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Undergraduate Thesis

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Alternate *Ipomoea* species as a diagnostic tool for enhancing virus detection in sweetpotato

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Declaration of Originality

The following thesis is the original work of the author, except where it is acknowledged. The work that is presented in this thesis has not been submitted previously for this degree, at this or any other institution.

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Abstract

Research into yield decline has proven virus to be one of the most limiting factors impacting sweetpotato production. To combat yield decline, Australian researchers developed a pathogen tested (PT) seed scheme to provide growers with first generation, virus free planting material (Dennien et al. 2013). Since the introduction of pathogen tested planting material in Australia, production has increased by about 1700% (Best et al. 2016). Sweetpotato plants with virus infection are typically difficult to visually assess for virus symptoms as they are often asymptomatic (Kashif 2009). Sweetpotato sap also contains inhibitors which can interfere with virus diagnostic methods (Dennien 2015).

The sweetpotato pathogen testing process at Gatton Research Facility uses a range of complimentary diagnostic procedures to overcome these issues. The use of *Ipomoea setosa* as an herbaceous indicator plant being the most important (Dennien et al. 2013). *Ipomoea setosa* is extremely susceptible to sweetpotato virus infection and although the grafting and plant monitoring procedure is time consuming, it allows rapid increase in virus titres which enhances the accuracy of Nitro Cellulose Membrane – Enzyme Linked Immunosorbent Assay (NCM- ELISA) testing (Aritua et al. 2007). Susceptibility and interaction of *Ipomoea setosa* to Sweetpotato Feathery Mottle Virus (SPFMV) is well documented in the literature, however, the susceptibility of other *Ipomoea* species is relatively unexplored (Valverde e al. 2007). This study investigated the interaction of SPFMV with 8 species of *Ipomoea*. Findings from this study indicate that *Ipomoea setosa* is the most reliable and consistent at producing yields and that cultivars of *Ipomoea nil* responded differently to infection of SPFMV.

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1. Introduction

Ipomoea batatas (sweetpotato) is currently the 8th most important food crop globally (Dennien et al. 2013) and is considered the fifth most important economic crop in developing countries (Thottapilly 2009). In countries like Africa and Papua New Guinea it is one of the most important staple food crops (Karuri et al. 2017). Australia is considered to have the highest yielding sweetpotato crops as yields have increased by 1700% in the last 16 years (Best el al. 2016). The biggest constraint on yields was infection of virus which in some instances would cause yield losses in excess of 50% (Gutierrez et al. 2002).

The Australian industry was able to combat yield decline issues with the introduction of Pathogen Tested (PT) 1st generation planting material. Sweetpotato growers in Australia now have access to clean planting material which has been the biggest contributing factor to increase in yields. An important process in generating PT material was the use of virus diagnostics. Herbaceous indicators complimented with NCM-ELISA and qPCR has been one of the key component to removing viruses.

Sweetpotato plants were initially difficult to deal with as they are often asymptomatic, have low titres making virus difficult to detect in serological testing. Also inhibitors in the sap would interfere, so the use of *I. setosa* removes these boundaries as it is quite vulnerable to virus so titres build quickly making it compatible for serological tests, no inhibitors are contained within the sap and when grafted show clear reliable and consistent expression of virus symptoms. In the virus diagnostic program, a suspect sweetpotato cutting is grafted onto *I. setosa* which is then monitored for symptoms and subsequently used for virus detection in NCM-ELISA and quantitative polymerase chain reaction (qPCR).

This study investigates the possible use of other *Ipomoea* in the virus diagnostic program. Eight relatively unexplored species of *Ipomoea* were graft inoculated with two SPFMV infected sweetpotato cultivars. Symptoms expression and suitability of grafting were observed and assessed to comment on the suitability of these species as herbaceous indicator plants.

1.2. Project Objectives

The research questions to be answered include; ‘‘Is there an alternative species of *Ipomoea* suitable for virus diagnostics?’’, ‘’Are the selected *Ipomoea* species susceptible to SPFMV?’’ and ‘‘How will each species respond to SPFMV infection?’’. To answer these questions a range of *Ipomoea* species were graft inoculated with sweetpotato material infected with SPFMV to determine whether there are other alternate species of *Ipomoea* that are suitable for use in SPFMV diagnostics. Assessment criteria of these grafted plants includes virus susceptibility, timing of symptom expression, severity of symptoms, suitability for grafting and subsequent use of the infected plants using NCM-ELISA. Results from this experiment will provide information on the suitability and effectiveness of alternate *Ipomoea* species as indicator plants for SPFMV.

2. Literature Review

2.1. Overview of Sweetpotato

Sweetpotato (*Ipomoea batatas*) belongs to the botanical family *Convolvulaceae* and is ranked globally as being the eight most important food crop with annual production of around 125 million tonnes across 100 countries (Dennien et al. 2013). In developing countries sweetpotato is ranked as being the fifth most important economic crop which this is not surprising due to high nutritional value (Thottappilly 2009). Sweetpotato is high in vitamin A & C, high in fibre and low in carbohydrates (Henderson et al. 2012). These nutritional advantages make sweetpotato one of the most important staple food crops in Africa (Karuri et al. 2017).

Until recent times sweetpotato was not seen as a crop of value in western countries but was grown more for livestock feed. It has only been within the last 20 or so years that production has increased to a commercial scale. A major driver in increased consumption by the consumer is the related health benefits. This 'newness' into the market has meant that research is still limited and a lot about this crop is unknown (Carey et al. 1998). One area where much work has occurred over the last two to three decades is the elimination of virus from planting material. It has been established that virus in planting material has been one of the most influential factors affecting yield (Henderson et al. 2012) and it is predicted that more than half of yield losses have been from virus infection (Gutierrez et al. 2002). Globally there are 35 reported sweetpotato viruses belonging to 10 families (Moyer & Salazar 1989), (Valverde et al. 2007), (Dennien 2016). Of these 35 there are to date five recorded sweetpotato viruses in Australia including Sweetpotato feathery mottle virus (SPFMV), Sweetpotato virus 2 (SPV 2), Sweetpotato chlorotic fleck virus (SPCFV), Sweetpotato leaf curl virus (SPLCV) Sweetpotato collusive virus (SPCV) and Phytoplasma *Candidatus aurantifolia* or Sweetpotato little leaf (SPLL). (Dennien 2015)

2.2. Sweetpotato Feathery Mottle Virus

SPFMV has been identified as one of the most widespread sweetpotato viruses being identified in Australia, Papua New Guinea, China, Solomon Islands, Africa, Korea, Europe, United States of America, Peru, Fiji and Taiwan (Dennien 2015). SPFMV was first found in the United States and described by Doolittle and Harter in 1945 (Karyeija et al. 1998). SPFMV is in the genus *Potyvirus* and found in the family *Potyviridae* (Dennien 2015) and symptoms

in sweetpotato are usually quite mild and might include vein clearing, feathered vein clearing and chlorotic spots on the leaves. The root might exhibit external cracking, and internal necrosis depending on the cultivar. Compared to other potyviruses SPFMV has a much more specific host range with infection only occurring in *Ipomoea* species with the exception of *Chenopodium amaranticolor, C. quinoa,* or *Nicotiana benthamiana* (Karyeija et al.1998). Insect vectors such as aphids are responsible for the spread of SPFMV in the field (Loebenstein 2009).

SPFMV can cause significant yield losses to sweetpotatoes with experiments finding that sweetpotato plants may only show mild symptoms of SPFMV infection but when comparing yield at harvest, virus free planting material yields 20 to 100% higher compared to infected SPFMV planting material (Dennien 2015). SPFMV also has the ability to form a synergistic relationship with other viruses. For example this has occurred in Peru where SPFMV has combined with Sweetpotato Chlorotic Stunt Virus (SPCSV) to create Sweetpotato Virus Disease (SPVD) (Gutierrez et al. 2002). SPVD arose in Peru from 1997 to 1998 after an increase in whitefly population which are vectors for SPCV. It is estimated that SPVD has resulted in yield losses of up to 80% (Carey et al. 1998). Other virus complex that exist include chlorotic wharf disease (CD) effecting mainly Argentina and the Philippines (Di Feo et al. 2000), In Uganda sweetpotato severe mosaic disease (SPSMD) can be responsible for yield losses of 80% (Mukasa et al. 2006) and Camote Kulot causing 50% yield losses in the Phillipines (Dennien et al. 2015).

2.3. Spread and Management of Sweetpotato Viruses in Australia

The spread of sweetpotato viruses occurs in a number of ways, the first being by insect vectors. Insects can spread sweetpotato viruses in two forms; via non-persistent and persistent transmission (Dennien 2015). Non-persistent transmission is when an insect is feeding on an infected sweetpotato leaf and viruses attach to the mouth of the feeding insect. Once this insect has virus particles present in the mouth piece it can quickly spread this virus when feeding on sweetpotato leaves of nearby plants. Persistent transmission occurs when an insect swallows virus particles into the gut and salivary glands. Once in the salivary glands the insect feeding on another leaf can transfer virus particles. This method of virus transmission is slower as the insect must feed for hours to transfer particles from the salivary gland (Dennien & Henderson 2016; Dennien 2015).

Once an aphid has fed on an SPFMV infected plant it has the potential to transfer the virus to any other *Ipomoea* in the surrounding environment. This includes any morning glory, bell vine (*Ipomoea plebia*), red and pink convolvulus, mile-a-minute or coastal morning glory. It is not currently known which species of *Ipomoea* are definite carriers of SPFMV and if any are asymptomatic like sweetpotato. Farmers are advised to use good hygiene to prevent the spread of viruses including, removing and monitoring *Ipomoea* weeds for any virus like symptoms, use of insecticides to control vectors, cleaning vehicles and machinery when moving between farms and being alert of people entering a farm who could potentially be carrying contaminants in soil or clothing (Dennien 2015).

2.4. Pathogen Tested Seed Scheme

SPFMV combining with other viruses to create a complex disease causes the greatest damage to yield, and the threat of incursions of new viruses in Australia and significant losses of yield led to the development and introduction of Pathogen Tested (PT), first generation planting material for growers. The commercial Australian Sweetpotato system is identified as being the most intensive and highest yielding in the world due to the introduction of PT planting material (Henderson 2015). The PT program involves firstly selecting a well yielding cultivar to be heat treated for 7 days at 25°C ,then 14 days at 29°C then lastly the plant is kept at 39°C for 28 days. This heat treatment is severe on plants and denatures virus particles present. Once heat treatment is complete the apical meristem is removed from the tip of the plant and meristem cells are extracted and placed onto nutrient media. Once plants grow 3 to 5mm in diameter they are potted and grown for at least three months. *I. setosa* is closely related to sweetpotato and is used as an herbaceous indicator as it is extremely susceptible to sweetpotato viruses displaying virus symptoms when sweetpotato ordinarily wouldn't. After grafting the plant is observed for symptoms then used in complimentary virus diagnostics tests, NCM-ELSIA and qPCR (Lovatt 2015).

I. setosa is used for these tests as the sap in sweetpotato has inhibitors making it incompatible with current virus diagnostic procedures (Kokkinos & Clark 2006). Also viruses are often present in sweetpotato plants at low titres but titres are able to rapidly increase in the highly susceptible *Ipomoea setosa* plants enabling more accurate detection of viruses. Once plants from meristems test negative to sweetpotato viruses they are planted and roots are harvested and provided to growers. These roots are planted into 'seedbeds' and each

individual root grows shoots that the farmer then uses as vegetative planting material (Lovatt 2015).

In previous farming systems growers would keep their own root stock and vegetative material but this was providing an extended period of time for material to become infected with sweetpotato viruses. Once infected, growers were then passing virus onto the subsequent crop by using old planting material (Dennien & Henderson 2016). In the last 16 years since the introduction of PT material it is estimated that sweetpotato yields have increased by 1700% in Australia (Best et al. 2016).

2.5. Herbaceous Indicator Plants

I. setosa is currently the most widely used herbaceous indicator plant for sweetpotato virus testing. To detect virus one of two methods are used for inoculating *I. setosa*. Shoots are grafted onto *I. setosa* or inoculation will occur by mechanically infecting *I. setosa* with sweetpotato sap (Dennien 2015). This method of virus detection is thought to be one of the most accurate methods as Sweetpotato sap contains inhibitors including latex, polyphenols and polysaccharides making it unsuitable in serological testing methods (Kokkinos & Clark 2006). Another issue is sweetpotato often won't display virus symptoms and issues often arise when testing sweetpotato tissue as they often have low virus titres. To overcome these hurdles the herbaceous indicator plant *I. setosa* is graft inoculated with a suspected virus infected sweetpotato cutting to initiate virus titre build up for more accurate detection in NCM-ELISA and qPCR (Valverde et al. 2007).

There has been some work using the 1000s of available *Ipomoea* species as indicator plants. Studies by Luan et al. (2006), Sonoda et al. (2000), Cali & Moyer (1981), Okada et al. (2001), Lotrakul et al. 1998 and Suoto et al. (2003) all successfully graft inoculated *Ipomoea nil* with sweetpotato viruses. However, only Sonoda et al (2000) and Lotrakul et al. (1998) stated the use of cultivar of *I. nil cv. Scarlet O'Hara'* for grafting. Another study by Guiterrez et al. (2003) used *I. nil cv. Roth.* This leaves some confusion around which cultivars have been used and whether all cultivars are susceptible to virus infection.

Nicotiana benthamiana (Guiterrez et al. 2003), Nicotiana clevelandii and *Chenopodium Wild* are other species that have also been utilised as virus indicators with some success (Moyer & Salazar 1990). Another species of *Nicotiana tabacus* has also been found to be successful at exhibiting symptoms of vein clearing, leaf curl and leaf distortion (Hollings et al. 1976).

I. aquatica was also trialled as an herbaceous indicator plant and findings indicate that this species of *Ipomoea* is not susceptible to SPFMV but is susceptible to SPLCV (Valverde et al. 2007; Lotrakul et al. 1998). *Ipomoea purpurea* was also trialled as an indicator plant by Cali & Moyer (1981) but found to be symptomless whereas Suoto (2003) found *Ipomoea tricolour* to be a successful indicator.

Other species which have not been used as an herbaceous indicator but have exhibited symptoms and been virus tested include a study by Banks et al. (1999) who found an *Ipomoea indica* plant with yellow vein symptoms which tested positive for *Ipomoea* yellow vein virus (IYVV). Like many other species of *Ipomoea, I. indica* was originally an ornamental plant in home gardens but at time of collection had escaped and was naturalised in the surrounding environment. One study in Uganda identified *I. hederifolia* and *I. tenuirostris* as being SPFMV virus infected. When *I. tenuirostris was* tested for SPFMV using NCM-ELISA, positive results were observed for leaves with and without symptoms (Karyeija et al.1998).

2.5.1. Herbaceous Indicator Plants in this study

Ipomoea aquatic (Swamp Morning Glory, Water Spinach) is popular for use in stir-fries and is abundant in the Caribbean and North America. *I. aquatic* has a white flower and is thought to have originated from the Caribbean Territories or in Continental US (Integrated Taxonomic Information System 2017).

Ipomoea coccinea (Mexican Morning Glory, Red Morning Glory) is thought to have originated from Continental US. This plant has a distinct red flower and is most often grown as an ornamental (Integrated Taxonomic Information System 2017).

Ipomoea nil is thought to have 1500 mutant lines (Hoshino et al. 2016). For this study 3 cultivars from mutant lines selected were Kidachi (sourced from Louisiana Southern University), Pink Morning Glory (from Australian Nursery Fair Dinkum seeds) and Red Speckled Splash (supplied by Australian nursery Herbalistics).

Ipomoea plebia (Bellvine) is a native weed to large parts of eastern and northern parts of Australia and has distinct white flowers and heart shaped leaves (Brisbane City Council 2015).

Ipomoea purpurea also referred to as common morning glory or tall morning glory has a light purple flower, is popular as an ornamental plant and originated from either Canada or Continental US (Integrated Taxonomic Information System 2017).

Ipomoea sloteri (Cardinal Climber) is a cross between *Ipomoea quamoclit* and *Ipomoea coccinea* and has heart shaped morning glory leaves and purple, trumpet shaped flowers (Iannoti 2017).

Ipomoea setosa (Brazilian morning glory) is the current universal indicator plant for sweetpotato virus diagnostics. It has a trumpet shaped light purple flower and heart shaped morning glory leaves (Integrated Taxonomic Information System 2017).

2.6. ELISA

The use of NCM -ELISA has become one of the most popular methods for testing sweetpotato viruses due to its simplicity, affordability sensitivity and adaptability (Valverde et al. 2007). This method uses antigens or antibodies and is manufactured to detect virus particles making it a very versatile test. NCM-ELISA is an antibody test which gives a positive or negative reaction when added antibodies attach to virus particles. When attachment occurs the substrate reacts with the conjugate antibody to give positive result in the form of a purple circle on nitrocellulose membrane (Dennien et al. 2013). An NCM-ELISA provides antibodies to test for 10 sweetpotato viruses; Sweetpotato feathery mottle virus (SPFMV), Sweetpotato mild mottle virus (SPMMV), Sweetpotato Mild Speckling virus (SPMSV), Sweetpotato chlorotic stunt virus (SPCSV), Sweetpotato collusive virus (SPCV), Sweetpotato C-6 virus (SPC-6), Sweetpotato chlorotic fleck virus (SPCFV), Sweetpotato virus V (SPVG), Sweetpotato latent virus (SPLV) and Cucumber Mosaic Virus (CMV).

The serological test and antibodies for sweetpotatoes were developed at the International Potato centre in Peru who now distribute NCM-ELISA tests in kits around the world (Dennien 2015). The use of *I. setosa* leaf tissue in this test has overcome barriers that originally existed when using sweetpotato. *I. setosa* overcame the issue of sweetpotato containing low and irregular distribution of virus titres and in some instances inhibitors in the sap. (Valverde et al. 2007).

2.7. Other Methods of Virus detection

2.7.1. Electron microscopy

Electron microscopy enables the researcher to view virus particles at a greater magnification than microscopes. This is achieved by staining leaf tissue, which surrounds virus particles revealing their structure (Wilson 2014). Issues identified with this method include being unable to identify virus strains easily, extensive training must be undertaken and equipment is expensive (Dennien 2015).

2.7.2. Nucleic Acid Spot Hybridization

Nucleic Acid Spot hybridization (NASH) is a nucleic acid based detection and hybridization of nucleic acid enables the nucleic acids and sequence to be detected. This denaturisation is completed on a gel, in solution or on nitrocellulose paper (Dennien 2015).

2.7.3. Polymerase Chain Reaction

Extracted DNA and RNA from plants is converted to complimentary DNA (cDNA) in a polymerase chain reaction (PCR). The PCR machine detects parts of the genome and specific primers attach. Once attached these strands go through thermocylcing (heating and cooling) where DNA strands are split and primers attach creating a new strand and replicating the DNA. The replication during thermocycling creates many thousands more strands of DNA which can be viewed on agarose gel (Wilson 2014).

2.7.4. Real Time Polymerase Chain Reaction

Real time polymerase reaction (RT-PCR) otherwise referred to as quantitative polymerase chain reaction (qPCR) uses the same principles as PCR but is more accurate. Accuracy is improved as qPCR compares the threshold value of an unknown sample with diluted series of a known sample. Fluorescent dyes are used to determine how much DNA is in the unknown samples (Wilson 2014). Advantages of qPCR over PCR include faster detection and higher accuracy. Disadvantages include high cost of equipment and currently unavailability of primers specific to sweetpotato viruses (Dennien 2015).

2.7.5. Loop-mediated isothermal amplification (LAMP)

LAMP uses a single tube technique to amplify DNA and detect specific nucleic acid sequences. The uses of 4 forward and reverse primers allows LAMP to replicate thousands of copies of the target sequence that fluoresce in the presence of virus (Dennien 2015).

2.8. Summary

Sweetpotatoes grown in Australia are the highest yielding in the world due to virus testing and ability to remove viruses. Having the capability to continue and expand this system is important to maintain high yields. The use of herbaceous indicator plants plays a vital role as other technologies like NCM-ELISA and qPCR are useful for detecting viruses but are very specific as reliance is on primers and antibodies. The use of herbaceous indicators is important as symptom expression will always present symptoms when viruses are present. So if a new virus incursion was to occur NCM-ELISA or qPCR may not test positive but *I. setosa* will exhibit symptoms.

Research using other species of *Ipomoea* is still limited leaving great opportunity to explore alternate species as many are naturalised in the environment, potentially creating a biosecurity threat as these provide a reservoir for virus. The other possibility is finding another species that could compliment *I. setosa* in the current virus diagnostics program.

3. Method

3.1. Design

There are a vast number of *Ipomoea* species available globally. Species evaluated for inclusion had to be not prohibited in Australia. The species that have been selected for this experiment are found in Figure 1. The attributes outlined below were used to determine their eligibility for this experiment.

The native plant *Ipomoea plebia* commonly referred to as 'Bellvine' was chosen for assessment due to its prevalence in Australian sweetpotato production areas. Improving understanding of virus expression in this species will contribute to the ongoing improvement of knowledge on important virus hosts in sweetpotato fields.

The internationally used herbaceous indicator plant *Ipomoea purpurea* (Cali & Moyer 1981) was incorporated to evaluate its compatibility in Australian environmental conditions to test reaction with local virus strain.

The popular home garden species *Ipomoea sloteri*, *Ipomoea Nil cv. Red Speckled Splash, Ipomoea Nil cv. Pink Morning Glory, Ipomoea Nil cv. Kidachi, Ipomoea coccinea* and food plant - *Ipomoea aquatica* (Kang kong or water spinach) were used due to their widespread distribution, readily availability and also because they are potential reservoirs of yield limiting sweetpotato viruses in the Australian environment. Inclusion of 3 cultivars of *I. nil* (*I. Nil cv. Red Speckled Splash, I. Nil cv. Pink Morning Glory, I. Nil cv. Kidachi*) was done consciously to determine if there is a cultivar effect when infected with virus.

A control plant is essential to ensure the graft inoculation of each plant is successful. *Ipomoea setosa* (Brazilian Morning Glory) was included as it is currently the universal herbaceous indicator plant used globally in sweetpotato virus detection (Dennien et al. 2013).

Ipomoea setosa 'Brazilian Morning Glory'

Ipomoea nil Kidachi

Ipomoea plebia 'Bellvine'

Ipomoea aquatic 'Water Spinach'

Ipomoea Sloteri

Ipomoea nil cv. Pink Morning Glory

Ipomoea nil cv. Red Speckled Splash

Ipomoea purpurea 'Gradnpa Ott'

Ipomoea coccinea 'Red Morning Glory'

FIGURE 1: IPOMOEA SPECIES USED

3.2. Graft Scions Selected

Ipomoea species were grafted with Department of Agriculture and Fisheries (DAF) Gatton Research Facility (GRF) sweetpotato cultivars 'Snowhite' and 'Lester's Original Beauregard' (LOB). The selected *Ipomoea* species were used as the rootstock and the two DAF sweetpotato cultivars were the scion. These two cultivars were selected as they have previously been used as a positive control plant for SPFMV in the virus diagnostics program at GRF for a decade (Dennien pers com.). For a negative control the actual grafting plants were grafted into themselves e.g. *Ipomoea purpurea* was grafted onto *Ipomoea purpurea.* This was also to verify there were no seed transmitted viruses. Listed in Table 1 was the schedule for plant graft inoculation. LOB was grafted on four occasions whereas Snowhite was only grafted on three.

TABLE 1: GRAFT INOCULATION SCHEDULE

	January	February	March	April
I. setosa	LOB x 4 Replications	LOB x 4 Replications Snowhite x 4 Replications	LOB x 4 Replications Snowhite x 4 Replications	LOB x 4 Replications Snowhite x 4 Replications
I .purpurea	LOB x 4 Replications	LOB x 4 Replications Snowhite x 4 Replications	Not grafted	LOB x 4 Replications Snowhite x 4 Replications
I. coccinea	LOB x 4 Replications	LOB x 4 Replications Snowhite x 4 Replications	Not grafted	LOB x 4 Replications Snowhite x 4 Replications
I. nil cv. Kidachi	LOB x 4 Replications	LOB x 4 Replications Snowhite x 4 Replications	Not grafted	Not grafted
I. nil cv. PMG	Not grafted	LOB x 4 Replications Snowhite x 4 Replications	LOB x 4 Replications Snowhite x 4 Replications	Not grafted
I.nil cv. RSS	Not grafted	Not grafted	LOB x 4 Replications Snowhite x 4 Replications	Not grafted
I.aquatica	Not grafted	Not grafted	LOB x 4 Replications Snowhite x 4 Replications	Not grafted
I. sloteri	Not grafted	Not grafted	Not grafted	LOB x 4 Replications Snowhite x 4 Replications
I. plebia	Not grafted	Not grafted	Not grafted	LOB x 3 Replications Snowhite x 3 Replications

4. METHODOLOGY

4.1. Growing Ipomoea Seedlings

Seeds of *Ipomoea* species were directly sown into individual pots. For the purpose of this report all seeded *Ipomoeas* will be referred to as I*pomoea* seedlings. As many of these species are considered weeds it was vital that they were grown in secure areas where they were unable to escape into the surrounding environment.

4.1.1. Sowing steps

The sowing and growing of *Ipomoea* seedlings was performed in a separate screened insect proof compartment within an insect free glasshouse. This was to prevent virus infection from other plants by insect vectors. New seeds were always selected for sowing to improve the success of germination. During the course of this experiment seed germination was difficult so different methods of scarification were trialled. After different treatments of scarification were applied to all species it was found that nicking the seed coat and soaking in water for 12 hours gave the best germination results. Using this method all seeds were sown successfully except *I. plebia*. Due to significant difficulty with germination this species was only grafted in April.

Scarified seeds were sown 1cm deep into an appropriate pot (approximately 10cm in diameter) and filled with new pasteurised UC mix with pearlite substituted for gravel. Seeds were watered in thoroughly. Growth differed due to species variation and environmental conditions, however, generally at around 4 weeks the first true leaf would open. Plants were deemed to be ready for grafting when the first two true leaves had opened.

4.2. Grafting Procedure

The procedures for grafting outlined below are adapted from Dennien et al. (2013) and Mukasa et al. (2003). Graft inoculation with SPFMV is important so each species can be assessed for reaction and susceptibility to SPFMV. The method of graft inoculation was chosen as it is similar to the graft inoculation experiment by Mukasa et al. (2003).

Furthermore, Sandra Dennien (2017), leader of the virus diagnostics program at the Gatton Research Facility was interviewed and she indicated that this method has been a consistent procedure in the virus diagnostic program she coordinates. Sandra explained that this

method of grafting onto *Ipomoea setosa* has given continuous reliable success rates for graft survival, symptom expression and success in ELISA testing.

This grafting technique allows two points for virus to enter the host plant. This is beneficial in the instance of one graft not surviving there is a second graft as a buffer. Another positive is the simplicity and affordability of tools and materials making it easily adaptable not just in Australia but in developing countries. There are two parts to this procedure. Table 1 provides the grafting schedule. A negative control (grafting the Ipomoea species onto itself or with known virus free cultivar for example PT Beauregard Clean) was completed first in the grafting procedure to avoid cross-contamination from either LOB or Snowhite. Then the SPFMV infected controls were grafted onto specified *Ipomoea* species. The first Step of the grafting procedure is as follows.

4.2.1. End Cleft Graft

All tools used in these steps were sterilised with methylated spirits and flamed. All working surfaces were thoroughly cleaned with methylated spirits. This is vital to ensure that all sap is denatured to prevent transmission of virus through sap contamination. The steps below are illustrated in Figure 2 by Dennien et al. (2013).

Using sterile scissors the plant was cut horizontally on the main stem above the third true leaf (usually just emerging) to remove the top portion of the plant. Where this cut was created the sterile scalpel then cut the main stem vertically 1 to 1.5cm down the middle to create two halves.

Using the same scalpel one node was cut from the SPFMV affected control ensuring 4cm of stem remained below the node. In this 4cm of stem a wedge was cut on either side of the stem to the size of the space created by the two parts of the cut *Ipomoea* seedling. The wedges of sweetpotato control scion were placed into the opening and secured in place using Teflon tape. Teflon tape was used as an alternative to grafting tape to seal the scion (SPFMV infected sweetpotato) to the root stock (Ipomoea seedling) and promote healing as it is flexible, cheap and readily available.

4.2.2. Side Veneer Graft

An incision was made in the stem below the cotyledons. This incision did not cut through the plant but instead created an opening for SPFMV infected sweetpotato to be wedged in.

After both grafts have been completed the plant was then secured in place using a stake and clearly labelled with date, species of seedling, and name of sweetpotato control grafted. To promote graft healing a moist humid environment was created for the grafted plant. This was achieved by placing a plastic bag over the grafted plant and ensuring leaves weren't touching the bag. A light mist was then sprayed inside the bag to increase humidity and promote healing.

FIGURE 2: DISPLAY OF END CLEFT AND SIDE VENEER GRAFT ADAPTED FROM DENNIEN ET AL. (2013)

Figure 21
A terminal (end cleft) graft. The shoot at the top is sweetpotato, with the stem cut to a wedge to fit the split *I. setosa* stem.

Figure 22
A close-up of a terminal (end cleft) graft

Figure 23
A side (veneer) graft. The lower part of the stem of the sweetpotato is cut as a wedge to fit into the diagonal cut made into the *I. setosa* stem.

Figure 24
A close-up of a side (veneer) graft

4.3. Care After Grafting

Plants were kept in a plastic bag for one week to encourage healing. After the first week the grafts had sufficient time to heal and the bag was removed. Each plant was randomly assigned to a replication and arranged in glasshouse. Each plant had a single pot tray and was watered twice weekly or as required. To eliminate nutritional deficiencies as a possible source of confounding leaf symptoms each plant was treated with an all-rounder liquid fertiliser (Aquasol ™) at 10g/8L of water fortnightly and a slow release fertiliser (Osmocote ® Plus trace Elements: Total All Purpose) was applied to each pot at a rate of 3g/pot once at the time of sowing.

4.4. Symptom Monitoring

Once the plastic bags had been removed the first collection of indexing data (symptom monitoring) occurred. The symptom descriptions for this experiment have been based on symptom examples in Growing Healthy Sweetpotato by Dennien et al. (2013).

The same style of symptoms are also displayed by Dennien & Henderson (2016) giving validity to this style of indexing. These symptoms were chosen as they are the only published symptoms in literature from Australian work. Use of Australian examples is thought to be the best fit as it is unknown what strains of SPFMV exist in Australia and whether these are the same as those in other parts of the world. Using this model is thought to give the truest plant expression of symptoms in our environment. These symptoms were also recorded in the same facility where this experiment took place. The grafted plants from this experiment had similar environmental conditions to those used to create the symptom guide in Growing Healthy Sweetpotato.

Symptoms were monitored and recorded twice weekly. This frequent checking over the 6 weeks was required as virus symptoms are often transient (Dennien et al. 2013). To determine how each species reacts to SPFMV the absence or presence of the following visual symptoms were assessed and recorded: mottle, vein clearing (VCL), chlorotic spots, chlorotic flecks, chlorosis pale leaf, leaf cupping, leaf curling/rolling, leaf balling, rugosity, leaf distortion, necrosis, flowering and the number of surviving or live grafts was also recorded.

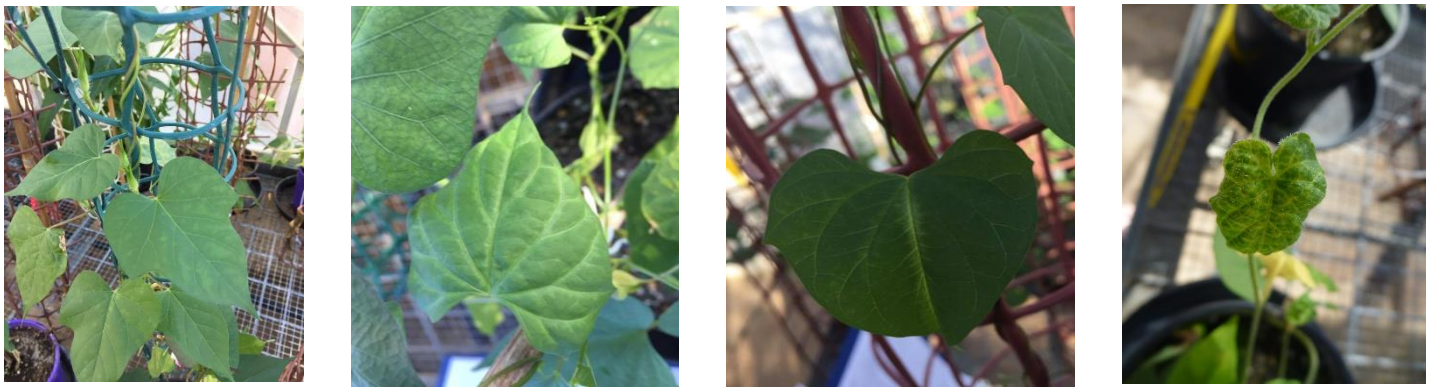

FIGURE 3: IN PHOTO ORDER LEFT TO RIGHT, PHOTO 1 CHLOROTIC SPOTS, PHOTO 2 AND 3 VEIN CLEARING, PHOTO 4 MOTTLE AND VEIN CLEARING

When recording these symptoms the severity and section of the plant exhibiting these symptoms was noted to understand how the virus was moving through the plant.

4.5. NCM-ELISA

To determine whether SPFMV had infected the graft inoculated *Ipomoea* plants an ELISA test was deemed to be the best method of detection. This method was chosen as in all literature, testing for SPFMV used the serological testing NCM-ELISA kit created by the International Potato Centre (CIP) in Peru. The ELISA kit uses specific antibodies to identify 10 viruses known to infect sweetpotato. This kit imported from CIP, Peru was used for testing *I. setosa* for the presence of SPFMV in work completed by Okpul et al. (2011), Kathurima et al. (2011), Dennien et al (2013), Dennien (2016) and Kashif (2009). Furthermore, this is the only kit available that can be imported into Australia.

4.5.1. Collection of Samples for Blotting

Three leaves with symptoms were sampled from the grafted plants, one from the bottom area of the plant, one from the middle and lastly the third leaf from the top. Sampling from these sections of the plant is important as the virus particles may not be evenly distributed throughout the plant. When collecting leaves the use of gloves was required. Gloves were changed between plants to prevent sap transmission of virus. These leaves were then placed into a labelled bag before moving onto the next plant. Once collected they were stored for no longer than 5 hours at 3°C to preserve leaf tissue. All leaves that were collected were blotted to membranes on the same day.

4.5.2. Blotting for NCM-ELISA

All bottles, beakers, dishes and forceps to be used in the NCM-ELISA tests were soaked in a 2% bleach solution for 20 minutes and then triple rinsed with distilled water and dried on paper towel. Extraction buffer was prepared as presented in the instruction manual of the NCM-ELISA kit. Fresh extraction buffer was made up before each blotting session as it expires after 12 hours. From each of the 3 leaves collected, a disc roughly 1-1.5cm in diameter was cut from the base of the mid rib. Using forceps each piece of these disks were placed into bags provided in NCM-ELISA kit. Each bag was labelled in the top right hand corner with plant number and 3mls of extraction buffer was added. Leaves were crushed and ground in extraction buffer within the plastic bag using a thick walled test tube supplied in the ELISA kit. The test tube were used to roll and manipulate the leaf disks until all leaf tissue was completely ground up and in the form of a green liquid

The membrane was then prepared for blotting and labelled with the date and virus – in this case SPFMV. As per ELISA kit instructions the membranes and filter paper were soaked in TBS. Each membrane was placed on 2 filter papers. This filter paper was required to keep the membrane moist. Using a pipette, 15µl of each ground sample was blotted onto the membrane. Membranes were then dried and stored as access to antibodies to perform the test were unavailable at the time.

4.5.3. Future Work

Other viruses were also tested for use with NCM-ELISA however due to time delays obtaining import permits they were not included in this experiment report. Further work involves evaluating other viruses for compatibility with Deoxyribonucleic acid (DNA) extraction in Quantitative Polymerase Chain Reaction (qPCR). Samples will also be assessed for suitability in Loop-mediated Isothermal Amplification (LAMP) later this year.

5. Data Analysis

Results were analysed using ANOVA in GenStat. The following results firstly investigate the average graft survival of each species. This result is the number of live grafts out of two. Secondly, each species and graft inoculated sweetpotato is listed separately to track symptoms development over the different environmental conditions. Lastly, the significant difference is listed in Table 2 and 4 to compare the statistical significance of each virus symptom, on each *Ipomoea* species, over each time period. Investigating these 3 criteria will give evidence to comment on success further on in the discussion.

6. Results

6.1. Graft Survival

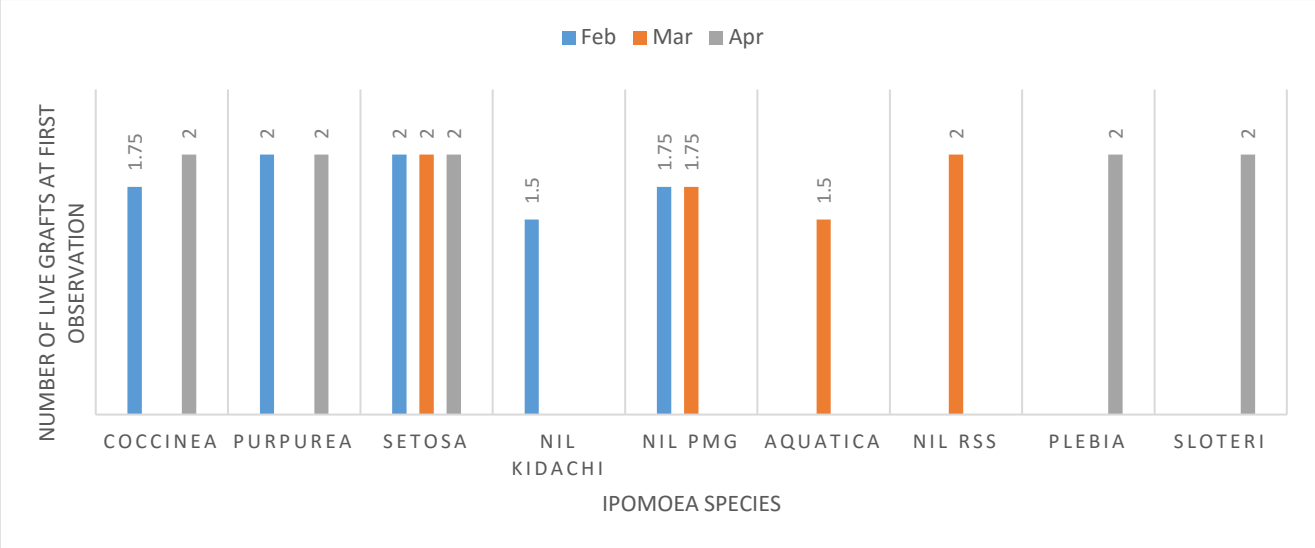

FIGURE 4: MEAN NUMBER OF LIVE GRAFTS AT FIRST OBSERVATION (7 DAYS AFTER GRAFTING)

All species grafted had on average more than one graft survive. This is illustrated in Figure 4. *I. setosa* and *I. purpurea* had a 100% graft success rate across all time periods. *I. plebia*, *I. nil RSS*, *I. sloteri* and *I. coccinea* all had 100% on at least one occasion. *I. nil cv. Kidachi*, *I. aquatica* and *I. nil PMG* all had more than one graft survive for successful inoculation.

6.2. Symptom Expression

6.2.1. Negative controls

All species grafted onto themselves and with known virus free sweetpotato cultivar 'Beauregard Clean' exhibited no symptoms throughout all observations. This indicates that grafting technique was correct and no cross contamination occurred, no presence of seed transmissible viruses and that no insect vectors were present to transmit virus within the glasshouse.

6.2.2. *I. Setosa*

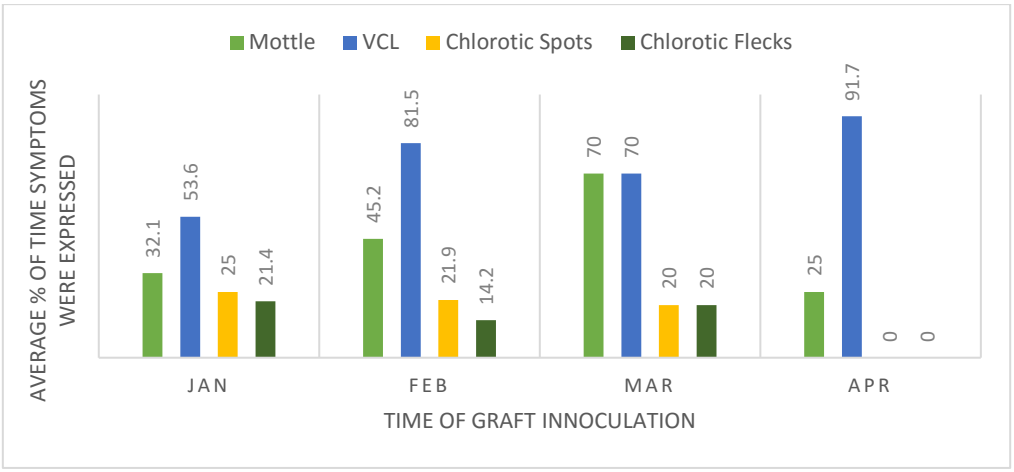

FIGURE 5: A COMPARISON OF MEAN SYMPTOMS OBSERVED OVER CONSECUTIVE MONTHS WHEN *I. SETOSA* WAS GRAFT INOCULATED WITH LOB

Figure 5 above demonstrates the total average percentage (%) of time each symptom was observed on *I. setosa* graft inoculated with LOB. *I. setosa* gave consistent high expression of VCL across all 4 months, although mottling, chlorotic spots and chlorotic flecks were also expressed but only in January, February and March but at lower percentages. In April only VCL and mottling were observed.

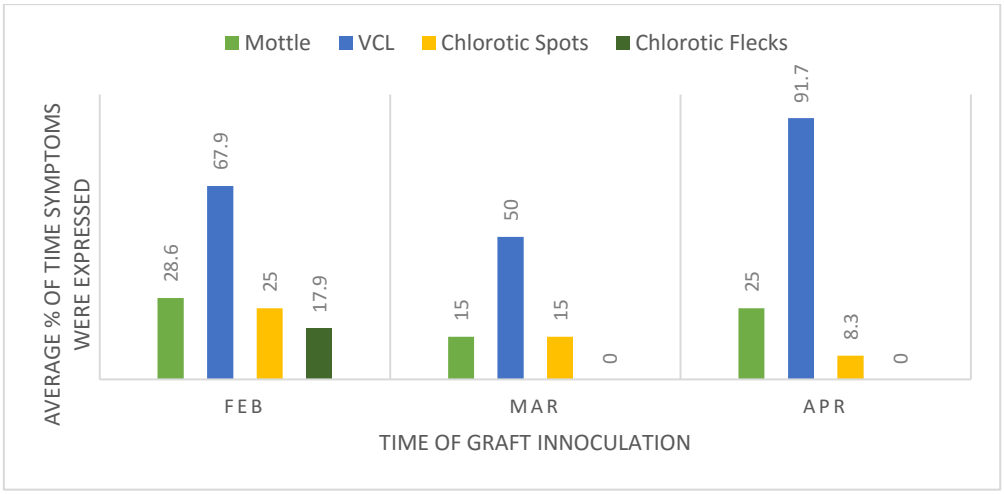

FIGURE 6: A COMPARISON OF MEAN SYMPTOMS OBSERVED OVER CONSECUTIVE MONTHS WHEN *I. SETOSA* WAS GRAFT INOCULATED WITH SNOWHITE

Figure 6 shows that *I. setosa* expressed all 4 SPFMV symptoms in February but not in March or April. Overall, VCL was the highest most common symptom for *I. setosa* followed by mottle which appeared most often in February.

6.2.3. *I. purpurea*

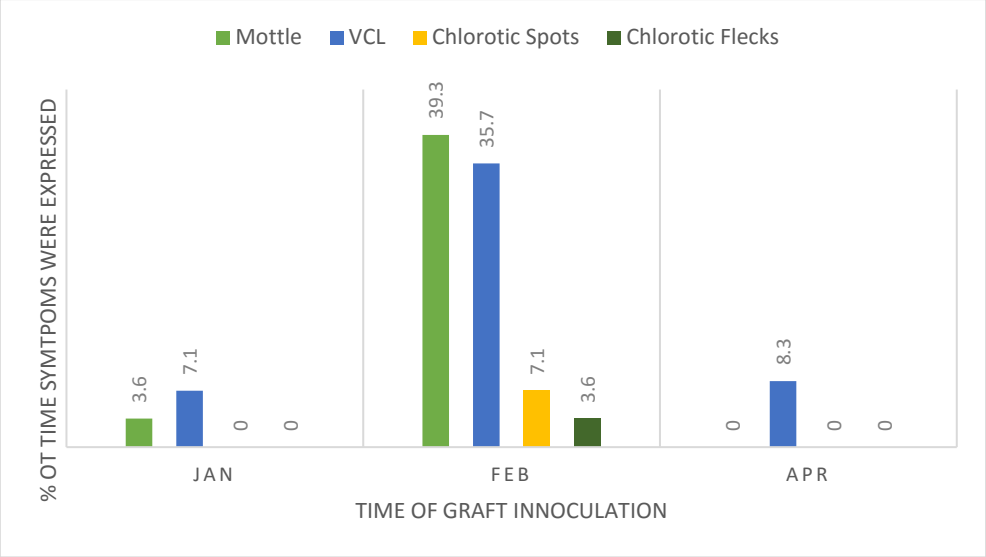

FIGURE 7: A COMPARISON OF MEAN SYMPTOMS OBSERVED OVER CONSECUTIVE MONTHS WHEN *I. PURPUREA* WAS GRAFT INOCULATED WITH LOB

I. purpurea graft inoculated with LOB (Figure 7) produced the highest percentage of symptoms during February. For plants grafted in January and April the percentage of VCL expression was low, 7.1% in January and 8.3% in April. In January, mottling was expressed at only 3.6%.

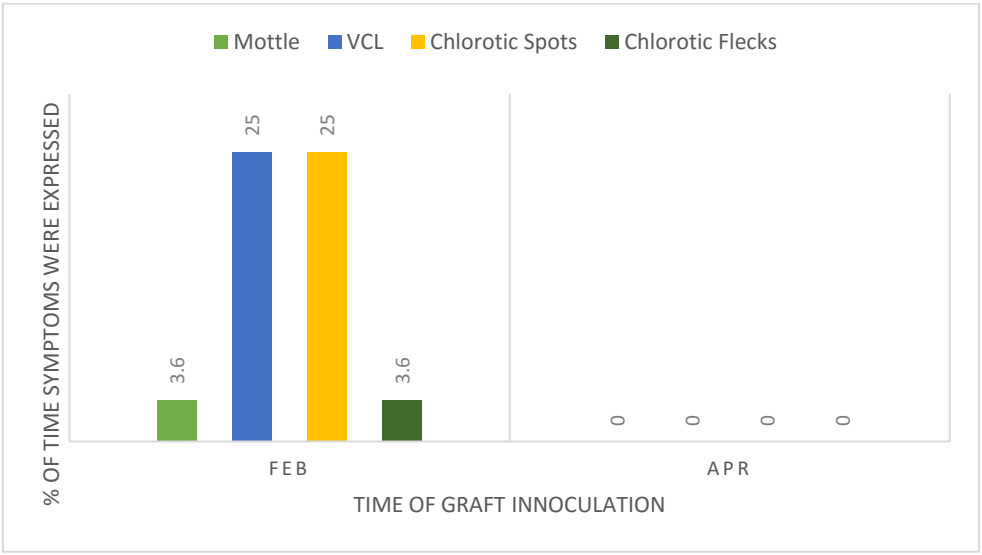

FIGURE 8: A COMPARISON OF MEAN SYMPTOMS OBSERVED OVER CONSECUTIVE MONTHS WHEN *I. PURPUREA* WAS GRAFT INOCULATED WITH SNOWHITE

Figure 8 illustrates that *I. purpurea* displayed symptoms for VCL and chlorotic spots 25% of the time but mottle and chlorotic flecks were only present for 3.6% of the observation period. In April *I. purpurea* did not show any symptoms as seen in Figure 8.

6.2.4. *I. coccinea*

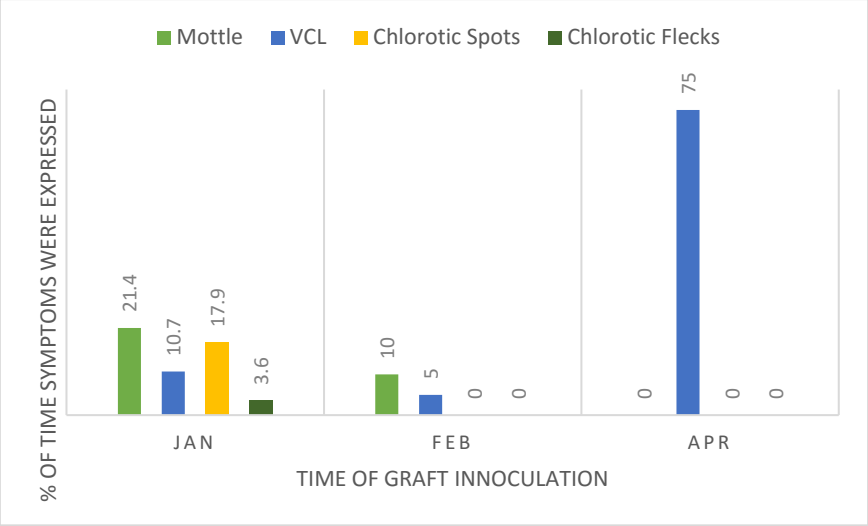

FIGURE 9: A COMPARISON OF MEAN SYMPTOMS OBSERVED OVER CONSECUTIVE MONTHS WHEN *I. COCCINEA* WAS GRAFT INOCULATED WITH LOB

Symptoms expressed by *I. coccinea* varied over the 3 time periods. In January it exhibited minimal amount of all symptoms (figure 9). However in February only mottle and VCL were present but at a low percentage, 10% and 5% respectively. However, in April *I. coccinea* expressed VCL symptoms 75% of the time but showed no signs of any other symptoms.

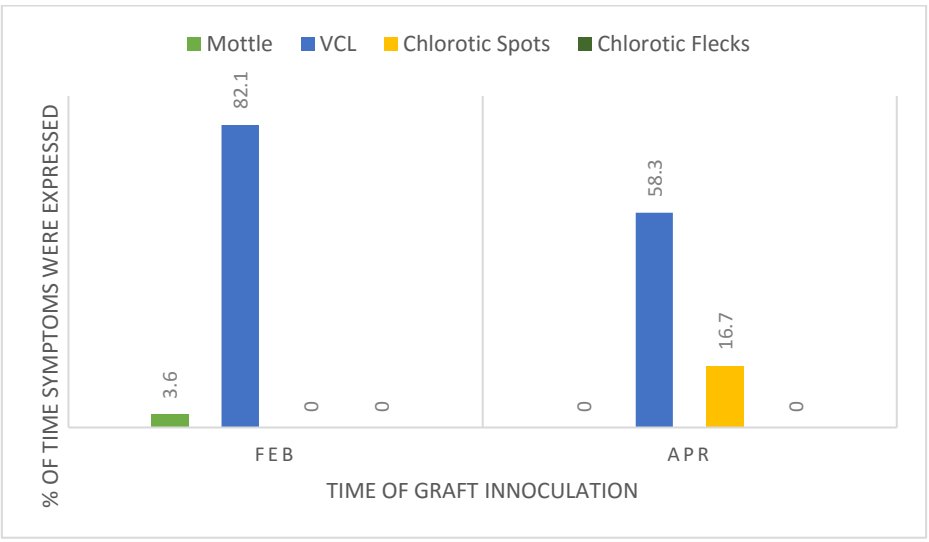

FIGURE 10: A COMPARISON OF MEAN SYMPTOMS OBSERVED OVER CONSECUTIVE MONTHS WHEN *I. COCCINEA* WAS GRAFT INOCULATED WITH SNOWHITE

In Figure 10, *I. coccinea* produced VCL symptoms 82.1% of the time in February and 58.3% in April. In February there was also a very minimal observation of mottle 3.6% of the time and in April chlorotic spots were observed on average 16.7% of the time.

6.2.5. *I. nil cv. Kidachi*

I. nil cv. Kidachi did not exhibit any symptoms when graft inoculated with LOB and snowhite.

6.2.6. *I. nil cv. Pink Morning Glory*

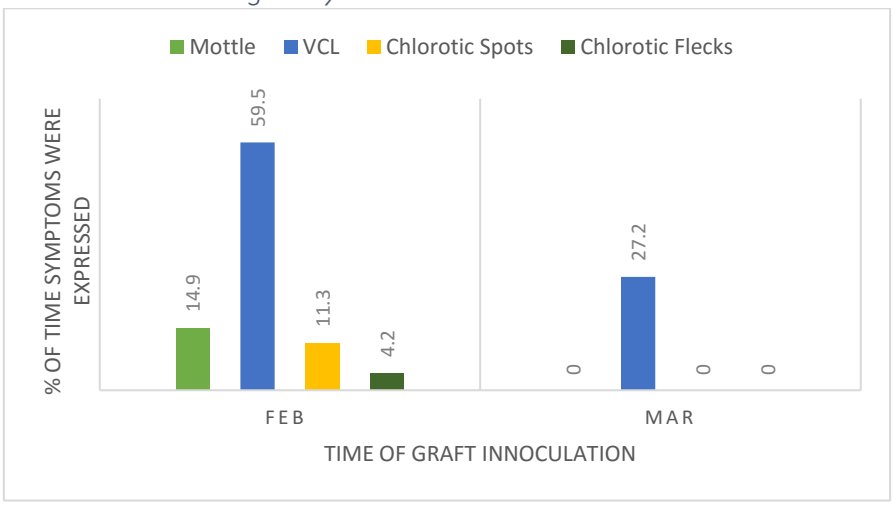

FIGURE 11: A COMPARISON OF MEAN SYMPTOMS OBSERVED OVER CONSECUTIVE MONTHS WHEN *I. NIL CV. PINK MORNING GLORY* WAS GRAFT INOCULATED WITH LOB

I. nil cv. Pink Morning Glory graft inoculated with LOB (Figure 11) expressed al 4 symptoms of SPFMV in February. However, in March only VCL was expressed. The average observation of VCL was higher in February at 59.5% compared to March where observation only presented VCL 27.2% of the time.

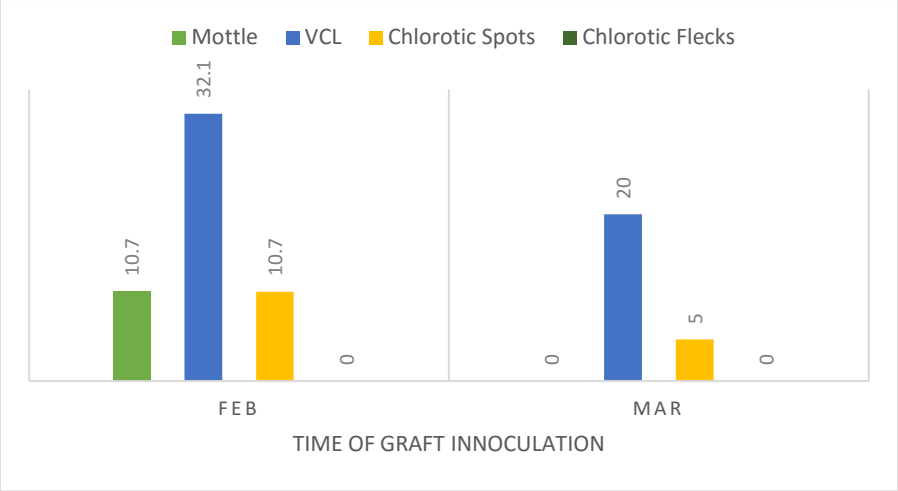

FIGURE 12: A COMPARISON OF MEAN SYMPTOMS OBSERVED OVER CONSECUTIVE MONTHS WHEN *I. NIL CV. PINK MORNING GLORY* WAS GRAFT INOCULATED WITH SNOWHITE

When *I. nil cv. Pink Morning Glory* was graft inoculated with Snowhite as presented in Figure 12, VCL was the most observed symptom, being observed 32.1% of the time in February and 20% in March. In February mottle, VCL and chlorotic spots were observed in greater numbers than VCL and chlorotic spots in March.

6.2.7. I. nil cv. Red Speckled Splash

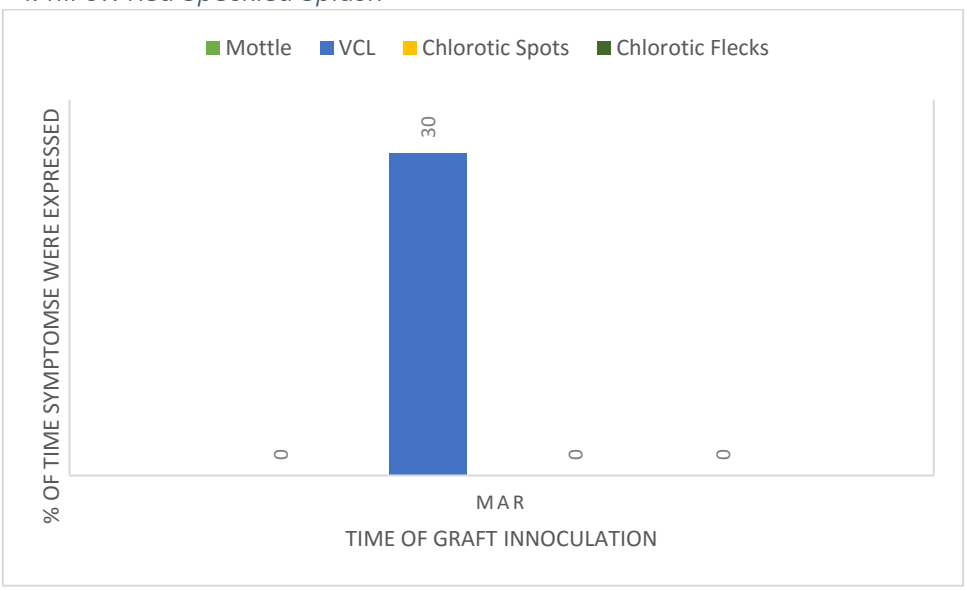

FIGURE 13: A COMPARISON OF MEAN SYMPTOMS OBSERVED OVER CONSECUTIVE MONTHS WHEN *I. NIL CV. RED SPECKLED SPLASH* WAS GRAFT INOCULATED WITH LOB

In Figure 13 *I. nil cv. Red Speckled Splash* only produced VCL symptoms 30% of the time when grafted with LOB. When *I. nil cv. Red Speckled Splash* was graft inoculated with Snowhite in March no symptoms were exhibited.

6.2.8. *I. sloteri*

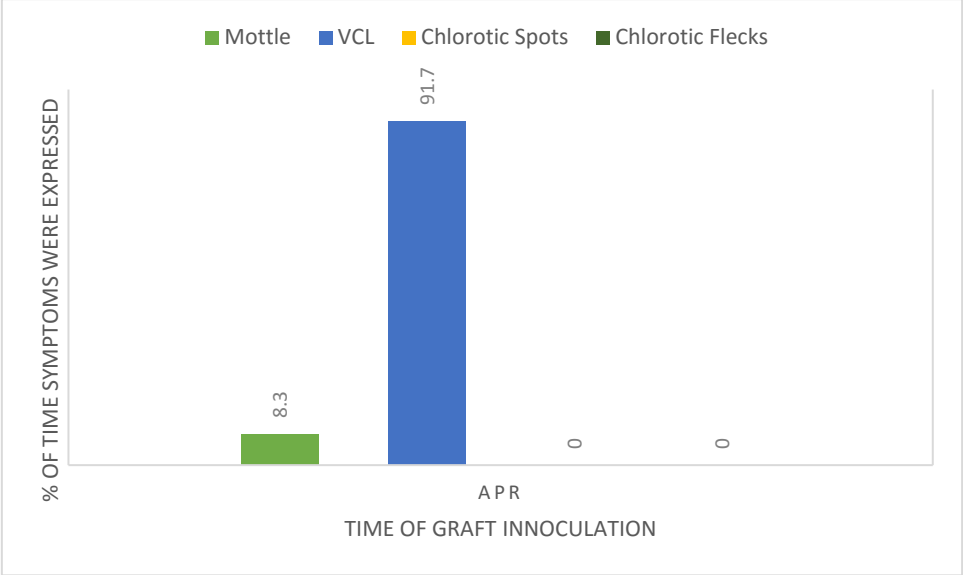

FIGURE 14: A COMPARISON OF MEAN SYMPTOMS OBSERVED OVER CONSECUTIVE MONTHS WHEN *I. SLOTERI* WAS GRAFT INOCULATED WITH LOB

In April when *I. sloteri* was grafted with LOB VCL symptoms were present during 91.7% of total observations and mottle 8.3% (Figure 14).

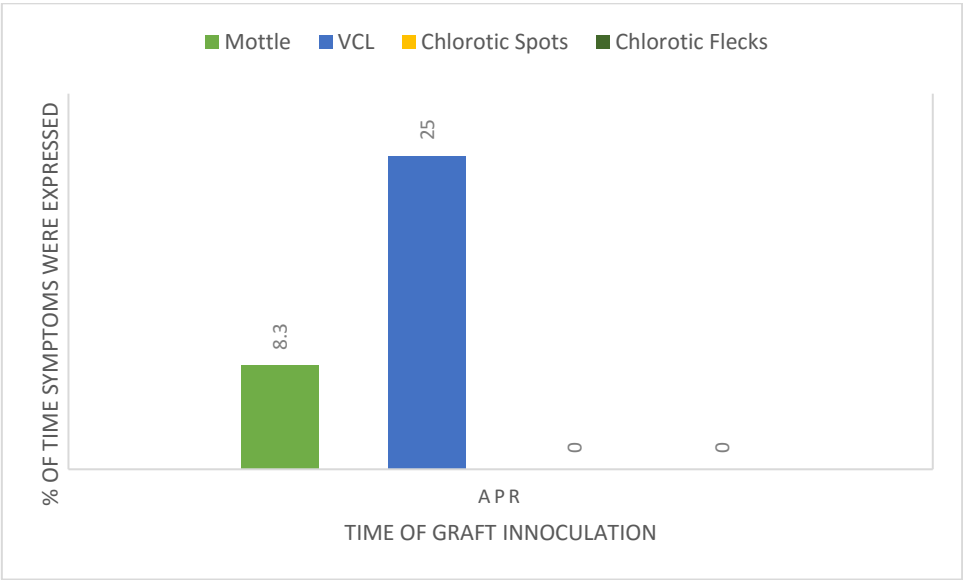

FIGURE 15: A COMPARISON OF MEAN SYMPTOMS OBSERVED OVER CONSECUTIVE MONTHS WHEN *I. SLOTERI* WAS GRAFT INOCULATED WITH SNOWHITE

Mottling was presented 8.3% of the time (Figure 15), the same as Figure 14. However, VCL was observed on average 25% of the time, significantly lower than Figure 14.

6.2.9. *I. plebia*

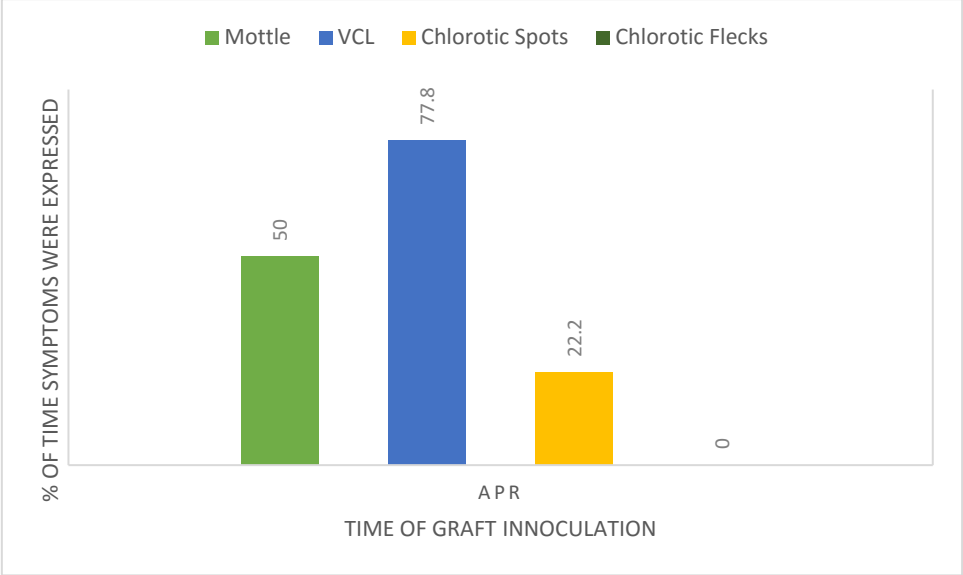

FIGURE 16: MEAN SYMPTOMS OBSERVED WHEN *I. PLEBIA* WAS GRAFT INOCULATED WITH LOB IN APRIL

In April (Figure 16) *I. plebia* displayed on average mottle 50%, VCL 77.8% and chlorotic spots for 22.2% of the time that observations were recorded.

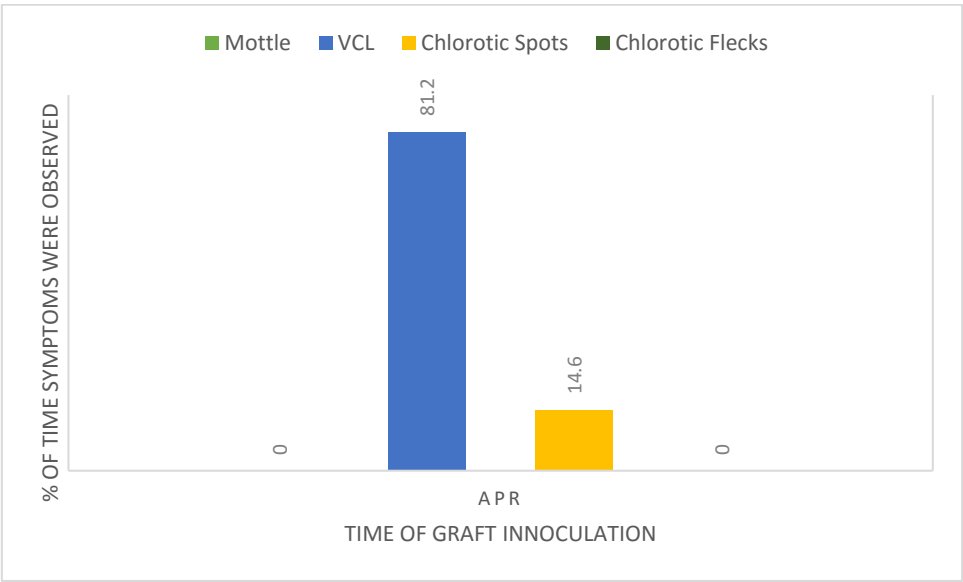

FIGURE 17: MEAN SYMPTOMS OBSERVED WHEN *I. PLEBIA* WAS GRAFT INOCULATED WITH SNOWHITE IN APRIL

Over the duration of observation dates (Figure 17), *I. plebia* displayed VCL 81.2% of the time and Chlorotic spots 14.6%.

6.2.10. I. Aquatica

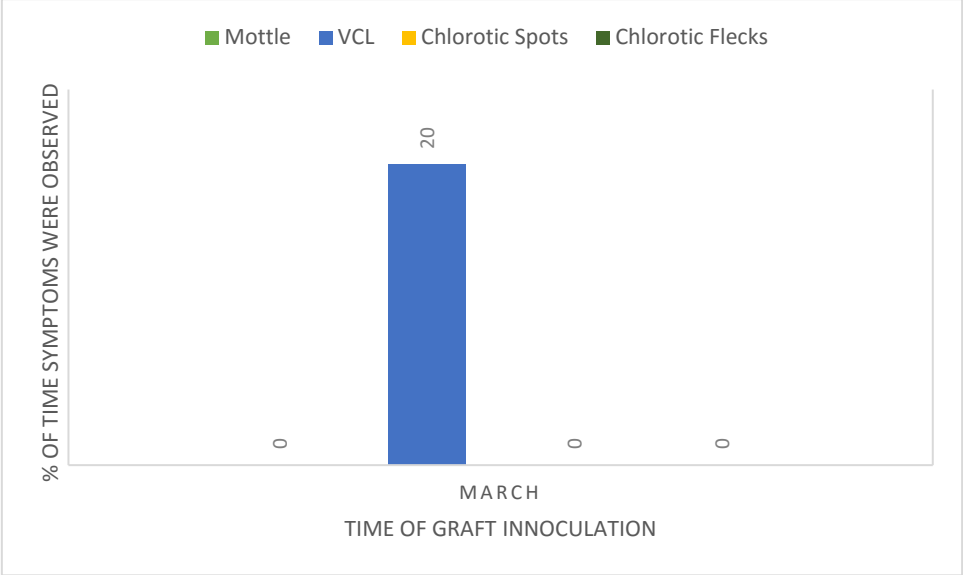

FIGURE 18: MEAN SYMPTOMS OBSERVED WHEN *I. AQUATICA* WAS GRAFT INOCULATED WITH LOB IN MARCH

In Figure 18 *I. aquatica* only expressed only VCL symptoms when graft inoculated with LOB.

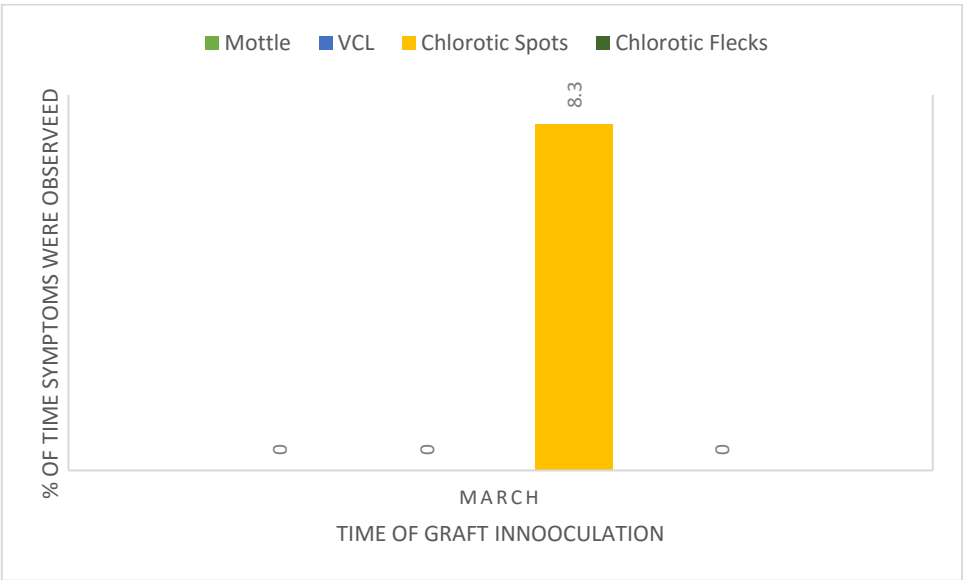

FIGURE 19: MEAN SYMPTOMS OBSERVED WHEN *I. AQUATICA* WAS GRAFT INOCULATED WITH SNOWHITE IN MARCH

I. aquatica exhibited chlorotic spots only and no other symptoms when graft inoculated with Snowhite in March (Figure 19).

6.3. Significant Difference across *Ipomoea* Species

TABLE 2: AVERAGE SYMPTOMS OBSERVED AND SIGNIFICANT DIFFERENCE FOR EACH SPECIES OF IPOMOEA SPECIES GRAFT INOCULATED WITH LOB

Time of Graft	*Ipomoea* Species	Mottle	VCL	Chlorotic spots	Chlorotic flecks
Jan	*I. setosa*	32.1 a	53.6 a	25.0 a	21.4 a
Jan	*I. coccinea*	21.4 a	10.7 b	17.9 a	3.6 b
Jan	*I. nil cv. Kidachi*	0 a	0 b	0 b	0 b
Jan	*I. Purpurea*	3.6 a	7.1 b	0 b	0 b
Feb	*I. setosa*	45.2 a	81.5 a	21.9 a	14.2 a
Feb	*I. coccinea*	10.0 a	5.0 c	0 b	0 b
Feb	*I. nil cv. Kidachi*	0 a	0 c	0 b	0 b
Feb	*I. nil cv. Pink Morning Glory*	14.9 a	59.5 ab	11.3 ab	4.2 b
Feb	*I. purpurea*	39.3 a	35.7 bc	7.1 b	3.6 b
Mar	*I. setosa*	70.0 a	70.0 a	20.0 a	20.0 a
Mar	*I. aquatica*	0 b	20.0 a	0 b	0 b
Mar	*I. nil cv. Pink Morning Glory*	0 b	27.2 a	0 b	0 b
Mar	*I. nil cv. Red Speckled Splash*	0 b	30.0 a	0 b	0 b
Apr	*I. setosa*	25.0 a	91.7 a	0 a	0
Apr	*I. coccinea*	0 a	75.0 a	0 a	0
Apr	*I. plebia*	50.0 a	77.8 a	22.2 a	0
Apr	*I. purpurea*	0 a	8.3 b	0 a	0
Apr	*I. sloteri*	8.3 a	91.7 a	0 a	0

Table 2 above presents each time of grafting and the symptoms associated with each species of Ipomoea. The significant difference is listed in letter combinations of a, b and c. Each time of grafting and symptom expression was treated as a distinct set of data and therefore analysed separately. For example in January of the first column all averages for mottle were followed by the same number meaning none of the 4 species were significantly different in displaying mottle symptoms as $p<0.01$.

Where a number is highlighted red this indicates significant difference and blue and purple are substantially different. In Table 2 *I. setosa* displays expression of VCL significantly more often than all other species in January, February and April. There is no significant difference in the expression of mottle symptoms in other *Ipomoea* species with the exception of *I. setosa* which differs once in March with 70% of mottle expression. Of the 4 times *I. setosa* was grafted, it exhibited chlorotic spot symptoms significantly more times than other *Ipomoea* species tested in January and February. The occurrence of chlorotic flecks was

similar to that of chlorotic spots except for the month of March where *I. setosa* was the only species to produce this symptom.

Of all other species graft inoculated *I. coccinea* exhibited significantly more chlorotic spot symptoms in January producing the second highest amount of symptom expression after *I. setosa*. *I. nil cv. Pink Morning Glory* displayed substantial difference in symptom expression difference in February with 59.5% expression of VCL and 11.3% of Chlorotic spots. Once again *I. setosa* performed better in both categories having 81.5% of total symptom expression for VCL and 11.3% for chlorotic spots.

In April *I. Coccinea*, *I. setosa*, *I. sloteri* and *I. plebia* were all significantly higher in displaying VCL symptoms than *I. purpurea*. *I. setosa* and *I. sloteri* both gave the highest average of VCL symptoms with 91.7% expression. They were followed by *I. plebia* with 77.8% and *I. coccinea* displaying symptoms 75.0% of the time.

TABLE 3: AVERAGE SYMPTOMS OBSERVED AND SIGNIFICANT DIFFERENCE FOR EACH SPECIES OF IPOMOEA SPECIES GRAFT INOCULATED WITH SNOWHITE

Time	Species	Mottle	VCL	Chl spots	Chl flecks
Feb	*I. setosa*	28.6 a	67.9 ab	25.0 a	17.9 a
Feb	*I. coccinea*	3.6 a	82.1 a	0 a	0 a
Feb	*I. nil cv. Kidachi*	0 a	0 c	0 a	0 a
Feb	*I. nil Pink Morning Glory*	10.7 a	32.1 bc	10.7 a	0 a
Feb	*I. purpurea*	3.6 a	25.0 c	25.0 a	3.6 a
Mar	*I. setosa*	15.0 a	50.0 a	15.0 a	0
Mar	*I. aquatica*	0 a	0 b	8.3 a	0
Mar	*I. nil cv. Pink Morning Glory*	0 a	20.0 ab	5.0 a	0
Mar	*I. nil cv. Red Speckled Spalsh*	0 a	0 b	0 a	0
Apr	*I. setosa*	25.0 a	91.7 a	8.3 a	0
Apr	*I. coccinea*	0 a	58.3 ab	16.7 a	0
Apr	*I. purpurea*	0 a	0 c	0 a	0
Apr	*I. plebia*	0 a	81.2 a	14.6 a	0
Apr	*I. sloteri*	8.3 a	25.0 bc	0 a	0

Table 3 above follows the same format as Table 2. Table 3 follows the symptom expression of all *Ipomoea* species graft inoculated with Snowhite. Only VCL expression gave significant different between species in each month. In February *I. coccinea* was significantly different with 82.1% of VCL symptom expression across all observation dates. *I .setosa* followed and was substantially different with 67.9%. *I. nil cv. PMG* produced VCL symptoms 32.1% of the time after *I.* setosa with VCL symptoms 62% of the time.

For the grafts in March *I. setosa* produced a significantly higher percentage of symptoms with 50% expression and *I. nil cv. Pink Morning Glory was* substantially different with VCL symptoms recorded for 20% of total observation. In April there was much variation of VCL symptoms. *I. plebia* and *I. setosa* showed significantly higher expression rate and *I. coccinea* and *I. sloteri* were only substantially different.

7. Discussion

7.1. Graft survival

As mentioned previously a plants ability to accept and sustain a graft is vital for sap transmission from the scion to infect the root stock with SPFMV. For sweetpotato viruses to infect a plant the graft must survive for 4 to 7 days. Figure 4 represents the average number of live grafts across each species on the first observation date. This first observation was recorded 7 days after grafting to give the graft inoculation sufficient time to infect the plant with SPFMV. At this first observation it was assumed if one graft survived this has given the virus sufficient time to enter the grafted plant (root stock).

As seen in Figure 4 all species had more than one graft survive on average so it was assumed that these all had ample opportunity to be infected with SPFMV. Not all species were grafted over the 4 time periods due to issues with germination. *I. setosa* had 100% graft success over all 4 time periods at first observation. *I .purpurea* was grafted in Feb and Apr and had 100% graft survival. *I. plebia* and *I. sloteri* were both graft inoculated in April and had successful graft survival (2/2 grafts survived on average). This is an important finding as this month began to cool but these cultivars still performed. *I .coccinea* saw 1.75 grafts survive in Feb but then improved in April with 2 grafts surviving. All plants from the last grafting in April had 100% graft success rate. This could either be due to the cooler weather or that the graft operator was by then more practiced at each species and had improved grafting technique and became more skilled at the grafting process.

7.2. Symptom Expression

7.2.1. I. setosa

I. setosa symptom expression is presented in Figure 5, Figure 6, Table 2 and Table 3. When *I. setosa* was graft inoculated with LOB in figure 5 during January, February and March, *I. setosa* exhibited all 4 symptoms. However, during April when the weather became cooler VCL expression increased, mottle decreased and chlorotic spots and flecks were absent. When *I. setosa* was graft inoculated with Snowhite (Figure 6) all symptoms were present in February. When comparing VCL results for LOB and Snowhite, symptoms were expressed in greater percentages for LOB in February, March and the same in April. It appears that during cooler weather VCL expression has increased but mottle has decreased and chlorotic spots and chlorotic flecks had also decreased or were not apparent.

When comparing significant differences of *I. setosa* graft inoculated with LOB to other species in Table 2 it was significantly different in expression of VCL, chlorotic spots and chlorotic flecks in the plants grafted in January and February. As it became cooler in March and April *I. setosa* was significantly different in the production of VCL symptoms in March and April. This suggests that as the weather cooled the effectiveness of mottle, chlorotic spot and chlorotic fleck symptom expression decreased. From these finding it can be deduced that *I. setosa* outperformed all other species for Mottle, VCL, chlorotic spots and chlorotic flecks in January and in February but did not produce symptoms as well in the two remaining months due to the cooler weather.

The data from graft inoculation of *I. setosa* with Snowhite (Table 3) differed from the observations made for plants grafted with LOB. VCL expression on *I. setosa's* in February was only substantially different at 67.9% and beaten by *I. coccinea* which was significantly different at 82.1%. However, in March and April *I.setosa* had higher incidences of VCL than all other species. This suggests that overall *I. setosa* has exhibited the highest percentages of VCL symptoms for Snowhite.

7.2.2. I. purpurea

When *I. purpurea* was graft inoculated with LOB and Snowhite in Figure 7 and 8 symptom expression was most apparent and successful in February. As for January and April *I. pupurea* plants grafted with LOB only expressed minimal mottle and VCL symptoms which were not significantly different when looking at Table 2. *I. purpurea* graft inoculated with

Snowhite did not produce any symptoms in April and Table 3 presents no findings of significance when compared to all the grafted *Ipomoea* species. Once again the influence of weather on symptom expression is realised. As the weather cools symptom expression decreases. Results from this study are the same as Suoto (2003) who also found that symptom expression of *I. purpurea* not significant.

7.2.3. I. coccinea

I. coccinea gave conflicting results for graft inoculation with LOB and Snowhite (Figures 9 and 10). In February *I. coccinea* graft inoculated with LOB expressed symptoms for vein clearing 5% of the time that observations were recorded compared to the plants grafted with Snowhite which displayed symptoms for 82.1% of the total observation time. VCL symptoms were expressed the most during April. This is similar to *I. setosa* which also saw the highest expression of VCL in April. Snowhite saw both VCL and chlorotic spots in April. VCL has decreased from February but chlorotic spots were only present during April. Once again this provides evidence that virus symptom expression changes as the months get cooler and all other symptoms generally fade whereas VCL expression remains consistent and sometimes increases. When comparing *I. coccinea* to all the species grafted it was only significantly different for *I. coccinea* plants grafted with LOB in April. Plants from this time period gave high incidences of VCL. Whereas, *I. coccinea* grafted with Snowhite was significantly different in the expression of VCL symptoms on plants grafted in February and substantially different for VCL symptoms in April.

7.2.4. I. nil cv. Kidachi

I. nil cv. Kidachi produced no symptoms when graft inoculated with both LOB and Snowhite during both January and February in Figure 10 and 11. As this plant exhibited no symptoms it is assumed to be either tolerant/resistant to SPFMV or asymptomatic. This will be established with further testing using NCM-ELISA and RT-qPCR.

7.2.5. I. nil cv. Pink Morning Glory

I. nil cv. Pink Morning Glory as observed in Table 2 had substantial differences for VCL symptoms recorded on plants grafted with LOB in February. *I. nil cv. Pink Morning Glory* followed behind *I. setosa* with 59.5% of symptoms observed across all observations. The same occurred in February for *I. nil cv. Pink Morning Glory* plants grafted with Snowhite. It was substantially different following behind *I. coccinea* also grafted with Snowhite for symptom expression. When tracking *I. nil cv. Pink Morning Glory* across the months and

climatic conditions it clearly performed better in February than March as see in Figures 11 and 12. This again supports the theory that the cooler months have changed the way in which these plants express virus symptoms.

7.3. Scoping Species

The following species have only been grafted on one occasion due to issues with germination. These species are included as a pilot study. This study will assess how each species performs to give an estimate of suitability if they were to be repeated.

7.3.1. I. nil cv. Red Speckled Splash

I. nil cv. Red Speckled Splash was grafted in March with both LOB (Figure 13) and Snowhite. For plants grafted with LOB 30% of the time observations were recorded VCL was present. For plants grafted with Snowhite in March no virus symptoms were present. In Table 2 and 4 *I. nil cv. Red Speckled Splash* was not significantly different from any other of the species. These results suggest this cultivar of *I. nil* may not be the most reliable. However, grafting of this species would need to be repeated over different time periods to qualify suitability.

7.3.2. I. sloteri

When *I. sloteri* was graft inoculated with LOB and Snowhite (Figures 14 and 15) the same results were observed with expression of mottle occurring 8.3% of the time. However, production of VCL symptoms varied between the 2 sweetpotato cultivars with LOB showing symptoms 91.7% of the time with Snowhite only 25% of the time. When comparing results in April with other species grafted at the same time with LOB, *I. sloteri* was significantly different for VCL symptoms. This has been observed with *I. setosa*, *I. coccinea*, *I. sloteri* and *I.* plebia which all gave high incidences of VCL expression but virtually no expression of any other symptoms. *I. sloteri* symptoms for graft inoculation with Snowhite were substantially different giving 25% symptom expression. During this time period *I. plebia*, *I. setosa* and *I. coccinea* out performed *I. sloteri*. As *I. sloteri* presented VCL symptoms in high proportions it would be beneficial to repeat this species in the warmer months to see if it follows the same patterns of *I. setosa*, *I. nil cv. Pink Morning Glory*, *I. coccinea* and *I. purpurea* which all displayed a greater variety of symptoms in the warmer summer months.

7.3.3. I. plebia

I. plebia graft inoculated with LOB produced a greater range and number of symptoms compared to *I. plebia* grafted with Snowhite (Figures 16 and 17). Those grafted with LOB

exhibited Mottle, VCL and chlorotic spots. Symptoms of VCL were presented 77.8% of the time that observations were recorded. Statistical analysis indicated that this was significantly different. The same occurred in Snowhite grafted in April. *I. plebia* was found to be significantly different following *I. setosa* with 81.2% of the time symptoms were expressed. Snowhite grafted with *I. plebia* did produce great VCL symptoms however, chlorotic spots were only present 14.6% of the time and mottle and chloric flecks were not present. The performance of *I.* plebia in expressing VCL symptoms is a finding beneficial to sweetpotato growers as this species of *Ipomoea* is present on majority of farms in the form of a weed. This plant could potentially act as an in paddock indicator plant as it should show VCL symptoms when infected with SPFMV.

7.3.4 *I. aquatica*

I. aquatica symptom expression (Figure 18 and 19) varied between inoculation with LOB and Snowhite. LOB presented VCL 20% of the time when observed and Snowhite saw chlorotic spots on average 8.3% of times it was indexed. Table 2 and 3 found that both of these results were not significantly different from any other results and did not perform as well as *I. setosa* and *I. nil cv. Pink Morning Glory* grafted in March. Results obtained in this study differ from findings of Valverde et al. (2007) and Lotrakul et al. (1998) who found that I*. aquatica* was not susceptible to SPFMV*.* This sample will need to be tested using NCM-ELISA and qPCR to confirm infection of SPFMV.

7.4. Summary

There were several significant findings from this study. The first was discovering that all species of *Ipomoea* grafted were successful at sustaining and supporting the growth of a sweetpotato graft. All species were on average capable of sustaining 1 to 2 grafts, seven days after graft inoculation. This is a vital component as virus diagnostic using herbaceous indicator plants solely relies on graft inoculation as the means of infecting the root stock with sweetpotato viruses.

The second noticeable result was the different reaction that each *Ipomoeas* species exhibited with expression of virus symptoms between the scion plants, LOB and Snowhite. For example *I. nil cv. Red Speckled Splash* exhibited symptoms when graft inoculated with LOB but did not show symptoms when graft inoculated with Snowhite. Also the severity of symptom expression appears to be lower. This was evident when looking at results for *I.*

sloteri which expressed symptoms of VCL 91.7% of the time when grafted with LOB in April but the same plant grafted with Snowhite only gave VCL symptoms 25% of the time.

The difference in symptom expression between cultivars of *I. nil* was quite remarkable. *I. nil cv. Kidachi* gave no expression of any symptoms so it is either asymptomatic or resistant to SPFMV infection. *Pink Morning Glory* and *Red Speckled Splash* behaved very differently exhibiting symptoms. Of these cultivars *Pink Morning Glory* was the most reliable producing symptoms of mottle, VCL, chlorotic spots and chlorotic flecks. *Red Speckled Splash* was the second best of these cultivars followed by *Kidachi* in which symptoms were absent.

All home gardener species excluding *I. nil cv Kidachi* displayed SPFMV symptoms. This is a valuable finding as these species are often wide spread in home gardens can easily establish outside of the backyard garden along roadsides and creek beds. The species that regularly produced positive symptoms could be useful to indicate the presence of virus and become an important tool for monitoring sweetpotato viruses in Australia and to teach growers and the public to monitor these plants for any changes in appearance. The same can be said for *I. plebia* which is a widespread weed on sweetpotato growers' farms and across the country. Having used this species in a replicated experiment gives good grounds to argue that this species will display virus symptoms and may be an important tool in the paddock when surveying for the presence of SPFMV. Further testing is necessary for *I. nil cv Kidachi* as widespread cultivation of this popular garden ornamental could also have biosecurity implications because if it is simply asymptomatic and not resistant to SPFMV, it could become a host for sweetpotato viruses without producing any symptoms.

Expression variation over the different times of the season was also another interesting learning. For most species grafted in the warmer months of January and February plants would show 3 or 4 of the SPFMV symptoms. Whereas when it was cooler in March and April VCL symptoms would remain constant or even increase but the incidence of mottle, chlorotic spots, and chlorotic flecks would decrease and in most instances cease.

Of all species grafted it was found that *I. setosa* gave the highest and most reliable expression of symptoms. When comparing each species for expression of a symptom *I. setosa* had the greatest amount of results that were significantly different. *I. setosa* was most reliable over all time periods expressing the greatest range of symptoms. As for other

species that could be used in conjunction with *I. setosa* include *I. purpurea*, *I. coccinea* and *I. nil cv. Pink Morning Glory.* However, before these species were to be implemented into the virus diagnostics program a similar experiment to this would need to be repeated. This would need to be completed from November to April with a larger number of replication and other sweetpotato viruses to assess reaction to other prevalent sweetpotato virus such as the Begomovirus Sweetpotato leaf curl virus (SPLCV). These species may be suitable as they performed well in this experiment but were however not as consistent and reliable as *I. setosa*.

8. Conclusion

8.1. Fulfilments of Objectives

The main objective of this study was to determine whether other species are susceptible to SPFMV and how each species reacted. The second objective was to determine if another one of these Ipomoea species could be used to compliment *I. setosa* in the virus diagnostic program. Findings have suggested that 7 of the 8 trialled species are susceptible and displayed classic SPFMV symptoms. Another important finding was that not all test plants show symptoms in the case *I. nil cv. Kidachi* which appeared to be asymptomatic. Unfortunately samples were not tested using NCM-ELISA and qPCR due to time constraints to verify if infection has occurred. However, symptoms observed on all other species give strong evidence to assume they are SPFMV positive.

Other findings not initially thought to be of importance was the need to scarify seeds. As mentioned in the methods section seed germination was a major constraint. Trialling different methods of scarification have found that nicking seed coat and soaking in water for 12 hours gave the best rates of germination across most species.

As for using other species of *Ipomoea* with *I. setosa* 3 may be may be suitable after the remainder of testing is completed to assess reaction to SPLCV. This study is continuing with NCM-ELISA, qPCR and LAMP tests to be carried out in the near future. Completion of these activities will give assessment of the suitability of each species for use as a part of the virus testing procedures at Gatton Research Facility for the Australian sweetpotato industry. Overall this study has found that *I. setosa* has produced the most reliable and the greatest range of virus expression across both cultivars of SPFMV infected sweetpotato plants used. This is an important finding to validate the use of *I. setosa* and continues to strengthen the importance of its use in any virus testing regime to produce clean or ‘PT’ planting material for sweetpotato growers.

8.2. Implications of Study

Results from this study give significant findings for the Australian sweetpotato industry. Firstly, establishing that *I. plebia* and other home gardener species display virus symptoms is important for biosecurity in Australia. This finding gives another means for sweetpotato virus surveillance as any of these species on farm or in the environment can be checked for symptoms as widespread distribution of these weeds could provide a virus sink especially with an incursion of a new virus that could pose a threat to the Australian sweetpotato industry. This study only investigated eight relatively unexplored species of *Ipomoea* but there are thousands of other species still untouched. This study gives validation to continue with investigation into *Ipomoea* species as there is great possibility that other species may react to virus as dramatically as *I. setosa* and could even build virus titres higher to allow for more accurate detection in qPCR and NCM-ELISA. The most valuable finding was determining *I. setosa* to be the most accurate and reliable for symptom expression. This gives increased validation to previous work as this species is still the global standard as an herbaceous indicator plant.

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