Hort Innovation

Final Report

Characterisation of a Carlavirus of French Bean

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Department of Agriculture and Fisheries

Project code:

VG15073

Project:

Characterisation of a Carlavirus of French Bean VG15073

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Content

Summary

A sudden and severe outbreak of a virus disease in fresh green beans in the Fassifern area of south east Queensland in the autumn of 2016 caused considerable economic impact in a matter of a few months.

The disease caused leaf mottling and, importantly, distortion of pods, resulting in considerable losses through yield reductions and downgrading of product.

Preliminary work identified the pathogen as a Carlavirus and a member of the Cowpea mild mottle virus (CPMMV) section of this virus group. Carlaviruses have not previously been identified from legumes in Australia. The detection of this virus at a high incidence in one important bean production area was significant and a potential economic threat to bean production in Queensland and other States. Green bean production in Australia has an annual value of $74 million with Queensland producing approximately 80% of the crop. The major Queensland production areas are spring to autumn crops in the Lockyer and Fassifern valleys in south Queensland and winter production in the Bowen-Burdekin area of north Queensland. Project VG 15073 was initiated to determine the cause, nature and management of the disease caused by this Carlavirus.

Project VG15073 met all four of the contracted outcomes:

1. Identify and characterise a new Carlavirus identified from beans crops in Queensland
2. Identify the potential distribution and incidence of the virus in other French bean production areas of Australia
3. Develop management strategies for the virus in and modify these as further information becomes available on virus host range, epidemiology, transmission and bean varietal reactions
4. Provide information on the virus to growers, the vegetable industry, biosecurity agencies and plant virologists

CPMMV occurs in all Queensland bean production areas, including the Lockyer and Fassifern valleys, Bowen and Bundaberg. Disease incidence recorded during surveys in these areas from 2016 to 2019 were usually less than 5%.

The exceptions were levels exceeding 50% in some crops in the Fassifern area growing during the autumn production period from March onward in 2016 and 2019. A consistent result from intensive surveying in this district over four years was no virus detections in bean crops grown from spring through to December. By contrast, virus did occur each year in crops growing into and during the autumn-early winter period, with low incidences in 2015 and 2017 and very high incidences in 2016 and 2019 Whitefly numbers varied throughout the district by location and potentially management practices. The numbers also varied by season and year with the highest populations on French bean generally observed during autumn, particularly in 2019.

CPMMV has not been found outside of Queensland and would only be a potential problem where the specific vector silver leaf whitefly (SLW) is established.

SLW can transmit the virus in relatively short feeding periods of about 10 minutes and the Fassifern outbreaks have demonstrated how quickly an epidemic can develop when whitefly populations are high, a source of virus is available and bean plants of a susceptible variety are at a high risk stage of growth.

Most host plants of CPMMV are in the legume family. In Queensland, the known field hosts are bean, soybean, mung bean, cowpea and the perennial legume species Siratro, Glycine and Phasey bean.

Glasshouse inoculation experiments have found that CPMMV has a relatively broad host range within the legume family, particularly in the genera Phaseolus, Glycine, Macroptilium and Vigna.

Some strains of CPMMV are known to be seed transmitted in other countries. However, extensive testing of commercial seed lots of bean, soybean and mung bean failed to demonstrate seed transmission. Grow out tests using seed produced from CPMMV inoculated bean and soybean plants also failed to detect seed transmission. Lack of virus detection in seedlings during intensive surveys of commercial bean crops in the Fassifern also indicate bean seed was not a pathway for virus introduction into these crops.

The major green bean varieties currently used are highly susceptible to the virusThirty four bean varieties were assessed for virus tolerance and agronomic characters in three field trials. Of these 18 were ranked as virus tolerant, with mild or no leaf or pod symptoms. Five of these varieties were selected for more extensive testing in several areas. These virus tolerant varieties are an important component of a management plan to reduce the economic impact of CPMMV in green beans.

Public summary

The carlavirus Cowpea mild mottle virus (CPMMV), has caused economic losses in Queensland's $70 million green bean industry. The virus is efficiently spread from plant to plant by the silverleaf whitefly (SLW), with early inoculation time found to have a very significant effect on pod yield and quality.

Symptoms include mottled leaves, discolouration and deformed pods. The disease has been most severe in autumn crops that are several weeks from harvest. Surveys in the Fassifern valley have indicated that bean crops grown during other windows over the spring-summer season are less likely to be affected by CPMMV.

In addition to French bean, the virus has been found in soybean, mung bean, cowpea, Siratro and Glycine.

Tests for seed transmission were negative.

Project VG15073 has provided the bean industry with the essential information to implement management tools specifically targeting CPMMV. Strategic insecticide applications as whitefly populations are detected, release of biological control agents which attack whitefly, and selection of tolerant bean varieties are all expected to minimise losses from this virus.

Keywords

Beans; Carlavirus; Cowpea mild mottle virus, Silverleaf whitefly; virus management; Queensland; tolerant varieties

Introduction

French bean (*Phaseolus vulgaris*) is one of five cultivated *Phaseolus* species, all of which have their centre of diversity in Mesoamerica or South America (Hancock 2014).

Beans are an important part of human diets throughout the world with Brazil and other countries of South America being major producers.

Australia produces approximately 34 000 t of green beans annually from approximately 6 800 Ha, with the majority of this production going to the domestic fresh market. The proportion of domestic production going to processing is small. The value of production was $76.4 million in 2017-18 (Anon 2018)

Over 50% of the crop is grown in Queensland with some production in Tasmania and Victoria (Anon 2018).

Industry estimates place the production area for green beans in Queensland at between 3844 and 4046 Ha.

The main growing areas in Queensland are spring to autumn production in south Queensland and winter production in the Bowen and Burdekin regions of north Queensland.

In south Queensland, the Fassifern and Lockyer valleys are the main growing areas with higher altitude, cooler areas around the Granite Belt and northern slopes of NSW being used during the hot mid- summer months.

In the autumn of 2016, a sudden and severe outbreak of a virus disease occurred in green bean crops in the Fassifern area. Affected plants developed leaf mottling and pods were distorted, reduced in size and had a greasy, discoloured appearance. Most crops in the area were affected with the percentage of infected plants exceeding 50 % in most crops surveyed. Severely affected crops were not harvested and the numbers of affected pods arriving at the packing facility resulted in considerably increased labor costs associated with culling and repacking the product.

A Carlavirus identified as a member of the Cowpea mild mottle virus clade was consistently associated with symptomatic plants using several independent tests, including electron microscopy, serology, reverse transcriptase- polymerase chain reaction (RT-PCR), sequencing of PCR products and transmission of selected isolates by the silver leaf whitefly (*Bemisia tabaci).*

Cowpea mild mottle virus (CPMMV) (*Carlavirus, Betaflexiviridae*) was first reported from Ghana infecting cowpea (Brunt and Kenten 1973). The virus is a member of the genus *Carlavirus*; family *Betaflexiviridae.* It is a filamentous virus approx. 650nm in length with a positive single-stranded RNA genome (Zanardo and Carvalho 2017).

Virus species within the Carlavirus genus are transmitted by aphids, with the exception of members of the CPMMV clade which are transmitted by the silver leaf whitefly *Bemisia tabaci* (Naidu et al. 1998).

The host range of CPMMV is largely confined to species within Fabaceae although several isolates infecting Solanaceae species were described from Israel and Jordan some years ago (Brunt 2016) and chia (*Salvia hispanica*) was recently reported as a host in Argentina (Celli et al.2016).

The virus has a wide geographical range and has been reported from at least 29 countries in Africa, Asia, Oceania and South America (Brunt 2016).

Seed transmission has been reported and is dependent on virus isolate, host species/variety and age when plants were infected. The original isolate from Ghana was reported to be seed transmitted in cowpea, soybean and bean (Brunt and Kenten 1973). Brito et al. (2012) demonstrated seed transmission in yardlong bean while Almedia et al. (2005) were unable to detect seed transmission of the virus in soybean seed.

CPMMV is currently important in bean and soybean in Brazil (Zanardo et al. 2014); Argentina (Laguna et al. 2006); Mexico (Chiquito-Almanza et al. 2018) and Puerto Rico (Brown et al.2014).

The virus has reached very damaging levels in soybean crops in Brazil (Zanardo and Carvalho 2017). Symptoms are variable and include severe stem necrosis, bud blight and dwarfing. There is also good evidence of considerable genetic diversity within the virus, including the identification of recombinant strains (Zanardo et al. 2014).

The virus has become more evident in French beans in Brazil following the release of a GM variety resistant to the whitefly transmitted begomovirus Bean golden mosaic virus which causes very severe symptoms and masked the presence of CPMMV (Faria et al. 2016).

The objectives of project VG15073 were:

- Characterise a new Carlavirus infecting beans and other Fabaceae crops in Queensland
- Identify potential distribution and incidence of the virus in other bean production regions of Australia
- Develop management strategies for the virus in bean production
- Provide information on the virus to growers, industry and other government agencies

Methodology

The major work areas were: virus identification and determination of relationships to other viruses infecting beans; virus host range and transmission; surveys for presence/ impact in major production areas; determination the reaction of bean varieties in glasshouse and field trials.

Detection and identification of carlavirus

The initial identification was based on isolate Q5288 collected from symptomatic French bean at Kalbar in south east Queensland.

The isolate was transmitted by manual inoculation and by *Bemisia tabaci* (MEAM1) from infected bean plants to uninfected soybean and bean plants. All test plants developed symptoms similar to the original samples. Flexuous virus particles 600-700 nm in length were observed by transmission electron microscopy and tested negative for potyvirus using an ImmunoStrip test (Agdia, USA). The particle morphology was also consistent with carlaviruses and the samples subsequently tested positive in DAS-ELISA with antibodies (RT-0907 DSMZ, Germany) for the carlavirus, *Cowpea mild mottle virus* (CPMMV).

Amplicons covering part of the coat protein gene through to the 3’ end of the genome were obtained by RT-PCR using the oligo-dT primer Poty 1 (Gibbs and Mackenzie, 1997) as the reverse primer and the forward primer Carla7190F (5’ GGNYTNGGNGTICCIACIGARCAYGT 3’; designed to a range of carlaviruses) for Q5288. The amplicons were directly sequenced and gave fragments of 916 nt (GenBank MK910291) for Q5288. The sequences were compared using BLASTN analyses (Zhang *et al*., 2000).

Complete genome sequence of the original isolate obtained from bean at Kalbar in 2016 (#5288) was obtained by Nanopore sequencing followed by primer walking to confirm the sequence. Viral genome specific cDNA was made using a specific primer in the coat

protein region which had been previously sequenced. The primer was designed with a Nanopore sequence tag on the 5' end, and the library was constructed using the Oxford Nanopore PCR cDNA sequencing kit, which enriches for full length cDNA. The complete genome reads were assembled by Canu (Koren et al. 2017). Manual editing of the assembly was required due to long stretches of repetitive nucleotides present in the genome. Twenty primers were then designed to create 10 overlapping PCR fragments spanning the complete genome. These fragments were Sanger sequenced and aligned to the NGS assembly to complete the genome.

Surveys for Carlavirus and whitefly vector

Fortnightly surveys were undertaken in the Fassifern production area from early November 2016 to June 2019. This area was selected for intensive surveying because of the severe outbreak of the virus in the area in 2016. Surveys consisted of inspecting 50 m per row of crop extending from the block edge for virus-like symptoms. This was repeated for a total of 16 rows. In the same survey area, 150 plants were inspected for adult silverleaf whitefly. Symptomatic plant samples were collected, photographed and indexed for CPMMV by RT-PCR using the primers Carla4973F/Carla5220R (M Sharman, unpublished) and newly designed forward primer CPMMVF3 used with Poty1 reverse primer (Gibbs and Mackenzie, 1997). Samples were also indexed for potyvirus by RT-PCR.

The other major bean production districts in south and north Queensland were surveyed during each year of the project: 2016 to August 2019. Each district was visited at least twice at appropriate times during the production cycle. Data was collected on stage of growth, variety, whitefly presence/ abundance. At least two areas were selected in each block and a minimum of 16 rows x 50m per row per area was assessed for symptomatic plants. Appropriate samples were collected for diagnostic tests which were done using a combination of electron, microscopy, serology, RT-PCR and sequencing of PCR products. Key isolates from all surveys have been lodged in the DAF plant virus collection.

Host range and bean variety screening

Species and varieties used in host range studies were sourced from industry contacts and the Australian grains genebank, Horsham.

Seeds were sown in UC steam pasteurized potting mix and maintained in a glasshouse. At least five plants of each species/ variety were tested.

For host range studies, inoculum of isolate Q5288 of CPMMV was propagated in French bean cv.Wyatt or soybean cv P791. Virus inoculum was then prepared by grinding infected leaf tissue in 0.1M potassium phosphate buffer pH 7 then adding celite abrasive to the inoculum before applying with the forefinger to young test plants. Most test species were inoculated when plants were at the first or second trifoliate leaf stage. Presence and types of symptoms were recorded over 3-4 weeks. Samples of all test plants were screened by RT-PCR at the conclusion of experiments.

Three field experiments were grown at Redlands Research Centre in 2018 and 2019 to assess bean varieties for tolerance to CPMMV in terms of symptom severity and marketable pod yields and other agronomic characters.

In two experiments in 2017 and 2018 (Table 2) one metre plots of 18 bean varieties were sown with plants in one half of each plot sap inoculated with CPMMV isolate Q5288, 14 days after sowing when plants were at the first trifoliate leaf stage.

Inoculated plants were rated for symptom type and severity using a zero to four scale.

The degree of pod damage due to virus was assessed and the number and weight of total and marketable pods measured in 10 plants per plot in both inoculated and uninoculated plots.

In 2019, 34 varieties (Tables 3 and 4) were sown in two trials. Two x three metre plots of each variety were planted. Plants in a one metre plot of each replicate x variety were inoculated with CPMMV isolate Q5288 in trial one and with a 2019 isolate Q5549 in trial two.

Virus severity ratings were made on three dates in both trials using a zero to four scale. At harvest 12 plants per variety were harvested from both inoculated and uninoculated as per the 2018 method. The data collected is given in appendix 2. Tolerance was defined as no to mild symptoms on leaves (rating 0-2) and few deformed pods. Tolerance did not imply no yield loss due to virus and the extent of yield loss in a variety was assessed in comparison with healthy plots of the same variety in the trials.

Seed transmission experiments

This work was undertaken by growing commercial seed lots of bean (cv.Wyatt), soybean (ZAM 1,P 791, Bunya) and mung bean (cv.Jade) in a greenhouse where no whitefly or sources of CPMMV were present. Young plants were inspected for virus symptoms over a four-week period and suspicious plants checked by electron microscopy and/ or RT-PCR. At the conclusion of each experiment random samples were collected from plants of each variety and tested for virus presence/ absence by RT-PCR.

To further investigate possible seed transmission of CPMMV, young plants of two bean varieties, Wyatt and Stanley and a soybean variety P 791 were inoculated with isolate Q5288 and plants grown to maturity. Seed was harvested and stored in a low humidity cool room before being planted in greenhouse free from whitefly and CPMMV.

The assessments and assays at the end of each experiment were as outlined above.

Outputs

Outputs listed in Research Agreement and M&E plan (Extension and capacity building) were provided as listed below:

Factsheets/ Conference abstracts

- Start of project statement
- Pest alert on Cowpea mild mottle virus
- Fact sheets on Cowpea mild mottle virus
- Management plan for CPMMV updated as necessary
- Two industry summaries of activities and progress
- Vegnote70 January/ February 2019 Characterisation of a carlavirus of French bean VG 15073
- Presentation" A disease of French beans and soybean caused by Cowpea mild mottle virus in Queensland"

  Science Protecting Plant Health Conference Brisbane September 2017
- Tolerance of French bean cultivars to Cowpea mild mottle virus to be presented at the APPS Conference Melbourne November 2019

Grower/ industry meetings

Grower meetings were held in 2016 to provide basic information on the virus and the planned response: Gatton August 12 (40 participants): Kalbar September 22 (10 participants); Aratula November 17 (25 participants).

The August 12 and September 22 meetings were organized by Denis Persley, Project Leader. The November 17 meeting was jointly organised by DAF, Farmcraft and Agrifoods. Presentations were made on CPMMV,

A grower/ industry seminar was held at Aratula on August 15 2017. A copy of the invitation is attached. The 30 participants were provided with an update on disease surveys, project activities and results. A management plan for CPMMV in beans was presented.

Grower and industry feedback was positive for both the meeting and project outcomes to date.

The intensive monitoring for virus and whitefly undertaken in the Fassifern area over four years by Peter Nimmo and Cherie Gambley enabled close contact with growers including regular updates on vector numbers, virus incidence and project progress.

The variety screening trials at Redlands over two years provided a valuable link with the green bean industry. In 2019, 23 people inspected trial one and visitors included the major green bean growers and all seed companies marketing bean varieties. Trial two was inspected by 14 people representing two growers and five seed companies.

A grower/ industry meeting will be held in south Queensland October 2019 to review project outcomes and 2019 results.

Outcomes

Vision	*Molecular and biological characterisation of a Carlavirus infecting beans in Queensland, determine distribution and risk in other bean production areas in Australia, develop and inform industry of management strategies for the virus*
Strategic Objective	*Farm productivity, resource use and management (to enable growers to defend themselves from emerging pests and diseases) (AusVeg Industry Strategic Investment Plant 2012-2017)*
Project Outcomes **Longer Term**	• *Australian bean growers adopt a range of integrated viral disease management strategies to minimise the impact of the Carlavirus Cowpea mild mottle virus on yield and quality* • *Effective management plans for the virus are developed based on comprehensive knowledge of the biology, epidemiology, genetic variability and transmission of CPMMV in Australia* • *Project work has provided the opportunity for the possible detection of other epidemic or biosecurity viral pathogens in Australian fresh bean crops and informing appropriate agencies of their occurrence and likely impact*
Project Outcomes **Intermediate**	• *Determine the reaction of commercially important bean varieties to CPMMV by September 2017* • *Determine host range of the virus to inform management plan by September 2017* • *Develop an industry management plan for the virus based on current knowledge of host range, epidemiology and transmission*
Project Activities **(immediate outcomes)**	• *Current scientific literature on the virus reviewed* • *Engage key stakeholders to advise, update, extend and evaluate research outputs*
Foundational Activities	• *Identify virus and assess relationship to strains internationally* • *Determine presence and distribution of CPMMV in major Queensland and Australian production areas* • *Determine source of bean seed planted in Queensland and Australia* • *Notify and advise biosecurity agencies of detection and risk assessments* • *Grower engagement communications activities and meetings*

Foundational activities

The initial identification of the virus was based on isolate Q5288 collected from symptomatic French bean at Kalbar in south east Queensland.
The isolate was transmitted by manual inoculation and by *Bemisia tabaci* (MEAM1) from infected bean plants to healthy soybean and bean plants. All test plants developed symptoms similar to the original samples. Flexuous virions 600-700 nm in length were observed and tested negative for potyvirus using an ImmunoStrip test (Agdia, USA) and a group-specific RT–PCR (Gibbs and Mackenzie, 1997; Langeveld *et al*., 1991). The virion morphology was also consistent with carlaviruses and the samples subsequently tested positive in DAS-ELISA with antibodies (DSMZ, Germany) for the carlavirus, *cowpea mild mottle virus* (CPMMV).

Amplicons covering part of the coat protein gene through to the 3' end of the genome were obtained by RT-PCR using the oligo-dT primer Poty 1 (Gibbs and Mackenzie, 1997) as the reverse primer for both isolates and the forward primer Carla7190F (5' GGNYTNGGNGTICCIACIGARCAYGT 3'; designed to a range of carlaviruses) for Q5288. The amplicons were directly sequenced and gave fragments of 916 nt (GenBank MK910291) for Q5288. Using BLASTN analyses (Zhang *et al*., 2000), the sequences most closely matched CPMMV from Brazil (GenBank KC884249) at 85% nt identity, and CPMMV from India (GenBank AF024629) at 76% nt, for Q5288.

The ICTV demarcation threshold for species within the *Carlavirus* genus is less than 72% nt identity between coat protein or polymerase sequences. A comparison of the partial coat protein nucleotide sequences indicated the Australian isolate fell within CPMMV with 78.9% identity to the type species originally reported from Ghana (Brunt and Kenten, 1973) (GenBank NC_014730).
This was the first report of a carlavirus infecting *Fabaceae* in Australia.

Determine presence and distribution of CPMMV in major Queensland and Australian production areas

In 2016 the virus was detected in the Fassifern production area in April 2016 where it caused extensive production losses. In ten crops examined virus disease incidence exceeded 50% in all crops and was close to 100% in at least three crops. Further surveys in the district from 2016 to mid-2019 found the virus in bean crops only during autumn plantings and virus incidence remained low until autumn 2019 where it returned to levels similar to that observed in 2016.

The virus was detected at an incidence of <1% in a bean crop at Bowen in August 2016. In 2017 and 2018 the virus was detected in several bean crops in the Lockyer valley and at Bundaberg. The incidence in these crops was < 5%
The virus has not been found in beans or other species in other Australian States.

Determine source of bean seed planted in Queensland and Australia

Introduction of bean seed into Australia for planting is prohibited and subject to quarantine.
Bean seed for planting is introduced into Australia from production areas in Idaho and Washington State in the USA under an agreement between the Australian government

and the United States Department of Agriculture (USDA). Seed production fields are inspected for freedom or incidence within specified tolerances for listed pathogens. CPMMV has not been reported from mainland USA.

Notify and advise biosecurity agencies of detection and risk assessments

Biosecurity Queensland was notified of the confirmed detection of the carlavirus infecting bean in May 2016 by a PIDS (Preliminary Information Data Sheet). The Department of Agriculture was notified soon after.
Speaking points about the detection were available on 30 June 2016. A CCEPP meeting was convened on 23 September 2016 where the consensus view was that eradication was not feasible, given the active vector and known distribution of the virus in Queensland.
A Pest Alert was issued by Biosecurity Queensland in October 2016.

Grower engagement and communication activities

Plant pathologists from DAF worked closely with growers in affected areas from the time the first sample was received and had discussions with seed companies and other industry staff to gain information on matters such as varieties, distribution, seed source and production areas.
Three grower information meetings were held in south Queensland in 2016, a Ref note on the virus was written and an interim management plan prepared in consultation with growers.

Immediate outcomes

Current scientific literature on the virus reviewed

Available literature was reviewed to assist project planning. Key information included specific detection protocols, current occurrence and importance of carlavirus in grain legumes worldwide, potential host species and reports of resistant or tolerant germplasm.

Engage key stakeholders

The main growers of green beans were engaged early in project development and have assisted with information and access to crops throughout the project. The major contacts have been Kalfresh, Rugby Farms and Mulgowie farming company.
In addition to annual surveys in each production area, fortnightly surveys were done in the Fassifern area throughout the season over four years to provide timely data on whitefly populations and virus incidence.
All seed companies supplying green bean seed have been engaged with the project and have made important contributions through supply of seed and variety data. The companies were HM Clause, Syngenta Australia, Bayer-Seminis, Lefroy Valley Seeds and Sunlands Seeds.

Intermediate outcomes

Determine the reaction of commercially important bean varieties to CPMMV by

September 2017

The reaction of 17 French bean varieties to CPMMV originally obtained from bean in south Queensland was determined by sap inoculation of young bean plants 12 days after planting. Plants were maintained in a glasshouse and assessed for symptom type and severity over a three week period.

The varieties inoculated included those currently used in large scale green bean production in Australia (Wyatt, Hickok, Aldrin, Excalibur). The other varieties are grown by smaller growers or are well established older varieties in the bean seed trade.

All varieties developed symptoms of CPMMV within 12 days of inoculation. Symptom severity varied between varieties with Jade, Simba and Borlotti bean developing mild leaf symptoms (1 to 2 on a 0 to 4 scale). Varieties expressing severe symptoms were Jackson, Excalibur and Aldrin. Detailed results are given in appendix 2.

None of the varieties expressed very severe symptoms which would include leaf blistering, distortion and necrosis.

These data informed future project work where up to 30 varieties were assessed for virus reaction/ tolerance over several field trials. Results are in Tables 2, 3 and 4.

Determine host range of the virus to inform management plan by September 2017

The known natural field hosts of CPMMV in Queensland are bean (*Phaseolus vulgaris*), soybean (*Glycine max*), mung bean (*Vigna radiata*), cowpea *(Vigna unquiculata),* Siratro (*Macroptilium atropurpureum*) and *Glycine* sp..

The experimental host range of CPMMV was established by sap inoculation of candidate species. Refer to Table 1 for results.

Develop an industry management plan based on current knowledge of host range, epidemiology and transmission

A management plan was developed based on the following information:

- Currently grown green bean varieties are very susceptible, including development of distorted pods, particularly when plants develop symptoms during the first four weeks of growth
- Project experimental work and surveys did not find evidence for seed transmission of the virus in bean or soybean seeds, including commercial lots of widely grown varieties.
- There are few alternative hosts but Siratro, Phasey bean and Glycine are known to host the virus and are often present along watercourses, fencelines etc.
- The white fly vector is a mobile and active. However, beans are not a preferred host and moderate to high populations seem to develop only when alternative preferred hosts are unavailable or unsuitable. This is a high risk situation if CPMMV inoculum is available.
- In these situations whitefly control alone will not be sufficient to provide adequate virus control and less susceptible varieties are required to minimize virus impact

Management plan for CPMMV

Purchase bean seed of a known variety from a reputable supplier. Seed of the major green bean varieties grown in Queensland is imported into Australia under an agreed protocol between Australia and the USA. This seed is produced in Idaho and Washington State. CPMMV has not been detected in the mainland USA hence the risk of seed borne CPMMV in this seed is extremely low.

Where possible, avoid planting bean crops adjacent to crops of soybean, cowpea and mung bean.

Monitor crops for whitefly weekly. Apply registered insecticides and/or biocontrol agents such as *Eretmocerus hayati*, as appropriate for whitefly control. Only low to moderate whitefly populations are needed to result in considerable virus spread if virus sources are available; these populations are less than those that would normally result in crop damage.

Crop damage through yield and quality reductions is likely to be greater when virus infection occurs in young crops. Disease close to harvest is unlikely to cause major damage.

Destroy crops as soon as practicable after harvest and this is particularly important if virus infection is suspected. If whitefly are present spray with an oil prior to destroying the crop as this will limit movement of whitefly to other crops.

Plant virus tolerant varieties in high risk periods or locations. The pods produced on these varieties are largely unaffected by virus, even when plants are infected early in their life. Select varieties based on performance and market requirement.

An alternative or complementary strategy is to plant a proportion of other crops such as pumpkin which is a preferred host for whitefly and should limit movement of whitefly into bean crops. Pumpkin is not a host of CPMMV and would have a positive impact of reducing virus spread into bean crops, particularly during high risk windows such as autumn in the Fassifern.

Project outcomes-longer term

- Australian bean growers adopt a range of integrated viral disease management strategies to minimize the impact of the Carlavirus Cowpea mild mottle on yield and quality. Refer to management plan above.

- Project work has provided the opportunity for the possible detection of other endemic or biosecurity viral pathogens in Australian fresh bean crops and informing appropriate agencies of their occurrence and likely impact

  About 30 virus diseases impact French bean production worldwide (Schwartz et al.2005; Singh and Schwartz 2010). The more important of these are diseases caused by begomoviruses, all of which are transmitted by the silver leaf whitefly Bemisia tabaci. The most important virus in this group is Bean golden mosaic virus (BGMV) which is a limiting factor to bean production in parts of Latin America.

  The potyviruses Bean common mosaic and Bean common mosaic necrosis virus occur in many countries but are generally well controlled through host resistance in commercial bean varieties. These viruses are both seed borne and aphid transmitted.

There are several seed and leaf beetle transmitted viruses infecting beans in the Americas but these have not been reported in Australia

Extensive surveys in Queensland bean production areas over four years of this project found very few virus infected plants, other than CPMMV infections which reached significant levels in one or more districts 2016 and 2019.

Trace levels of the potyvirus Bean yellow mosaic virus, confirmed by viral sequence, were found on two occasions during surveys.

Sporadic outbreaks of bean summer death disease caused by a leafhopper transmitted Mastrevirus occur in Australia but none were recorded during this project. This disease is linked to susceptible varieties and conditions that favour the movement of the vector into bean crops.

Monitoring and evaluation

Project activities and outcomes	Monitoring / evaluation activity
Aspects of the project to be monitored / evaluated	*What monitoring or evaluation activity will be undertaken and when?*
Identify virus and determine phylogenetic relationships to other CPMMV clade members	• *Identify virus by molecular, serological and biological tests* • *Obtain complete genome sequence and compare with available sequences on gene bank and elsewhere*
Determine natural and experimental host range of the virus	• *Determine host range by manual inoculation of Fabaceae and other plant species in glasshouse experiments* • *Collect and test candidate potential hosts during field surveys*
Determine the reaction of commercial and other French bean varieties to CPMMV	• *Obtain and test by manual inoculation a range of French bean cultivars for CPMMV reaction* • *Test at least some of these varieties by inoculation via the whitefly vector*
Determine the mode of transmission of CPMMV	• *Confirm whitefly as vector of Queensland CPMMV* • *Undertake grow-out tests with bean and other grain legumes to determine if seed transmission of the virus can be demonstrated*
Determine distribution of CPMMV in Queensland and develop risk index for Australian bean production areas	• *Undertake surveys in all green bean production areas of Queensland in each year of project and record presence/ incidence and impact of the virus* • *Identify fresh bean production areas in other States and determine risk by surveys and/or vector distribution*
Evaluate diagnostic methods for CPMMV *Management plan produced and amended as necessary*	• *Compare available diagnostic assays and ensure appropriate assays available to identify virus strains within CPMMV carlavirus clade* • *Plan produced in consultation with bean growers and amended as necessary e.g as new information is available*
Extension and capacity building	• *Communications strategy and plans devised* • *Grower meetings* • *Ref notes / newsletter contributions produced and distributed*

All four project contracted outcomes were met:-

- The cause of the disease was found (CPMMV) and characterised.
- The distribution and incidence was determined through surveys in all Queensland bean growing areas over four years and the risk to the bean industry outside of Queensland was assessed
- A management plan was developed and modified over the life of the project in light of new information from project work.
- Consultation and information with growers, industry and government was initiated and maintained from project inception onwards

There has been practice change by growers and industry as a result of awareness and results obtained during this project.

Confirmation that the virus disease in beans in Queensland was caused by a whitefly transmitted virus changed the perception of growers from regarding whitefly as a sporadic nuisance pest to realising that monitoring and management was critical to minimise potential losses from CPMMV.

Intensive monitoring for whitefly and virus in the Fassifern area over four years by the project team identified specific high risk periods for whitefly increase and virus spread. Growers accepted that whitefly management with insecticides, although useful, was not providing the level of control needed in terms of vector management during this high risk period.

Demonstration through project field trials that good virus tolerance existed in some currently available commercial bean varieties has resulted in a change in the variety mix used by growers and evaluation of a wider range of varieties to assist virus management.

Growers and seed companies have also recognised the need to include screening for virus tolerance early in the evaluation phase for potential new commercial varieties.

Discussions to facilitate this activity are in progress.

All project activities and outcomes listed above have been met when evaluated against the listed monitoring and evaluation activity. The specific results obtained are provided in the project outcomes section and as an appendix to the report.

In evaluating extension and capacity building the following evaluations and comments are made:-

There were three grower/ industry meetings during the project in south Queensland; all were well attended with over 20 people at each, including representatives of all important bean growers. Feedback was positive but could have been more accurately judged with a formal evaluation form. This will be used for end of project meeting in November.

The disease surveys in all districts over four years have allowed personal contact with growers/ agronomists on all important bean production farms. This has been a valuable means of discussing the problem and providing information on the project.

The weekly surveys in the Fassifern area have provided close contact with growers in the area where the virus has been most prevalent and damaging. These surveys have provided real time data to growers on whitefly populations and virus incidence.

The relationship with seed companies required some effort to establish because of early issues surrounding possible seed transmission of the virus, biosecurity concerns and susceptibility of widely grown varieties.

Work with biosecurity agencies allayed these concerns quickly and a good relationship developed with all seed companies who were and are very willing to have varieties tested and work with growers to provide the most appropriate range of varieties for a region.

The virus screening trials at Redlands provided an excellent opportunity for grower/ industry interaction. In 2018 and 2019 all bean growers and seed companies visited the trials and gained valuable information which was translated into further testing on farm of promising varieties.

The DAF Redlands research facility is an excellent site for work with CPMMV as it is remote from all bean growing areas, has low whitefly populations and has an extended growing season and is easily accessible. Undertaking the virus screening work in a bean growing area would be unacceptable given the ease of whitefly transmission and the high risk of initiating an outbreak in a production area.

Recommendations

- Growers adopt the management plan provided during the project to minimise potential losses from carlavirus
- Growers continue to evaluate virus tolerant bean varieties identified in the project to decide which are best suited to their location and market requirements
- Potential new commercial bean varieties be screened for reaction to carlavirus early in the evaluation cycle and these results be used as a selection criteria for possible future commercial release
- Bean crops in south and north Queensland be monitored to determine if the incidence and impact of carlavirus has significantly altered compared with data obtained over the four years of the project

Refereed scientific publications

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Intellectual property, commercialisation and confidentiality

No IP, commercialization or confidentially issues to report

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Table 1. Experimental host range of Cowpea mild mottle virus #Q5288*

Family	Species/Cultivar	Symptoms #
Cucurbitaceae	*Cucurbita pepo* Squash	–
Fabaceae	*Arachis hypogoea (peanut)*	–
	Cicer arietinum (chickpea)	–
	Desmodium intortum	–
	Glycine canescens	+ Mo
	G. cytoloba	+ Mo
	G. max (soybean 6 cultivars)	+ Mo, Mos, Vc
	Lablab purpureus	–
	Medicago sativa (Lucerne)	–
	Macroptilium atropurpureum (Siratro)	+ Mo
	M. bracteatum (Burgundy bean)	+ Mo
	M. latyroides (Phasey bean)	+ Mo
	Pisum sativum (Pea)	–
	Phaseolus acutifolius	+ Mo
	P. coccineus (runner bean)	+ Mo
	P. dumosus	+ Mo
	P. filiformis	+ Mo
	P. lunatus (lima bean)	+ Mo
	Stylosanthes spp.	–
	Vicia faba (broad bean)	–
	Vigna luteola	+ Mo
	V. radiata (mung bean)	+ Mo
	V. unguiculata subsp. *unguiculata*	+ Mo, Mos
Solanaceae	*Capsicum annuum*	–
	Solanum lycopersicum (tomato)	–

* Plants sap inoculated with isolate Q5288 CPMMV

\# Key to symbols: – = no symptoms and virus not detected in new growth leaves by RT-PCR

Symptoms: Mo = mottle; Mos = mosaic

Table 2

REACTION OF BEAN VARIETIES TO CPMMV FOLLOWING INOCULATION – REDLANDS 2017/18

Variety	Severity leaf/plant symptoms*	Symptom severity pods/marketable yield#	Warrants further testing##
Excalibur	3	3	N
Sybaris	2	2	Y
Lawrence	4	4	N
Jackson	4	4	N
Jade	1	3	Y
Aldrin	4	4	N
Simba	2	2	Y
Prarie	4	4	N
Wyatt	3.5	3	N
Hickok	3.4	4	N
Coulter	3	3	N
Tasman	1	2	Y
Windsor long pod	3.5	2	Y
Voltage	3.5	2	Y
Labrador	2	3	Y
Greenleaf	1	2	Y
New Pioneer	1	2	Y
Venice	2	3	Y

* Severity rating 0-4 with 0= no symptoms and 4= very severe mottling/ mosaic

1= pods mildly affected with most marketable 4= pods badly distorted, discoloured and largely unmarketable

Y=yes worth further trialling N= severely affected; further trialling not warranted

Table 3 Evaluation of green bean varieties in trial 1 Redlands 2019. These include healthy versus infected pod weight, total yield loss (%), healthy versus infected pod uniformity characteristics, healthy versus infected pod straightness, pod length measurements and pod colour ratings (G = green, LG = light green, DG = dark green, Y = yellow) . 1=good quality; 5= poor quality

		Yield				Pod uniformity (1-5) (b)		Pod straightness (1-5) (c)			
Variety	Virus severity rating – plant/ leaves (a)	Healthy weight (g)	Infected weight (g)	Difference weight (g)	% Yield loss	Healthy	Infected	Healthy	Infected	Pod length (cm)	Pod colour
Outlaw	0-1	950	555	395	41.6	2.1	2.1	2.1	2.1	11-15	DG
Cahill	0-1	1205	870	335	27.8	2	2.1	2	2	15	G
Sybaris	0-1	910	510	400	44	2	2	2.5	2.5	16	DG
Jaguar	0-1	805	685	120	14.9	2	1.8	2	1.8	17	DG
Seminis BA 0958	0-1	720	495	225	31.1	2.5	2.5	2.1	2.5	16	LG-DG
Excalibur	4	775	455	320	41.3	2.5	2	2.5	3	15	G
Syngenta 4735	0-1	670	325	345	51.5	1.9	2	1.9	2	14-16	DG
Messi	0-1	675	635	40	5.9	1.5	2	1.5	1.8	18	DG
Syn 4734	0-1	705	475	230	32.6	2	2	2.9	2.4	11-16	G
Venice	0-1	735	550	185	25.2	1.5	1.9	1.5	1.9	14	G-DG
Colter	4	710	485	225	31.7	2.2	3	2.2	3	16	DG
Cabot	4	660	350	310	47	2.1	3.1	2.1	2.4	12-17	LG
Hickok	4	695	425	270	38.8	1.5	2.3	1.5	2.8	13	G-DG
Aldrin	4--5	555	255	300	54.1	2	2.4	2	2.4	12-14	DG
Wyatt	3--4	600	320	280	46.7	1.5	3	1.5	3	17	DG
Jackson	4	740	450	290	39.2	2.2	2.5	2.2	2.3	15	DG
Labrador	1	810	370	440	54.3	2.2	2.5	2.2	2.5	15	LG
Stanley	0-1	535	320	215	40.2	2	2.4	2	2.4	14	LG
New Pioneer	0-1	550	255	300	54.1	2.3	2.8	2.3	2.5	22	LG
Tasman	0-1	600	525	75	12.5	2	2	3	3	12-15	LG
Simba	0-1	590	530	60	10.2	2	2.5	2.3	2.9	16	LG
Greenleaf	0-1	580	430	150	25.9	1.5	2.5	1.5	2.3	18-20	LG
Jade	0-1	720	500	220	30.6	2.3	2.8	2.3	3	18	LG
Voltage	2--3	685	305	380	55.5	2.1	2.3	2.1	2.3	16	Y

Table 4 Field screening of bean varieties for Carlavirus
Redlands Research Centre 2019

Variety	Trial 1 rating #5288	Trial 2 rating #5549
Outlaw	0-1	1.5
Cahill	0-1	1
Sybaris	0-1	1.5
Jaguar	0-1	1
Sem BA0958	0-1	1.5
Excalibur	4	5
Syn 4734	0-1	1
Syn 4735	0-1	1.5
Messi	0-1	1
Venice	0-1	1
Colter	4	5
Cabot	4	4
Hickok	4	5
Aldrin	5	5
Wyatt	4	5
BED 95321	-	1.5
Sonesta	-	1.5
Goldplay	-	2
Caprice	-	5
HMX 8615	-	1
HMX 8617	-	1
HMX 0175724	-	4
Voltage	3	4
Greenleaf	0-1	1.5
Stanley	1	1.5
Jackson	4	4
Labrador	1	1
Jade	1	1.5
New Pioneer	0-1	1
Lawrence	-	4
Simba	1	1.5
Tasman	1	1

Virus scores

0 = no symptoms

1 = very mild symptoms

2 = mild symptoms

3 = leaf mottling/mosaic evident

4 = severe leaf mottling/mosaic

5 = very severe symptoms/plant stunting evident

Trial 1 planted: 20 March 2019

Inoculated CPMMV 1 April 2019

Virus ratings 24 April 2019; 3 May 2019

Trial 2 planted: 15 May 2019

Inoculated 30 May 2019

Virus rating 10 July 2019; 24 July 2019

Appendix 1

Detailed results from experimental work and surveys VG 15073

Virus identification and phylogenetic relationships

Virus isolates, particularly # Q5288 were identified using several independent molecular, serological and biological tests as detailed in the Methodology and Outcomes sections.

The complete genome of isolate # Q 5288 was obtained and compared with available full sequences of related Carlaviruses on GenBank.

A complete genome sequence of the original isolate was obtained from bean at Kalbar in 2016 (#5288) by Nanopore sequencing followed by primer walking to confirm the sequence.

Sequencing of the coat protein gene of other virus isolated collected from beans in southern and northern Queensland since 2016 generally show a nucleotide variation of less than 5%. The exception to this are several isolates from bean and legume weeds collected in the Fassifern area which have genetic differences of about 30%. Interestingly, the original 2016 isolate from bean (#5288) and #5294 originally obtained from soybean in south Queensland in 2016 show only 71% nt identity over the sequenced region. These isolates are also only around 70% identical with the third virus strain which was found in bean and legume weeds in the Fassifern. All appear to represent distinct variants of CPMMV in Queensland. This may reflect more than one introduction of the virus into Australia.

A phylogenetic comparison to a range of CPMMV isolates from various global regions indicates that the Australian isolates fall within the CPMMV cluster and are genetically distinct from each other and from other isolates included in the analysis. Comparison of the #5288 isolate with the full genomes yields the closest nucleotide homology of 76.8% with an Indian isolate of CPMMV (MH345698). Recently collected and sequenced CPMMV isolates from several countries, including Brazil, USA, India and Australia, are only distantly related to the original CPMMV type isolate described from cowpea in Ghana in 1973. Analysis of the complete amino acid sequences of the RDRP region only shows a ~60% identity with the type species, though the coat protein sequences have ~95% identity. This conservation of the coat protein sequence is probably due to a function of the whitefly transmission.

All Australian isolates tested have reacted in double antibody sandwich ELISA with antibodies to CPMMV (DSMZ Germany Catalogue RT -0907), as can be expected due to the amount of homology in the coat protein between CPMMV isolates.

Natural and experimental host range

The host range of isolate Q5288 was determined by sap inoculation of some 26 species and 35 cultivars/accessions across Fabaceae and Solanaceae.

The experimental host range was confined to Fabaceae with host species being largely confined to the genera

Phaseolus, Macroptilium, Glycine and *Vigna*.

The natural hosts of the virus found in Queensland were bean, soybean, mung bean,

cowpea, Siratro, Phasey bean and Glycine.

Siratro, a twinning legume species originally introduced into tropical Australia as a pasture legume, occurs widely along roadsides, fencelines and watercourses in much of Queensland. Isolates of CPMMV have been collected from this species at widely separated locations in south, central and north Queensland. These data support the conclusion that this species is an important perennial alternative host of the virus.

Reaction of bean varieties to CPMMV

In initial work 17 green bean varieties were screened for virus reaction by sap inoculation. Most were susceptible but several older varieties developed milder symptoms. This work was expanded with three field trials assessing varieties for virus reaction, yield and quality. A total of 30 varieties were evaluated which included all current commercial varieties, some new experimental varieties and older varieties with pedigrees dating back some 50 years. Grower experience in the Fassifern area was that insecticide applications, while lowering whitefly populations, were not very effective in reducing virus spread when populations were high and virus inoculum was available. Tolerant varieties provide an effective option for management and growers and seed companies were active participants in the work. Five varieties were selected for semi-commercial evaluation based on trial results in 2018/19. These have or are being evaluated in north and south Queensland. These varieties provide an alternative planting option during high risk periods when the preferred but highly susceptible varieties have suffered major yield and quality losses. Although tolerant varieties may have a yield reduction following infection they do not develop pod twisting and deformity which is a major issue with susceptible varieties because of the increased labor costs and time delays in culling large numbers of deformed pods in the packing shed.

Virus transmission

Almost all Carlaviruses are transmitted by aphids, except for members of the CPMMV clade and another unrelated virus infecting cucurbits (Melon yellowing-associated virus)

Transmission of two CPMMV isolates to several bean and soybean varieties by *Bemisia tabaci* (MEAM1) was confirmed in cage tests

Work overseas has demonstrated that at least some isolates of CPMMV are transmitted through the seeds of several Fabaceae species, including French bean, soybean and cowpea. However, reports of seed transmission are contradictory and depend on factors such as host variety, virus strain, time of infection, environmental influences such as temperature and seed storage conditions.

We have not demonstrated seed transmission of CPMMV in Queensland where commercial seed lots of bean, soybean and mung bean were sown and inspected/ tested for the virus. Further, many thousands of young bean plants have been inspected in fortnightly surveys in the Fassifern area and no virus has been detected until natural spread begins in late summer/ autumn crops.

In this work the potential for seed transmission was also examined by growing plants of French bean, soybean and mung bean in a greenhouse free from whitefly and sources of CPMMV. Young plants were examined for symptoms over a four week period. Random samples and any individuals showing suspicious symptoms were tested by PCR from each experiment.

CPMMV was not detected in any of 1864 plants grown from bean variety Wyatt. The seed sample was from two seed lots sown in the Fassifern area in the 2016 and spring 2017 seasons.

The virus was not detected in 588 plants of soybean variety ZAM 1, 564 plants of

variety P 791 and 930 plants of variety Bunya. Samples of P 791 and Bunya were from commercial seed lots sold in the Fassifern and other regions while CPMMV had been found in ZAM 1 in the Lockyer Valley in 2016.

CPMMV was not detected in 1768 plants of the mung bean variety Jade, the main variety grown in Queensland.

In further work, seed was harvested from two bean varieties, Wyatt and Stanley and one soybean variety P 791, which were inoculated with a Queensland isolate of CPMMV at the first trifoliate leaf stage isolate and grown to maturity.

CPMMV was not detected in 1110 Wyatt plants,1080 Stanley plants or 776 P791 soybean plants

These results do not exclude the possibility of seed transmission of CPMMV in one or more species and seed transmission remains as the most likely pathway for the incursion of CPMMV into Australia. However, seed transmission rates are likely to be very low and infrequent. The results also provide confidence that the virus is most unlikely to be present in commercial seed lots of bean and soybean.

Distribution of CPMMV in Queensland

The important bean production areas in Queensland were surveyed for CPMMV in each year of the project. Although detected in the Bowen winter production in each year, virus incidence has been less than 1%. The components for an epidemic are present in this area-whitefly are common and sometimes abundant; highly susceptible bean varieties are grown and the virus is present in alternative hosts. Factors which are assisting in preventing significant outbreaks most likely include concentration of bean production in large areas on several properties where known whitefly hosts such as cucurbits and tomato are not grown adjacent to beans which are not a preferred host of the insect. Infected alternative hosts, although present, are not abundant which limits initial inoculum available to the whitefly vector.

Some or all of these conditions could change hence vigilance is recommended in what is a major and important crop in the region.

The district where the virus has had greatest impact is the Fassifern in south east Queensland where the virus was first detected as a result of a major epidemic in the autumn crop in 2016.

Intensive surveys for the virus and vector have been done in this area over four seasons. Beans are produced in the area from spring through to late December then a break over the hot, humid summer months and planting resuming in February and the last crops harvested in May-June.

Beans are grown in a mixed cropping situation with lucerne, cucurbits, soybean, grain legumes and corn being other important crops.

The fortnightly surveys over four years have identified consistent patterns with virus and vector.

Whitefly numbers are low in bean crops in the production period up to December and CPMMV has not been found in bean crops during this time.

Whitefly numbers increase in bean crops from late February onwards and can remain high into May. In each of the four yeas virus has first been found in bean crops in March. In two of the years (2017 and 2018) virus levels remained low with little impact. However, in 2016 and 2019 disease levels of between 50% and 100% occurred in several crops, resulting in considerable economic losses. There has been a strong association between severely affected crops and proximity to watercourses, fencelines etc where infected virus infected Siratro and Glycine is prevalent and where adult whitefly have been consistently found during surveys. Sampling data also found that pumpkin and soybean crops were good hosts of whitefly and were an important factor in maintain populations from spring through to late autumn.

In 2019 surveys commenced in the Fassifern in late October 2018 and continued until June 2019. The first virus records from beans were made on January 17. A large whitefly population developed throughout the district in late February with all bean crops surveyed having carlavirus- infected plants. Whitefly numbers on beans at 17 sites during the first three weeks of March averaged 250 insects per 150 leaves with a range of 21 to 1000insects. Virus incidence ranged from 7% to 100% across six crops surveyed.

The survey work has found that at least in the Fassifern area the autumn production period is the high risk period for virus spread and where virus tolerant varieties should be deployed to reduce disease impact.

An assessment of the economic impact of CPMMV is made with the following background:

- Queensland is the major producer of green beans
- CPMMV occurs in all production areas in Queensland and has the potential to be economically damaging if virus/ vector dynamics favors spread and a high virus incidence in crops
- The major green bean varieties used in Queensland are highly susceptible to CPMMV

A major economic impact of CPMMV in beans is the development of curled, discoloured pods on infected plants, particularly if plants are infected during the first four weeks of growth.

The major green bean producers do a once over machine harvest and if the percentage of defective pods is in the 10 to 15% range or above then the crop is frequently not harvested. The cost of growing one acre (2.47Ha) of beans to the harvest stage is approximately $1600 so the direct cost of not harvesting is considerable. If a decision is made to harvest at this or higher defect level then there are significant indirect costs at pack out as additional labor is needed to remove low grade beans and the volume through the packing shed per hour is reduced. As labor is always one of the major costs for growers these additional costs can quickly reduce margins, particularly if prices are only average.

Actual example of losses due to CPMMV in beans. In the late summer/autumn of 2016 around 150 acres of beans were affected by CPMMV in one district of south Queensland. The epidemic was linked to high populations of silver leaf whitefly and a very susceptible variety.

The following estimates of losses were made by growers closely involved with the business:

Moderate losses-25% of value of beans lost through quality downgrade and extra labor in the packing shed to cull defected beans. Sixty two acres affected with an estimated loss of $95 000.

Serious losses-50 % of value of beans lost through quality losses and additional labor costs. 75 acres affected with estimated losses of $230 000.

Complete crop loss- crops not harvested due to severe virus damage. 12 acres with estimated loss of $90 000.

Total loss over this period: $415 000

Estimates of industry wide losses from CPMMV:

The economic impact will depend on factors such as virus incidence in each production area, time if infection with a high level of disease early in crop cycles causing most damage, prevailing prices, potential yields and labor costs to cull defected beans.

A one per cent loss based on a Queensland industry value of $70M is $700 000; a 5% loss is $3.5 M while a 10% loss is $7M.

The estimated and actual losses due to CPMMV certainly provide an economic background to develop management systems which minimize disease levels. Components of this system include vector control, less susceptible varieties and control of perennial alternative hosts.

Although Queensland is the major producer of green beans in Australia, smaller production areas are also planted in Victoria, NSW and WA.

A risk assessment for CPMMV in these States hinges on the presence of the silver leaf whitefly (*Bemisia tabaci*), the only vector of the CPMMV clade of Carlaviruses. Although the virus can be seed borne at varying levels in several grain legume species, secondary spread will depend on the presence of the white fly vector. The vector has not been recorded in Victoria or WA hence the risk of CPMMV becoming a significant problem in beans in these States is very low to negligible.

The vector does occur in bean production areas in the Sydney basin and western NSW hence there is the potential for spread if the virus is introduced via seed or other means. There is no evidence for the presence of the virus in these areas at present.

Bean Carlavirus-

Cowpea mild mottle virus

Cowpea mild mottle virus (CPMMV) is a member of the Carlavirus group of plant viruses. When viewed by electron microscopy virus particles of CPMMV are slightly flexuous filamentous rods, approx. 600-700 nm in length. Considerable genetic diversity exists among the CPMMV group and virus strains now prevalent in the Americas and Australia are considerably different from the original virus reported from Ghana in 1973.

Unlike other Carlaviruses, which are aphid transmitted, CPMMV is transmitted by the silver leaf whitefly (*Bemisia tabaci*).

Distribution: CPMMV has a very wide geographical range, occurring in most countries where grain legumes are grown. These include countries of Asia, the Middle East, Africa, Central America and Caribbean, South America and Oceania.

CPMMV was first detected in Australia in 2016 on bean and soybean in south- east Queensland. This was the first record of the virus in Australia.

Hosts: The majority of host species for CPMMV are legumes (family Fabaceae) with French bean, soybean and cowpea being the species most frequently infected.

Other hosts include mung bean, asparagus bean, adzuki bean and lima bean. Several weed species in Fabaceae are also hosts.

In Queensland, the known natural hosts of CPMMV are French bean, soybean, mung bean, cowpea, Siratro (*Macroptilium atropurpureum*), *Glycine* and Phasey bean(*Macroptilium lathyroides*)

Symptoms: A wide range of symptoms can develop on infected plants, depending on the host species, variety and time of infection. Most infected plants develop leaf mottling and mosaic. Seed pods on beans may be deformed and the surface discoloured. This is most likely to occur on susceptible varieties infected in the first month of growth. The disease in beans is sometimes called angular mosaic because of the yellow, angular leaf spots seen against a normal green background.

Symptoms of CPMMV on bean

Infected plants with twisted pods in the field

CPMMV symptoms on cowpea

VG16086: Area wide management of vegetable diseases: viruses and bacteria

Economic impact: In Queensland the main economic impact on beans is poor pod development and the development of distorted, discoloured pods. Crops may not be harvested because of severe damage while considerable extra labour is required on the packing line to cull distorted pods from consignments.

The crops most severely affected overseas are soybean and bean. The virus was identified in soybean in Argentina and Brazil in 2001 with losses from stem and bud blight being as high as 85%. CPMMV is now the most widespread and economically important virus infecting soybean in Brazil.

Pods from infected and healthy bean plants. Note distorted pods and reduced yields from infected plants on left.

Spread: The virus is transmitted by the silverleaf whitefly (*Bemisa tabaci*). Transmission is non- persistent with feeding times of only a few minutes needed to obtain virus from infected plant and transmit to another plant. About 10 minutes of feeding are required to efficiently obtain a virus charge and 5 minutes of feeding to inoculate another plant with virus. The virus is retained by whitefly for only one to two hours if it does not feed on another infected plant.

Healthy and infected plots of a susceptible bean variety

At least some strains of CPMMV are seed borne to varying levels, depending on the host species and variety.

Seed transmission rates in cowpea were reported as 1% to 3 % in India and up to 20% in Uganda.

Seed transmission rates in soybean in Egypt were 6.75% for variety Clark and 10.75% for Crawford.

In Queensland, seed transmission has not been found in grow out tests of bean, soybean and mung bean. In addition, seed transmission was not found when seeds from CPMMV- infected bean and soybean plants were grown and young plants tested for virus.

Management:

- In terms of virus vector management, whitefly control with insecticides is unlikely to be very effective because of the very short feeding times for transmission. However, management to reduce population levels is sensible and may prevent major population peaks which contribute to rapid virus spread and high disease levels.
- Destruction of annual crops after harvest and a period without major hosts and whitefly should reduce virus levels/ survival.
- Several commercially available green bean varieties are tolerant to the virus. When infected, even when young, plants develop few if any symptoms. Pods develop normally with very few deformed pods as occurs in susceptible varieties. These varieties are tolerant, not highly resistant, and varying levels of yield decrease may still occur compared with healthy plants of the same variety. The very low cull level of pods in the packing shed is a key advantage.
- Use of virus tolerant varieties combined with knowledge of likely whitefly populations throughout the production period provide the tools to minimise the economic impact of the virus.
- Another strategy is to plant a proportion of other crops, such as pumpkin, which is a preferred host for the whitefly and should result in limiting movement of whitefly into bean crops. Pumpkin is not a host of CPMMV and would have a positive impact in reducing virus spread into bean crops, particularly during high risk periods, for example autumn in the Fassifern area.

A tolerant bean variety with no leaf symptoms despite inoculation with CPMMV

Symptoms of CPMMV on a susceptible bean variety

Source: Denis Persley, DAF Queensland, email: Denis.Persley@daf.qld.gov.au

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Screening bean varieties for reaction to CPMMV

Infected (right) and healthy bean var. Hickok

INDUSTRY UPDATE. BEAN CARLAVIRUS 2017

VG 15073 Characterisation of a carlavirus of French bean

Background

The carlavirus Cowpea mild mottle virus (CPMMV) was first detected in Australia in April 2016 when it caused severe disease in beans in the Fassifern area. Affected plants were mottled and stunted. Pods were curled and deformed, resulting in considerable loss of product and expensive culling and sorting in the packing shed.

Since the initial outbreak the virus has been detected in beans in the Lockyer valley, Bowen and Bundaberg.

As is the case with most viruses, CPMMV requires a specific vector or carrier, and this is the silver leaf whitefly (SLW). The virus is not spread by aphids, jassids or thrips. SLW can transmit the virus in relatively short feeding periods of about 10 minutes and the Fassifern outbreak demonstrated how quickly an epidemic can develop when whitefly populations are high, a source of virus is available and bean plants are at a high risk stage of growth.

Most host plants of CPMMV are in the legume family. In Queensland, the virus has been found naturally infecting bean, soybean, mung bean, cowpea and several perennial legume species including Siratro, Glycine and Phasey bean.

Glasshouse inoculation tests have also found that CPMMV has a relatively broad host range within the legume family, particularly in species of Phaseolus, Glycine and Macroptilium and Vigna.

Some strains of the virus can be carried in the seed of several host species. Transmission rates through seed are often very low (one per cent or less) and depend on host variety, the time plants were infected, virus strain and seed storage and maturity.

The main aims of the project have been to determine the distribution and levels of the virus and SLW vector in green bean production areas, determine the host range of the virus, investigate seed transmission and develop a management plan to minimise the impact on the bean industry.

What has happened recently?

Virus/ whitefly levels in the 2017/18 season in south Queensland

Surveys of bean, soybean and weeds commenced in the Fassifern area in late August 2017 and continued until May 2018.

CPMMV was first detected in bean crops in early March 2018, a similar time frame to that found in 2017. This also correlates with the time of year for the initial severe outbreak which occurred in autumn 2016 in this district.

Virus levels were monitored in late autumn to determine the severity of diseases outbreaks for the season. Of the six bean crops evaluated in early May, virus levels ranged from 0% to 41%, with only two sites having levels exceeding 10%.

Although CPMMV was detected in several volunteer soybean plants growing among infected beans in 2018, the virus has not been detected in soybean crops in the Fassifern area in either 2017 or 2018, using the same survey techniques that are applied to bean crops.

The incidence of adult SLW observed in pumpkin, soybean and French bean crops during the 2017-18 season was lower than during the previous season. Regular suction sampling of creek bank vegetation yielded low but consistent numbers of adult whitefly from early August onwards. During this period whitefly numbers in early pumpkin crops were low to moderate. In late May 2018, after all pumpkin and bean crops had been removed, SLW continued to be detected by suction sampling of creek bank vegetation. French bean crops surveyed before Christmas yielded low numbers of both adults and pupae. The only significant French bean infestation occurred in mid-November near a heavily infested pumpkin patch at Tarome. This whitefly infestation was short-lived, presumably due to insecticide intervention. Whitefly numbers increased steadily in pumpkin crops during spring and early summer with very little parasitism by the parasitic wasp *Eretmocerus hayati.* Despite the lack of parasitism, SLW populations in pumpkin crops were below pest thresholds and thus did not require insecticide treatment. This may have been due to relatively cool, wet climatic weather.

As in the previous season whiteflies were most prevalent in bean crops during late February, March and April 2018. Parasitism by *E. hayati* increased during March with up to 50% of pupa affected as whitefly numbers declined during late-April. In March and April, soybean crops that are drying off, appear to be unattractive to SLW at a time when there are young French bean crops nearby. Populations in the French bean crops never appeared out of control and significant parasitism of whitefly by *E. hayati* (40.7%) was observed in samples taken on April 10.

Again, as observed in the previous season, the populations of SLW are not a good predictor of low disease incidence. The important finding from the two seasons of monitoring data is that the autumn production of French bean is at the most risk of virus impact in the Fassifern valley. Bean crops grown during other windows over the spring-autumn season are less likely to be infected based on data collected so far. The second important finding from the 2017/18 survey work is the detection of key environmental reservoirs of CPMMV, namely, leguminous weeds e.g. Siratro found throughout the Fassifern area. The infected weeds were detected along creek banks where SLW is also often found.

Surveys in the Lockyer production area in 2017/18, including surveys of late season crops in areas where the virus occurred in 2017, failed to detect CPMMV.

The virus was detected at a low level in green bean crops at Bundaberg in May 2018.

Survey work for the 2018 winter crop at Bowen detected a very low level of infection in bean crops.

Reaction of bean varieties to CPMMV

Two field trials were planted at the Redlands research centre in 2017/18 to assess bean varieties for tolerance to CPMMV in terms of symptom severity and marketable pod yields when infected at an early growth stage.

One metre plots of 18 bean varieties were sown with plants in one half of each plot inoculated with CPMMV 14 days after sowing. Inoculated plants were rated for symptom severity, degree of pod damage from the virus and an overall assessment made regarding the need for further more detailed testing. Ten varieties were selected for further work, and although some of these are better suited to processing or lack the quality required for current use, a few of these virus tolerant lines may fit into a management program where virus is a problem at certain times of the season

In a second trial in March/ May 2018 we also investigated the effect of time of viral infection on the yield of variety Wyatt, one of the principal green bean varieties grown.

A trial was established with five replicates and four treatments-inoculation with CPMMV at two, four and six weeks after planting and a non-inoculated control. Ten plants were harvested from each plot and total and marketable yield data obtained. Results have yet to be statistically analysed but the early inoculation time clearly had a very significant effect on pod yield and quality. Virtually no marketable pods were produced while yield and quality in the plants inoculated six weeks was similar to the non-inoculated controls.

These data emphasise the importance of virus/ vector management during early crop stages to minimize yield and quality losses.

This time of infection trial will be repeated and new bean varieties have been sourced to extend the tolerance screening work.

Seed transmission tests with CPMMV

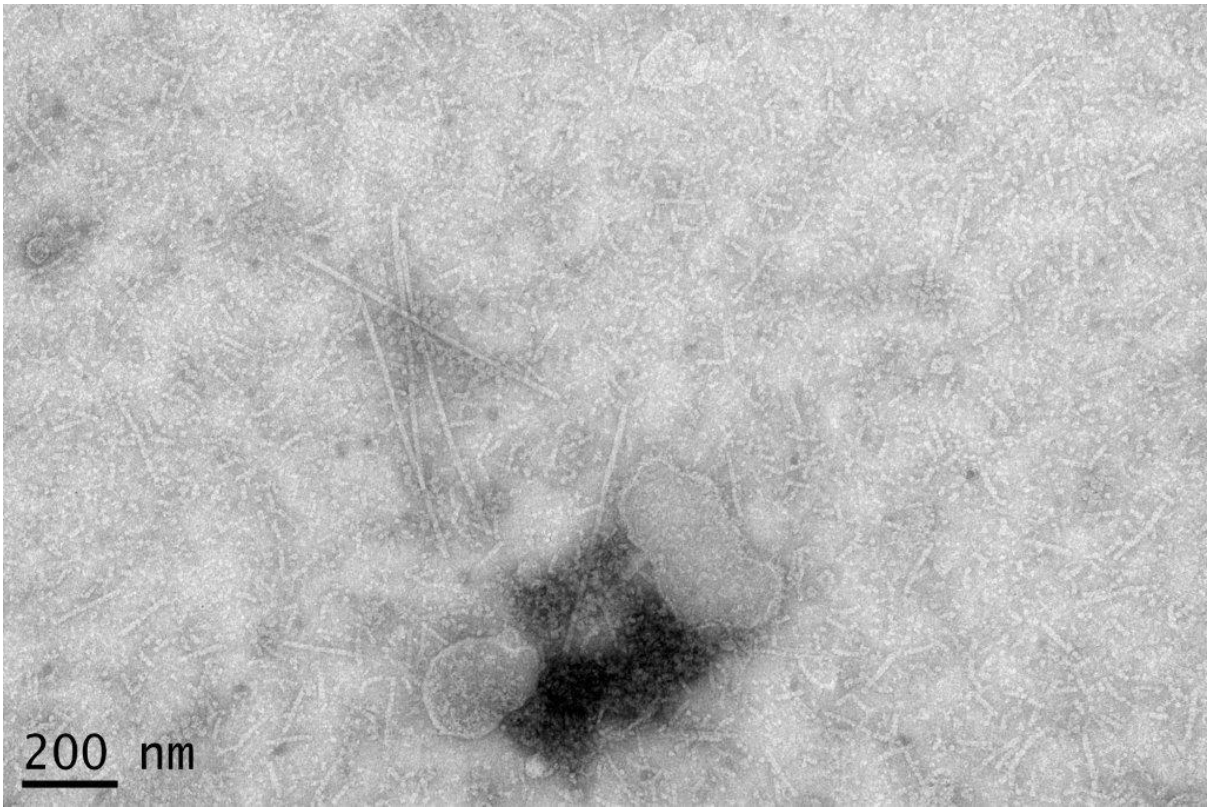
Overseas work has shown that at least some strains of CPMMV can be transmitted through the seeds of several legume species, including French bean, soybean and cowpea. However, reports of seed transmission are contradictory and depend on factors such as host variety, virus strain, time of infection and environmental influences such as temperature.

We have not demonstrated seed transmission of CPMMV in Queensland where commercial seed lots of bean, soybean and mung bean have been sown and inspected/ tested for the virus. Further, many thousands of young bean plants have been inspected in fortnightly surveys in the Fassifern Valley for two seasons and no virus has been detected until natural spread begins in autumn crops.

In order to further investigate this aspect of virus epidemiology we have produced seeds from virus inoculated plants to determine the potential for seed transmission in selected varieties.

Seed of the bean varieties Wyatt and Stanley and of the soybean variety P791 has been produced for grow out trials in a greenhouse which are currently underway. To date, seed transmission has not been found

The work has been funded by Hort Innovation using the vegetable research and development levy and funds from the Australian government



Thread-like particles of carlavirus from bean as viewed in a transmission electron microscope

Symptoms of carlavirus on bean variety Wyatt

Screening bean varieties for carlavirus reaction at Redlands research facility 2018

Growers inspecting variety virus screening trial

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Carlavirus tolerant bean variety. Plants have been inoculated with virus but show no symptoms

Virus tolerant varieties under commercial test in north Queensland