635	0.703
lsd	-	-	-	-

^Transformation of data did not improve residuals. [A] Fungal disease rated on a 0-4 scale. [B] Fruit virus rated on a 1-3 scale: 1 – unaffected, 2 - moderate distortion and 3 - severe distortion. [C] Vine virus rated on a 1-5 scale; 1 – healthy, 2 - slight mottle, 3 - moderate mottle, 4 - distortion & vein clearing, 5 - tip blight

Experiment 8: Tissue culturing

Tissue culturing has previously been used to 'clean-up' viruses from planting material (ref). The possibility of using meristem tissue culturing to produce virus free passionfruit was discussed with Dr Mike Smith, Principal Horticulturist, DPI&F. Dr Smith considers that it could be a successful method as researchers at QUT have had some success with the tissue culture of passionfruit. However tests of micropropagation were not carried out due to the discovery of a badnavirus infecting passionfruit from north Queensland (note section in virus section of report).

It is possible that badnavirus sequences may be integrated in the passionfruit genome as with banana streak virus and banana. With the banana streak virus/banana pathosystem the expression of virus diseases is heightened by tissue culture of banana. Work has begun to determine if the badnavirus is integrated into the passionfruit genome (meaning that it could be present in all passionfruit), and if the virus is transmissible between plants. Further work on micropropagation of passionfruit may be undertaken once the importance of the new badnavirus is determined.

Other areas of research carried out under PF04001

Interaction between *Phytophthora nicotianae* and *Fusarium solani*

A number of passionfruit growers experienced major losses of vines to due a disease caused by the interaction of *Phytophthora nicotianae* and *Fusarium solani*. We investigated the disease, determined the causal agents and produced a fact sheet for growers (Appendix 2) as well as submitting an article to 'The Passion Vine'.

Propagation of rootstocks by cuttings

As reported previously, the 'Selection 1' and *P. incarnata* crosses with 'Heuston' have good cold tolerance, good virus tolerance and good foliar disease resistance; however, they do not set seed which is a requirement for the development of a seedling rootstock. We intend to test these potential rootstocks by using cuttings. We have developed a protocol for the striking of cuttings. We have had a high success rate for root development using the new protocol.

Presence of *Fusarium oxysporum* f.sp *passiflorae* in trial site

During our assessments of vines in block C in July 2006 we identified *Fusarium oxysporum* infecting an ungrafted *P. edulis* vine. We do not know if this strain of *F. o.* f.sp. *passiflorae* is the same strain which devastated the passionfruit industry in the 1950's prior to the development of the *F. o.* f.sp. *passiflorae* resistant rootstocks. We will use molecular tests to determine the relationship between isolates from the BRIP Herbarium and the isolate recovered from the field throughout the trial. This is evidence that growers need to continue grafting to Fusarium wilt resistant rootstocks.

Recommendations

For industry and growers:

New varieties

Some of the varieties assessed in this project ('Sweetheart', 'Jumbo Gem' and 'Pandora') have been already released to industry, feedback from growers are that the varieties are growing well. If growers are thinking of planting new varieties they are advised to test a small number of vines on their farm before committing to large scale planting. Grafted 'Pandora' vines are performing poorly during winter in northern NSW and south-east Queensland.

Control of severe vine decline caused by an interaction of *F. solani* and *P. nicotianae*

The aim is to control *Phytophthora* not *Fusarium*:
* **Graft high (at least 30cm)** - 'DPI' and 'Heuston' rootstocks are resistant to *Phytophthora*, so high grafting to prevent soil splash onto the scion will discourage the disease.
* **Use phosphonate fungicides** (e.g. Fosject, Agrifos, Aus-phos). These slow the growth of *Phytophthora* and activate natural defence mechanisms in the host. There is an APVMA permit for the use of phosphonate to control *Phytophthora* of passionfruit.
* **Use cultural control practices to prevent splash dispersal of infested soil.** Some suggestions are; plant sweet smother grass under vines, use collars/guard around base of vine, mulching. Improve plantation drainage. Note that heavy applications of lime will increase *Phytophthora* activity.

For future research:

Rootstock and scion breeding

Peter Beal has recommended that a test cross of 50 plants of 'Misty Gem' x 'Selection 1' is planted in the field for assessment of field performance of vines and to determine if the cross is useful for introducing cold and virus tolerance in future scion breeding programs.

The cutting propagation method developed in this project should be used to propagate the 'Heuston' x *P. incarnata* cross and determine if 'Heuston' x *P. incarnata* could be used as a clonally propagated rootstock.

Seedlings of the seed produced from the ('Heuston' x *P. incarnata*) x 'DPI' cross should be planted out to examine of they maintain the cold and virus tolerance of the 'Heuston' x *P. incarnata* cross but also have the ability to set seed.

Virus research

- Mild strain cross protection appears to be ineffective, and unlikely to assist in virus control in the short term, using the virus isolates currently available.
- Passionfruit badnavirus sequences appear to be integrated into the passionfruit genome, but the incidence appears to be extremely low, and there is no evidence that the virus is being activated in the current breeding program. This virus should be considered a low priority at present.
- Progeny of the breeding program should be protected from field infection by viruses where possible and clonal material of promising lines maintained in virus-free glasshouse conditions. Reinfection rates are very high under natural cropping conditions, and levels of 100% have been recorded within 6 months of planting out of virus-free material.
- The individual effect of the different viruses infecting passionfruit still needs to be determined, as this will likely have a significant effect on the direction of any future cross-protection control strategies.

- It would be useful to study the genetics of resistance to viruses in *P. incarnata*, as this species could provide a source of resistance in future breeding work. *P. incarnata* has remained virus-free at the field trial site throughout this project, despite high inoculum pressures. F_1 hybrids of crosses with *P. edulis f sp flavicarpa* have also remained symptomless.

Defence activator work and analysis of defence related compounds

The techniques developed in this project can be used to better examine effect of putative defence activators for passionfruit.

Technology Transfer

'Passion Vine' Articles

- Anderson, J. M. and Pegg, K. G (2004) Anthracnose of Passionfruit 'The Passion Vine' December 2004 Pg 14-16
- Anderson, J. M. (2005) Update of HAL project PF04001 – Genetic improvement of passionfruit to achieve improved disease resistance 'The Passion Vine' March 2005 Pg 10-11
- Pegg, K., Anderson, J., Thomas, J. & Geering, A. (2006) Passionfruit Viruses 'The Passion Vine' January 2006 Pg 10-11
- Anderson, J. & Pegg, K. (2006) *Phytophthora nicotianae* and *Fusarium solani* interact to cause heavy vine losses. 'The Passion Vine' March 2006 Pg 14-15.
- Anderson, J., Pegg, K., Thomas, J., Willingham, S., Parmenter, K., Cooke, T., Dean, J., Smith, L., Peasley, D. & Beal, P. (2006) Genetic improvement of passionfruit to achieve improved disease resistance. 'The Passion Vine' March 2006 Pg 16.
- Anderson, J., Pegg, K., Thomas, J., Parmenter, K., Cooke, T., Dean, J., Smith, L., Peasley, D., and Beal, P. (2007) Passionfruit Pathology Update 'The Passion Vine' January 2007
- Anderson, J., Cooke, T., Pegg, K., Dean, J., and Smith, L. (2007) Use of fungicides to control diseases of passionfruit April 2007 Pg 14-15

Visits/Meetings

- 9th February 2005 - An update of project work was presented to the Australian Passionfruit Industry Association R & D committee meeting held at Growcom
- 26th August 2005 Update on project activities and planned future work. AGM and passionfruit levy payers meetings, Cairns.
- 27th August 2005 – 'Plant defence promoters' talk to growers APIA Field/fun day, Brampton Beach.
- 1st December 2005 – participated in Passionfruit Strategic Planning workshop
- 13th February 2006 – visit to John McLeod and Errol Hale to investigate severe vine decline
- 1st March 2006 – R&D meeting with Industry at Duranbah
- 11th April 2006 – follow-up visit to Errol Hale's property
- 20th – 21st April 2006 – field visit to Tony Kelly's farm to examine decline of vines
- 25th August 2006 – Presentation of project results and information at annual levy payers meeting
- 26th August 2006 – Attendance at APIA field and fun day at Woombye
- 26th September 2006 – Presentation at R&D committee meeting
- 21st February 2007 – Presentation at Passionfruit Industry R&D Committee Meeting at Duranbah

Reports

- Anderson, J. Pegg, K. & Peasley, D. (2006) Preliminary report on Visit to Tony Kelly's North Gregory Passionfruit Farm 21st April 2006. Report to APIA Executive meeting.
- Annual Reports to HAL (July 2005, July, 2006, July 2007)
- Milestone reports to HAL

Commercialisation /IP

A draft commercialisation plan was developed in conjunction with APIA (Appendix 3).

Acknowledgements

- David and Sue Peasley – Peasley Horticultural Services
- Mr Peter Beal
- Mr Bill Mumford – assistance with maintenance of the trial block and useful discussions
- Members of APIA executive who prepared block C art Duranbah for planting
- Dr Elizabeth Dann and Ms Jan Dean (DPI&F) for assistance with development of defence related compounds assays

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Yang IC, Hafner GJ, Revill PA, Dale JL, Harding RM (2003) Sequence diversity of South Pacific isolates of Taro bacilliform virus and the development of a PCR-based diagnostic test. Arch Virol 148: 1957-1968

Appendices

Appendix 1

Passionfruit Genetic Improvement Update

P R Beal 1.7.2004

1. Introduction

A detailed background to the breeding of *P. edulis* and *P. edulis* x *P. incarnata* hybrids is now provided. A table of the species and forms of special interest to the current breeding program is also presented. Implications for breeding are discussed.

2. The breeding of *P. edulis* and of *P. edulis* x *P. incarnata* hybrids 1955-1984.

2.1 *P. edulis* x *P. edulis* f *flavicarpa* (1955 – 1968)

This initial cross was made by Groszmann in 1955 using the GP50 accession of *P. edulis* f *flavicarpa*. The F_1 population involved 56 plants generally intermediate in characteristics to the parental forms. The F_1 clones E23, Lacey etc were selected. The F_2 population of 216 plants was most variable and the F_2 clones 3-1, 3-26 etc were selected (Groszmann pers comm.)

Groszmann and Meurant released selections to industry from around 1960. These selections combined high yield, winter and summer cropping, tolerance to PWV, root knot nematodes and Fusarium wilt.

Beal selfed and intercrossed selections from this program from 1963-1968. Continued selfing lead to loss of vigour and of desirable features and intercrossing of superior lines was found preferable in breeding. Also, Beal established yields for lines 3-1, 3-26 and *P. edulis* in replicated yield trials at Redlands Research Station over 1966-1968 of around 25, 30 and 10 tonnes/ha/yr respectively.

2.2 The hybrid *P. edulis* (F_1) x *P. incarnata* (1965-1968).

The cross *P. edulis* x *P. edulis* f *flavicarpa* was repeated by Beal in 1965. This *P. edulis* (F_1) hybrid as female was used in a subsequent cross with *P. incarnata* in 1967 to give the interspecific hybrid *P. edulis* x *P. edulis* f *flavicarpa* (F_1) x *P. incarnata* (Beal 1968).

The interspecific F_1 hybrid population totalled 35 plants with many chlorophyll – deficient and only three of normal appearance and only one of these latter plants being fertile. Fertility was low in this plant with few fruit set and very few seed per fruit. Beal recognised the potential value of this fertile hybrid in breeding with its freedom from foliar disease and acknowledged cold tolerance in south-east Queensland.

2.3 *P. edulis* (F_1) x *P. incarnata* hybrid derivatives (1968 – 1984)

2.3.1 General

The original *P. incarnata* clone was lost and this germplasm was resought (Farlow, 1981 DPI report). Seedlings of a new accession of *P. incarnata* were evaluated for PWV reaction by QDPI Plant Pathology Branch. No symptoms were seen after repeated inoculations with PWV. Also virus particles were only found in one plant.

2.3.2 The F_1 hybrid *P. edulis* (F_1) x *P. incarnata*

The fertile plant of the hybrid *P. edulis* x *P. edulis* f *flavicarpa* (F_1) x *P. incarnata* was observed by Farlow to have vigorous growth and be floriferous. Fruit set was generally very low and little seed was produced from bagging/selfing (Prytz pers. comm.). Natural fruit set improved when *P. edulis* f *flavicarpa* was adjacent and flowering well at the same time (Farlow pers. comm.). Fruit of the hybrid was small, 2-5 cm long, thin skinned, yellow green in colour, with <10 seed per fruit and with a small amount of light yellow pulp.

This fertile accession of *P. edulis* (F_1) x *P. incarnata* F_1 was found to be resistant to PWV and also to be cold tolerant. In addition it had resistance to Fusarium oxysporum (46% of plants) in seedling progeny tests documented by Farlow et al (1983).

2.3.3 *P. edulis* (F_1) x *P. incarnata* x E23

The first generation of plants from the outcross of the *P. incarnata* hybrid to E23 is complex. It may be best considered equivalent to a BC_1 generation. The size of this BC_1 generation which was available for selection is unclear. It seems most likely it was limited in size and thus in its genetic potential possibly because of low seed production from the cross to E23. This contention is supported by only SPS 1, 4, 5, 7 & 8 being retained as worthy (Farlow, 1982 DPI report).

Plants in this BC_1 generation were very vigorous while natural fruit set and yield was low (20% of normal commercial expectations) (Winks, Prytz pers. comm.).

Pulp content was recalled as being fair to good for the low yield of naturally set fruit (Winks pers. comm.). This contrasts with other observations of poor fruit and seed set. It also emphasises issues with such hybrids of the need to improve fertility, to understand their floral biology and the influence of pollen source, and compatibilities and environmental constraints.

Resistance to *Fusarium oxysporum* in seedling samples from the *P. edulis* (F_1) x *P. incarnata* x E23 BC_1 generation ranged from Selection 1 (45%) to 4 (75%) and 7 (80%) (Farlow et al 1983).

Farlow, Dale and Gilllespie (1979, DPI report) described field evaluation of the BC_1, and subsequent generation after inoculation with severe PWV. Resistance to PWV was recorded in both BC_1 and the subsequent generation as in the *P. edulis* (F_1) x *P. incarnata* hybrid. Few plants showed PWV symptoms, although resistant plants generally had PWV particles. Also, while there was segregation for resistance and high susceptibility in the BC_1 generation ratios were not documented.

Cold tolerance was observed in the BC_1 selections and the subsequent generation.

Vines from crossing *P. edulis* (F_1) x *P. incarnata* x E23 to commercial hybrids varied considerably in fruit size and colour (purple to yellow). Also, they had PWV symptoms and poor vigour and died after their second winter (Farlow, 1981- DPI report).

2.3.4 *P. edulis* (F_1) x *P. incarnata* x E23 x *P. edulis* f. *flavicarpa*

Several SPS (single plant selections) of the BC_1 (referred to in Section 3.3) were outcrossed to *P. edulis* f *flavicarpa* to produce effectively a BC_2 generation. It seems only a limited population was available and subsequently selected with only SPS 2-4,-6,-10,-30 & -37 and 3-10,-19 and 10-4,-10 considered worthy of retention. The number of plants established for each SPS in the BC_2 generation was uncertain. It was generally preferred as being a minimum of 10 (Prytz pers. comm.) but was obviously larger than this for further SPS within original selections 2 and 3. This may indicate better inherent fertility in Selections 2 and 3, now lost. Also SPS of Selection 1 in BC_2 were not apparently selected or retained.

Plants of the BC_2 selections had vigorous growth and were generally similar to f. *flavicarpa* as may be expected (Table 2) although there was considerable variation between selections. (Farlow, 1981 DPI report). Fruit set/yield was generally low around only 20% of normal commercial expectations (Winks, Prytz pers. comm.).

Resistance to *Fusarium oxysporum* in seedling samples from BC_2 selections was generally low ranging from 2-4 (19%), 2-10 (20%) to 3-19 (21%) (Farlow et al, 1983). Seedlings were believed to be from selfings (Winks pers. comm.) for seedling tests at each generation of selection. This contrasts with views that an open pollinated source was more likely (Prytz pers. comm.), The latter is supported by the substantial variability observed in seedlings of Selection/Line 3-19 and discussed later in this section. Also, such low levels of resistance are not consistent with a controlled seed source and the f. *flavicarpa* parentage (see Table 2).

Clearly, it is necessary to use a controlled seed source (selfed or specific cross) or even a clone with effective and reliable expression of a necessary attribute so the results of assessments may be uniform, repeatable and well understood.

PWV resistance and cold tolerance were observed in the BC_2 lines as in the BC_1 lines from which they were derived although any relevant ratios (of resistance and tolerance : susceptiblity) were not documented (Farlow, Dale and Gillespie, 1979 DPI report).

The stock potential of seedlings of Line 3-19 vs f *flavicarpa* was evaluated in field trials using selected commercial scions E23, Purple Gold and Lacey. Line 3-19 generally performed as well as f. *flavicarpa* over two years (Farlow, 1983 DPI report). The effect of rootstock on individual plants was highly variable particularly within the 3-19 treatments. This is believed to be due to the heterozygous nature of the material (Menzell et al, 1984). They considered it useful to breed further lines from 3-19 to reduce variability and select superior progeny in any future work. Alternatively, clonal propagation of rootstocks was another possibility.

3. The *Passiflora* species and forms of interest in genetic improvement.

A table of selected *Passiflora* species and forms and the attributes of value required in passionfruit genetic improvement has been developed (Table 1). This initial table with continued updating as required, should serve as a basic working reference for the current

genetic improvement program. As progress is made in obtaining and evaluating new accessions the table can be modified accordingly and made more complete.

4. Issues and Implications

4.1 Utilisation of *P. incarnata* hybrid derivatives

Only a small progeny of Selection 1 remains viable from the retained refrigerated seed collection from Maroochy Horticultural Research Station retained since 1985 for use in breeding. Moreover, the *P. edulis* x *P. incarnata* hybrid populations originally available for selection seem likely to have been small and not allowing maximum gains. *P. incarnata* with its unique PWV resistance still deserves a high priority for use in breeding. It would be beneficial in the long run to repeat the original cross viz by crossing *P.* 'Misty Gem' x *P. incarnata* to produce large populations with maximum variation and opportunities for selection gains. High productivity, including the components of high fruit and seed set, should be a priority selection criterion.

4.2 Low seed fertility in crossing in 2003/2004.

The low level of success in crossing in 2003/2004 was associated with periods of high (40°C) maximum day temperatures in summer and also with emphasis on Selection 1 as a prospective parent. The very low natural fruit set in Selection 1 plants is of concern. Plants in this small (9 plants) population need to be managed and evaluated as individual plants to identify those with superior performance and for their use in crossing. Additional available Selection 1 progeny should be established in the field for evaluation. Consideration should be given to more closely examining the floral biology and fruit and seed set of select/superior plants and factors which may be involved. The probability of useful gains in productivity using Selection 1 and its progeny in breeding should be continually evaluated. These measures should be used as well as the proposal to repeat the original cross as in 4.1.

4.3 Identifying Accessions of Potential Value

The accessions of interest to the genetic improvement program can be identified by continuing to establish resistances/adaptations etc that they may possess as shown in Table 1. Priority should be given to evaluating *P. alata* and *P. coccinea* as such germplasm (Table 1) may be directly useful as rootstocks or through hybridisation. Also, the strategic opportunity should be taken to cross *P.* 'Misty Gem' with each of *P. alata* and *P. coccinea*, and seed from crossing held, while the species are fully characterised.

4.4 Procedures for use in evaluation

The evaluation of an accession should include where possible both clonal material and seedling progeny from controlled pollination. Genetic ratios should be determined and interpreted from relevant progenies. An open pollinated seed line does not normally provide the required uniformity, repeatability and genetic understanding of a line based on selfing or intercrossing of known parents. In addition, appropriate differential / check varieties should also be included for comparison.

Table 1 **Selected *Passiflora* species and forms and their attributes of value for use in genetic improvement of commercial passionfruit (Beal, Pegg, Peasley etc 7/04 Draft)**

Attributes / Species and forms	Cold tolerance (5.1)	Tropical adaptation (5.1)	Non-suckering	Self-compatible	Resistance to *Fusarium oxysporum* (5.2)	Resistance to *Fusarium solani* (5.3)	Resistance to PWV (5.4)	Resistance to *Meloid. javanica* (5.5)	Produces fertile hybrids with *P. edulis* (5.6)
P. edulis	+		+	+	–	⊘	–	–	not applic.
P. edulis f *flavicarpa*		+	+	–*	+	⊘	+	+	+
P. edulis x *P. edulis* f *flavicarpa* F_1								+	+
'Misty Gem'				+	⊘	⊘			+
P. incarnata	+		–	–	+	⊘	+	–	+***
"Selection 1" (P. edulis (F_1) x P. incarnata x E23)					+ **	⊘	+	⊘	⊘
P. alata	⊘				⊘	⊘	⊘	⊘	+
P. coccinea	⊘		–	–	⊘	⊘	⊘	⊘	+
P. caerulea	+		-		+**			+	
P. ligalaris	+			⊘				–	

* Some lines self-compatible
** Moderate resistance
*** Barriers to crossing – some hybrid weakness

\+ yes
– no
⊘ worth testing

Table 2 **Estimated genetic contribution from specific parental forms to hybrids under evaluation (percentage)* Beal 7/04**

Species/hybrid	*P. edulis*	*P. edulis* f. *flavicarpa*	*P. incarnata*	Comment
P. edulis x *P. edulis* f. *flavicarpa* F_1	50	50		
P. edulis x *P. edulis* f. *flavicarpa* (F_1) x *P. incarnata*	25	25	50	
P. edulis x *P. incarnata* (F_1) (Ellison)	50		50	mid lavender fruit colour
P. edulis x *P. edulis* f. *flavicarpa* (F_1) x *P. incarnata* x *E23*	37.5	37.5	25	Sel. 1 parentage
P. edulis x *P. edulis* f. *flavicarpa* (F_1) x *P. incarnata* x *E23* x *P. edulis* f. *flavicarpa*	19 app	69 app	12 app	generally f. *flavicarpa* like
P. 'Misty Gem'	50 app	50 app		equal contributions assumed
P. 'E23'	50 app	50 app		equal contributions assumed

* ignores selection gains/losses and assumes random assortment of genes from each parental form.

5. Literature

5.1 Adaptation

5.1.1 (Geographic distribution of species)

Killip E. P. (1938). The American species of *Passifloraceae*. Field News, Nat. Hist. Bot. Series 19. Parts 1 and 2 public. 407 and 408, Chicago.

5.1.2 (Cold tolerance)

Patterson, B. D., Murata and Graham D. (1976). Electrolyte leakage induced by chilling in *Passiflora* species tolerant to different climates. Aust. J. of Plant Physiology 3, 435.

5.2 Resistance of species to Fusarium wilt

5.2.1 Purss, G. S. (1958). Studies of the resistance of species of *Passiflora* to Fusarium wilt (*F. oxysporum* f *passiflorae*). Queensland Journal of Agricultural and Animal Sciences 15, 95.

5.2.2 Purss, G. S. (1958). DPI project report.

5.2.3 Farlow P. J., Winks, C. W., Lanham, T. E. & Mayers, P. E. (1983). Genetic Improvement in Passionfruit, p. 96-98. Maroochy Horticultural Research Station Research Report No. 3 1981-1983, QDPI.

5.3 Resistance of species to *F. solani.*

5.4 Resistance of species to PWV

5.4.1 Winks C. W. (1982). Passionfruit Genetic Improvement, Open Day, Maroochy Horticultural Research Station 12.11.82, DPI, p. 49.

5.4.2 Winks, C. W., Menzell, C. M., Lanham, T. E. and Simpson D. (1988). Passionfruit Plant Improvement, progress report. Maroochy Horticultural Research Station Report No. 5, p 111 QDPI.

5.5 Resistance of species to *M. javanica* root knot nematode.

5.5.1 DPI trial (1967). Inoculation of *P. edulis, P. edulis* f *flavicarpa*, the F_1 hybrid, an F_2 population and hybrid 3-26 – assessment of root galling – (Beal unpub.).

5.5.2 DPI trial (1967) inoculation of 17 *Passiflora* accessions – assessment of root galling and presence of eggs. – (Colbran unpub.).

5.6 Species producing fertile interspecific hybrids with *P. edulis.*

5.6.1 (*P. edulis* x *P. caerulea, P. edulis* x *P. incarnata*)

Beal, P. R. (1968). Two new interspecific hybrids in the genus *Passiflora* Sabrao Newsletter 4, 113.

5.6.2. Beal P. R. (1975). Hybridisation of *Passiflora edulis* Sims and *Passiflora edulis* Sims f *flavicarpa* Degener. Queensland Agricultural and Animal Sciences Vol 32 (1) 101-111.

5.6.3 (*P. edulis* f *flavicarpa* x *P. alata*)

Nakasone H. Y. and Paull R. E. (1998). "Passionfruit" (p 280) in Tropical Fruits – Crop Production Science in Horticulture Series Ed. J Atherton and A Rees. CAB Intern. 445 pp.

5.6.4 (*P. edulis* and *P. coccinea*)

Success in hybridisation of *P. edulis* and *P. coccinea* : (T. Kehoe pers. comm.)

5.7 Other

Menzel C. M., Winks, C. W. and Simpson D. R. (1984). in Effect of Cultivar and Rootstock on Growth and Development of Passionfruit, p 160-162. Maroochy Horticultural Research Station Report No. 4, QDPI.

Appendix 2

***Phytophthora nicotianae* and *Fusarium solani* interact to cause heavy vine losses.**

Jay Anderson and Ken Pegg, Department of Primary Industries and Fisheries, Queensland.
Contact: Jay.Anderson@dpi.qld.gov.au or (07) 38969511
Prepared February 2006.

In recent weeks a number of growers have had major problems with vine decline, collapse and death. These notes are intended to give information on the interaction between the two fungi causing this decline and options for the control of the decline.

The extremely hot summer months with frequent tropical downpours have been tailor made for the soilborne fungi *Phytophthora nicotianae* and *Fusarium solani.* These fungi are very common in tropical and subtropical soils. Both fungi can be dispersed by the splashing of infested soil particles onto the stems of passionfruit vines.

Phytophthora will rapidly invade susceptible plant tissue. As both 'DPI' and 'Heuston' rootstocks are resistant to *Phytophthora* the fungus can only infect stems above the graft union (Photo 1).

Fusarium solani alone is unable to initiate attack on sound tissue or vigorous vines. Plants subjected to stress as a result of other diseases, wounding, old age, poor growing conditions, and insect attack are the most susceptible. In humid weather *Fusarium solani* may be seen on the stems of vines as a white mass (Photo 2).

Previous infection by *Phytophthora* provides an ideal entry point for *Fusarium solani.* The two fungi then interact to cause an extensive and serious stem rot which quickly kills the vine.

Disease management

The aim is to control *Phytophthora* not *Fusarium.*

1. Graft high (at least 30cm) - 'DPI' and 'Heuston' rootstocks are resistant to *Phytophthora*, so high grafting to prevent soil splash onto the scion will discourage the disease.

2. Use phosphonate fungicides (e.g. Fosject, Agrifos, Aus-phos). These slow the growth of *Phytophthora* and activate natural defence mechanisms in the host. There is an APVMA permit for the use of phosphonate to control *Phytophthora* of passionfruit.

3. Use cultural control practices to prevent splash dispersal of infested soil. Some suggestions are; plant sweet smother grass under vines, use collars/guard around base of vine, mulching. Improve plantation drainage. Note that heavy applications of lime will increase *Phytophthora* activity.

Photo 1. The *Phytophthora* invades only the susceptible part of the plant above the graft union.

Photo 2. The white fungal mass of *Fusarium solani* growing on a passionfruit stem.

Other information

During the sexual stage of *Fusarium solani* small red fruiting bodies (perithecia) form abundantly on infected stems (Photo 3.). Spores from these perithecia rarely initiate an attack alone on healthy tissue. They will only infect through wounds.

In the recent investigation we found that the rootstocks of affected vines were healthy; cankers were not present at the root collar and there was no root decay. This was despite the presence of both pathogens in the soil where the vines were growing.

***Fusarium* confusion**

Base rot is often confused with Fusarium wilt. Base rot is caused by *Fusarium solani* while Fusarium wilt is caused by the xylem invading pathogen *Fusarium oxysporum* f.sp. *passiflorae.* Any wilted plant from which any *Fusarium* was isolated has been often regarded as having the vascular disease *Fusarium* wilt. They are totally different fungi and they infect in different ways and produce different diseases.
The *Fusarium* wilt pathogen is a species without a sexual stage and it has no airborne phase. It only infects through the roots.

Photo 3. Red perithecia forming on a piece of stem.

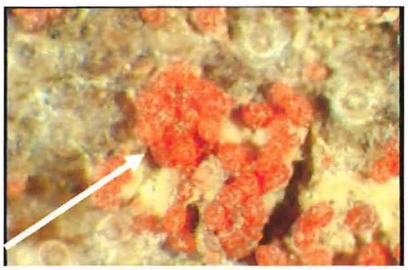

Photo 4. Close up photo of red perithecia forming on a piece of stem.

Appendix 3

Draft commercialisation Plan for material arising from DPI&F, HAL and APIA Passionfruit Breeding Program part of project PF04001

Queensland Government
Department of Primary Industries and Fisheries

HAL
Know-how for Horticulture™

Australian Passionfruit Industry Association

1

Commercialisation Plan for material arising from DPI&F, HAL and APIA Passionfruit Breeding Program

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Introduction

The aim of PF04001 is to increase the yield of quality fruit by improving vine health and increasing their tolerance to cold. The lack of cold tolerance and virus resistance in rootstocks and scions is impacting heavily on production. The fungal diseases Alternata spot and anthracnose continue to seriously affect vine health and downgrade or destroy fruit. Vines can be infected with up to five viruses and these viruses are severely affecting winter/spring fruit production.

The project will breed rootstocks with superior cold and virus tolerance. Rootstocks will be screened for resistance to the important soil borne pathogens. The project will continue with the intercrossing of commercial hybrids to improve disease resistance and increase yields of high quality fruit. The project intends to investigate mild-strain cross protection to reduce the severity of PWV.

This project will focus in the following areas:

1. Breeding and propagating rootstocks with superior cold tolerance and virus resistance
2. Breeding and selecting scion varieties with superior disease resistance and high productivity of quality fruit
3. Using mild-strain cross protection to reduce the impact of PWV

Industry will benefit from:

1. Rootstocks with improved virus resistance and cold tolerance
2. Hybrid scion varieties with increased disease resistance and high yields of quality fruit
3. More efficient management of foliar and fruit diseases

Rootstock improvement

Since 1958 (Grozmann & Purss, 1958) *Passiflora edulis* f. *flavicarpa* has provided a rootstock for the industry in terms of overall production and resistance to Fusarium wilt, nematodes and *Phytophthora nicotianae* root and stem rot. However, its performance during the winter in sub-tropical production areas is unsatisfactory due to its lack of cold tolerance

(*P. e.* f. *flavicarpa* is the least cold tolerant of *P. edulis* forms), which results in cessation of growth, poor production and severe virus infections. The *P. e.* f. *flavicarpa* rootstock itself is highly susceptible to virus diseases, and succumbs to base rot (caused by *Fusarium solani*) when it is below optimum health. In this project we identified a source of cold and virus tolerance in interspecific hybrids between *P. e.* f. *flavicarpa* and *Passiflora incarnata*. The best selection (C16V3 – *P. e.* f. *flavicarpa* 'Heuston' x *P. incarnata* cross made on 10th January 2005), which shows exceptional cold and virus tolerance, does not sucker, flowers well but fruit does not set seed suggesting a genetic incompatibility problem (Peter Beal pers comm., 2006). Without sufficient seed set this line will not be suitable as a rootstock in current propagation systems.

Two approaches could be taken to addressing the issue of seed set.

Firstly, backcrossing C16V3 to *P. e.* f. *flavicarpa* 'Heuston' may retain the characteristics of cold and virus tolerance as well as improving fruit set.

Secondly, the use of cuttings is a possibility. The Western Australian passionfruit industry, based on 'Sunshine Special', propagates planting material using semi-hardwood cuttings (Patricia Woods, pers comm., 2006). The passionfruit industry in Florida, USA also propagates planting material from cuttings (Jonathan Crane, pers comm., 2001).

For the passionfruit industry to adopt rootstocks propagated from cuttings a few questions may need to be answered:

a) Do rooted plants derived from physiologically mature stem cuttings root well enough for commercial production in nurseries and optimum performance in the field? This may require more research into the mechanisms of rooting in stem cuttings (e.g. callus tissue may be a barrier to root emergence).
b) Does the selected line have adequate commercial resistance to Fusarium wilt, *F. solani* base rot, nematodes and Phytophthora root and stem rot? This will require glasshouse testing for resistance to the various pathogens.
c) Does the selected line confer high sustainable yields of uniform fruit quality? Will there be a physiological incompatibility between the rootstock and the scion?

A good rootstock not only protects against the development of soil borne diseases but also needs to be physiologically compatible with the scion. Rootstock/scion interactions may mediate an effect on yield, quality, and disease susceptibility. This may occur through uptake and distribution of nutrients and carbohydrate flow to roots.

Scion improvement

Current commercial passionfruit production in Australia is based on:

a) hybrids between *P. edulis* f. *edulis* and *P. e.* f. *flavicarpa* (Rigden and Newett, 2006) and
b) selections within *P. e.* f. *flavicarpa* for more tropical production areas (Rigden and Newett, 2006)

Previous scion breeding work has concentrated on the inter-crossing of commercially accepted hybrids to recombine the genetic variability that exists in the genotypes. Progeny has been selected for good agronomic performance and resistance to fruit and foliar diseases with particular emphasis on resistance to alternata spot (caused by *Alternaria alternata*). Useful types resulting from previous scion breeding include 'Sweetheart' and 'Jumbo Gem'. Some hybrids are more tolerant of virus diseases than others.
However, this program did not provide genetic resistance to virus diseases or superior cold tolerance. For these traits it is necessary to investigate novel material.

In this project interspecific hybrids between *P. edulis* and *P. flavicarpa* hybrids and *P. incarnata* have shown healthy foliage and vigorous growth during the winter months while other lines not incorporating *P. incarnata* were severely affected by cold and virus. This material will now be incorporated in to the scion improvement program.

Current systems for distribution of scions and rootstocks

Rootstocks

P. edulis f. *flavicarpa* 'DPI' rootstock seed is currently distributed by Department of Primary Industries & Fisheries (DPI&F). The rootstocks have resistance to nematodes, Phytophthora root rot (caused by *Phytophthora nicotianae*) and Fusarium wilt (caused by *Fusarium oxysporum* f. sp. *passiflorae*).

The rootstocks are grown in isolation at the Maroochy Horticultural Research Station (MHRS) at Nambour. The resistance to Fusarium wilt is thought to be through a single dominant gene. The vines are maintained in isolation to reduce the likelihood of pollination from plants which do not carry resistance to Fusarium wilt.

Staff at MHRS check the integrity of each batch of seed by testing for resistance to Fusarium Wilt. Approx 100 plants are screened for resistance for each batch of seeds collected. *P. edulis* f. *edulis* seedlings are included as susceptible checks.

Each batch is assigned a batch number and the percentage germination and percentage resistance to Fusarium wilt is recorded.

Rootstock seed is sold to passionfruit growers and nurserymen on a cost recovery basis.

Scions

The scion material distribution scheme is administered by Australian Passionfruit Industry Association (APIA). Vines are sold through APIA licensed nurseries. Royalties are charged on a per vine basis. Members of APIA pay a lower royalty fee than non-members. If a non-member places an order of less than 200 vines the order is subject to an administration fee. The vines are sold to growers after they have signed a non-propagation agreement.

References

Grozmann, H.M. and Purss, G.S. (1958) Beating passionfruit wilt. Queensland Agricultural Journal **84**: 214-226

Ridgen, P and Newett, S. (2006) Passionfruit Growing Guide. Department of Primary Industries & Fisheries, Brisbane.

Project equity

The equity split is based on the contributions by Horticulture Australia Ltd (HAL), DPI&F and APIA for the projects PF04001 and PF04002. The equity split is as follows; HAL 68% (to be added to passionfruit levy pool), DPI&F 24% and APIA 8%.

IP Exploitation

Scion material

A license will be provided to APIA to manage distribution of any new scion material through APIA licensed nurseries to growers who have signed a growers agreement.

Estimated cash flow

In 2005/2006 approx. 62,500 vines were sold through APIA licensed nurseries. According to the Secretary for APIA this is approximately the usual number of vines distributed each year. A royalty of 35c per vine (approx. 12% of cost of vine) would be charged to members of APIA. Non-members would be charged 70c per vine. Non-members who order less than 200 plants will be charged a $100 administration fee. The cost of management of the scheme would be subtracted prior to the split of the royalties. This cash flow estimation has been made on the basis that most orders for vines will be made by members of APIA.

62,500 vines x $0.35per vine	= $21875.00
Minus 10% management fee	= $ 2187.50 (to be paid to APIA)
	= $19687.50
To be split between	
HAL (68%)	= $13387.50(to be added to passionfruit levy pool)
DPI&F (24%)	= $ 4725.00
APIA (8%)	= $ 1575.00

Rootstock material

If this project is successful in identifying or producing superior rootstocks, the material will be released commercially through the existing DPI&F commercial distribution scheme. The fee for the material will be to cover the cost of maintaining and providing the material. The price will be reviewed annually.

IP Protection / Plant Breeders Rights

Between HAL, APIA and DPI&F investigate the economic feasibility of registering several scions with plant breeders rights (PBR). However, at this stage it is felt that the licensing regime used by APIA provides sufficient IP protection.

International

DPI&F and APIA would collaborate to investigate the feasibility of protecting the material in an overseas market (such as South Africa) and having a distribution agent for it in the country which it is being sold. The distribution agent would register the material with PBR.