Characterisation and management of Fusarium wilt of watermelon

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VM12001

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Summary

Fusarium wilt is one of the most severe diseases of watermelon and is caused by the soil-borne fungus, *Fusarium oxysporum* f. sp. *niveum* (*Fon*). Although found in other Australian States, *Fon* was first detected in the Northern Territory (NT) in 2011. Following the outbreak, this project VM12001, commenced in November 2012 with the following objectives;

1. Identify the NT *Fon* race(s) and compare with other Australian and international (USA) *Fon* isolates
2. Screen rootstocks and grafted watermelon seedlings for resistance to *Fon*
3. Raise awareness of Fusarium wilt, deliver outcomes and propose management options to industry through extension strategies

Despite delays due to three biosecurity incursions in the NT- banana freckle, *Cucumber green mottle mosaic virus* (CGMMV) and myrtle rust, the project's outcomes were achieved and the findings are summarized below.

Temperature has a significant effect on the observed wilt symptoms in cultivars 'Sugar Baby', 'Royal Armada' and 'Kalahari' during the 'build up' months of November and December. Wilt symptoms were either delayed, reduced or completely suppressed depending on aggressiveness of the isolate or the cultivar tested. Our study showed that *Fon* isolate NTP-Dc 36955 caused a higher percentage of wilt compared to NTP-Dc 36953. 'Kalahari' was the most susceptible cultivar, followed by 'Royal Armada' then 'Sugar Baby'.

Race differentials across six cultivars clearly showed that the two NT *Fon* isolates were identified as race 3, the most aggressive and virulent race, previously only recorded in Maryland, USA. Race typing using isolates from Queensland, Western Australia, Victoria and New South Wales showed that race 2 and race 3 were also present across the states. The work conducted in this project is the first comprehensive race differential research to be completed in Australia for *Fon*.

A glasshouse trial using three *Cucurbita* rootstocks ('RTX1', 'RS 841' and 'Carnivore') grafted with 'Royal Armada', were 100% resistant to the two NT *Fon* isolates compared with the highly susceptible non-grafted watermelon 'Royal Armada'. Two seedless cultivars 'Kalahari' and 'Bullseye' showed low tolerance to both NT isolates. The grafted *Cucurbita* rootstocks could provide resistance in *Fon*-infested field. However, further field trials to assess agronomic characteristics and scion compatibility would be necessary.

An in-field rootstock agronomic trial showed that, under NT conditions, *Cucurbita* rootstocks significantly reduced fruit weight compared to non-grafted controls. Although fruits were harvest prematurely due to rain events and magpie geese destruction, grafting had no effect on the fruit quality (firmness and sweetness) compared to the non-grafted treatment. *Cucurbita* rootstocks grafted with the pollinator 'Red Tiger' did not perform well in the field which may be due to scion and rootstock incompatibility. Further rootstock trials are required to evaluate and assess suitability of *Cucurbita* rootstocks in southern states using other *Fon* races.

Finally, the specific *Fon* PCR assay was able to detect the NT isolates and some other Australian and USA *Fon* isolates, however it failed to detect all isolates used in this study. In the circumstances where *Fon* is suspected, but the PCR assay is negative, than it is impertinent that pathogenicity and/or race differential trials are performed to confirm *Fon* is the causal agent of the wilt symptoms observed. Putative effector 'secreted in xylem' genes were detected in the *Fon* isolates included in this study. However, sequence similarity analyses between the *Fon* races showed minimal diversity, thereby the usefulness of the SIX genes, as a diagnostic marker, is questionable. As such, additional Australian and USA *Fon*

isolates are currently undergoing next generation sequencing to expand the genetic data and enable diagnostic markers to be evaluated.

Since the lifting of quarantine measures for CGMMV in late February 2016, all NT growers are now able to return to growing watermelons. As part of the extension activities, NT growers were asked for feedback on whether they would use grafted or seedless watermelons for production. The consensus among growers is that grafted watermelons are expensive and growers would move ahead with seedless watermelons.

The project results were presented at the 2016 Australian Melon Association Industry conference in Mildura and project outcomes will be uploaded on the project website. The project results will be published in peer-reviewed journals as well as inclusion in Victor Puno's, University of Sydney, PhD dissertation.

Keywords

Watermelon, *Fusarium oxysporum* f. sp. *niveum*, Fusarium wilt, rootstock, race differentials, seedless watermelon, grafted and PCR.

Introduction

Fusarium wilt is one of the most severe diseases in watermelon (*Citrullus lanatus* (Thun.) Matsum & Nakai) and is caused by *Fusarium oxysporum* formae specialis *niveum* (*Fon*). This f. sp. is only pathogenic on watermelons and can be divided into four races (0, 1, 2 and 3) (Crall 1963, Elmstrom & Hopkins 1981. Netzer 1976, Netzer & Weintall 1980, Zhou *et al.* 2010). Two of the races have been detected in Australia (Horlock 2004) but there is limited information on what these races are. Fusarium wilt of watermelon occurs on every continent except Antarctica (Egel and Martyn 2007). The disease is one of the major yield limiting factors in production, worldwide (Zhang *et al.* 2005). Symptoms include damping-off, seedling wilt or disease during any stage of plant development (Bruton *et al.* 2007). The fungus can survive many years in the soil as chlamydospores and can spread by soil, plant debris, farm machinery and seeds (Martyn and Bruton 1989, Bruton *et al.* 2007). Since 2000, there has been an increase in the triploid seedless watermelon industry in the USA, Europe and Australia. Prior to this, only resistant melon varieties were grown in Australia (Horlock 2004). However, these triploid melons had little or no resistance to Fusarium wilt (Paulus *et al.* 1976, Bruton *et al*. 2007). It was not until Fusarium wilt became a major limiting factor before grafted watermelon seedlings were used as an alternative. Grafting watermelon seedlings onto resistant cucurbit rootstocks is common practice in many countries as a management option (Lee 1994, Lee and Oda 2003, Boughalleb *et al.* 2007, Besri 2008, Dau *et al.* 2009). In Australia, using grafted watermelon is becoming a common commercial practice (S. Smith and B. Condé, pers. comm.).

In May 2011, there was an outbreak of Fusarium wilt of watermelon in the NT. Triploid seedless watermelon seedlings and plants from six locations in the NT expressed symptoms such as leaf necrosis, necrotic blotching and seedling deaths in seedling trays, as well as, wilting and vine collapse in the field (Tran-Nguyen *et al.* 2012). No vascular browning was observed except for one mature field plant; similar observations were previously recorded in Vietnam (Dau *et al.* 2009).

Races of *Fon* are not as clearly defined as other *Fo* where races are based upon gene-gene relationship on differential cultivars (Larkin *et al.* 1990, Risser *et al.* 1976). As such, *Fon* races are differentiated according to their aggressive variability on differential cultivars. Investigations have indicated that there are no distinct *Fon* races which could be clearly defined (Zhou *et al.* 2010 and references therein). When race classification is based upon virulence alone, then specific assay methods are used which would indicate that races 0 and 1 are the same but different to race 2 which is more virulent (Zhou *et al.* 2010 and references therein). Race 0 causes wilt in susceptible cultivars such as 'Florida Giant' and 'Sugar Baby'. Race 1 was considered more virulent than race 0 because it was pathogenic to some moderately resistant varieties (Cirulli *et al.* 1972). Race 2 was pathogenic to race 1 resistant varieties such as 'Calhoun Gray', 'Summit' and 'Smokylee' (Netzer 1976, Netzer and Dishon 1973). Martyn and Netzer (1991) suggested using PI-29631-FR as a differential to classifying *Fon* races. Using this system, race 0 only infects 'Sugar Baby'; race 1 infects 'Sugar Baby' and 'Charleston Gray' but not 'Calhoun Gray' and PI-29631-FR; race 2 causes wilt in all differentials except PI-29631-FR. A newly described race 3 from Maryland, USA was found to be highly virulent to PI-29631-FR as well as the three other differentials (Zhou *et al.* 2010).

Methodology

The research encompassed several areas – race differentials, effect of temperature on *Fon*, evaluation of rootstocks and seedless watermelons, and improved molecular characterization and extension and building capacity. All glasshouse and field trials and laboratory work were conducted at Berrimah Research Farm unless stated otherwise. For each trial, two NT isolates were included, NTP-Dc 36953 and 36955. For the full methodology, refer to Appendix 1.

Experiment 1: Temperature glasshouse trials

A pot trial was conducted to evaluate the effect of temperature on *Fon* symptom expression in three watermelon cultivars: 'Sugar Baby', 'Kalahari' and 'Royal Armada' (Table 1). Two experiments were conducted simultaneously in dual locations in a 'hot' ambient temperature screen house and a 'cold' temperature-controlled glasshouse, average daily maximum temperature of 43.3 and 31.7°C, respectively. The trial was conducted over 40 days, from 23rd October to 2nd December 2015, during the build-up season in the NT. Plants were inoculated and monitored daily for symptoms of *Fon* and if symptoms were observed, isolations were conducted to confirm the presence *Fon*. At 40 days, all remaining inoculated but asymptomatic plants had isolations conducted to confirm if *Fon* was present.

Table 1. Watermelon cultivars and treatments evaluated in the temperature glasshouse trials.

Cultivar	**Temperature**	***Fon* isolate/treatment**
'Sugar Baby'	'Hot'	NTP-Dc 36953, NTP-Dc 36955, PDA control
	'Cold'	NTP-Dc 36953, NTP-Dc 36955, PDA control
'Kalahari'	'Hot'	NTP-Dc 36953, NTP-Dc 36955, PDA control
	'Cold'	NTP-Dc 36953, NTP-Dc 36955, PDA control
'Royal Armada'	'Hot'	NTP-Dc 36953, NTP-Dc 36955, PDA control
	'Cold'	NTP-Dc 36953, NTP-Dc 36955, PDA control

Experiment 2: Race differentials

Six watermelon cultivars were evaluated for their susceptibility to two NT isolates to determine the *Fon* race of the isolates. Race differentials of *Fon* are based according to variability in aggressiveness on differential cultivars, where a resistant reaction is <33% wilt and a susceptible reaction is ≥ 33% wilt (Martyn and Bruton, 1989; Zhou *et al.* 2010) (Table 2). Race differential trials in the NT were conducted using a modified method of Zhou *et al.* (2010). Modifications included the use of mycelial cultures instead of spore suspensions for the plant inoculations and the inclusion of additional cultivars 'Crimson Sweet' and 'All Sweet' (Keinath and DuBose, 2009). Due to the unavailability of PI-296341-FR, 'SP-4' was used as a suitable replacement due to its resistance to *Fon* race 2 which is derived from PI-296341-FR by Syngenta plant breeders (Zhang, 2009).

Each cultivar was subjected to three inoculation treatments using mycelial cultures of two NT *Fon* isolates and an un-inoculated PDA control. Experiments were conducted over 28 days in a temperature controlled glasshouse and air temperatures were recorded. Each cultivar was tested once in 2014, and then all trials were repeated between March and September in 2015. 'SP4' was tested three times in total, once in 2014 and twice in 2015.

Table 2. Revised race differential cultivars used to determine *Fon* race in the NT trials.

Genotype	Race 0	Race 1	Race 2	Race 3
'Sugar Baby'	S	S	S	S
'Crimson Sweet'	R	S	S	S
'Charleston Gray'	R	S	S	S
'All Sweet'	R	R	S	S
'Calhoun Gray'	R	R	S	S
'SP-4'	R	R	R	S

Experiment 3: Rootstock and seedless watermelons resistance trial

In milestone report 104, we reported the preliminary findings from experiments using *Cucurbita* and *Lagenaria* rootstock, their vigour and success rate of grafting watermelon scions onto the rootstocks. We found that *Cucurbita* rootstocks showed greater early vigour and scions 'took' faster compared to *Lagenaria*. Unfortunately, further investigations using *Lagenaria* was halted due to the *Cucumber green mottle mosaic virus* (CGMMV) outbreak which greatly restricted access to available rootstocks that were certified as CGMMV-free.

A 40-day glasshouse trial was conducted to evaluate *Cucurbita* specific hybrid rootstocks and seedless watermelon cultivars for resistance to *Fon*. Three grafted rootstocks (RTX), two seedless lines and non-grafted 'Royal Armada' as a control, were subjected to three inoculation treatments including two NT *Fon* isolates, and an un-inoculated PDA control (Table 3).

Table 3. Grafted rootstocks and watermelon cultivars assessed for resistance to *Fon* in the glasshouse resistance trial in 2015. See Appendix 4 for the full list of rootstocks and seedless lines used in this study.

Cultivar	Species	Treatment
'RTX1'	*C. maxima x C. moschata*	Grafted with 'Royal Armada'
'RS 841'	*C. maxima x C. moschata*	Grafted with 'Royal Armada'
'Carnivore'	*C. maxima x C. moschata*	Grafted with 'Royal Armada'
'Kalahari'	*Citrullus lanatus*	Non-grafted
'Bullseye'	*Citrullus lanatus*	Non-grafted
'Royal Armada'	*Citrullus lanatus*	Non-grafted

Experiment 4: Rootstock agronomic field trial

Due to the limited number of rootstocks that were CGMMV-free and available for trials, it was decided to include an extra trial. Based upon the glasshouse trials, we confirmed that the *Cucurbita* rootstocks were resistant to *Fon*. A field trial was conducted to evaluate the effect of grafting with *Cucurbita* specific hybrid rootstocks on the agronomic characteristics of the watermelon crop (Table 4). 'Red Tiger', a seeded diploid cultivar, was used as the scion and as a pollinator for fruit production. The trial was planted at Berrimah Farm in a paddock with a history of vegetables, cover crops and watermelons in a Kandasol sandy loam soil. The trial was conducted over 72 days, from 8th September to 19th November 2015, and the grafted and non-grafted seedlings were planted at 55 and 32 days old, respectively, with 1 m spacing's (Figure 1).

Grafted plants were observed for growth and compatibility between rootstocks and the scion. Crop nutrient levels were monitored by taking leaf samples at weeks 3, 6 and 9 post-planting for analysis by CSBP Laboratories and levels were compared to industry standards. The harvest was brought forward due to rain causing fruit splitting. Four harvest events were undertaken at 66, 70, 71 and 72 days post planting. At the first harvest, ten fruit per treatment were assessed for fruit firmness using a fruit pressure tester (mod FT 011) and fruit sweetness using the Brix test. Statistical analyses are provided in Appendix 1.

Table 4. Treatments evaluated in the rootstock agronomic field trial in 2015.

Cultivar	**Species**	**Treatment**
'RTX1'	*C. maxima x C. moschata*	Grafted with 'Red Tiger'
'RS 841'	*C. maxima x C. moschata*	Grafted with 'Red Tiger'
'Carnivore'	*C. maxima x C. moschata*	Grafted with 'Red Tiger'
'Red Tiger'	*Citrullus lanatus*	Non-grafted

Figure 1. Planting of seedlings in the rootstock agronomic field trial in 2015.

Experiment 5: Molecular characterisation of Fon isolates

Molecular characterisation of *Fon* isolates included testing isolates using the specific *Fon* PCR assay (Lin *et al.* 2010); 'secreted in xylem' (SIX) genes derived from previous studies on other Fusarium pathosystems such as *F. oxysporum* f.sp. *lycospersici*, Fusarium wilt of tomato and *F. oxysporum* f. sp. *cubense*, and Fusarium wilt of banana (Rep *et al.* 2004, Meldrum *et al.* 2012). Isolates used in this study are listed in Table 5.

Table 5. Molecular characterisation of *Fon* isolates.

Race	Sample Identifier	Location
0	F-121-2	Maryland, USA
1	F-016-1	Maryland, USA
1	F-079-1	Maryland, USA
1	F-107-1	Maryland, USA
2	F-17B-1-29	Maryland, USA
2	F-17B-1-3	Maryland, USA
2	VP 088	Queensland, Australia
3	MDZE-6221A	Maryland, USA
3	VP 0457	New South Wales, Australia
3	VP 0583	Western Australia, Australia
3	VP 0585	Queensland, Australia
3	NTP-Dc 36953	Northern Territory, Australia
3	NTP-Dc 36955	Northern Territory, Australia

NB. USA samples provided by Dr. Kathryne L. Everts (University of Maryland/University of Delaware). VP samples were provided by Victor Puno, University of Sydney.

In collaboration with the University of Queensland and the Australian Genome Research Facility, Brisbane, NT *Fon* isolates underwent whole genome sequencing using MiSeq next generation sequencing (NGS) in search for SIX genes. The NGS study was expanded to include USA and additional Australian isolates from QLD, NSW, WA and VIC. At the time of this report, the final data for the NGS of the wider range of *Fon* isolates was not yet available. These will be analysed later and included in a publication in a peer-reviewed journal.

Outputs

- Australasian Plant Pathology Society newsletters.
- Presentation at the NT DPIF and Northern Australian Quarantine Service in November 2015.
- Conference posters at the Australasian Plant Pathology Biennial conference in Auckland (2013) and Fremantle (2015) – Appendix 2.
- Posters at the Fred's Pass Rural Show and the Royal Darwin Show.
- Article in Grow NT Edition 5 August 2013.
- Numerous grower meetings for Fusarium wilt of watermelon and *Cucumber green mottle mosaic virus*.
- Presentation at the Bayer 2016 Australian Melon Industry Conference and Field Days March 16-18 2016.
- NT grower survey to raise the question whether grafted seedlings would be adopted. Feedback indicates that after the CGMMV quarantine period ended, growers will use seedlings and not the costly grafted seedlings option.
- Draft manuscript detailing the results achieved from VM12001 with the intention to submit into Australasian Plant Pathology journal.
- *Website (*http://www.nt.gov.au/d/Primary_Industry/index.cfm?header=Characterisation%20and%20management%20of%20Fusarium%20wilt%20of%20watermelon).
- PhD dissertation (Victor Puno – University of Sydney) is currently ongoing with due date in 2017/2018.

Outcomes

The project outcomes across all activities are listed below.

Experiment 1: Temperature glasshouse trials

- There was a significant (P<0.001) effect of temperature on observed wilt symptoms in 'Sugar Baby', 'Royal Armada' and 'Kalahari' cultivars
- Average daily soil and air temperatures greater than 30°C, in the 'hot' treatment either delayed, reduced or completely suppressed wilt symptoms depending on the isolate and cultivar tested
- 'Kalahari' was the most susceptible cultivar; followed by 'Royal Armada' then 'Sugar Baby'; *Fon* isolate 36955 caused higher percent wilt than *Fon* isolate 39653
- Results in 2015 confirmed previous glasshouse observations in 2014, whereby wilt symptoms were suppressed in infected 'Sugar Baby', 'Royal Armada' and 'Nightshade' which were grown in hot temperatures during the build-up months (Nov-Dec)

Experiment 2: Race differential glasshouse trials

- Results from glasshouse race differential trials in 2015 showed that all six cultivars ('All Sweet', 'Calhoun Gray', 'Charleston Gray', 'Crimson Sweet', 'SP-4' and 'Sugar Baby') were susceptible (≥ 33% wilt) to the two NT *Fon* isolates, indicating *Fon* race 3 is present in the NT
- Race differentials were also conducted using *Fon* isolates from Victoria, Queensland, New South Wales and Western Australia and identified races 2 and 3 (Victor Puno)
- This is the first comprehensive race differential work for *Fon* to be conducted in Australia and the first record of the highly aggressive race 3 outside of Maryland, USA and now in Australia

Experiment 3: Rootstock and seedless watermelons resistance trial

- 'Royal Armada' grafted with *Cucurbita* rootstocks ('RTX1, 'RS 841' and 'Carnivore') were resistant to the NT *Fon* isolates (0% wilt), compared to non-grafted 'Royal Armada' which were highly susceptibility (100% wilt)
- Seedless cultivars ('Kalahari' and 'Bullseye') showed low tolerance (80-100% wilt) to both NT *Fon* isolates
- *Cucurbita* rootstocks grafted with 'Royal Armada' could provide resistance in *Fon* infested fields, however field trials are needed to assess the agronomic characteristics and scion compatibility under field conditions in the NT

Experiment 4: Rootstock agronomic field trial

- *Cucurbita* rootstock treatments significantly (P<0.0001) reduced fruit weight at harvest compared to the non-grafted control treatment
- There was no detectable effect of grafting on fruit quality (fruit firmness or sweetness)
- *Cucurbita* rootstocks grafted with 'Red Tiger' scion did not perform well in the field, which was potentially due to incompatibility between the rootstock and scion

Experiment 5: Molecular characterisation of Fon *isolates*

- Current *Fon* specific PCR assay does not detect all *Fon* isolates. Pathogenicity and race differential assays will need to be conducted to confirm *Fon* for isolates that are negative in the PCR test but clearly displays Fusarium wilt symptoms

Evaluation and Discussion

Experiment 1: Temperature glasshouse trials

There was a significant effect of temperature ($P<0.001$) on the observed wilt symptoms for all three cultivars (Table 6, model 2). There was no significant effect of *Fon* isolate on observed wilt symptoms, except for 'Kalahari' (Table 6, model 1). Figures 2 and 3 depict temperature glasshouse trial results for the NT isolates. In the 'cold' treatment, all plants had wilted by day 40 for both of the NT *Fon* isolates, except 'Sugar Baby' where 90% of plants had wilted. For 'Royal Armada', wilt was reduced in the 'hot' treatment by 70% and 40% for *Fon* isolates 36953 and 366955, respectively, compared to the 'cold' treatment. For 'Kalahari', wilt was reduced in the 'hot' treatment by 60% for *Fon* isolate 36953 compared to the 'cold' treatment; however, wilt was not reduced for *Fon* isolate 36955. For 'Sugar Baby' no wilt was observed at 40 days post inoculation for both isolates. Plants with symptoms of wilt were confirmed as *Fon* by isolations and PCR. After 40 days, *Fon* was isolated and confirmed by PCR from 100% of all remaining inoculated, but asymptomatic plants, for all three cultivars, except 'Sugar Baby' where *Fon* was isolated from 90% of plants in the 'hot' treatment.

'Sugar Baby' began to wilt in the 'cold' treatment at 10 days, and by 40 days 90% of the plants had wilted compared to no wilt observed in 'hot' treatment, for both *Fon* isolates (Figure 4). In the 'hot' treatment, observed wilt in 'Kalahari' was both delayed and reduced when inoculated with the *Fon* 36953 isolate, and wilt was delayed but not reduced with the *Fon* 36955 isolate (Figure 5). For 'Royal Armada', the 'hot' treatment slightly delayed wilt symptoms with *Fon* isolate 36955 and significantly reduced wilt for both *Fon* isolates (Figure 6). The isolation of *Fon* from all asymptomatic plants in the 'hot' treatment indicates that despite being infected with *Fon*, plants grown in the 'hot' treatment either delayed, reduced or completely suppressed symptom expression. Results from the 2015 trials confirmed previous glasshouse trials conducted in 2014. 'Sugar Baby', 'Royal Armada' and 'Nightshade' cultivars inoculated with *Fon* 36953 and 36955 had both delayed and reduced observed wilt when grown in the 'hot' screen house during the build-up months (November to December 2014) compared to plants grown in the 'cold' glasshouse. In the 'hot' treatment, wilt in 'Sugar Baby' was reduced by 70% and 60% when inoculated with *Fon* 36953 and 36955, respectively, compared to plants grown in the 'cold' treatment (data not shown). Similarly, wilt was reduced in 'Nightshade' by 70% and 40% when inoculated with *Fon* 36953 and 36955, respectively, and wilt in 'Royal Armada' was reduced by 90% for both isolates in the 'hot' treatment compared to the 'cold' treatment (data not shown). In each case, *Fon* was re-isolated from the asymptomatic plants in the 'hot' treatment.

Although there is little literature on the effect of temperature on *Fon,* it has been reported that wilt caused by *Fon* was reduced at soil temperatures above 30°C, and wilt was absent at soil temperatures above 33°C (Walker 1941 and Holliday 1980). Similar results were observed in the trial conducted in this project, where the average daily soil temperature of 30.9°C reduced symptoms in 'Royal Armada', reduced or delayed symptoms in 'Kalahari', and completely suppressed wilt symptoms in 'Sugar Baby'. Average daily air temperatures were over 7°C higher in the 'hot' treatment (31.7°C) compared with the 'cold' treatment (24.4°C) (Table 7). The average daily maximum air temperature of 43.3°C experienced in the 'hot' treatment was 11.6°C hotter than the conditions experienced in the 'cold' treatment (Table 7). As expected, temperatures were slightly buffered by the soil with temperatures on average a few degrees cooler in soil than the ambient air temperatures (Table 7). The average daily soil temperatures were over 8°C higher in the 'hot' treatment (30.9°C)

compared with the 'cold' treatment (22.2°C) (Table 7). The average daily maximum soil temperature of 37.5°C experienced in the 'hot' treatment was 11.7°C hotter than the conditions experienced in the 'cold' treatment (Table 7). Our results clearly show that both high soil and air temperatures can delay, reduce or completely suppress wilt symptoms despite plants being infected, and this temperature effect is influenced by both the *Fon* isolate and cultivar being tested.

Table 6. Model results for the effect of temperature and *Fon* isolate on the numbers of plants wilting up to 40 days post inoculation. Model 1 includes both effects and the interaction. Model 2 includes only the Temperature effect. Degrees of Freedom (d.f.) is shown for each with the Maximum Likelihood (ML) deviance (Appendix 1) and the probability of the effect being a significant effect in the model.

'Cultivar' / Effect	**Model 1:**			**Model 2:**		
	Wilt~ Temperature * Isolate			**Wilt ~ Temperature**		
	d.f.	ML deviance	Pr(>Chi)	d.f.	ML deviance	Pr(>Chi)
'Sugar Baby'						
NULL	38	307.608				
Temperature	36	280.986	<0.001	37	281.013	<0.001
Isolate	37	307.605	0.959			
Temperature * Isolate	35	280.961	0.876			
'Kalahari'						
NULL	38	294.021				
Temperature	36	245.67	<0.001	37	267.405	<0.001
Isolate	37	278.541	<0.001			
Temperature * Isolate	35	245.255	0.519			
'Royal Armada'						
NULL	38	300.52				
Temperature	36	261.224	<0.001	37	268.863	<0.001
Isolate	37	297.773	0.01			
Temperature * Isolate	35	259.758	0.226			

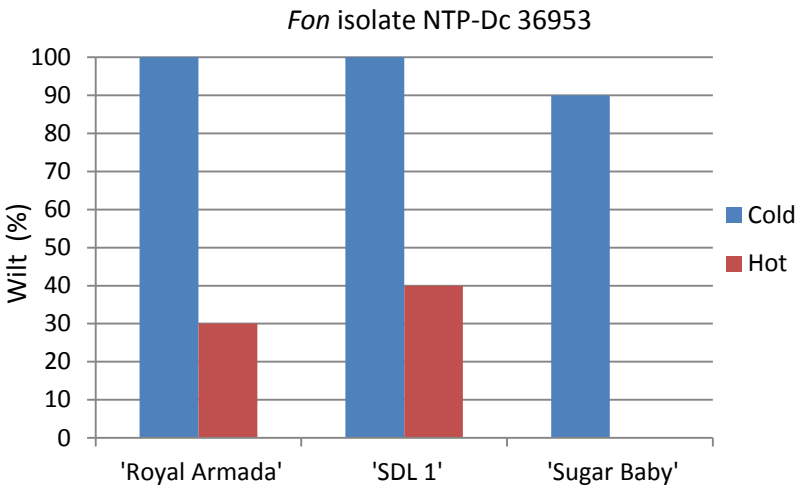

Figure 2. Percent wilt in watermelon seedlings at 40 days post inoculation using *Fon* isolate NTP-Dc 36953 in the temperature glasshouse trials (Experiment 1). 'SDL1' is 'Kalahari'.

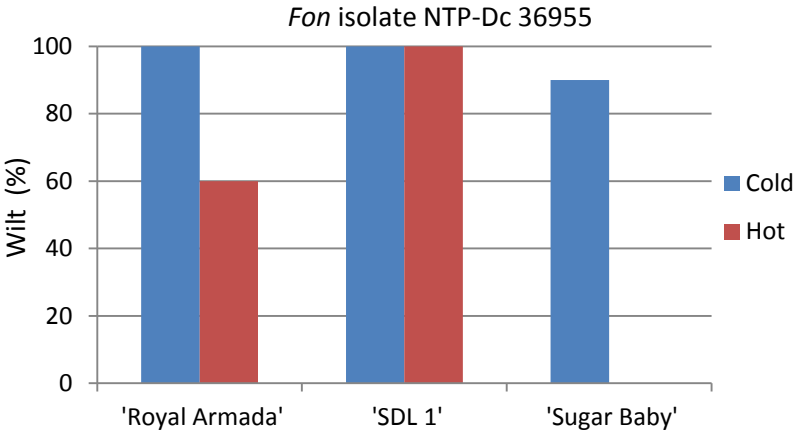

Figure 3. Percent wilt in watermelon seedlings at 40 days post inoculation using *Fon* isolate NTP-Dc 36955 in the temperature glasshouse trials (Experiment 1). 'SDL1' is 'Kalahari'.

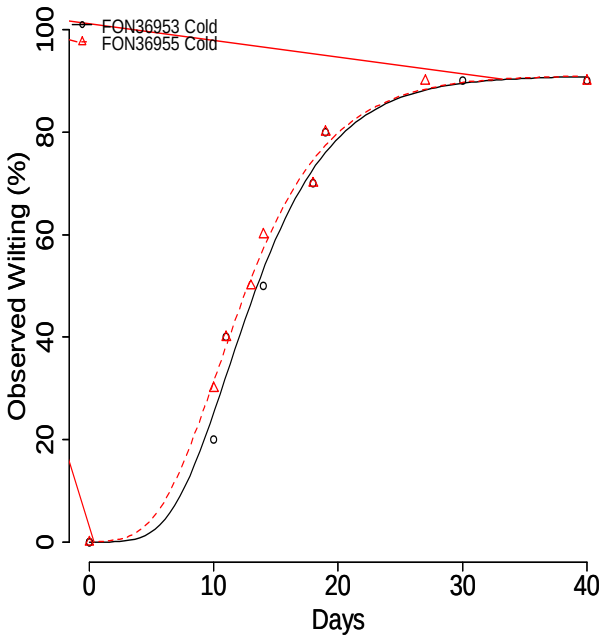

Figure 4. Percent wilt observed in 'Sugar Baby' seedlings at 40 days post inoculation in temperature glasshouse trials (Experiment 1) (see Table 6, Model 2). No wilt was observed in the 'hot' treatment'.

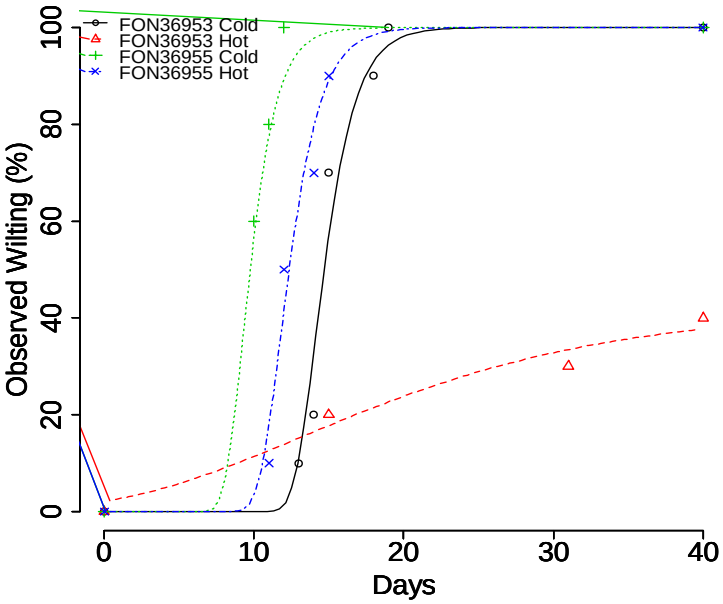

Figure 5. Percent wilt observed in 'Kalahari' seedlings at 40 days post inoculation in the temperature glasshouse trials (Experiment 1) (see Table 4, Model 2).

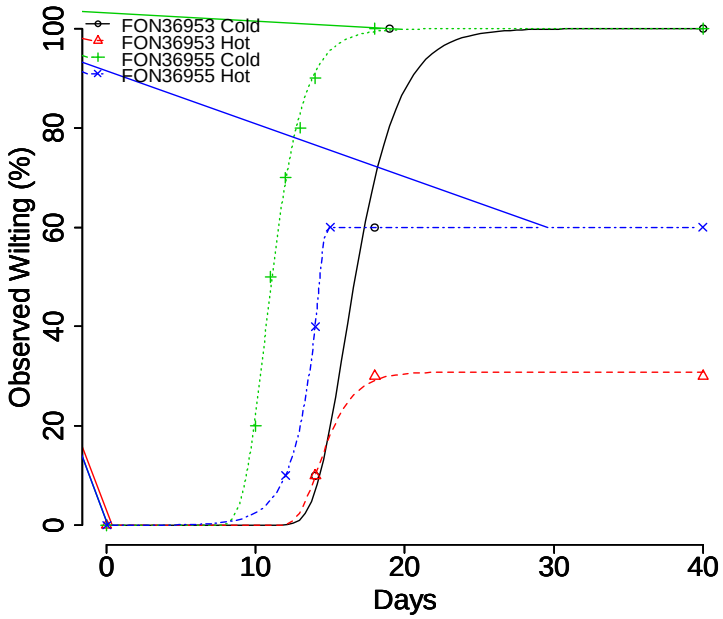

Figure 6. Percent wilt observed in 'Royal Armada' seedlings at 40 days post inoculation in in the temperature glasshouse trials (Experiment 1) (see Table 6, Model 2).

Table 7. Average daily soil and air temperatures in the glasshouse trials (Experiment 1) between October and November 2015.

	Soil Temperature (°C)			Ambient Temperature (°C)	
	'cold'	'hot'		'cold'	'hot'
Average Daily Maximum	25.8	37.5		31.7	43.3
Average Daily Minimum	20.9	26.5		21.8	26.1
Average Daily Temperature	22.2	30.9		24.4	31.7

Experiment 2: Race differentials

All six cultivars were susceptible to both NT *Fon* isolates tested with wilt ranging from 45% to 100% depending on the *Fon* isolate and cultivar (Table 8). The susceptibility of 'Crimson Sweet' and 'Charleston Gray' eliminated race 0, and the susceptibility of 'All Sweet' and 'Calhoun Gray' eliminated race 1. The susceptibility of 'SP-4' (Figure 7) helped to delineate between races 2 and 3, and these results were confirmed by the NSW results for 'SP-4' as well as repeating the trial twice in the NT in 2015. All PCR tests conducted on the single-spored cultures obtained from isolated material were positive for *Fon*, confirming that the symptoms observed were caused by *Fon*.

Table 8. Percent wilt and susceptibility reaction of six cultivars to two NT *Fon* isolates in the race differential trials (Experiment 2) conducted in the NT in 2015.

Cultivar	NTP-Dc 36953	Reaction		NTP-Dc 36955	Reaction
'All Sweet'	100%	S		98%	S
'Calhoun Gray'	88%	S		85%	S
'Charleston Gray'	45%	S		63%	S
'Crimson Sweet'	98%	S		88%	S
'SP-4' (1)*	53%	S		65%	S
'SP-4' (2)*	62%	S		63%	S
'Sugar Baby'	98%	S		92%	S

S indicates a susceptible reaction (≥ 33% wilt), R indicates a resistant reaction (<33% wilt).
*SP-4 was tested once in 2014 and twice in 2015

'Calhoun Gray', 'Charleston Gray' and 'SP-4' were previously reported in milestone 104 as having a resistant reaction to *Fon* in the 2014 trials. Pathogenicity results can be greatly affected by factors including concentration and method of inoculation, age of plants being inoculated, environmental conditions, and source of the differential genotypes (Zhou *et al.* 2010). Issues with the glasshouse temperatures during the 'Calhoun Gray' trial in 2014 may have affected the results, whereby hotter temperatures may suppress symptom development as alluded to by Walker (1941) and Holliday (1980). The effect of 'hot' temperatures on wilt suppression is previously reported (Experiment 1) and Martyn (2014) also alluded to temperature as a factor which can affect the reliability and reproducibility of *F. oxysporum* pathogenicity tests. After repeating the 'Calhoun Gray' trial in 2015, results clearly show a susceptible reaction (88% and 85% wilt for *Fon* isolates 36953 and 36955, respectively), which is consistent with published literature (Zhou *et al.* 2010). Trials conducted in NSW also confirmed a susceptible reaction (83% wilt) in 'Calhoun Gray' to the *Fon* isolate 36955 in (data not shown). 'Charleston Gray' is notorious for not reacting the way it should in differential host trials, therefore it is important to source genuine seed (Martyn 2014). 'Charleston Gray' can also be affected by the level of infestation. Stevenson (1957) showed 'Charleston Gray' was resistant to wilt when infestation in the field was light, yet wilt ranged from 25% to 100% when infestation was moderate to high. In 2015, care was taken to use cultures freshly isolated from diseased plants to ensure the virulence of the cultures was maintained and the results clearly showed a susceptible reaction (45% and 63% wilt for *Fon* isolates 36953 and 36955, respectively) in 'Charleston Gray' (Table 4).

'SP-4' has 'intermediate resistance' to *Fon* race 2 which is comparable to 'SP-5' or 'SP-6' cultivars (Zhang and Brusca, 2011; Brusca and Zhang, 2012). Despite initial results in 2014 indicating a resistance reaction, after repeating the 'SP-4' experiments twice in 2015 we obtained a consistent susceptible (53-65% wilt) reaction. These results were also confirmed in the NSW trials which showed a susceptible reaction (50% wilt) to *Fon* isolate NTP-Dc36955 (data not shown). Based on all of these results, we were able to confidently delineate between races 2 and 3, and conclude that race 3 is present in the NT which documents the first record of *Fon* race 3 in Australia. Race 3 is a new and highly virulent race of *Fon*, previously only reported in Maryland in the USA (Zhou *et al.* 2010). Our trials show consistency in results between using mycelial cultures compared to spore suspensions for plant inoculation, and these results were validated by repeating experiments multiple times.

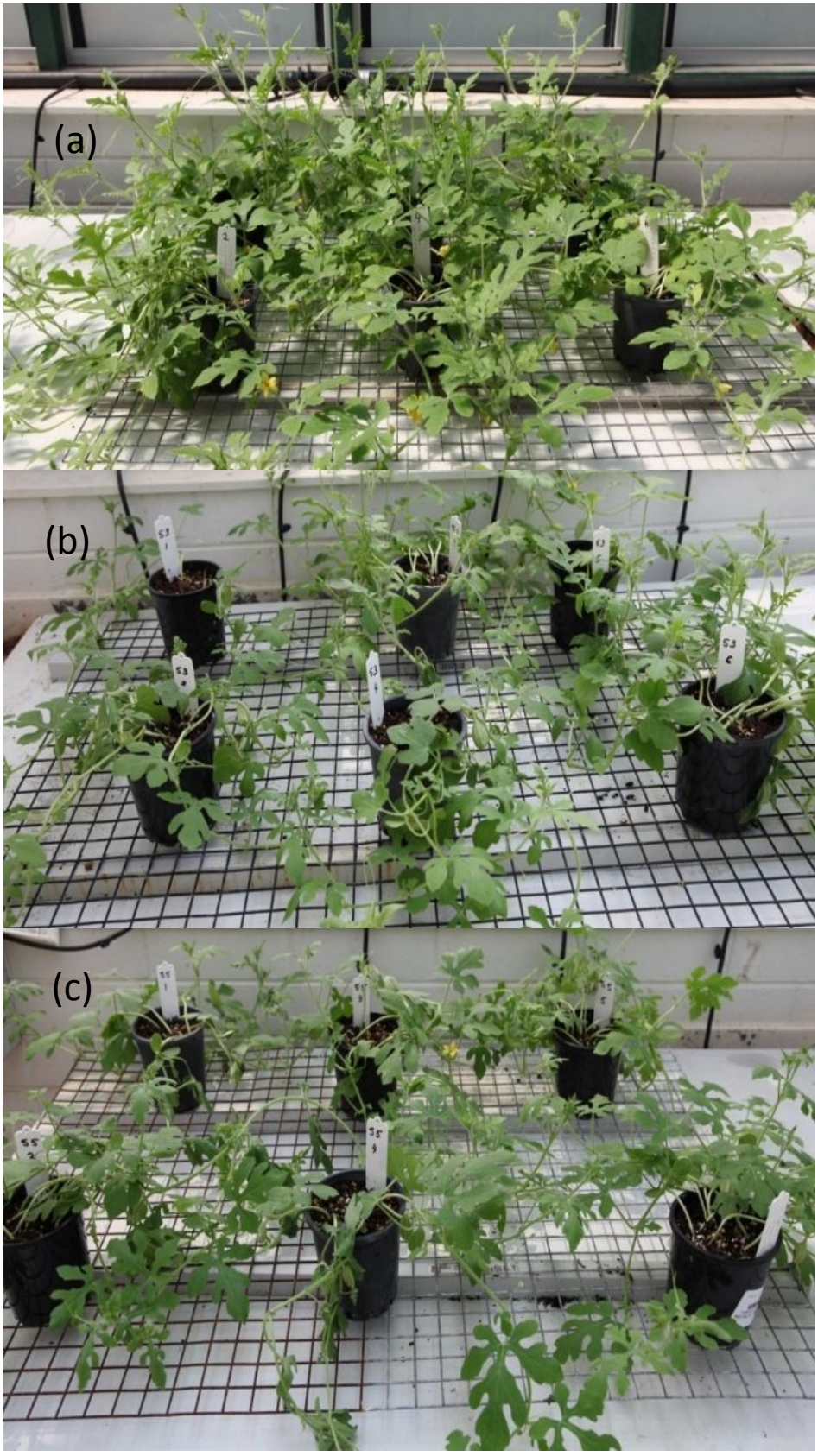

Figure 7. 'SP-4' plants at 20 days post inoculation in the (a) un-inoculated PDA control compared to plants (b) inoculated with *Fon* isolates NTP-Dc 36953 and (c) NTP-Dc 36955 (Experiment 2).

Experiment 3: Rootstock and seedless watermelons resistance trial

Results for the rootstock and seedless watermelons resistance trial are presented in Table 9. No *Fon* was isolated from control plants in all treatments. No *Fon* was isolated from inoculated plants in the rootstock treatments 'RTX1', 'RS 841', and 'Carnivore', indicating total resistance (0% wilt) to both NT *Fon* isolates. The resistance of the *Cucurbita* rootstocks is likely due to the inability of the pathogen to penetrate and colonise the rootstocks vascular system given the host specificity of *Fon* to watermelon (Martyn 2014). However, stunting and plant death observed in some 'RTX1' and 'RS 841' plants from both control and inoculated pots may indicate minor incompatibility between the rootstock and scion, given that no *Fon* was isolated from these plants.

Fon was isolated from all wilted 'Royal Armada' and seedless 'Kalahari' and 'Bullseye' plants inoculated with both *Fon* isolates. 'Kalahari' and 'Bullseye' showed a low tolerance (0-80% wilt) to both NT *Fon* isolates. Similarly, 'Royal Armada' showed low tolerance (100% wilt) to both NT *Fon* isolates (Table 9) which was consistent with results presented in Experiment 1. These results show that 'Royal Armada' and the two seedless cultivars have low tolerance to *Fon* race 3 which has significant implications for the management of *Fon* in watermelon production in Australia.

This study shows that grafting a commercially desired cultivar which is highly susceptible to *Fon* (i.e. 'Royal Armada') onto a resistant *C. moschata* x *C. maxima* rootstock can provide total resistance to the highly aggressive *Fon* race 3. However, poor development and plant death in some of the grafted plants in the glasshouse may be an indication of incompatibility with the scion (Edelstein *et al.* 2004). Therefore, field trials should be done to assess the agronomic qualities and compatibility of *Cucurbita* rootstocks grafted with 'Royal Armada' in field conditions in the NT.

Table 9. Percentage *Fon* isolated from three rootstocks treatments, two seedless cultivars and the susceptible control 'Royal Armada' in the resistance trial (Experiment 3).

Cultivar	NTP-Dc 36953	NTP-Dc 36955	Control	Pr (>Chi)
'RTX1'	0	0	0	na
'RS 841'	0	0	0	na
'Carnivore'	0	0	0	na
'Kalahari'	100	90	0	~1.00
'Bullseye'	90	80	0	~1.00
'Royal Armada'	100	100	0	na

Experiment 4: Rootstock agronomic field trial

Plant vigour was reduced in the grafted treatments compared to the non-grafted treatment. Yellowing and dying of the older leaves was observed in the grafted treatments (Figure 8) which was likely due to new leaves extracting nutrients from older leaves caused by nutrient stress. Leaf analysis indicated deficiencies in phosphorus, potassium and calcium in all treatments, and boron deficiency in the grafted treatments (Table 10). Typically, potassium, calcium and magnesium are important nutrients at the time of planting. Whole root systems were excavated at the end of the trial to assess root development. Grafted plants showed

root galling and reduced lateral roots compared to the non-grafted plants. Inadequately sized seedling trays can effect seedling root development (Handreck *et al*. 2010), and may have caused the observed root galling and reduced root development in the grafted plants given that the grafted plants were 20 days older than the non-grafted plants old at the time of planting. The additional time that the grafted plants were grown in the seedlings trays may have caused root damage which contributed to poor root development and performance in the field. While rootstocks can sometimes provide a stronger root system that is capable of increased nutrient uptake, this was not necessarily the case in the current rootstock trial.

These differences were also reflected in the yield, with a significant difference detected in fruit weight for harvest events 1 ($F_{3,48}$=9.6, P<0.0001), 2 ($F_{3,115}$=71.0, P<0.0001), 3 ($F_{2,77}$=15.4, P<0.0001) and 4 ($F_{3,50}$=17.2, P<0.0001). A post-hoc Tukey's test indicated that the non-grafted plants had significantly higher fruit weight than all three grafted treatments (Table 11). These results were similar to Yetisir *et al.* (2003) who also found that grafting watermelon with *Cucurbita* rootstocks reduced yield. Reduced leaf cover and deficiency of boron at 3 weeks post planting may have affected fruit set in the rootstocks treatments in the current trial. Napier (2011) showed that growing grafted rootstocks in a sandy loam soil where fusarium wilt is absent resulted in reduced yields; however, their study used *Lagenaria* (bottle gourd) rootstocks and was conducted in the Riverina in NSW which is very different climatic conditions to the NT. While the rootstock agronomic field trial was only observational, the results clearly indicate that *Cucurbita* rootstocks may not be suitable in the NT.

Unlike Yetisir *et al.* 2003, our study did not detect negative effects of grafting caused by *Cucurbita* rootstocks on fruit quality (firmness and sweetness). There was no significant difference in fruit firmness ($F_{3,40}$=71.0, P=0.208) or fruit sweetness ($F_{3,40}$=2.2, P=0.101) between the grafted and non-grafted treatments (Table 11). Napier (2011) also showed no difference in total soluble sugar (TSS) content in grafted plants; however in *Lagenaria* rootstocks. The planting of the current trial was delayed due to complications caused by the CGMMV outbreak in the NT. Consequently, the trial was planted late in the Dry season; however, a typical watermelon crop in the NT would be planted in staggered plantings throughout the Dry season and crops are usually harvested prior to the build-up season (Sept to Nov). However, our trial was conducted over a 3-month period during the NT's build-up season, in a non-*Fon* infested sandy loam soil During the trial, the average daily minimum and maximum air temperatures were 22.6°C and 35.6°C, respectively, with an average daily air temperature of 28.5°C. These temperatures and growing conditions clearly did not suit the *Cucurbita* rootstocks, given that the non-grafted plants grew quite well in comparison. In addition, because the fruit was harvested prematurely (at 66-72 days post planting) due to rain in November which caused fruit splitting, and the early harvest may have affected fruit sweetness. Further trials need to be conducted in cooler growing months to evaluate true growing conditions and evaluate the fruit sweetness in comparison. Further field trials to assess more *Cucurbita* rootstocks may provide evidence on whether this type of rootstock is suitable for the NT. Similar evaluations in southern states also need to be assessed as well as the evaluation of *Lagenaria* rootstocks.

In the rootstock agronomic field trial, reduced plant vigour and yield in the grafted plants indicated a stress (i.e. nutrient deficiency, heat or water stress) that were potentially caused by incompatibility between the rootstock and 'Red Tiger' scion and also possibly damage caused from the seedling trays, as previously alluded to. Similar observations showing incompatibility between the rootstocks and the 'Royal Armada' scion were seen in the glasshouse trial. Edelstein *et al.* (2004) described incompatibility between squash rootstocks and melons expressed as poor development and leaf necrosis in the glasshouse, delayed or

poor development in the field, or plant collapse enhanced by fruit load even in disease free soils. Several of these symptoms were observed in the NT trial. Rootstock-scion compatibility can depend on specific combination of rootstock and scion, and compatibility can vary under different environmental conditions (Lee and Oda 2003). Yetisir and Sari (2003) showed *Cucurbita* type rootstocks promoted vegetative growth compared to non-grafted controls, and lower graft affinity and incompatibility with the scion caused lower yield and fruit quality in *Cucurbita* type rootstocks compared to *Lagenaria* type rootstocks. We showed that *Cucurbita* rootstocks reduced vegetative growth and yield, but did not detect any negative effects on fruit quality. This work needs to be repeated using *Lagenaria* rootstocks.

It may be recommended that NT growers to grow watermelons without grafting in non-*Fon* infested fields or with grafting onto rootstocks other than *Cucurbita* rootstocks in infested fields. Grafting onto a well-selected non-host rootstock can have many benefits including resistance to soil borne disease, increased water and nutrient uptake, salinity tolerance, low soil temperature tolerance, increased fruit size and number and extended harvest duration (Lee 1994; Davis *et al.* 2008). Of these, control of soil borne disease including Fusarium wilt, is the most common and important reason for grafting watermelons (King *et al.* 2008). In some cases, grafting on *Cucurbita* rootstocks may be required when watermelon resistance to a certain disease, such as *Fon* race 3, is not available. Further replicated field trials are needed to evaluate other rootstocks types including *Lagenaria* to determine compatibility with commercially desired scions and suitability to the NT's growing conditions i.e. soil type and temperature.

Due to the later planting date of the trial, magpie geese on their seasonal migration began damaging fruits. A large netted structure was built to cover the entire trial and the number of damaged fruit was very low and not considered to affect the harvest results. In addition, the harvest was brought forward due to rain events in November which began to cause fruit splitting, something which would not be encountered if the trial was planted during a typical NT watermelon season. In routine sampling conducted after the harvests, CGMMV was detected in two of the grafted rows and the non-grafted row, despite the seeds and seedlings being negative for the virus prior to planting. No symptoms of the virus were observed during the trial, and its occurrence is not thought to have impacted trial results due to the virus being detected in both grafted and non-grafted plants.

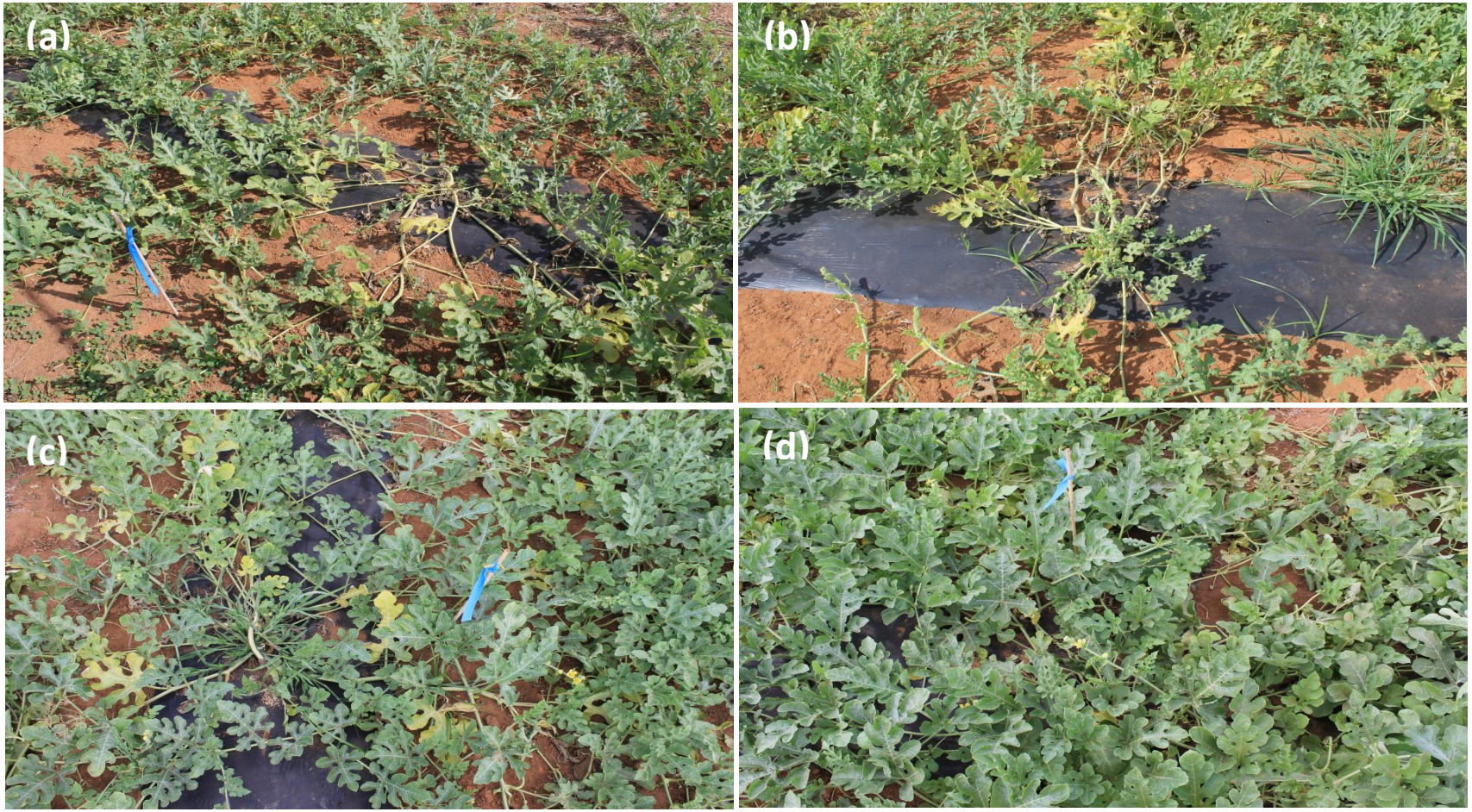

Figure 8. Watermelon grafted with *Cucurbita* rootstocks treatments 'RTX1' (a), 'RS 841' (b) and 'Carnivore' (c) compared to non- grafted watermelons at 8 weeks post planting in the agronomic field trial (Experiment 4) at Berrimah Farm, NT.

Table 10. Leaf nutrient analysis in watermelon grafted with *Cucurbita* rootstocks (treatments 'RTX1', 'RS 841' and 'Carnivore') compared to non-grafted watermelons in the agronomic field trial at Berrimah Farm, NT.

		N (kjeldahl)	P	K	S	Ca	Mg	Mn	Fe	Cu	Zn	B
Treatment	Week	%	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Non-grafted	3	6.7	0.57	3.94	0.39	1.14	0.6	56.15	359.97	12.27	42.99	31.87
'RTX1'	3	6.88	0.54	4.69	0.39	1.39	0.84	103.82	396.33	12.02	51.35	22.64
'RS 841'	3	6.43	0.52	3.99	0.39	1.38	0.71	124.67	379.52	10.33	42.62	24.73
'Carnivore'	3	6.3	0.5	4.45	0.38	1.19	0.81	83.2	409.81	12.2	41.58	22.66
Non-grafted	6	4.68	0.28	2.15	0.32	1.56	0.75	57.18	347.99	8.21	37.43	59.97
'RTX1'	6	4.59	0.25	2.14	0.31	1.07	0.59	69.47	246	8.06	38.21	37.96
'RS 841'	6	4.5	0.26	1.99	0.31	0.96	0.57	60.15	148.68	7.66	36.04	48.29
'Carnivore'	6	4.64	0.27	1.93	0.3	1.36	0.77	66.42	272.5	7.74	33.12	47.4
Non-grafted	9	6.7	0.29	1.62	0.36	3.39	1.02	91.17	668.88	10.38	36.17	198.84
'RTX1'	9	6.88	0.22	1.32	0.33	2.61	0.97	94.34	413.32	8.93	35.04	114.46
'RS 841'	9	6.43	0.24	1.43	0.32	2.68	1.06	96.27	276.27	7.99	28.28	108.97
'Carnivore'	9	6.3	0.28	1.55	0.36	2.49	1.02	77.83	381	8.53	28.77	107.36

Yellow = levels below industry standard; Green = levels within industry standard; and Blue = levels exceeding industry standard.

Table 11. Fruit weight, firmness and sweetness of watermelon grafted with *Cucurbita* rootstocks compared to non-grafted (control) plants in the agronomic field trial (Experiment 4) at Berrimah Farm, NT.

	Fruit weight (kg) at harvest				Fruit assessment	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Firmness	BRIX
Non-grafted	8.81 b	9.25 b	7.48 b	6.26 b	28.41 a	9.25 a
'RTX1'	5.89 a	5.85 a	-	4.65 a	27.43 a	8.88 a
'RS 841'	6.85 a	6.0 a	5.49 a	4.77 a	27.67 a	8.43 a
'Carnivore'	6.35 a	6.75 a	5.96 a	5.07 a	27.25 a	7.88 a

Treatments sharing a letter within a column are not significant different by Tukey's test at P=0.05

Experiment 5: Molecular characterisation of Fon isolates

Molecular tools are often used to provide a rapid diagnostic test for plant pests. The current specific *Fon* PCR assay (Lin *et al.* 2010) is able to detect all *Fon* isolates found in the NT, however, the *Fon* PCR test failed to detect all *Fon* isolates from the USA and Australia assessed in this study. This is not surprising as the assay targets a very small section on the *Fon* genome. Using putative effector, secreted in xylem (SIX), genes that were found in other Fusarium pathotype systems, we identified homologous within the two NT isolates using NGS. Initially NGS identified several SIX genes within the NT isolates (Table 12).

Table 12. Molecular characterisation of *Fon* isolates

			SIX genes					
R*	**Sample/ Site**	***Fon***	**4**	**6**	**8**	**9**	**11**	**13**
0	F-121-2/ USA	-	-	+	+	-	-	-
1	F-016-1/ USA	+	-	-	+	+	+	+
1	F-079-1/ USA	+	+	+	+	+	+	+
1	F-107-1/ USA	-	-	-	+	-	+	+
2	F-17B-1-29/ USA	+	+	+	+	+	+	+
2	F-17B-1-3/ USA	+	+	-	+	+	+	+
2	VP 088/ QLD	-	-	-	+	-	+	+
3	MDZE-6221A/ USA	+	-	-	+	+	+	+
3	VP 0457/ NSW	-	-	-	+	-	+	+
3	VP 0583/ WA	+	+	+	+	+	+	+
3	VP 0585/ QLD	+	+	+	+	+	+	+
3	36953/ NT	+	+	+	+	+	+	+
3	36955/ NT	+	+	+	+	+	+	+

*Based upon race differential studies. R= race

Based upon these sequences, new primers were designed to target homologs within the *Fon* USA and Australian isolates. Gap analysis of the individual SIX genes across all *Fon* isolates used in this study showed very high sequence similarity (>99%) with other *Fon* isolates but only 82% compared with the same gene in *Fo* f.sp. *lycopersici* and *Fo* f.sp *vasinfectum* (Appendix 3) NTP-Dc 36955 compared to NTP-Dc 36953. Two SIX8 genes were identical

between the two NT isolates but the third copy within NTP-Dc 36955 shared only 83% similarity with the remaining *Fon* isolates. NGS is still underway for the remaining *Fon* isolates. The expansion of genetic data for all *Fon* races should identify diverse regions within their genomes and may be suitable to develop a new specific *Fon* test that can distinguish and differentiate all *Fon* races.

Recommendations

- Further research is needed to identify other rootstock types (i.e. *Lagenaria*) which are suitable in the NT growing conditions and are compatible with commercially desired scions
- The published specific *Fon* PCR test failed to detect all isolates used in this study. It is necessary to conduct pathogenicity or race differential trials using a high susceptible cultivar such as 'Sugar Baby' if the PCR fails and Fusarium wilt is suspected. This is critical particularly if the Fusarium wilt is suspected on previously healthy ground
- Race 3 has clearly spread throughout the NT and other Australian States, this highly virulent race has never previously been recorded outside of Maryland, USA. Seed companies now need to focus on breeding for resistance to this aggressive strain of *Fon*
- *Cucumber green mottle mosaic virus* was detected in the NT in 2014 and in QLD in 2015, future breeding programs should consider resistance to not only *Fon* but CGMMV as well

Scientific Refereed Publications

Draft manuscript

Tran-Nguyen LTT, McMaster CA, Condè B, Nguyen, VT, Puno V, Cook SE, Czislowski E, Fraser-Smith S, Everts KL. Characterisation and management options for Fusarium wilt of watermelon in the Northern Territory, Australia.

Intellectual Property/Commercialisation

No commercial IP generated.

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Appendices

Appendix 1 – Full methodology

Appendix 2 – Conference posters

Appendix 3 – SIX gene matrix

Appendix 4 – Full list of rootstocks and seedless lines used in this study

Appendix 1 Full methodology

Glasshouse plant inoculations

Potting mix containing peat 20L; vermiculite 20L; dolomite 340g; mono ammonium phosphate 5g; trace element mix (e.g."Micromax") 6g; potassium nitrate 6g; and water 3-4L, was pasteurised at 60°C for 30 mins. Four inch plastic pots were ¼ filled with pasteurised potting mix then 10 day old mycelial cultures of *Fon* grown on Potato Dextrose Agar (PDA) were added mycelial side up and seedlings were planted directly on top. PDA plates without *Fon* were used in the control pots.

Pathogen isolations to confirm Fon

Stem sections were cut from symptomatic plants then surface sterilised in 100% ethanol for 1 min and rinsed in sterile water. Four smaller sections were cut then plated onto PDA plus lactic acid and incubated at 25°C for 3-5 days before being assessed for *Fon* growth. An isolation plate from each treatment was single spored and subjected to *Fon*-specific PCR tests. One plant from each control pot had isolations conducted to confirm that controls were free of *Fon*.

Experiment 1: Temperature glasshouse trials

A pot trial was conducted to evaluate the effect of temperature on *Fon* symptom expression in three watermelon cultivars: 'Sugar Baby', 'Kalahari' and 'Royal Armada' (Table 1). 'Kalahari' and 'Royal Armada' seedlings were grown at Trandos Hydroponics Nursery, Western Australia (WA) and were 25 days old at inoculation. 'Sugar Baby' seedlings were grown at Berrimah Farm and were 18 days old at inoculation. Two experiments were conducted simultaneously in dual locations in a 'hot' ambient temperature screen house and a 'cold' temperature-controlled glasshouse, with ten replicate pots per treatment. The trial was conducted over 40 days, from 23rd October to 2nd December 2015, during the build-up season in the Northern Territory (NT) when air temperatures in the 'hot' screen house were very high. Air temperatures were recorded using TinyTag®Plus loggers and soil temperatures were recorded using Escort Junior probes in the control pots. Plants were monitored daily for symptoms of *Fon* and if symptoms were observed, isolations were conducted to confirm the presence *Fon*. At 40 days, all remaining inoculated but asymptomatic plants had isolations conducted to confirm if *Fon* was present. One plant from each control pot had isolations conducted to confirm that controls were free of *Fon*.

Table 1. Watermelon cultivars and treatments evaluated in the temperature glasshouse trials.

Cultivar	Temperature	Fon isolate/treatment
'Sugar Baby'	'Hot'	NTP-Dc 36953, NTP-Dc 36955, PDA control
	'Cold'	NTP-Dc 36953, NTP-Dc 36955, PDA control
'Kalahari'	'Hot'	NTP-Dc 36953, NTP-Dc 36955, PDA control
	'Cold'	NTP-Dc 36953, NTP-Dc 36955, PDA control
'Royal Armada'	'Hot'	NTP-Dc 36953, NTP-Dc 36955, PDA control
	'Cold'	NTP-Dc 36953, NTP-Dc 36955, PDA control

Experiment 2: Race differentials

Six watermelon cultivars ('All Sweet', 'Calhoun Gray', 'Charleston Gray', 'Crimson Sweet', 'Sugar Baby' and 'SP-4') were evaluated for their susceptibility to two NT isolates to determine the *Fon* race of the isolates. Race differentials of *Fon* are based according to variability in aggressiveness on differential cultivars, where a resistant reaction is < 33% wilt and a susceptible reaction is ≥ 33% wilt (Martyn and Bruton, 1989; Zhou *et al.* 2010). Race differential trials in the NT were conducted using a modified method of Zhou *et al.* (2010). Modifications included the use of mycelial cultures instead of spore suspensions for the plant inoculations and the inclusion of additional cultivars 'Crimson Sweet'

and 'All Sweet' (Keinath and DuBose, 2009). Due to the unavailability of 'PI-296341-FR', 'SP-4' was used as a suitable replacement due to its resistance to *Fon* race 2 which is derived from 'PI-296341-FR' by Syngenta plant breeders (Zhang, 2009).

Each cultivar was subjected to three inoculation treatments using two NT *Fon* isolates (NTP-Dc 36953 and 36955) and an un-inoculated PDA control. Experiments were conducted over 28 days in a temperature controlled glasshouse at Berrimah Farm and air temperatures were recorded using TinyTag®Plus loggers. All seedlings were grown at Berrimah and were inoculated at the second true leaf-stage (~14 days old) with mycelial cultures. Each cultivar was tested once in 2014, and then all trials were repeated between March and September in 2015. 'SP-4' was tested in June and again in July in 2015, resulting in 'SP-4' being tested three times in total. For each cultivar there were a total of 60 plants in six pots, with ten plants per pot. Plants were monitored daily for symptoms of wilt, and plants with symptoms were harvested for isolation to confirm the presence of *Fon*. At 28 days, one plant from each control pot was also harvested for isolation to confirm the absence of *Fon*.

Table 2. Revised race differential cultivars used to determine *Fon* race in the NT trials.

Genotype	Race 0	Race 1	Race 2	Race 3
'Sugar Baby'	S	S	S	S
'Crimson Sweet'	R	S	S	S
'Charleston Gray'	R	S	S	S
'All Sweet'	R	R	S	S
'Calhoun Gray'	R	R	S	S
'SP-4'	R	R	R	S

Experiment 3: Rootstock and seedless watermelons resistance trial

A glasshouse trial was conducted to evaluate *Cucurbita* specific hybrid rootstocks and seedless watermelon cultivars for resistance to *Fon*. Three grafted rootstocks, two seedless lines and non-grafted 'Royal Armada' as a control, were subjected to three inoculation treatments including two *Fon* isolates (NTP-Ds 36953 and 36955), and an un-inoculated PDA control (Table 3). The experiment was conducted over 40 days, from 22nd October to 1st December 2015, in a temperature controlled glasshouse at Berrimah Farm. Air temperatures were recorded using Tiny Tags®Plus loggers. Plants were monitored daily for symptoms of wilt, and plants with symptoms were harvested for isolations to confirm the presence of *Fon*. At 40 days, all asymptomatic grafted plants and one plant from each control pot were harvested for isolation to confirm the absence of *Fon*.

Table 3. Grafted rootstocks and watermelon cultivars assessed for resistance to *Fon* in the resistance trial in 2015.

Cultivar	Species	Treatment
'RTX1'	*C. maxima x C. moschata*	Grafted with 'Royal Armada'
'RS 841'	*C. maxima x C. moschata*	Grafted with 'Royal Armada'
'Carnivore'	*C. maxima x C. moschata*	Grafted with 'Royal Armada'
'Kalahari'	*Citrullus lanatus*	Non-grafted
'Bullseye'	*Citrullus lanatus*	Non-grafted
'Royal Armada'	*Citrullus lanatus*	Non-grafted

Experiment 4: Rootstock agronomic field trial

A field trial was conducted to evaluate the effect of grafting with *Cucurbita* specific hybrid rootstocks on the agronomic characteristics of the watermelon crop (Table 4). 'Red Tiger' ('Sangría'), a seeded diploid cultivar, was used as the scion and as a pollinator for fruit production. The trial was planted at Berrimah Farm in a paddock with a history of vegetables, cover crops and watermelons in a Kandasol sandy loam soil. The paddock was prepared three months prior to planting by rotary hoeing then laying plastic mulch in the planting rows. All seedlings were grown at Trandos Hydroponics and delivered to Darwin by refrigerated air freight to minimise seedling losses and duration in transport. The trial was conducted over 72 days, from 8th September to 19th November 2015, and the grafted and non-grafted seedlings were planted at 55 and 32 days old, respectively, with 1 m spacing's. Basal fertiliser was applied at planting, and drip tape was used for irrigation and fertigation. Weekly applications of Diamond 19 containing Nitrogen 13kg/ha, Phosphorus 5.6kg/ha, and Potassium 10.7kg/ha was applied. At 6 weeks, Calcium 5kg/ha and Boron 1.7kg/ha were included to enhance fruit set, then Magnesium was applied at 2 kg/ha due to a suspected deficiency after poor growth in the non-grafted treatments.

Grafted plants were observed for growth and compatibility between rootstocks and the scion. Crop nutrient levels were monitored by taking leaf samples at weeks 3, 6 and 9 post-planting for analysis by CSBP Laboratories and levels were compared to industry standards. The harvest was brought forward due to rain causing fruit splitting. Four harvest events were undertaken at 66, 70, 71 and 72 days post planting. At the first harvest, ten fruit per treatment were assessed for fruit firmness using a fruit pressure tester (mod FT 011) and fruit sweetness using the Brix test.

Table 4. Treatments evaluated in the rootstock agronomic field trial in 2015.

Cultivar	**Species**	**Treatment**
'RTX1'	*C. maxima x C. moschata*	Grafted with 'Red Tiger'
'RS 841'	*C. maxima x C. moschata*	Grafted with 'Red Tiger'
'Carnivore'	*C. maxima x C. moschata*	Grafted with 'Red Tiger'
'Red Tiger'	*Citrullus lanatus*	Non-grafted

Statistical analysis

For Experiment 1, temperature data, time to wilting and the effects of *Fon* isolate and temperature was analysed with parametric survival regression models (R Core Team 2015). Given that wilting is more likely to show when infection accelerates with time, models were fitted with a Weibull error distribution to allow for a non-constant hazard with age. Censoring was used as not all plants had wilted by day 40. Maximum Likelihood (ML) Deviance, or -2*Log-Likelihood, was used to assess the strength of an effect, where a larger decrease in the ML deviance from the Null model indicates a stronger effect. Note that the Null ML deviance is the same for both models 1 and 2. Experiment 2 (Race differentials) data was not analysed because results were determined by percent thresholds (< 33% wilt or ≥ 33% wilt). Experiment 3 (Rootstock resistance trial) data was analysed with a Chi test using a two-sample test with continuity correction for equality for the proportion of plants infected by each *Fon* isolate for each cultivar tested (i.e. controls not included). Experiment 4 (Rootstock field trial) data was analysed using single-factor Analysis of variance with a 95% type 1 error rate, and means were compared using Tukey test with a 95% family-wise confidence level.

Experiment 5: Molecular characterisation of **Fon** *isolates*

PCR testing
All DNA extractions were conducted using mycelial growth from two PDA plates with single spore *Fon* cultures using a commercial DNA extraction kit. PCR assays were assembled as below in Table 5 and PCR conditions in Table 6. SIX PCRs were based upon published primers as well as those designed within this study (Table 7), the PCR conditions for the SIX PCRs were identical (Table 8).

Table 5. Reagents for PCR assembly to detect *Fon*.

PCR Reagents	**1x (µL)**	**5.5x(µL)**
2 X MyFi	12.5	68.75
10 µM Fon1	0.5	2.75
10 µM Fon2	0.5	2.75
SDW	11	60.5
TOTAL	24.5	134.75

Fon1 (5′ CGA TTA GCG AAG ACA TTC ACA AGA CT 3′)
Fon2 (5′ ACG GTC AAG AAG ATG CAG GGT AAA GGT 3′)

Table 6. PCR assay conditions to detect *Fon* DNA.

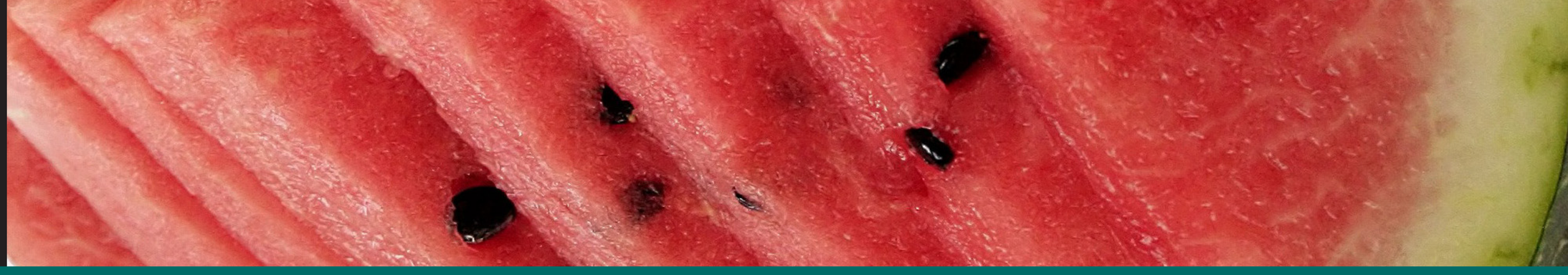
Step	**Temp. (ºC)**	**Time**	
Initial denaturing	95	1m	
Denaturing	94	30s	30 cycles
Annealing	60	30s	
Extension	72	1m	
Final Extension	72	10m	

Table 7. SIX primers used in this study.

Primer name	**Sequence (5′ – 3′)**	**Reference**
FonSIX4 F	TCTCAAAGCACTCATTGTTATTGC	This study, S. Cook pers. com
FonSIX4 R	TGTGCCTTGAACGCGAAATG	This study, S. Cook pers. com
SIX6 F	GGCTGCGTAGCTGGTCCCCT	Meldrum et al. 2012
SIX6 R	CATGTCATGAATGTACGCATGTCCCT	Meldrum et al. 2012
FonSIX 8 F	AGCCGTCTCTGTGGCTACTA	This study, S. Cook pers. com
FonSIX 8 R	TCCACCACTTCAGTTACCGC	This study, S. Cook pers. com
FonSIX 9 F	CCTTGGCCGTCTTCTCTACC	This study, S. Cook pers. com
FonSIX 9 R	TCTGTAAGCGCAAGGGTACG	This study, S. Cook pers. com
FonSIX 11 F	TGATGTTCTCCAAAGCCATCTCA	This study, S. Cook pers. com
FonSIX 11 R	TCAAATGCAGGGTCTATTGCG	This study, S. Cook pers. com
FonSIX13 F	ACCACTACTTTTCTCCTGGTTCT	This study, S. Cook pers. com
FonSIX13 R	GATCTCGGTGAGCTTCGTCG	This study, S. Cook pers. com

Table 8. PCR assay conditions to detect *Fon* SIX genes.

Step	**Temp. (ºC)**	**Time**	
Initial denaturing	95	1m	
Denaturing	95	30s	35 cycles
Annealing	60	45s	
Extension	72	1min	
Final Extension	72	10	



FUSARIUM WILT OF WATERMELON IN THE NORTHERN TERRITORY, AUSTRALIA

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Figure 1. Watermelon plant displaying signs of Fusarium wilt.

Figure 2 (above) and 3 (below). Vascular browning in watermelon stem.

Figure 4. Pathogenicity trial with wilted (background) and non wilted (foreground) seedlings.

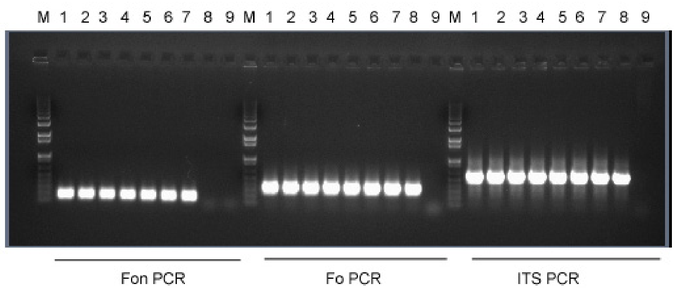

Figure 5. Agarose gel electrophoresis of PCR products amplified from specific *Fon, Fo* and ITS PCR tests. M. 1 Kb Plus marker, 1-7. *Fon* isolates, 8. *Fusarium oxysporum* f. sp. *tracheiphilum*, 9. Sterile distilled water.

Summary

The watermelon industry in the Northern Territory (NT) of Australia has a production value of approximately $47.8 million and covers approximately 900 hectares. All NT watermelons are sold within Australia, catering to interstate retail markets during the cooler months of the year.

In May 2011, there was an outbreak of Fusarium wilt of watermelon in the NT. Triploid seedless watermelon seedlings and plants from six locations in the NT expressed symptoms such as leaf necrosis, necrotic blotching and seedling deaths in seedling trays, as well as, wilting (Fig. 1) and vine collapse in the field. No vascular browning was observed except for one mature field plant (Fig. 2 and Fig. 3). Pathogenicity trials of two NT *Fon* isolates on 'Sugar Baby' plants confirmed the fungus was the causal agent of Fusarium wilt of watermelon in the NT.

Background

Fusarium wilt is a severe disease in watermelon and is caused by the soilborne fungus, *Fusarium oxysporum* f. sp *niveum (Fon).* The fungus is only pathogenic to watermelons and is divided into four races (0, 1, 2 and 3) (1-5). The disease is one of the major yield limiting factors in production, worldwide (6).

Symptoms include damping-off, seedling wilt or disease during any stage of plant development (7). The fungus can survive many years in the soil as chlamydospores and spread by soil, plant debris, farm machinery and seeds (7, 8). Fusarium wilt of watermelon occurs on every continent except Antarctica; and Australia is known to have 2 races of *Fon*, though there is limited information as to what these races are (9).

Methods

Isolation and Identification

Excised sections from symptomatic watermelon seedlings were surface sterilised and embedded in potato dextrose agar plates supplemented with 1% lactic acid. After two to three days incubation at 25°C, *Fusarium*-like spores were observed and subcultured. Single spore *Fusarium* cultures were used for pathogenicity tests and DNA analyses.

Fusarium DNA was extracted using a commercial kit and subjected to three different PCR amplification tests. Primers included those that targeted the ITS region (10), specific-*Fon* RAPD marker and specific-*Fo marker* (11).

Results

Pathogenicity trials

Typical wilt symptoms were observed on inoculated young seedlings after eight days post inoculation and up to 25 days in older seedlings (Fig. 4). *Fon* was confirmed by re-isolation and PCR.

Molecular identification

All NT isolates except *Fusarium oxysporum* f. sp. *tracheiphilum* was positive in the *Fon*-specific test. While all isolates were positive in the *Fo* and the ITS PCR tests (Fig. 5).

Current work

A Horticulture Australia Limited national research project is currently underway with the aims to:

- Determine what *Fon* races exist in the NT and Australia
- Develop molecular protocols for race identification
- Screen commercial varieties for resistance

Race differentials

Race differentials is based upon levels of aggressiveness, where resistant reactions are < 33% wilt and susceptible reactions are ≥ 33% wilt and will utilise the matrix of cultivars listed in Table 1 (5). Due to lack of true to type seed availability of certain cultivars, an alternative list of race differential cultivars was devised (Table 2).

Two NT isolates (NTP-Dc 36953 and NTP-Dc 36955) are being used for race differentials. Trials will include 60 plants for each isolate as well as 60 plants in a control group. Seedlings will be planted into pots containing a small amount of potting mix and *Fon* culture on agar, or plain agar for the control group.

Trials will run for 28 days, stem segments will be cut from symptomatic plants and isolated in an attempt to re-isolate *Fon*. This will confirm that *Fon*, is the causal agent, of plant wilt displayed during the trials.

Table 1: Disease reaction of watermelon genotypes used to differentiate races of *Fon.*

	***Fon* Races**			
Genotype	**0**	**1**	**2**	**3**
'Sugar Baby'	**S**	**S**	**S**	**S**
'Charleston Gray'	**R**	**S**	**S**	**S**
'Calhoun Gray'	**R**	**R**	**S**	**S**
'PI-296341-FR'	**R**	**R**	**R**	**S**

Table 2: Revised disease reaction of watermelon genotypes used to differentiate races of *Fon.*

	***Fon* Races**			
Genotype	**0**	**1**	**2**	**3**
'Sugar Baby'	**S**	**S**	**S**	**S**
'Crimson Sweet'	**R**	**S**	**S**	**S**
'Allsweet'	**R**	**R**	**S**	**S**
'SP-4'	**R**	**R**	**R**	**S**

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Biological races of *Fusarium oxysporum* f.sp. *niveum* in Australian watermelon production regions

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Introduction

Fusarium wilt of watermelon is a soil borne disease that reduces watermelon yields by 75% or more and is a major limiting factor to industry growth. The causal organism is *Fusarium oxysporum* f.sp. *niveum* (*Fon*) with four known races (race 0,1,2, and 3), defined by their virulence to differential resistant cultivars. The disease has been a consistent problem and continues to spread throughout Australia (Tran-Nguyen et al. 2012). Main symptoms include chlorosis, dwarfing, unilateral wilt, vascular discolouration and death (Figure 1). The aim of this study is to determine the distribution and race composition of the Australian *Fon* population.

Figure 1. Top: (L) Vascular discolouration; **(R)** healthy seedlings vs. inoculated**. Bottom: (L)** Severe field symptoms vs. **(R)** healthy.

Figure 2. Sampling locations of wilted watermelon plants.

Materials & Methods

Fusarium oxysporum isolates were recovered from stem sections of wilted watermelons from various growing regions in Australia (Figure 2). All isolates were single spored. Pathogenicity was determined for all isolates using 'Sugar Baby', a universal susceptible cultivar, followed by race determination using cultivars based on Keinath and DuBose (2009) and Zhou et al. (2010) (Table 1). Eight isolates from Queensland (QLD), five from New South Wales (NSW), four from Western Australia (WA) and one from the Northern Territory (NT) were tested. A 3 mL spore suspension of concentration 10^6 spores/mL for each isolate was used to inoculate 60 plants of each differential cultivar (6 reps of 10). Plants displaying typical wilt symptoms, with pathogen re-isolation, were scored as susceptible. Race differentiation was determined by >33% susceptibility.

Table 1. Race Differential Cultivars (Keinath & DuBose 2009, Zhou et al. 2010).

Cultivar	Race 0	Race 1	Race 2	Race 3
'Sugar Baby'	Susceptible	Susceptible	Susceptible	Susceptible
'Crimson Sweet'	Resistant	Susceptible	Susceptible	Susceptible
'Allsweet'	Resistant	Resistant	Susceptible	Susceptible
'PI-296341-FR/SP4*'	Resistant	Resistant	Resistant	Susceptible

Results & Discussion

Five isolates from QLD and one isolate each from NSW, WA and the NT were pathogenic on watermelon (Table 2). Preliminary results for race differential trials indicate that the four isolates from Qld are race 2, with ongoing trials being conducted to differentiate between race 2 and 3 as well as the remaining Australian isolates. This is the first report of *Fon* race 2 or 3 in the country and has important implications for disease management strategies which rely largely on host resistance.

Table 2. Percentage seedling wilt of *F. oxysporum* isolates (5 QLD, 1 NSW, 1 WA, 1 NT).

Cultivar	Isolate Location							
	QLD					NSW	WA	NT
	VP016	VP051	VP071	VP088	VP0585	VP0457	VP0583	VP0584
'Sugar Baby'	91%	82%	48%	79%	100%	95%	100%	93%

Table 3. Race type of four QLD *Fon* isolates based on susceptibilities of inoculated differential cultivars

Cultivar	QLD Isolates			
	VP016	VP051	VP071	VP088
'Crimson Sweet'	85%	83%	34%	73%
'Allsweet'	77%	70%	34%	66%
Race Type	**Race 2**	**Race 2**	**Race 2**	**Race 2**

Reference:
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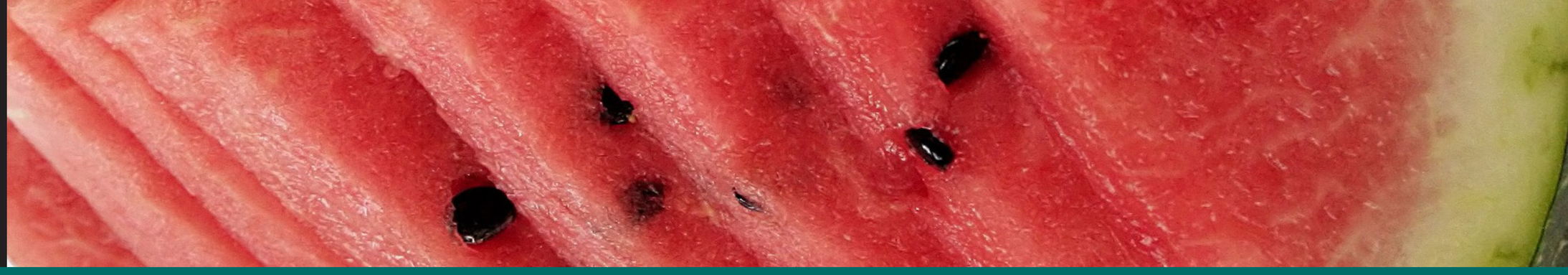

Characterisation of *Fusarium oxysporum* f. sp *niveum* by race differentials and molecular markers

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BACKGROUND

Fusarium oxysporum f. sp. *niveum (Fon)* causes a severe wilt of watermelon plants and is a major yield limiting factor in production worldwide. In Australia the disease was first detected in the Northern Territory (NT) in May 2011 causing leaf necrosis, necrotic blotching and seedling death on triploid watermelon seedlings and plants (1).

Fon is divided into four races (0, 1, 2 and 3), according to their aggressiveness on differential cultivars (2,3). There is limited information on the *Fon* races found in Australia, but based upon race differential typing conducted in 1969-71, race 0 and 1 was identified in Queensland (4). Our objectives were to determine the race structure of Australian *Fon* isolates from extensive sampling of different watermelon growing regions and inoculating differential cultivars, and to improve the diagnostic test for *Fon* by targeting the putative effector secreted in xylem (SIX) gene(s).

METHODOLOGY

Race differentials

Race differential trials were conducted using six cultivars (Table 1). Trials consisted of three treatments (two NT *Fon* isolates plus an agar control) each with six pots of ten plants. Isolations were made from symptomatic plants, and one single-spore culture was obtained from each pot.

Molecular characterisation

Mycelium was scraped from cultures grown on PDA plates, and DNA was extracted using the DNeasy Plant kit (Qiagen, Australia). All cultures were subjected to the *Fon*-specific PCR test (5). Putative SIX genes were targeted using published methods (6, 7) and primers designed in-laboratory.

SUMMARY

All isolates used in this study were pathogenic to watermelon. Race differential trials (Fig. 1) conducted in the NT, over two years, have indicated that the NT isolates are likely to be race 3 (Table 1). Each isolate was subjected to the specific *Fon* PCR test and all were positive except for two isolates from USA and two from Australia (Table 2). All *Fon* races were positive for the presence of SIX 8. No correlation of the *Fon* races and the SIX genes were observed (Fig. 2). Bioinformatics also did not show significant differences in the sequences (data not shown). Next generation sequencing using MiSeq technology is currently underway to generate a comprehensive whole genomic data set that will allow diagnostic markers to be designed and developed to distinguish between *Fon* races.

Table 1: Disease reaction of watermelon genotypes used to differentiate races of *Fon*. 'Sugar baby' results for 2014 trial only.
R= resistant (<33% wilt) and S = susceptible (>33%) (3)

Fon isolate/ treatment	'Sugar baby'	'Crimson Sweet'	'Charleston Gray'	'Allsweet'	'Calhoun Gray'	'SP-4'
NTP-Dc 36953	S*	S	To do	S	S	S
NTP-Dc 36955	S*	S	To do	S	S	S
Race 0	R	R	R	R	R	R
Race 1	S	S	S	R	R	R
Race 2	S	S	S	S	S	R
Race 3	S	S	S	S	S	S

Table 2: Molecular characterisation of *Fon* isolates.

Race	Sample Site	*Fon*	4	6	8	9	11	13
0	F-121-2/USA	-	-	+	+	-	-	-
1	F-016-1/ USA	+	-	-	+	+	+	+
1	F-079-1/ USA	+	+	+	+	+	+	+
1	F-107-1/ USA	-	-	-	+	-	+	+
2	F-17B-1-29/ USA	+	+	+	+	+	+	+
2	F-17B-1-3/ USA	+	+	-	+	+	+	+
2	VP 088/ QLD	-	-	-	+	-	+	+
3	MDZE-6221A/ USA	+	-	-	+	+	+	+
3	VP 0457/ NSW	-	-	-	+	-	+	+
3	VP 0583/ WA	+	+	+	+	+	+	+
3	VP 0585/ QLD	+	+	+	+	+	+	+
3	NTP-Dc 36953/ NT	+	+	+	+	+	+	+
3	NTP-Dc 36955/ NT	+	+	+	+	+	+	+

Figure 1: 'Allsweet' race differential trial – a) control, b) NTP-Dc36953 and c) NTP-Dc36955 treatments, 11 days post inoculation.

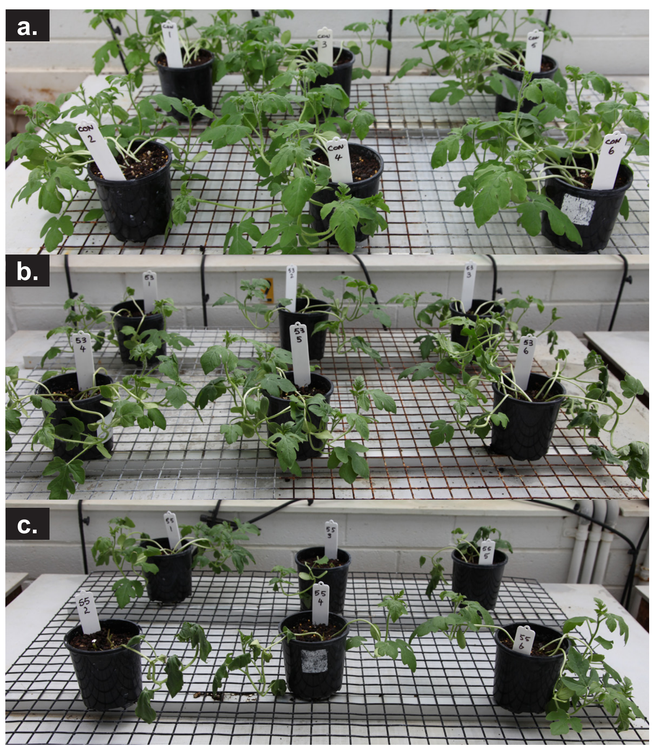
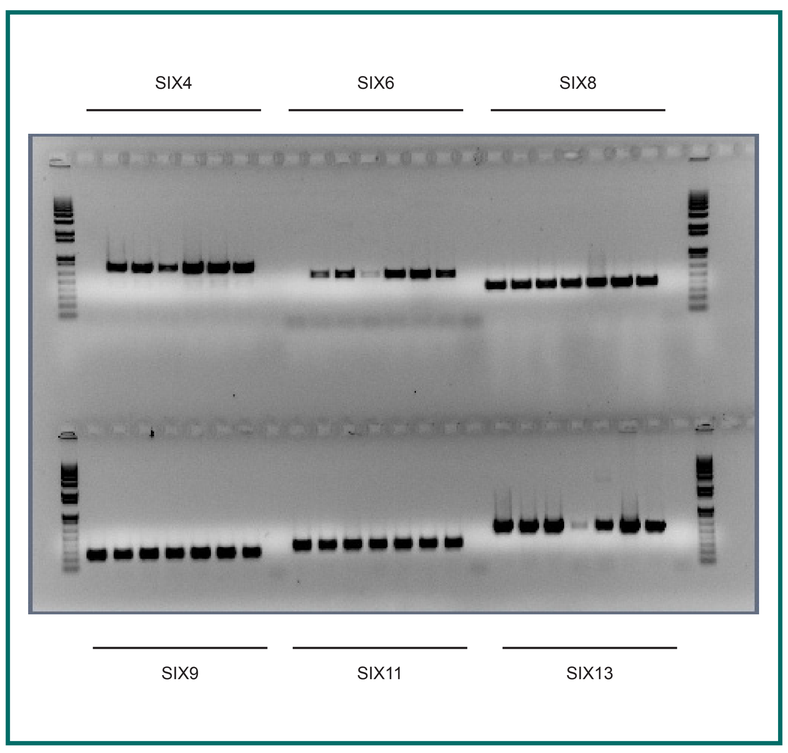


Figure 2: SIX PCRs – 1. F-016-1/USA R1, 2. F-079-1/USA R1, 3. F-097-1/USA R1, 4. F-17B-1-29/USA R2, 5. F-099-1/USA R2, 6. NTP-Dc 36953, 7. NTP-Dc 36955, 8. Sterile distilled water.



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Images courtesy of Barry Condé, Cassie McMaster, Lois Ulyatt, Stacey Cook and Lucy Tran-Nguyen.

Appendix 3. SIX gene matrix

SIX4	F-17B-1-29	F-079-1	F-097-1	F-099-1	Fon36953	Fon36955	HQ260605 At	VP 0583	VP 0585
F-17B-1-29		99.865	99.207	99.864	99.009	99.075	97.421	99.339	99.075
F-079-1	99.865		99.865	100	99.797	99.865	97.421	99.865	100
F-097-1	99.207	99.865		100	98.485	98.551	97.282	99.339	99.604
F-099-1	99.864	100	100		99.797	99.864	97.414	99.864	100
Fon36953	99.009	99.797	98.485	99.797		99.937	97.321	98.613	98.483
Fon36955	99.075	99.865	98.551	99.864	99.937		97.39	98.679	98.549
HQ260605 At	97.421	97.421	97.282	97.414	97.321	97.39		97.421	97.421
VP 0583	99.339	99.865	99.339	99.864	98.613	98.679	97.421		99.472
VP 0585	99.075	100	99.604	100	98.483	98.549	97.421	99.472	

SIX6	F-17B-1-29	F-079-1	F-097-1	F-099-1	FJ755835 Fol	Fon36953	Fon36955	HM467128 Fov	VP 0583	VP 0585
F-17B-1-29		99.497	100	99.497	92.195	97.761	99.337	83.955	99.168	100
F-079-1	99.497		100	100	91.79	97.236	98.827	83.306	100	100
F-097-1	100	100		100	92.359	97.881	99.492	83.775	100	100
F-099-1	99.497	100	100		91.79	97.236	98.827	83.306	100	100
FJ755835 Fol	92.195	91.79	92.359	91.79		90.393	91.831	82.963	91.68	92.321
Fon36953	97.761	97.236	97.881	97.236	90.393		98.534	84.691	97.088	98.041
Fon36955	99.337	98.827	99.492	98.827	91.831	98.534		85.068	98.669	99.659
HM467128 Fov	83.955	83.306	83.775	83.306	82.963	84.691	85.068		83.252	83.86
VP 0583	99.168	100	100	100	91.68	97.088	98.669	83.252		100
VP 0585	100	100	100	100	92.321	98.041	99.659	83.86	100	

SIX8	F-016-1	F-17B-1-29	F-079-1	F-097-1	F-099-1	Fon36953-p1	Fon36953-p2	Fon36955-p1	Fon36955-p2	Fon36955-p3	VP 088	VP 0457	VP 0583	VP 0585
F-016-1		99.751	99.757	99.274	99.752	100	99.757	100	99.757	83.495	99.499	99.752	100	99.757
F-17B-1-29	99.751		100	100	99.504	99.751	100	99.751	100	82.878	100	100	99.751	99.751
F-079-1	99.757	100		99.517	99.505	99.757	100	99.757	100	83.293	99.749	100	99.757	99.513
F-097-1	99.274	100	99.517		99.505	99.279	99.519	99.279	99.519	82.974	99.75	100	99.274	99.515
F-099-1	99.752	99.504	99.505	99.505		99.752	99.505	99.752	99.505	83.416	99.744	99.505	99.752	99.752
Fon36953-p1	100	99.751	99.757	99.279	99.752		99.809	100	99.809	80.762	99.499	99.752	100	99.757
Fon36953-p2	99.757	100	100	99.519	99.505	99.809		99.809	100	80.571	99.749	100	99.757	99.513
Fon36955-p1	100	99.751	99.757	99.279	99.752	100	99.809		99.809	80.762	99.499	99.752	100	99.757
Fon36955-p2	99.757	100	100	99.519	99.505	99.809	100	99.809		80.571	99.749	100	99.757	99.513
Fon36955-p3	83.495	82.878	83.293	82.974	83.416	80.762	80.571	80.762	80.571		84.211	82.921	83.495	83.252
VP 088	99.499	100	99.749	99.75	99.744	99.499	99.749	99.499	99.749	84.211		100	99.499	99.749
VP 0457	99.752	100	100	100	99.505	99.752	100	99.752	100	82.921	100		99.752	99.751
VP 0583	100	99.751	99.757	99.274	99.752	100	99.757	100	99.757	83.495	99.499	99.752		99.757
VP 0585	99.757	99.751	99.513	99.515	99.752	99.757	99.513	99.757	99.513	83.252	99.749	99.751	99.757	

SIX9	F-016-1	F-17B-1-29	F-079-1	F-097-1	F-099-1	Fon36953	Fon36955	KC701447 Fol	VP 0583	VP 0585
F-016-1		100	100	100	100	99.18	99.18	88.934	100	100
F-17B-1-29	100		100	100	100	99.18	99.18	88.934	100	100
F-079-1	100	100		100	100	99.588	99.588	89.3	100	100
F-097-1	100	100	100		100	99.18	99.18	88.934	100	100
F-099-1	100	100	100	100		99.18	99.18	88.934	100	100
Fon36953	99.18	99.18	99.588	99.18	99.18		100	91.884	99.18	99.588
Fon36955	99.18	99.18	99.588	99.18	99.18	100		91.884	99.18	99.588
KC701447 Fol	88.934	88.934	89.3	88.934	88.934	91.884	91.884		88.934	89.3
VP 0583	100	100	100	100	100	99.18	99.18	88.934		100
VP 0585	100	100	100	100	100	99.588	99.588	89.3	100	

SIX11	F-17B-1-29	F-079-1	F-097-1	F-099-1	Fon36953-p1	Fon36953-p2	Fon36955-p1	KC701449 Fol	VP 088	VP 0457	VP 0583	VP 0585
F-17B-1-29		100	100	100	99.702	98.958	99.702	96.131	99.704	99.704	99.704	100
F-079-1	100		100	100	99.702	98.958	99.702	96.131	99.704	99.704	99.704	100
F-097-1	100	100		100	99.702	98.958	99.702	96.131	99.704	99.704	99.704	100
F-099-1	100	100	100		99.702	98.958	99.702	96.131	99.704	99.704	99.704	100
Fon36953-p1	99.702	99.702	99.702	99.702		99.256	100	96.429	99.407	99.407	99.407	99.702
Fon36953-p2	98.958	98.958	98.958	98.958	99.256		99.256	95.685	98.665	98.665	98.665	98.958
Fon36955-p1	99.702	99.702	99.702	99.702	100	99.256		96.429	99.407	99.407	99.407	99.702
KC701449 Fol	96.131	96.131	96.131	96.131	96.429	95.685	96.429		95.846	95.846	95.846	96.131
VP 088	99.704	99.704	99.704	99.704	99.407	98.665	99.407	95.846		99.41	99.704	99.704
VP 0457	99.704	99.704	99.704	99.704	99.407	98.665	99.407	95.846	99.41		99.41	99.704
VP 0583	99.704	99.704	99.704	99.704	99.407	98.665	99.407	95.846	99.704	99.41		99.704
VP 0585	100	100	100	100	99.702	98.958	99.702	96.131	99.704	99.704	99.704	

Appendix 4. Rootstock and seed lines used in trials (CONFIDENTIAL)

Product name	Generic label	Company	Type
Cobalt	**RTX1**	Rijk Zwaan	*C. maxima* x *C. moschata*
RS 841	**RTX2**	Monsanto	*C. maxima* x *C. moschata*
Carnivore	**RTX3**	Syngenta	*C. maxima* x *C. moschata*
Kalahari	**SDL1**	Monsanto	*Citrullus lanatus*
Bullseye	**SDL2**	Hm Clause (Clause Pacific)	*Citrullus lanatus*