# VM12001 Milestone Report 104

**Project Title**
Characterisation and management of Fusarium wilt of watermelon

**Milestone**
104

**Description**
Third progress report

**Achievement criteria**
Project activities (1) Temperature studies – Year 1 trials completed and progress of trials reported (2) Race differentials - Continue experiments in NT - Commence experiments in NSW. Progress of trials reported (3) Extension - Update project web page - Consult/liaise with AMA – combine activities – melon meeting (local and national), newsletter - Produce posters (Darwin, Fred's Pass, Katherine shows and the Katherine Farm and Garden Day) (4) Molecular studies - Characterisation of Fon races (NSW/NT) initiated (5) Rootstocks and grafted melons - Start trials – Lagenaria and pumpkin rootstocks (field and shadehouse) (6) NSW postgraduate student to visit Darwin lab.

**Due date**
31/10/2014 – extension provided to 30/11/2014 due to cucumber green mottle mosaic virus response

**Summary**
- Temperature glass house trials were delayed due to the later onset of Darwin build up season. But were completed on 20 December. On farm observations indicate a very low incidence of Fusarium in the field. This may be due to higher temperatures observed for 2013 and 2014 which may have suppressed Fon.
- Race differentials were conducted in the NT to determine the *Fusarium oxysporum* f.sp. *niveum* (*Fon*) race found in the NT using six differential cultivars. Results are inferring that the NT isolates may be race 2 but there is a discrepancy with two lines with published data. Therefore race differential trials need to be repeated to clearly determine NT Fon races.
- Close links with NSW post graduate student (Victor Puno) to ensure nationwide research on Fusarium wilt of watermelons maintained. Teleconferences are held monthly. NSW, QLD, Vic and WA melon growing areas were surveyed and samples collected. Preliminary results indicate race 2 and 3 are found in interstate watermelon growing regions.
- Dr Len Tesoriero (DPI NSW) and Mrs Dianne Fullelove (AMA) visited Darwin and the Katherine region and held discussions with Lucy Tran-Nguyen and Stuart Smith, NT DPIF.
- Extension activities included the update of the department's website dedicated to the project, a poster presented at the Royal Darwin, Katherine and Fred's Pass Show and the Katherine Farm and Garden Day.
- Molecular work continuing in the NT to differentiate between *Fon* isolates in collaboration with University of Queensland. Links with Dr. Katherine Everts (University of Maryland) maintained to obtain DNA references from the USA to cover all *Fon* races and vegetative compatibility groups.

**Progress since the last milestone report**

## 1.) Temperature trials

*Glasshouse trials:*

Glasshouse observations in 2011 suggested that 'Sugar Baby' watermelon plants inoculated with Fon in the hotter build-up months showed a degree of tolerance to the fungus. Inoculated plants were either symptomless or expressed symptoms very late in the trial. In each case, we were able to re-isolate the pathogen from the inoculated plants. 'Sugar Baby' plants are generally very susceptible to the pathogen and symptoms can express within 5-6 days post inoculation. Although there is little literature on the effect of temperature on Fon, it has been documented (Walker 1941 and Holliday 1980).

Due to the late onset of the warmer build up season in Darwin this year, the temperature glasshouse 40 day trial did not commence until November and concluded 20 December 2014. The temperature trial includes the susceptible cultivar 'Sugar Baby' and two seedlines varieties 'Nightshade' and 'Royal Armada' in dual locations (hot ambient biocontrol screenhouse and cool temperature-controlled glasshouses). Treatments include ten plants of each variety inoculated with NT Fon isolates NTP-Dc 36953 and NTP-Dc 36955 and the control treatment included ten plants inoculated with potato dextrose agar. The air temperature is monitored using Tiny Tags®, and "soil temperatures" in representative control pots using temperature probes. Plants are evaluated daily and harvested when wilt is evident. Isolations are made from plants to confirm the presence of FON in the stem, 5 cm above the potting mix surface. At the conclusion of the trial, all remaining surviving plants will also be isolated to determine the presence of Fon. Nineteen days post inoculation, there are a large number of plants dying in the cooler glasshouse compared to the hot biocontrol screenhouse (Fig 1).

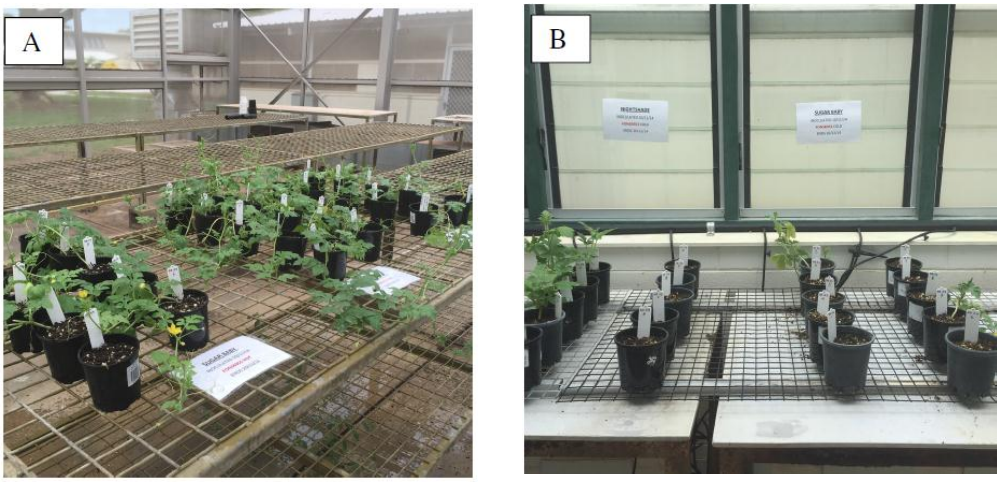


Fig 1. Temperature trial showing inoculated 'Sugar Baby' and 'Nightshade' watermelon seedlings in the hot screenhouse (A) and cool glasshouse (B), 19 days post inoculation.

*On-farm observations:*

Negotiations were held with a local watermelon grower for 'Royal Armada' watermelon plantings to be assessed regularly for Fusarium wilt. The grower decided to delay planting in known Fusarium wilt affected area until later in the season when the weather is warmer. However, arrangements were made for watermelon "sentinel plants' to be planted amongst a pumpkin planting in a known Fon affected area.

Observations were made weekly from mid-June until early October, assessing the six sentinel plants and also 100 – 200 plants in normal blocks of plantings. By the end of the 2014 season, the sentinel watermelon plants growing in the pumpkin block (Fon- infected site) were all doing well with no symptoms of wilt observed.

Sampling was undertaken on three occasions from all the plants on all blocks throughout the whole season. Isolations followed by molecular analyses from one wilted plant collected in mid-July from Block "C" confirmed that Fusarium wilt (Fon) was the cause of the wilt. Fusarium was also isolated from one yellowing plant collected from a patch in Block "C" in early September, and from plants collected from a yellowing patch in Block "F" in late September.

Due the Cucumber Green Mottle Mosaic virus (CGMMV) outbreak reported in Katherine in early September, all watermelon plants with unusual symptoms were collected. In mid- September, a sample was collected from a stunted plant with distorted leaves. This was tested and found to be negative for Fusarium wilt, Phytoplasma and CGMMV. It may have been a genetic off-type.

Altogether there were only three plants with Fusarium from the on-farm observations and no Fusarium detected in the sentinel plants. It could well be that the warmer weather during the 2013 and 2014 growing season has led to the suppression of Fusarium wilt symptoms. The Fusarium isolated from samples collected in September may have invaded the plants because they were stressed. The observed yellowing symptoms are probably due to the watermelon bearing plants being stressed on poorer soil.

## 2) Race differential trials

Since the last milestone report (103), there has been significant progress with the race differential trials. Trials have been completed in 'Sugar Baby', 'Crimson Sweet', 'Allsweet', 'SP-4' and 'Charleston Grey' and 'Calhoun Grey' cultivars. We experienced a delay in the 'Calhoun Grey' race differential trial due to poor germination of seeds. Consequently the 'Calhoun Grey' trial ran simultaneously with the temperature trial.

For each race differential trial, there are three treatments, each with 60 plants in six pots of ten plants each. The treatments consist of two Fon NT isolates (NTP-Dc 36953 and NTP-Dc 36955) and one agar-only control. We conducted a 28-day race differential trial using seedlings that had started growing a second true leaf. Seeds are germinated in 'squat' pots which are half the height of the 4"pots used in the trial, typically 15 seeds are sown (30 in the case of Calhoun Grey due to poor germination) and seedlings are thinned to 10 per pot. Trial pots are filled a quarter of the way with steam sterilised potting mix. On top of this, whole cultures are placed fungus side up and the seedling mass is simply transferred from the squat pot and placed on top of the culture, this method eliminates damage to roots during the inoculation process. Wilted plants were sampled and isolated to confirm Fon was present; an isolate from each pot is single spored and subjected to Fon-specific PCR tests.

Results for each of the trials can be seen in the following tables.

As indicated in Milestone report 102, it is critical to undertake race differentials to determine the NT and interstate Fon races prior to conducting temperature and rootstock trials. Race differentials of Fon are based according to variability in aggressiveness on differential cultivars (Table 1), where a resistant reaction is < 33% wilt and susceptible reactions are ≥ 33% wilt (Martyn and Bruton, 1989, Zhou et al 2010). A small amount of commercially genuine 'Charleston Grey' and 'Calhoun Grey' seeds were mass multiplied for race differential trials to be conducted in the NT and NSW. In the interim, additional differentials ('Crimson Sweet' and 'Allsweet') have been sourced which could be used (Keinath and DuBose 2009) (Table 2). The 'SP-4' cultivar was included in the revised race differential study because initial studies have indicated that it is resistant to race 2 (Condé pers. comm; Zhang 2009) and a suitable replacement for 'PI-296341-FR'. SP4's resistance to Fon was derived from 'PI-296341-FR' by Syngenta plant breeders (Zhang 2009).

Table 1. Race differential cultivars for Fon.

| Genotype | Race 0 | Race 1 | Race 2 | Race 3 |
|---|---|---|---|---|
| 'Sugar Baby' | S | S | S | S |
| 'Charleston Grey' | R | S | S | S |
| 'Calhoun Grey' | R | R | S | S |
| 'PI-296341-FR' | R | R | R | S |

Table 2. Revised Race differential cultivars for Fon

| Genotype | Race 0 | Race 1 | Race 2 | Race 3 |
|---|---|---|---|---|
| 'Sugar Baby' | S | S | S | S |
| 'Crimson Sweet' | R | S | S | S |
| 'Allsweet' | R | R | S | S |
| 'SP-4' | R | R | R | S |

Table 3. Shows the primary data of total symptomatic plants for Race Differential varieties inoculated with the two NT FON isolates, NTP-Dc36953 and NTP-Dc36955. Sixty plants of each cultivar were inoculated with either isolate NTP-Dc36953 or NTP-Dc36955 using PDA plate cultures, one plate per pot.

| Fon isolate treatment | 'Sugar Baby' | 'Crimson Sweet' | 'Charleston Grey' | 'Allsweet' | 'SP4' | 'Calhoun Grey' |
|---|---|---|---|---|---|---|
| NTP-Dc 36953 | 42 (70%) | 30 (50%) | 9 (15%) | 33 (55%) | 7 (12%) | 0 (0%) |
| NTP-Dc 36955 | 33 (55%) | 39 (65%) | 18 (30%) | 32 (53%) | 19 (32%) | 2 (<1%)[#] |
| Control | 0 | 0 | 0 | 0 | 0 | 0 |

# Fon to be confirmed using molecular analyses.

Table 4. Results of FON-confirmed symptomatic plants for the FON race differential varieties converted from percentage values to Resistance or Susceptible values compared with the published race differential table for these varieties

| FON isolate treatment | 'Sugar Baby' | 'Crimson Sweet' | 'Charleston Grey' | Allsweet | 'Calhoun Grey' | 'SP-4' |
|---|---|---|---|---|---|---|
| NTP-Dc 36953 | S | S | R | S | In progress | R |
| NTP-Dc 36955 | S | S | R | S | In progress | R |
| | | | | | | |
| Race 0 | S | 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 |

From Table 4, both isolates NTP-Dc36953 and NTP-Dc36955 gave Susceptible reactions on 'Sugar Baby', 'Crimson Sweet' and 'Allsweet' and Resistant reactions in 'SP-4'. This equates to Race 2. The numbers of symptomatic 'Charleston Grey' plants confirmed to be Fon- infected for both isolate NTP-Dc 36953 and NTP-Dc 36955 were 15 and 30 percent respectively, much lower than 'Crimson Sweet' and 'Allsweet'. This result for 'Charleston Grey' is unexpected because published results (Zhou et al 2003) indicate 'Charleston Grey' susceptibility to Fusarium wilt lies between 'Crimson Sweet' and 'Allsweet'. Our experimental results in the above tables 3 and 4 places 'Charleston Grey' between 'Allsweet' and 'SP-4'. These differing results compared with literature and those completed in NSW clearly highlights the importance of replicating the race differential trial which is planned for 2015.

## 3) Communication/Extension Activities

*Website*

The project web page was updated in September 2014, with current results added.
http://www.nt.gov.au/d/Primary_Industry/index.cfm?header=Characterisation%20and%20management%20of%20Fusarium%20wilt%20of%20watermelon.

Provide project update at melon grower meeting: There haven't been any melon grower meetings to provide an update on the project to. However since the Cucumber Green Mottle Mosaic Virus outbreak in September 2014, there have been several meetings with melon growers on CGMMV biosecurity matters. These meetings with melon growers were facilitated by the Department's Biosecurity Division and the NT Chief Plant Health Manager. Project members were indirectly involved with the CGMMV meetings. .

Consult – Liaise with AMA: Dianne Fullelove and Len Tesoriero visited the NT in July looking for diseases, and visiting major melon growers. They met with Lucy Tran-Nguyen to discuss the melon industry in the NT. The discussion touched on the expansion of the industry in the NT with melons surpassing mango as the largest industry in the NT (2012) and interstate melon growers expanding their business in the NT.

Stuart Smith hosted them in the Darwin region, taking them to two melon growers properties and a number of Asian Melon and cucumber growers. Dianne and Len also visited Katherine, taking samples of suspect plant diseases. Len was able to discover Cucumber

Green Mottle Mosaic Virus in Katherine, for which there is now a NT emergency plant pest response. Very little Fusarium was found on this tour.

Produce posters (Darwin, Fred's Pass, Katherine Shows and the Katherine Farm and Garden Day) – The Fusarium Poster was taken around all these shows and included as a static display. A picture of the poster is attached (Appendix 1).

*Project meetings*

Teleconference meetings are held monthly between NT project members, Mr Puno and his supervisory panel to provide project updates and ensure activities align with milestones.

## 4) Molecular Studies

Due to the difficulty of importing live Fusarium cultures into Australia and the lack of QC3 glasshouse facilities in the NT, it was decided that DNA reference material be imported into the laboratory to enable molecular characterisation work. An agreement was reached with Dr. Katherine Everts (University of Maryland, USA) to send DNA of at least two isolates from each of the four *Fon* races and vegetative compatibility group for inclusion in the comparative molecular studies of *Fon* isolates. This is in progress and no DNA to date has been received for research. Continual contact with Dr Everts throughout the year has been maintained. The delay has been due to technical complications and lack of resources in Dr Everts laboratory. It is anticipated that isolates will be received in the new year and molecular characterisation of the overseas and interstate isolates can commence.

Investigations into molecular characterisation of *Fon* isolates has commenced by searching for pathogenicity genes such as 'secreted in xylem' (SIX). Previous studies on other Fusarium pathosystems such as *F. oxysporum* f.sp. *lycospersici*, Fusarium wilt of tomato and *F. oxysporum* f. sp. *cubense*, Fusarium wilt of banana indicated the presence of SIX genes which may be linked to pathogenicity (Rep et al 2004). In collaboration with the University of Queensland and the Australian Genome Research Facility, Brisbane, NT isolates NTP-Dc 36953 and NTP-Dc 36955 underwent whole genome sequencing using MiSeq next generation sequencing (NGS) in search for SIX genes. Collaborations with the University of Sydney, with the project's post graduate student, Mr Puno has also been initiated with a list of interstate Fon isolates from NSW, Qld, WA and Vic to be included in the NGS work. DNA preparations are currently being prepared with purified DNA to be sent to NT DPIF for SIX PCR tests but also for the NGS work in collaboration with UQ and AGRF.

Using the data obtained from the NGS work for NTP-Dc 36953 and NTP-Dc36955, primers are being designed against the SIX genes thus far identified. These include SIX 1-14. All Australian and overseas Fon isolates will be included in this SIX study.

## 5) Rootstocks and grafted melons

**Watermelon grafting – preliminary observations**

*Rootstock Germination*

Ten seeds were sown of each of the five rootstock seed lines that are to be used in the watermelon grafting trials. After ten days, the number of germinated seeds in the nursery was noted and the percentage germination is presented in Table 5. Allowances for poor germination will need to be made when sowing seeds for future work.

Table 5. Percentage of watermelon seed germination.

| Rootstock line* | Type | % Germination |
|---|---|---|
| NTX1 | *Cucurbita* sp. Hybrid squash | 100 |
| NTX2 | *Cucurbita* sp. Hybrid squash | 80 |
| NTX3 | *Cucurbita maxima* – F1 hybrid | 20 |
| NTX4 | *Cucurbita maxima* – F1 hybrid | 40 |
| NTX5 | *Lagenaria siceraria* – bottle gourd | 100 |

*Generic numbers assigned by NT DPIF

*Rootstock Vigour*

Seedlings of all of the *Cucurbita* lines showed good strong vigour with thick Hypocotyls compared to the *Lagenaria* seedlings. Rootstock seedlings are ready for grafting when plants have their first fully expanded leaf. The *Cucurbita* seedlings reached the first fully expanded leaf stage at 13 to 14 days from sowing while the Lagenaria seedlings took 17 to 18 days to reach this stage.

*Grafting*

Due to the lack of seedless watermelon seedlings, the variety 'Charleston Grey' watermelon was used as the scion in this preliminary grafting work. These seedlings had the first leaf beginning to emerge when grafted.

There are a range of grafting methods used for grafting watermelons. Most methods allow the rootstock to continue to function after grafting by leaving one or both cotyledons intact. The side insertion graft was used in this initial trial which leaves the entire rootstock intact and the watermelon scion is inserted into the side of the rootstock Hypocotyl. This scion is held in place using a grafting clip until the graft heals. Grafted plants were placed in a mist house (daytime misting for 8 seconds every 2 minutes and 15 seconds every 20 minutes at night) and their progress monitored.

At 7 days after grafting, all rootstock growth above the cotyledons was removed and callus had begun to form around the graft union. At this stage the scions on the *Cucurbita* rootstocks looked stronger than those grafted onto the Lagenaria rootstock. Half strength liquid fertiliser (aquasol) was applied to all the plants.

At 10 days after grafting, the rootstock cotyledons were removed thus eliminating any possibility of re-growth from the rootstock. The plants were moved from the mist house in to the shaded nursery to harden off.

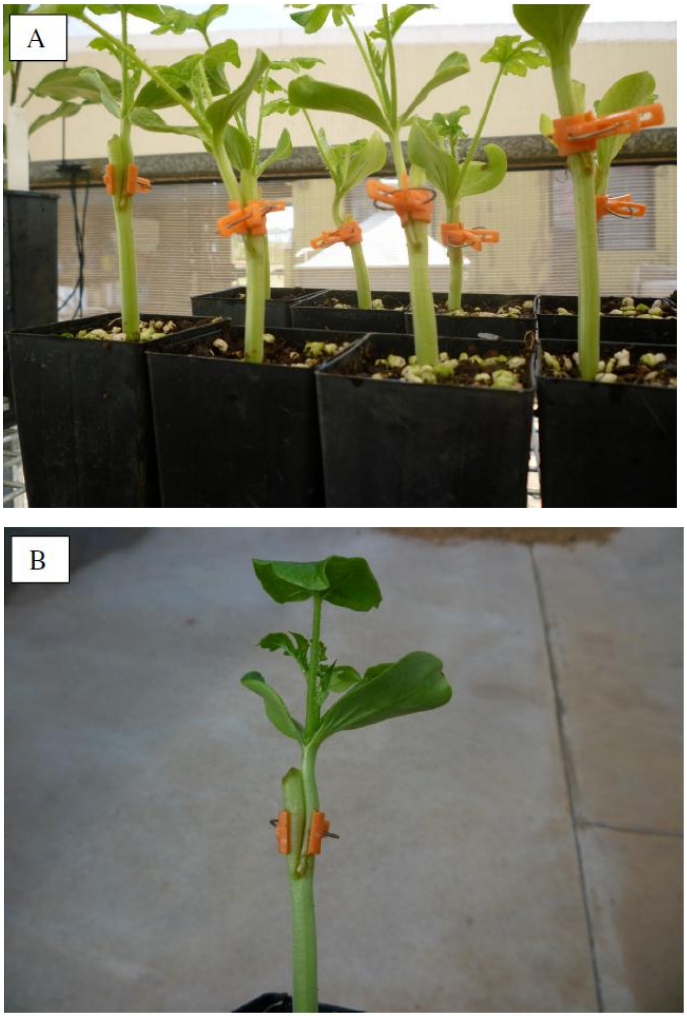


Fig. 2. A successful side insertion graft (A, B) from the preliminary grafting trial.

*Graft Success*

While the success rates between the grafts on *Cucurbita* and *Lagenaria* rootstocks were similar, the scions on the *Cucurbita* rootstocks appeared to "take" more quickly and showed greater early vigour compared to the scions on the Lagenaria rootstock.

Table 6. Results from the graft preliminary trial for *Cucurbita* and *Lagenaria*.

| Rootstock line | Type | No. of grafts | Success |
|---|---|---|---|
| NTX1 | *Cucurbita* sp. Hybrid squash | 8 | 7 |
| NTX2 | *Cucurbita* sp. Hybrid squash | 5 | 4 |
| NTX3 | *Cucurbita maxima* – F1 hybrid | 0 | 0 |
| NTX4 | *Cucurbita maxima* – F1 hybrid | 3 | 3 |
| NTX5 | *Lagenaria siceraria* – bottle gourd | 10 | 8 |

Further trials are planned over the next few months to test other grafting methods and the effect of rootstock age on grafting success.

## 6) Collaboration with New South Wales

Victor Puno, University of Sydney Masters Student did not visit the NT during this milestone period (Milestone 102 has previously reported a visit to the NT by Victor). It was decided that the money to pay for his visit would be better used to support his field surveys to melon growing regions in NSW and Qld. He collected samples from NSW, QLD and WA for race differential trials and molecular characterisation studies. Similar techniques were used for the trials as the NT experiments except inoculations were conducted with $10^6$ spores/ml spore suspensions on cultivars outlined in Table 2.

Mr Puno's first race differential preliminary trial results are shown in Table 7. These results indicate that the aggressive race 3 (previously thought to be only found in Maryland, USA) is now found in Australia. The NT isolate (NTP-Ds36955) used by in the NSW trial was renamed as VP0584 and preliminary data indicates this is race 3. This differs to the results obtained in the NT, thus highlighting the need to repeat the race differential trials in 2015. The two cultivars 'Charleston Grey' and 'Calhoun Grey' still needs to be conducted in NSW for interstate Fon isolates.

Table 7. Race differential trials conducted in NSW for interstate Fon isolates.

| | | Percentage Death of Differential Cultivars (%) | | | | |
|---|---|---|---|---|---|---|
| Isolate | Location | Sugar Baby' | Crimson Sweet' | Allsweet' | SP-4' | Race Designation |
| VP0583 | WA, Cookernup | 100 | 91 | 91 | 91 | Race 3 |
| VP0586 | WA, Broome | 88 | 75 | 72 | 21 | Race 2 |
| VP0457 | NSW, Griffith | 86 | 44 | 60 | 52 | Race 3 |
| VP0603 | NSW, Mildura | 100 | 95 | 45 | 42 | Race 3 |
| VP051 | QLD. Chinchilla | 80 | 91 | 78 | 15 | Race 2 |
| VP016 | QLD, Oakey | 75 | 90 | 86 | 18 | Race2 |
| VP071 | QLD, Gatton | 41 | 47 | 36 | 0 | Race 2 |
| VP088 | QLD, Chinchilla | 79 | 73 | 66 | 20 | Race 2 |
| VP0585 | QLD, Bundaberg | 85 | 76 | 73 | 34 | Race 3 |
| VP0624 | QLD, Bundaberg | 96 | 89 | 82 | 47 | Race 3 |
| VP0584 | NT | 72 | 98 | 100 | 50 | Race 3 |

Mr Puno successfully applied to upgrade his Masters to a PhD degree in late 2014. He will continue to work on Fusarium wilt of watermelons to gain a better understanding of the disease and pathogen to aid the management of the disease in Australia.

**Commercialisation & Intellectual Property Issues**
Nil

**Next Steps**
Activities for the next progress report will include the continuation of race differential testing in the NT and NSW; progress of temperature trials both glasshouse and field; commence rootstock trials when seedlines obtained from Monsanto Australia and Rijk Zwaan. Extension activities will include maintaining the project website, article for the industry newsletter and continual molecular characterisation of *Fon.*

**Other Issues**

A major issue arose in the NT in August 2013 when banana freckle disease caused by *Phyllosticta cavendishii* was discovered on the popular Cavendish cultivar. All departmental resources including all personnel involved in this project were reallocated to help with the emergency plant pest incursion. This response is ongoing. A second exotic disease outbreak occurred in September 2014 with the detection of Cucumber Green Mottle Mosaic Virus on watermelon and pumpkin crops. A majority of staff involved in this project was reallocated to help with the emergency plant pest incursion. Although the glasshouse trials and on farm observations continued for this project, resources were fully stretched. The CGMMV response is still ongoing. It is unknown if this will affect resources for this project in 2015.

**Appendices**

- Conference and show poster

**Acknowledgements**

We would like to thank Horticulture Innovation Australia for funding; Monsanto Australia and Rijk Zwaan for funding and providing seedlines for experiments. Syngenta for providing seedlines. Local NT grower for allowing NT DPIF to conduct on farm observations Fon trial.

Contributors to the milestone report and research are Lucy Tran-Nguyen, Barry Condé, Stacey Cook, Stuart Smith, Mark Traynor, Heather Wallace, Lois Ulyatt, Peter Bergin, Paige Richter and Victor Puno (NSW).

**References**

Hassell, R. "Continuation of improved grafting method for watermelons transplants." South Carolina–USA (2008).

Hassell, Richard L., Frederic Memmott, and Dean G. Liere. "Grafting methods for watermelon production." HortScience 43.6 (2008): 1677-1679.

Holliday, P (1980). "Fungus diseases of Tropical Crops". Dover Publications, New York., pp 180 – 181, "Fusarium oxysporum f. sp. niveum"

Keinath, A. P. and DuBose, V. (2009) First report of *Fusarium oxysporum* f. sp. *niveum* race 2 in South Carolina watermelon fields. *Phytopathology* 99:S63

Memmott, F.D. and Hassell, R.L. 2010. Watermelon (Citrullus lanatus) grafting method to reduce labor cost by eliminating rootstock side shoots. *Acta Hort.* (ISHS) 871:389-394 http://www.actahort.org/books/871/871_53.htm

Martyn, R. D., and Bruton, B. D. 1989. An initial survey of the United States for races of

*Fusarium oxysporum f. sp. niveum. HortScience* 24:696-698.

Rep M, van der Does HC, Meijer M, van Wijk R, Houterman PM, Dekker HL, de Koster CG (2004) A small, cysteine-rich protein secreted by *Fusarium oxysporum* during colonization of xylem vessels is required for I-3-mediated resistance in tomato. *Mol Microbiol* 53:1373– 1383.

Walker, M.N. (1941). "Fusarium wilt of watermelons. 1. Effect of soil temperature on the wilt disease and growth of watermelon seedlings". Bulletin Florida Agriculture Research Station 363.

Zhang (2009). Watermelon Pollenizer SP-4. United States Patent. Patent No. US 7,550,652 B2. Date of Patent: Jun. 23, 2009.

Zhou, X. G., and Everts, K. L. 2003. Races and inoculum density of Fusarium oxysporum f. sp. niveum in commercial watermelon fields in Maryland and Delaware. Plant Dis. 87:692-698.

Zhou XG, Everts K, Bruton BD. (2010) Race 3, a new and highly virulent race of *F. oxysporum* f.sp. niveum causing Fusarium wilt in watermelon. *Phytopathology* 94(1):92-98.