Final Technical Results Report

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Quantifying the yield risk of planting CMV infected lentil seed in the southern region

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REPORT SENSITIVITY

Does the report have any of the following sensitivities?

Intended for journal publication	YES ☐ NO ☒
Results are incomplete	YES ☐ NO ☒
Commercial/IP concerns	YES ☐ NO ☒
Embargo date	YES ☐ NO ☒ If Yes, Date: 31 May 2024

Contents

ABSTRACT

Lentil is an important food crop originating from the Middle East and well adapted to dry-land production such as the Mediterranean-type environments of the Australian continent. Viral diseases such as cucumber mosaic virus (CMV) are among the major limiting biotic factors hindering lentil production and their negative impact on lentil crops is often underestimated and poorly understood. CMV is both seed and aphid transmitted and high yield losses can result when aphids transmit CMV from infected seedlings early in the growing season. Quantifying the yield risk to lentil crops from sowing CMV-infected seed enabled the development a risk guide matrix to be used by growers to better manage CMV disease in lentils. The Risk Guide Matrix focused on lentil cultivar susceptibility to CMV infection and the levels of CMV infected seed planted at sowing and recommended growers sow lentil seeds with zero levels of CMV and that sowing seed with 1% or more levels of CMV infected seed significantly increases the risk of significant yield losses. It is recommended that a disease resistance score for CMV infection in lentils be developed and that the levels of disease resistance to CMV in existing lentil germplasm be determined.

EXECUTIVE SUMMARY

Lentil is one of the oldest domesticated crop species originating from the Middle East. It is an important food crop and is also considered to be one of the main protein sources in many countries including Australia. It is well adapted to dry-land production such as the Mediterranean-type environments of the Australian continent. However viral diseases are among the major limiting biotic factors hindering lentil production and their negative impact on lentil crops is often underestimated and poorly understood. In Australia, cucumber mosaic virus (CMV) has been found to infect commercial seed stocks and a range of legume species such as chickpea, lupin and lentil, as well as some non-legumes growing on broadacre farms. CMV is seed transmitted in lentils and is also transmitted by aphids, including green peach aphid (*Myzus persicae*), in a non-persistent manner. CMV infected seedlings generated from sowing CMV-infected seed is therefore a damaging primary source of CMV inoculum for aphids and can result in rapid dissemination of the virus throughout a crop if left unchecked.

Quantifying the yield risk to lentil crops from sowing CMV-infected seed was deemed critical to better understanding the CMV-Lentil patho-system and determining risk levels for growers when sowing their lentil crops. Our results showed that the mean incidence of CMV in treatment plots with 1%, 2% and 3% levels of CMV infected seed ranged from 8 to 26%, compared to an average incidence of just 1% in the "healthy" lentil seed plots. The yield in treatment plots sown with 1, 2 or 3% levels of CMV infected seed was significantly lower than treatment plots sown with healthy lentil seed. Yield comparisons between healthy and CMV-infected plants in the glasshouse showed a similar trend with a reduction in yield ranging from 10% to 93%. CMV was readily transmissible by aphids to 20 of 24 cultivars, but with variable rates of transmission ranging from 1 to 71%, while four cultivars were not infected with CMV. The rate of seed transmission of CMV also varied between cultivars. These results clearly demonstrate variation in tolerance of lentil cultivars to CMV infection with four cultivars potentially showing resistance to CMV infection.

Based on the field and glasshouse experiments generated by this study, together with our knowledge of CMV, the pathogen, lentils as the plant host, and seasonal conditions for lentil production in southern Australia, including the presence of aphid vectors of CMV, a risk matrix guide was developed. This is to inform growers the risk of yield potential of planting CMV infected lentil seed in the southern growing region of Australia. This risk matrix focused on two key biological variables in the CMV-Lentil pathosystem: lentil cultivar susceptibility to CMV infection; and the levels of CMV infected seed planted at sowing. The Risk Matrix Guide for the control of CMV in lentils provides the following guidelines for growers:

1. An effective control strategy for growers is to plant lentil cultivars that are resistant to CMV infection.

2. An effective control strategy for growers is to sow lentil seeds with zero levels of CMV infected seed. In this scenario the only source of CMV inoculum is from migratory aphids moving from CMV infected alternate hosts onto the lentil crop, and for this infection event to happen early in the growing season to have a negative impact on lentil yield.

3. There is a moderate risk to growers to plant lentil seed with 1% CMV infection for moderately resistant, tolerant or moderately susceptible lentil cultivars, while susceptible cultivars will have a measurable reduction in yield. These impacts will be influenced by the abundance of aphid vectors in the crop, particularly early in the season.

4. There is a major risk to growers to sow tolerant, moderately susceptible or susceptible cultivars of lentil with 2-3 % infection levels of CMV in seed at sowing.

5. There is a major risk to growers to sow moderately resistant, tolerant, moderately susceptible or susceptible cultivars of lentil with greater than 3% infection levels of CMV in seed at sowing.

Informed management strategies driven by a Risk Matrix guide will have a long-term outcome of reducing the levels of, and yield losses resulting from, the viral disease caused by CMV in the lentil cropping system. Further work is required however to better understand the CMV-lentil pathosystem and further optimize the disease risk matrix guide. A numbers of recommendations are proposed including developing a disease resistance score for CMV infection in lentils, determining the levels of disease resistance to CMV in existing lentil germplasm and better understanding the epidemiology of CMV and associated aphid vectors.

BACKGROUND

Lentil is one of the oldest domesticated crop species originating from the Middle East. It is an important food crop and is also considered to be one of the main protein sources in many countries including Australia. It is well adapted to dry-land production such as the Mediterranean-type environments of the Australian continent. Viral diseases are among the major limiting biotic factors hindering lentil production. However, their negative impact on lentil crops is often underestimated and poorly understood due to several factors including:

- the persistent confusion of viral symptoms with those of nutrient deficiencies,
- herbicide damage,
- insect direct damage
- environmental factors and
- the occurrence of non-symptomatic infection.

In Australia, cucumber mosaic virus (CMV) has been found to infect commercial seed stocks and a range of legume species such as chickpea, lupin and lentil, as well as some non-legumes growing on broadacre farms. Symptoms of CMV include reduced internodes, a bunchy appearance and plant stunting. If the virus is carried in the seed, the entire plant may be stunted and may die before maturity. A plant infected late in the season may have normal sized leaves at the bottom but those near the top, above the point of infection, can be less than half the normal size. Infected plants may occur randomly throughout paddocks, or appear in small patches 1-2 metres in diameter.

CMV is transmitted by aphids, including green peach aphid (*Myzus persicae*), in a non-persistent manner. With this mode of virus transmission, the aphids acquire the CMV virus particles within seconds to minutes of feeding on the CMV-infected plant (either CMV-infected crop plants or weeds) and can then transmit CMV to a healthy plant within seconds, minutes or hours of feeding on the healthy plant.   Aphids lose infectivity after a few probes because non-persistently transmitted viruses such as CMV are not kept long on an aphid's mouth parts and aphids do not remain infective throughout their life. Insecticide sprays are generally less effective against non- persistent than persistent viruses because a non- persistent virus would typically have already been transmitted in the time that it takes an aphid to be affected by the insecticide, due to the short transmission time.

A primary source of infection in lentils for CMV is infected lentil seed. If the seed infection level is one per cent, one plant in every 100 will be virus-infected, therefore the virus will be randomly distributed across the field. Alternate non-crop hosts, such as weeds, are also an important primary source of inoculum for CMV. For the broad acre cropping system for lentils, volunteer crop hosts that survive in the green bridge between harvest and sowing each year are also an important primary source of inoculum. The secondary spread of CMV by aphids is from either CMV-infected seedlings (germinated from CMV infected seed) or from CMV infected weeds or volunteer crop hosts located in nearby green bridge.

CMV is of economic importance in a range of crops worldwide. In areas where large aphid populations occur, crop losses can be high due to reduced herbage production and grain yield. However, accurate data on yield losses associated with CMV infection in lentils, and pulses in general, is not well understood. In Western Australia, high levels of CMV infection in lupin crops have resulted in subsequent yield losses of up to 60 per cent, leading to the development of integrated management packages to control the disease (Jones, 2000). In Western Australian field experiments, CMV infection in Matilda lentils was shown to diminish shoot dry weight by 72 to 81 per cent, seed yield by 80 to 90 per cent and individual seed weight by 17 to 25 per cent (Latham et al., 2004). Virus infection typically causes more severe yield losses when plants are infected in the early growth stages.

Yield losses associated with pulse viruses are often crop- and virus- specific and season- dependent. Based on survey results (Victorian Surveillance Project - DJP1907-004RTX), turnip yellows virus (TuYV, previously known as beet western yellows virus) and cucumber mosaic virus (CMV) are the most commonly detected and important viruses affecting pulse crops in Victoria and considerable year-to-year variation in virus prevalence and incidence is observed. Although this variation can be complex and difficult to define, it is influenced by differences in environmental conditions, availability of virus and vector reservoirs, aphid populations, aphid activity, long-distance migration of aphids, the abundance of natural enemies and interactions between these factors.

There is a data gap relating to the plant health impacts and actual yield risk from sowing lentil seeds with known levels of infected cucumber mosaic virus (CMV). As the yield risk is unknown, growers are not seed testing and are unable to make informed choices on planting virus infected lentil seeds.

PROJECT OBJECTIVES

The aim of this project is to quantify the yield risk associated with planting CMV infected lentil seed in the Southern region. To do this, field experiments were conducted in key lentil growing areas over 2 years. This was done by:

- determining the impact of sowing different levels of CMV-infected seed on:
    - Disease progress throughout the season
    - Grain yield
    - Subsequent incidence of virus level in harvested seed for all treatments.
- Assessing the rate of CMV transmission via seed for different lentil cultivars, and
- Investigating lentil varietal susceptibility to CMV infection

Based on these findings and an understanding of the lentil-CMV pathosystem, a Risk Matrix Guide was developed to inform Southern region lentil growers, advisers and commercial seed businesses of the yield risk associated with planting various levels of CMV infected lentil seed.

The long-term outcome of this project is to reduce the levels of plant viruses such as CMV and minimise yield losses resulting from these plant pathogens in cropping systems in south-eastern Australia.

METHODOLOGY

Identification of CMV-infected seed lots

To quantify the level of seed infection in seed lots or propagated infected plants, representative samples of 300-400 seeds were grown under controlled environmental conditions in insect-free cages and maintained in a glasshouse at 22°C. Four to six weeks post germination, each individual plant was blotted onto nitrocellulose membrane and tested for CMV using the tissue blot immunoassay (TBIA) as described by Freeman et al. (2013). Lentil seeds belonging to 68 varieties were tested for CMV using tissue blot immunoassay in 2022 to find CMV infected seed (Table 1).

Field Trials 2022-2023

Eight field trials were run in across both years (2022, 2023) four in South Australia and four in Victoria.

Lentil seed lots with known but variable levels of CMV-infected seed were used to establish field experiments in the 2022 and 2023 growing seasons. Seed lots with three different levels of seed infection (low, medium and high), as well as non-infected seed lots, were sourced from field grown seeds from commercial or breeding trials or from plants propagated in the glasshouse (See section 1 Materials and Methods). All field trials were machine sown. During the growing season, field trials were maintained using typical agronomical practices.

The field trials were established using a randomised block design with four replicates for each level of seed infection treatment and non-infected control treatment. The trials were sampled twice during the season in 2022 (once in October and once in November(and once during the season (November) in 2023. Symptoms were recorded if present. Twenty samples were collected randomly from each treatment plot and from selected control plots. Stems were bundled in batches of 10, then each bundle was blotted onto nitrocellulose membrane and tested for CMV using tissue blot immunoassay (TBIA).

Yield assessment of field trial treatments:

Detailed non-destructive assessments were performed on 10-15 representative plants from each replicate of each treatment and included visual symptom assessment, seed germination counts, and plant height measurements. At harvest, the following metrics were calculated or recorded:

- Yield of seed: The trials were machine harvested and seed from each plot was packed into separate polythene bags. The harvested seed was cleaned, transferred into plastic containers and weighed.
- Two hundred grain weight was obtained by counting 200 seeds from each plot using a Numigral seed counter and weighing them.
- The number of seeds per plot was estimated using the calculation “yield/[200 grain weight/200]”

Data analysis

All data from 2022, 2023 and each individual site in each year were analysed by ANOVA using GenStat 22nd Edition. Means and standard errors of the means were also calculated using GenStat. Differences between treatments were considered statistically significant at $p < 0.05$.

2022 Field trials

Four sites were selected that align with the National Pulse Breeding Programme (see details of latitude and longitude in the table provided in the "Location" section below).

Seed was sown at a rate of 20 plants per square metre and each trial was 603.5 m^2 in size, containing 48 plots: 24 plots for treatments and controls and 24 buffer plots. Each plot was 8.75 m^2, has five rows (Fig.1) and the row- to- row distance was 25 centimetres (Figure 1). The distance between plot rows was 1 metre and the distance between plot columns was 50 centimetres. The trial design at each field site was a randomised complete block with four replications (Figure 1).

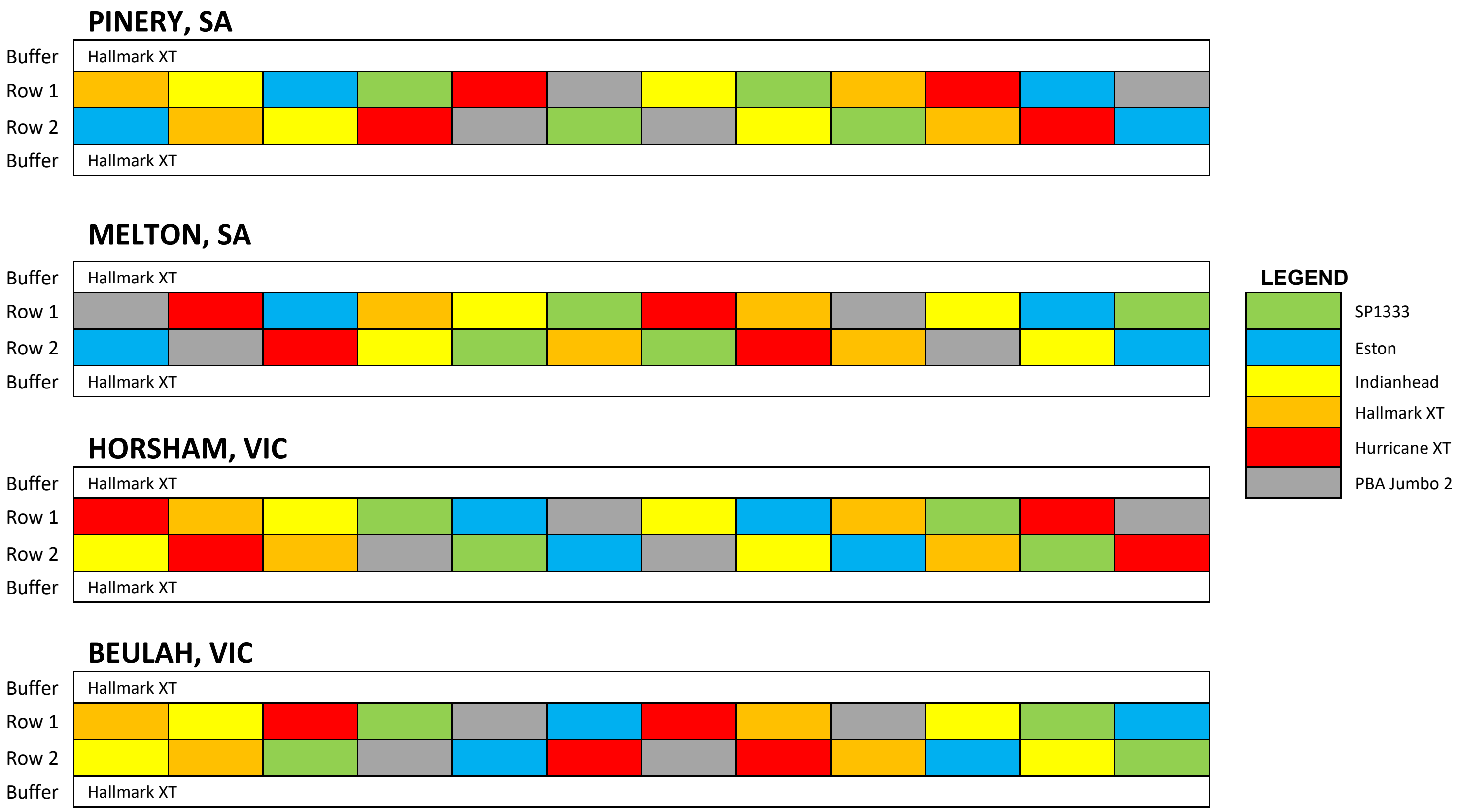


Figure 1. Design and layout of the field trials carried out in South Australia (SA) and Victoria (VIC) during 2022.

Three lentil varieties (SP1333, Eston and Indianhead) with different levels of CMV-infected seed were selected for "virus-infected" treatments and three virus-free controls (Hallmark, Hurricane and Jumbo2) were selected for control plots. For outer buffer Hallmark was used. The seed for the lentil varieties SP1333, Eston and Indianhead had 1%, 2% and 3% CMV infected seed respectively. The seed rate was at 200 plants per square metre and each trial was 603.5 m2 containing 48 plots: 24 plots for treatments and controls and 24 plots for buffer plots.

The Horsham field trial was sown on 9/6/2022 and Beulah trial was sown on 8/7/22. The Melton trial was sown on 2/6/22 and Pinery trial was sown on 19/6/22. Please note, nine plots within the Beulah trial were not sown due to a problem with the sowing machine.

The Horsham trial was harvested on 15/1/23 and Beulah trial on 5/1/23. The Melton trial was harvested on 7/12/22 and Pinery trial on 30/12/22.

2023 field trials:

A total of four field trials were selected; two in Victoria that align with National Pulse Breeding Program (Horsham and Curyo) and two in South Australia subcontracted by Global Grain Genetics (Blyth and Kadina) (see details of latitude and longitude in the table provided in the "Location" section below). The trial design at each field site was a randomised complete block with four replications (Figure 2).

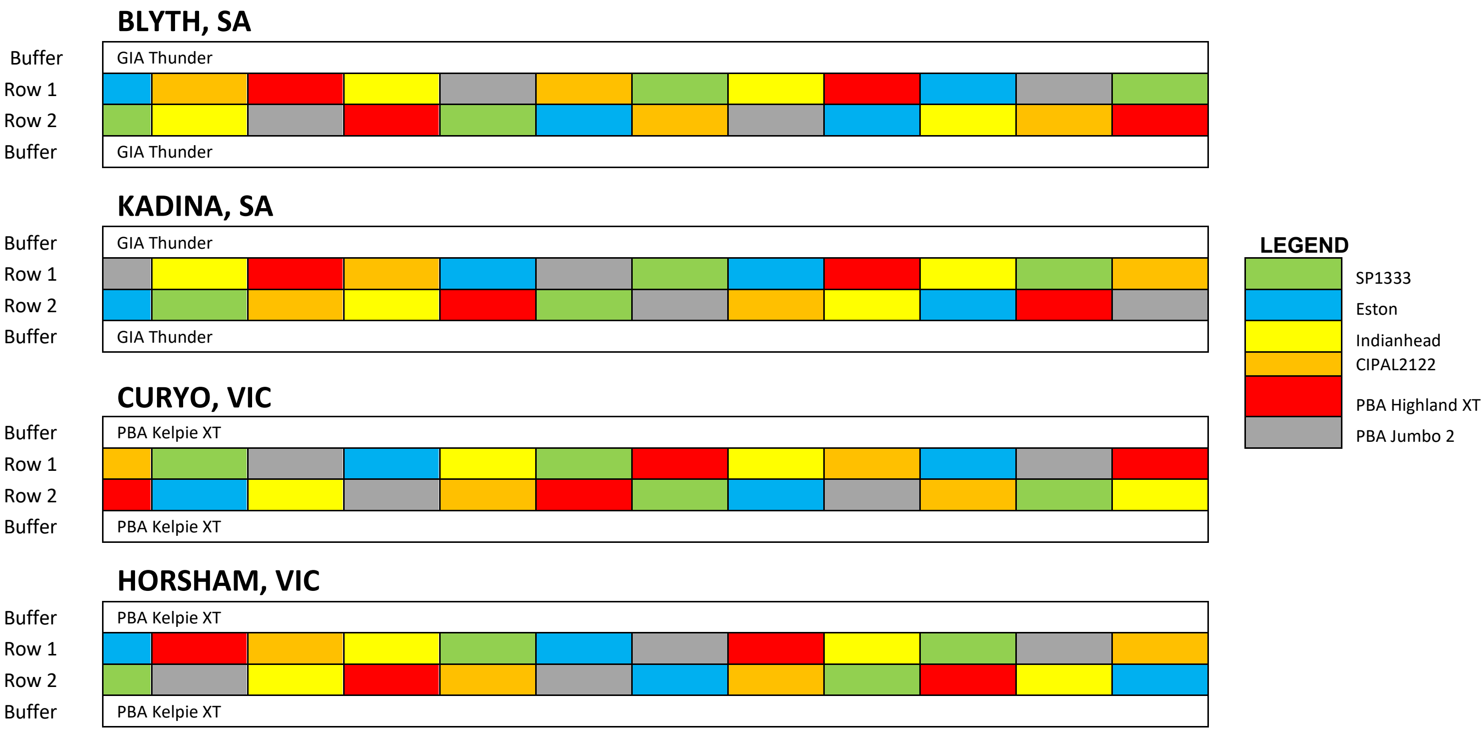


Figure 2. Design and layout of the field trials carried out in South Australia (SA) and Victoria (VIC) during 2023.

The Eston, Indianhead and SP1333 seed used for sowing was derived from the plants from the 2022 field trials with the aim of sowing seed with known levels of CMV infection in the seeds. It was anticipated that similar levels of CMV would be detected in the seed of all three CMV-infected varieties (SP1333 (1%), Eston (2%) and Indianhead (3%)) from the 2022 field trial. However, CMV was only detected in SP1333 (2.6%) and was not detected in the Eston or Indianhead seed collected from the trial.

The control plots in 2023 field trial were Jumbo2, CIPAL and Highland. The buffer plots in Victoria were PBA Kelpie and in SA were GIA Thunder.

The seed rate was at 200 plants per square metre and each trial was 603.5 m^2 containing 48 plots: 24 plots for treatments and controls and 24 plots for buffer plots. Each plot was 8.75 m^2, has five rows (Figure 2) and the row-to-row distance was 25 centimetres. The distance between plot rows was 1 metre and distance between plot columns was 50 centimetres.

Due to continuous wet weather, the Horsham field trial was sown on 20/7/23 but the Curyo trial was sown on 15/5/23. The Kadina trial was sown on 30/5/23 and the Blyth trial was sown on 3/6/23. Seed germination counts were conducted six-eight weeks after the germination of crop. The plants were counted on a metre length in two separate rows in each plot. The field trials were monitored and assessed as for the 2022 field trials.

Plant heights were measured at flowering stage and at the same time, samples were collected for virus testing. The trial samples were tested for the presence of CMV using TBIA. Any CMV symptoms present were recorded. At harvest, grain yield and 200-grain weight were recorded for each plot and the number of seeds per plot was estimated as described previously.

Determination of CMV-infected seed transmission rates

Ninety virus-free seeds from each of 24 lentil cultivars (Aldinga Boomer, Cassab, CIPAL0714, CIPAL2122, Cobber, Commando, Digger, Eston, Giant, Greenfield, Hack, Hallmark, Herald, Highland, Hurricane, Indianhead, Jumbo, Jumbo2, Kelpie, Matilda, Northfield, Nipper and Nugget) were sown in insect-free cages in the glasshouse. The plants were inoculated with CMV one to two weeks after germination using 5-10 *Myzus persicae* aphids per plant. Three days after inoculation, the aphids were killed by systemic insecticide and the plants were grown to maturity for seed production. At the flowering stage, a sample from each inoculated plant was collected and individually blotted onto nitrocellulose membrane, the blots were then tested using TBIA. To validate the TBIA testing three healthy and three CMV-infected plants of PBA Greenfield and one healthy and one CMV-infected plant of PBA Giant were also tested by PCR.

Comparison of seed numbers and seed weight from healthy and CMV-infected lentil plants grown in the glasshouse.

Seeds were collected from aphid inoculated CMV positive and negative lentil plants belonging to six cultivars: Aldinga, Boomer, Cassab, Commando, Eston and Hack. The CMV-infected plants were inoculated at the 2-week stage in October 2022 and the plants harvested in January 2023. The number of seeds produced by healthy and CMV-infected lentil plants were counted and seed weight was recorded for each cultivar.

Testing of CMV transmission in seedlings germinated from seeds collected from aphid-inoculated CMV positive and negative plants maintained in the glasshouse.

A variable number of seeds harvested from the glasshouse-grown, aphid-inoculated CMV positive and negative plants were collected and sown in the glasshouse, and the germinated seedlings were tested for using both TBIA and PCR. These cultivars included Aldinga, Boomer, Cassab, Commando, CIPAL0714, Hack, Indianhead and PBA Hallmark.

Bulk up of seed lots of lentil cultivars with known levels of CMV-infected seed.

In September 2022, plots within the buffer zone of the field trial were inoculated with CMV using GPA that had been feeding on virus-infected plants to generate more virus-infected seed. Two rows in each of the 13 outer PBA Hallmark buffer plots at the Horsham trial were inoculated using CMV virulent green peach aphids. In November, 20 samples from each inoculated buffer plot of Hallmark were randomly collected, bundled into batches of 10 and blotted onto nitrocellulose membrane for CMV testing using TBIA. The inoculated plants were hand harvested, thrashed and seed was cleaned. To assess the level of CMV infection in the resulting seed, one hundred seeds from each inoculated plot was sown in the insect-proof cages in the glasshouse. Approximately six weeks after sowing, seedlings were tested for the presence of CMV by TBIA and then later by PCR.

Lentil seeds harvested from the 2022 trial treatment plots of Eston, Indianhead and SP1333 were also sown in the glasshouse and the levels of CMV infection assessed in the same way.

LOCATION

Trial site locations and description

Field experiments were located in key lentil growing areas of the Southern Region, in South Australia and Victoria. In total, eight field experiments will be conducted over two consecutive years at four sites. Each site was located in a number of different lentil growing regions, including the York Peninsula and Mid North of South Australia, and the Wimmera and Mallee of Victoria.

2022 Field sites:

Site #	**Latitude (decimal degrees)**	**Longitude (decimal degrees)**	**Nearest town**
Trial Site #1	-36.012	142.00258	Horsham
Trial Site #2	-35.9994	142.5735	Beulah
Trial Site #3	-34.0739	138.0196	Melton
Trial Site #4	-34.2432	138.6102	Pinery

Research	**Benefiting GRDC region (select up to three)**	**Benefitting GRDC agro-ecological zone**	
CMV lentil field trial	Southern Region National Choose an item.	☐ Qld Central ☐ NSW NE/Qld SE ☐ NSW Vic Slopes ☐ Tas Grain ☒ SA Midnorth-Lower Yorke Eyre ☐ WA Northern ☐ WA Eastern ☐ WA Mallee	☐ NSW Central ☐ NSW NW/Qld SW ☐ Vic High Rainfall ☒ SA Vic Mallee ☒ SA Vic Bordertown-Wimmera ☐ WA Central ☐ WA Sandplain

2023 field trials:

Site #	Latitude (decimal degrees)	Longitude (decimal degrees)	Nearest town
Trial Site #1	-36.012	142.00258	Horsham
Trial Site #2	-35.807833	142.794609	Curyo
Trial Site #3	-34.45366	137.482008	Kadina
Trial Site #4	-33.483822	138.292480	Blyth

Research	Benefiting GRDC region (select up to three)	Benefitting GRDC agro-ecological zone	
CMV lentil field trial	Southern Region National Choose an item.	☐ Qld Central ☐ NSW NE/Qld SE ☐ NSW Vic Slopes ☐ Tas Grain ☒ SA Midnorth-Lower Yorke Eyre ☐ WA Northern ☐ WA Eastern ☐ WA Mallee	☐ NSW Central ☐ NSW NW/Qld SW ☐ Vic High Rainfall ☒ SA Vic Mallee ☒ SA Vic Bordertown-Wimmera ☐ WA Central ☐ WA Sandplain

RESULTS

Identification of CMV infected seed lots

Lentil seeds belonging to 69 varieties were tested for CMV using TBIA in 2022 to identify CMV infected seed (Table 1). Of the 143 seed lots belonging to 69 varieties that were tested for CMV, only three varieties (Indianhead, Eston and SP1333) were positive with virus levels of 3%, 2% and 1% respectively.

Table 1. Testing of seed lots to identify CMV infected seed for field trials.

Seed lot ID	Variety/line	Date sown	Number of seeds sown	Number of CMV infected seedlings	% of CMV infected seedlings
1	12H022L-2-16HH14002	2/02/2022	177	0	0
2	12H024L-2-16HH14005	3/03/2022	184	0	0
3	12H040L-2-16HH14002	3/03/2022	166	0	0
4	12H1200L-1-16HH14002	3/03/2022	100	0	0
5	12H655L16HH14003	3/03/2022	134	0	0
6	12H656L-16HH14005	3/03/2022	141	0	0
7	12H824L-2-16HH14009	3/03/2022	118	0	0
8	13H235L-16HH14014	2/02/2022	155	0	0
9	13H308L-3-16HH14013	3/03/2022	170	0	0
10	13H382L-3-15HSH13009	3/03/2022	177	0	0
11	CIPAL2122	2/02/2022	180	0	0
12	14H048L-16HSH14005	2/02/2022	132	0	0
13	14H087L-16HSH14001	3/03/2022	146	0	0
14	14H101L-16HSH14002	3/03/2022	155	0	0
15	14H139L-16HSH14001	3/03/2022	149	0	0
16	14H167L-16HSH14004	3/03/2022	161	0	0
17	14H235L-15HSH12022	3/03/2022	161	0	0
18	14H325L-16HSH14002	3/03/2022	150	0	0
19	14h329l-16hsh14004	3/03/2022	153	0	0
20	14H358L-16HSH14001	3/03/2022	144	0	0
21	14H359L-16HSH14001	3/03/2022	134	0	0
22	15H015L-16HSH13008	2/02/2022	209	0	0
23	15H026L-16HSH13002	3/03/2022	158	0	0
24	15H085L-16HSH13006	3/03/2022	160	0	0
25	15H155L-2-16HSH13005	3/03/2022	196	0	0
26	Aldinga	2/02/2022	155	0	0
27	Boomer	2/02/2022	172	0	0
28	Cassab	2/02/2022	149	0	0
29	CDCruby	2/02/2022	176	0	0
30	CIPAL0714	2/02/2022	160	0	0
31	CIPAL0717	3/03/2022	142	0	0
32	CIPAL0719	3/03/2022	159	0	0
33	CIPAL0901	3/03/2022	160	0	0
34	CIPAL1522	3/03/2022	172	0	0
35	CIPAL2001	3/03/2022	153	0	0
36	CIPAL1821	3/03/2022	157	0	0

37	Indianhead	3/03/2022	133	0	0
38	ILL2024	3/03/2022	154	0	0
39	CIPAL2121	3/03/2022	136	0	0
40	CIPAL1901	1/04/2022	150	0	0
41	Cobber	2/02/2022	108	0	0
42	Commando	2/02/2022	233	0	0
43	Cumra	2/02/2022	168	0	0
44	Digger	2/02/2022	132	0	0
45	Eston	2/02/2022	185	3	2
46	Hack	2/02/2022	158	0	0
47	ILL2024	2/02/2022	160	0	0
48	ILL7537	2/02/2022	174	0	0
49	Indianhead	2/02/2022	181	5	3
50	Matilda	2/02/2022	123	0	0
51	Nipper	2/02/2022	179	0	0
52	Northfield	2/02/2022	175	0	0
53	Nugget	2/02/2022	156	0	0
54	PBA ACE	2/02/2022	132	0	0
55	PBA BLITZ	2/02/2022	161	0	0
56	PBA Bolt	2/02/2022	167	0	0
57	PBA Flash	2/02/2022	163	0	0
58	PBA Giant	2/02/2022	144	0	0
59	PBA Greenfield	2/02/2022	155	0	0
60	PBA Hallmark XT	3/03/2022	118	0	0
61	PBA Herald XT	2/02/2022	176	0	0
62	PBA Highland XT	2/02/2022	171	0	0
63	PBA Hurricane XT	2/02/2022	189	0	0
63	PBA Hurricane XT	2/02/2022	147	0	0
63	PBA Hurricane XT	2/02/2022	172	0	0
63	PBA Hurricane XT	2/02/2022	170	0	0
64	PBA Jumbo	2/02/2022	155	0	0
65	PBA Jumbo2	3/03/2022	150	0	0
66	PBA Kelpiext	2/02/2022	172	0	0
67	Precoz	2/02/2022	148	0	0
68	SP1333	2/02/2022	155	1	0.7
69	CIPAL1921	1/04/2022	150	0	0

Field Trials

2022 field trials

Four field trials were run across Victoria and South Australia. The Victorian trials were located in Horsham and Beulah while SA trials were located in Melton and Pinery.

Three lentil varieties (SP1333, Eston and Indianhead) with different levels of CMV-infected seed were selected as "virus-infected" treatments and three virus-free controls (Hallmark, Hurricane and Jumbo2) were selected as control plots. PBA Hallmark XT was sown as buffers. The seed for the lentil varieties SP1333, Eston and Indianhead had 1%, 2% and 3% CMV infected seed respectively.

The Horsham field trial was sown on 9/6/2022 and Beulah trial was sown on 8/7/22. The Melton trial was sown on 2/6/22 and Pinery trial was sown on 19/6/22. The Horsham trial was harvested on 15/1/23 and Beulah trial on 5/1/23. The Melton trial was harvested on 7/12/22 and Pinery trial on 30/12/22.

Seed germination count

There was no significant difference in plant numbers between treatment and control plots (Table 2).

CMV incidence

CMV infected lentil plants showed a range of viral symptoms: CMV infected Eston plants showed stunting; CMV infected Indianhead showed yellowing; and CMV infected SP1333 plants showed stunting and yellowing.

The field trials were sampled and tested for CMV infection using TBIA twice during the growing season, with the first sampling occurring in October and the second sampling in November. The incidence of CMV in the treatment plots at all four trial sites was higher in the November sampling than the October sampling, suggesting aphid activity at the field trial sites were spreading CMV to healthy lentil plants. The incidence of CMV was highest in Horsham compared to all other locations (Table 2). SP1333 had the highest incidence of CMV at each site during 2022, while Eston and Indianhead varied between sites. Overall across all sites during 2022, the mean incidence of CMV in the treatment plots was highest in SP1333 (26%), followed by Indianhead (8%) and Eston (15%), while the mean incidence in control plots (PBA Hallmark, PBA Hurricane and PBA Jumbo 2) was 1% (Figure 1B). This result demonstrates the risk associated with sowing CMV-infected seed.

Plant height measurements

In the virus infected treatment plots the average height of Eston and SP1333 plants was significantly lower when compared to Indianhead and all control varieties. However, there was no significant difference in plant height between the virus-infected Indianhead treatment and the virus-free control plots (Table 2). Of the three control varieties, Hallmark had the highest plant height (Table 2).

Yield loss

In Horsham, Beulah and the Pinery trials, the yield of control plots was generally higher compared to CMV treatment plots (Table 2). For example, at the Horsham site, the yield for the control plots (PBA Hallmark, PBA Hurricane and PBA Jumbo 2) was 3,380, 3,035 and 3,658 grams per plot, respectively, and was significantly higher than the treatment plots of SP1333 (1% CMV-infected seed), Eston (2% CMV-infected seed) and Indianhead (3% CMV-infected seed) which had yields of 1,635, 1,589 and 2,163 grams per plot, respectively.

However, accurate comparisons are compromised due to the lack of virus-free seed sown for Eston, SP1333 and Indianhead, as the differences in yield observed could be related to cultivar traits rather than presence /absence of CMV infection. For example, in Melton, the yield of the Hurricane control (1946 grams per plot) was not significantly different to the SP1333 (2147 grams per plot) and Indianhead (2158 grams per plot) treatments. However, overall, mean yield across all trial sites was significantly higher in control plots than the CMV infected treatment plots. On a 200 grain weight basis, SP1333 weighed the highest and Indianhead lowest. This is most likely because the SP1333 seed size was larger than the Indianhead seed size.

Table 2. Data from individual sites included in the field trials in 2022. Within a parameter, different letters denote varieties that are significantly different from each other.

Site	Cultivar	Treatment	% CMV infection in seed (TBIA)	Mean incidence of CMV (%) per plot	Mean number of plants per plot that germinated	Mean plant height (cm)	Mean yield per plot (g)	Mean 200 grain weight (g)	Mean estimated number of seeds per plot
Beulah (Mallee, VIC)	PBA Hallmark	Control	0	0	38 [a]	67 [e]	1,944 [ab]	8 [bc]	75,444 [c]
	PBA Hurricane	Control	0	0	40 [a]	61 [d]	2,194 [ab]	7 [b]	65,034 [c]
	PBA Jumbo2	Control	0	10	39 [a]	64 [b]	3,187 [b]	9 [c]	70,832 [c]
	SP1333	CMV- infected	1	28	41 [a]	53 [b]	1,049 [a]	13 [d]	24,103 [a]
	Eston	CMV- infected	2	3	40 [a]	48 [a]	916 [a]	7 [b]	41,344 [b]
	Indianhead	CMV- infected	3	13	44 [a]	N/A	1,079 [a]	4 [a]	82,165 [c]
Horsham (Wimmera, VIC)	PBA Hallmark	Control	0	0	25 [a]	76 [d]	3,380 [cd]	8 [d]	85,332 [cd]
	PBA Hurricane	Control	0	5	26 [a]	66 [cd]	3,035 [c]	7 [c]	88,646 [cd]
	PBA Jumbo2	Control	0	3	36 [a]	65 [c]	3,658 [d]	10 [e]	76,072 [bc]
	SP1333	CMV- infected	1	36	31 [a]	53 [ab]	1,635 [ab]	13 [f]	24,493 [a]
	Eston	CMV- infected	2	20	29 [a]	44 [a]	1,589 [a]	6 [b]	55,017 [b]
	Indianhead	CMV- infected	3	16	32 [a]	60 [bc]	2,163 [b]	4 [a]	105,710 [d]
Melton (York Peninsula, SA)	PBA Hallmark	Control	0	3	30 [a]	77 [b]	2,779 [c]	8 [c]	71,003 [bc]
	PBA Hurricane	Control	0	0	25 [a]	71 [b]	1,946 [b]	7 [b]	60,044 [b]
	PBA Jumbo2	Control	0	0	27 [a]	66 [ab]	3,968 [d]	10 [d]	77,666 [c]
	SP1333	CMV- infected	1	28	26 [a]	56 [a]	2,147 [b]	12 [e]	35,823 [a]
	Eston	CMV- infected	2	21	30 [a]	56 [a]	1,259 [a]	6 [b]	42,445 [a]
	Indianhead	CMV- infected	3	0	26 [a]	71 [b]	2,158 [b]	5 [a]	94,331 [d]
Pinery (Mid North, SA)	PBA Hallmark	Control	0	0	26 [a]	74 [b]	3,060 [b]	7 [c]	83,614 [d]
	PBA Hurricane	Control	0	0	26 [a]	67 [ab]	1,325 [a]	6 [b]	47,375 [bc]
	PBA Jumbo2	Control	0	3	25 [a]	64 [ab]	2,645 [b]	9 [d]	60,450 [bc]
	SP1333	CMV- infected	1	13	24 [a]	57 [a]	1,187 [a]	13 [e]	17,998 [a]
	Eston	CMV- infected	2	11	26 [a]	56 [a]	1,124 [a]	6 [b]	37,338 [ab]
	Indianhead	CMV- infected	3	3	28 [a]	66 [ab]	1,286 [a]	4 [a]	67,375 [cd]

2023 field trials

In 2023, seed sown in the field trials was collected from CMV-infected plots from the previous season (2022) and while CMV was detected in seed collected from the SP1333 plots, it was not detected in the Indianhead or Eston plots by TBIA. Therefore, only one variety (SP1333) from the 2023 field trial was confirmed as containing CMV infected seed. However, some of the results obtained (e.g.- CMV incidence in the plots) suggest that Indianhead and Eston may have actually contained some CMV-infected seed but none was detected by the TBIA test (Table 3). The three control treatment plots used in the 2023 field trials were CIPAL2122, PBA Highland XT and PBA Jumbo2.

Seed germination count

Although some varieties had a significantly higher germination rate than others, it did not appear to be related to CMV infection (Table 3).

CMV Incidence

Overall across all sites during 2023, the mean incidence of CMV in the treatment plots was highest in SP1333 (20%) followed by Indianhead (13%) and Eston (10%), while the mean incidence in control plots (CIPAL 2122, PBA Highland XT and PBA Jumbo 2) was 3% (Figure 3B). Along with the similar result from 2022, this further demonstrates the risk associated with sowing CMV-infected seed.

The mean incidence of CMV present in field plots was also highest in SP1333 at each individual site during 2023, while the incidence of CMV in Eston and Indianhead field plots varied between sites. For example, the incidence of CMV in both Eston and Indianhead was lower at Curyo (Mallee) and Kadina (York Peninsula) than Horsham (Wimmera) and Blyth (Mid North) (Table 3). At three of the four sites the incidence of CMV in Indianhead and Eston treatment plots was much higher than the control plots and suggests that the TBIA test did not detect CMV in the seedlings tested, or enough seedlings were not grown out to detect a CMV seed transmission rate of 0.5 % (e.g. 200 seeds) or 0.1 % (e.g. 1000 seeds). The overall mean CMV incidence was particularly low in the field trial at Kadina, SA in 2023 and this could be due to lower aphid activity at this site during the growing season (Table 3).

Plant Height measurements

Across all sites, the average height of Eston, Indianhead and SP1333 plants were significantly lower when compared to the control plots (Table 2023 overall) and there was also a site effect on the height of the plants across the four sites (Table 3).

Yield loss

At two sites (Curyo and Horsham), the mean yield was significantly lower in all three CMV-infected treatment plots of SP1333, Indianhead and Eston when compared to the than the three control plots (Table 3). Although no CMV was detected in the Indianhead and Eston seed that was sown in the 2023 treatment plots, there is the possibility that CMV infection may have been present in those seed lots but not detected by the TBIA test.

The mean yield of SP1333 (which was the only variety in which CMV seed infection was confirmed to be present in 2023) was significantly lower than the three control varieties at all four sites in

2023, even though SP1333 has the largest/heaviest seed of all six varieties (Table 3). SP1333 also produced significantly fewer seeds than the three control varieties (Table 3).

Table 3. Data from individual sites included in the field trials in 2023. Within a parameter, different letters denote varieties that are significantly different from each other.

Site	Cultivar	Treatment	% CMV infection in seed (TBIA)	Mean incidence of CMV (%) per plot	Mean number of plants per plot that germinated	Mean plant height (cm)	Mean yield per plot (g)	Mean 200 grain weight (g)	Mean estimated number of seeds per plot
Curyo (Mallee, VIC)	CIPAL 2122	Control	0	3	27 ^{ab}	39 ^{ab}	1,956 ^{b}	8 ^{c}	48,682 ^{c}
	PBA Highland XT	Control	0	5	39 ^{b}	44 ^{b}	2,055 ^{b}	8 ^{c}	48,735 ^{c}
	PBA Jumbo2	Control	0	0	24 ^{ab}	37 ^{a}	1,862 ^{b}	11 ^{d}	33,942 ^{b}
	SP1333	CMV- infected	1	24	18 ^{a}	36 ^{a}	1,292 ^{a}	15 ^{e}	17,843 ^{a}
	Eston	CMV- infected	0	3	21 ^{ab}	34 ^{a}	1,220 ^{a}	7 ^{b}	36,322 ^{bc}
	Indianhead	CMV- infected	0	5	40 ^{b}	36 ^{a}	1,055 ^{a}	5 ^{a}	40,667 ^{bc}
Horsham (Wimmera, VIC)	CIPAL 2122	Control	0	0	38 ^{ab}	35 ^{c}	1,521 ^{b}	8 ^{c}	39,772 ^{c}
	PBA Highland XT	Control	0	5	38 ^{ab}	38 ^{d}	1,726 ^{b}	8 ^{d}	41,566 ^{c}
	PBA Jumbo2	Control	0	11	31 ^{a}	37 ^{cd}	1,523 ^{b}	11 ^{e}	28,650 ^{b}
	SP1333	CMV- infected	1	18	26 ^{a}	32 ^{b}	860 ^{a}	14 ^{f}	12,709 ^{a}
	Eston	CMV- infected	0	18	31 ^{a}	29 ^{a}	1,080 ^{a}	7 ^{b}	31,791 ^{b}
	Indianhead	CMV- infected	0	14	47 ^{b}	30 ^{ab}	846 ^{a}	5 ^{a}	35,236 ^{bc}
Kadina (York Peninsula, SA)	CIPAL 2122	Control	0	0	27 ^{a}	51 ^{c}	1,576 ^{c}	8 ^{c}	38,002 ^{b}
	PBA Highland XT	Control	0	0	31 ^{ab}	48 ^{bc}	1,477 ^{c}	8 ^{c}	36,910 ^{b}
	PBA Jumbo2	Control	0	0	33 ^{ab}	50 ^{bc}	1,534 ^{c}	10 ^{d}	29,723 ^{b}
	SP1333	CMV- infected	1	6	25 ^{a}	42 ^{ab}	863 ^{b}	14 ^{e}	12,219 ^{a}
	Eston	CMV- infected	0	1	29 ^{ab}	39 ^{a}	570 ^{ab}	7 ^{b}	16,775 ^{a}
	Indianhead	CMV- infected	0	5	40 ^{b}	42 ^{ab}	348 ^{a}	5 ^{a}	13,720 ^{a}
Blyth (Mid North, SA)	CIPAL 2122	Control	0	15	29 ^{ab}	45 ^{b}	1,287 ^{d}	8 ^{c}	30,531 ^{c}
	PBA Highland XT	Control	0	5	37 ^{bc}	49 ^{b}	1,083 ^{c}	8 ^{c}	26,452 ^{bc}
	PBA Jumbo2	Control	0	0	26 ^{ab}	46 ^{b}	1,358 ^{d}	11 ^{d}	24,868 ^{b}
	SP1333	CMV- infected	1	31	25 ^{a}	39 ^{a}	771 ^{b}	14 ^{e}	10,869 ^{a}
	Eston	CMV- infected	0	20	29 ^{ab}	38 ^{a}	431 ^{a}	7 ^{b}	12,310 ^{a}
	Indianhead	CMV- infected	0	28	44 ^{c}	40 ^{a}	367 ^{a}	6 ^{a}	13,311 ^{a}

Overall results across 2022/23

When comparing the yield data across the two seasons it is important to note that CMV-infected seed of the variety SP1333 was available for both years of the trial, while CMV was not detected using the TBIA test in the Eston and Indianhead seed lots that were sown in the 2023 field trials. SP1333 had 1% and 3% CMV seed infection in 2022 and 2023, respectively.

There was no significant difference in seed germination rates across all cultivars and field sites during 2022, but there were some differences in 2023. Plant height was significantly lower in the SP1333 and Eston plots than the control plots in 2022, and was lower in SP1333, Eston and Indianhead than the control plots in 2023. Based on the mean 200 grain weight of each lentil cultivar used in the field trials during 2022 and 2023, SP1333 had the biggest seeds, Indianhead had the smallest seeds, while Eston had average seed size, which was likely primarily due to variety differences (Table 4 and Table 5).

In both 2022 and 2023, the mean incidence of CMV infection in trial plots was highest in SP1333, which had 1% and 3% CMV seed infection in 2022 and 2023, respectively (Table 4 and Table 5). Overall, the mean yield across all trial sites was significantly higher in the control plots with 0% CMV-infected seed treatment plots that were sown with CMV-infected seed levels ranging from 0 to 3% (Figure 3D) that may have been infected with CMV in 2023 (Figure 3B). However, the mean yield of the three CMV-infected treatment plots was only significantly lower than two of the three control treatment plots in 2022 (Figure 3C).

Table 4 Data from the 2022 lentil field trials. Within a parameter, different letters denote varieties that are significantly different from each other.

Cultivar	Treatment	% CMV infection in seed	Mean incidence of CMV (%) per plot	Mean number of plants per plot that germinated	Mean plant height (cm)	Mean 200 grain weight (g)
PBA Hallmark	Control	0	1	29 [a]	74 [c]	7.7 [c]
PBA Hurricane	Control	0	1	29 [a]	66 [b]	6.4 [b]
PBA Jumbo2	Control	0	2	30 [a]	64 [b]	9.5 [d]
SP1333	CMV- infected	1	26	30 [a]	55 [a]	12.9 [e]
Eston	CMV- infected	2	15	31 [a]	51 [a]	6.1 [b]
Indianhead	CMV- infected	3	8	32 [a]	64 [b]	4.1 [a]

Table 5. Data from the 2023 lentil field trials. Within a parameter, different letters denote varieties that are significantly different from each other.

Cultivar	Treatment	% CMV infection in seed	Mean incidence of CMV (%) per plot	Mean number of plants per plot that germinated	Mean plant height (cm)	Mean 200 grain weight (g)
CIPAL 2122	Control	0	3	29 [a]	44 [b]	8.1 [c]
PBA Highland XT	Control	0	4	37 [b]	43 [b]	8.2 [c]
PBA Jumbo2	Control	0	0	28 [a]	43 [b]	10.7 [d]
SP1333	CMV- infected	3	20	24 [a]	37 [a]	14.1 [e]
Eston	CMV- infected	0	10	27 [a]	35 [a]	6.8 [b]
Indianhead	CMV- infected	0	13	43 [b]	37 [a]	5.2 [a]

Despite having the largest seed of all the cultivars examined, the yield of SP1333 was significantly lower than two of the three controls in 2022 (Figure 3A) and was significantly lower than all three controls in 2023 (Figure 3B). Additionally, SP1333 also produced the lowest number of seeds per plot, which was estimated using the calculation "yield/[200 grain weight/200]" (Figures 3E, and 3F).

Although there was a lower level of CMV in the seed of SP1333 than Eston and Indianhead in 2022, these results suggest that CMV infection has a greater impact on SP1333 than Eston and Indianhead as the incidence of CMV in the SP1333 treatments prior to harvesting is higher and the percentage of yield loss is greater. This trend of increased susceptibility to CMV infection by SP1333 cannot be confirmed from this dataset as we don't have any non-infected SP1333, Indianhead and Eston control plots to compare to. However, the yield of Eston (2% CMV seed infection) and Indianhead (3% CMV seed infection) was also lower than the yield of the controls in 2022.

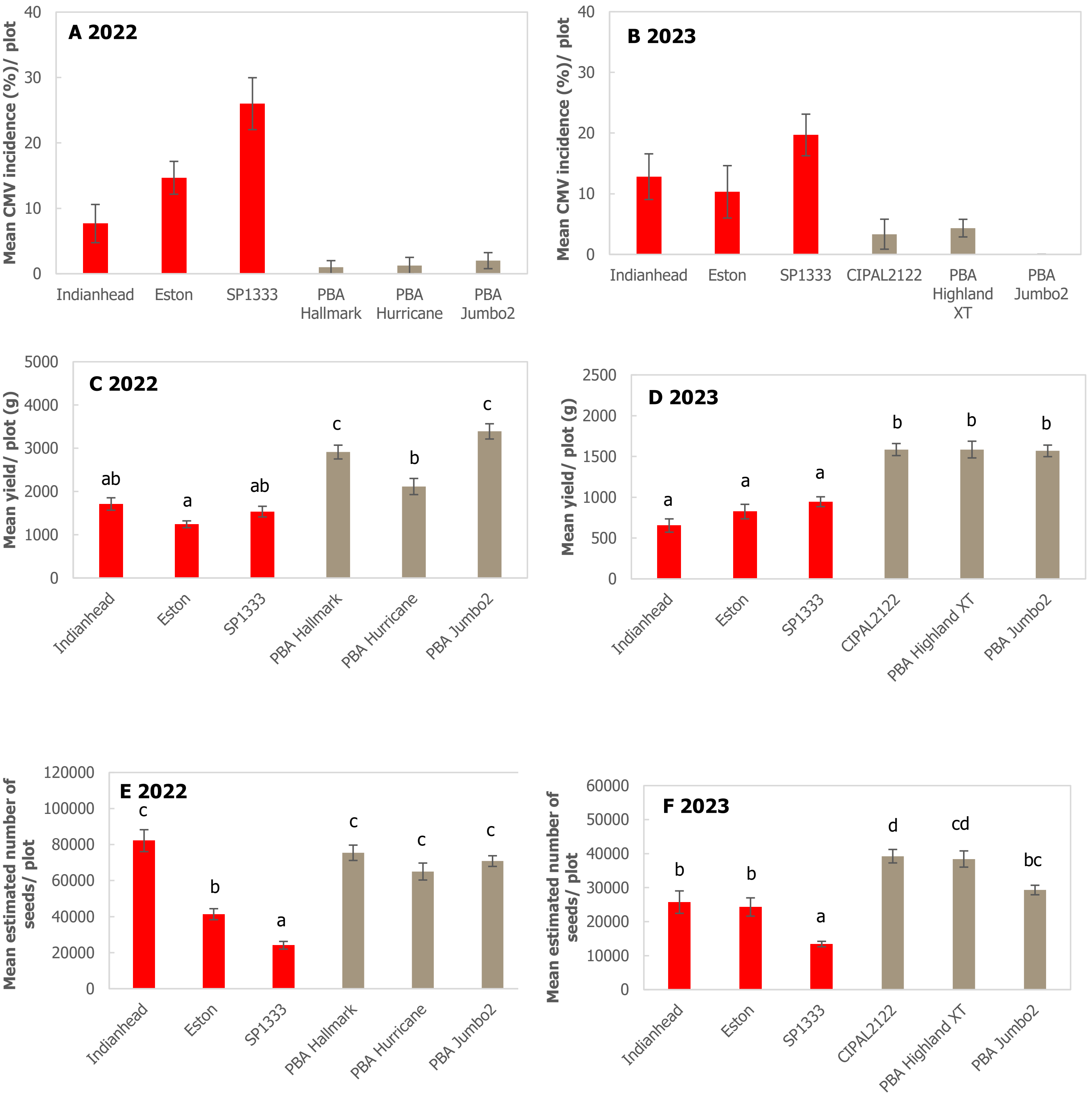


Figure 3. Mean incidence of CMV (A, B), yield (C, D) and estimated number of seeds (E, F) per plot for each variety examined (overall) during 2022 and 2023. Error bars represent the standard error; means with the same letter are not significantly different at $p < 0.05$.

Determination of CMV-infection rates of lentil cultivars by aphids

Thirty nine to 103 plants of 24 lentil cultivars (Aldinga Boomer, Cassab, CIPAL0714, CIPAL2122, Cobber, Commando, Digger, Eston, Giant, Greenfield, Hack, Hallmark, Herald, Highland, Hurricane, Indianhead, Jumbo, Jumbo2, Kelpie, Matilda, Northfield, Nipper and Nugget) were challenged in the glasshouse with CMV infection using aphids to see the difference in rate of transmission (Table 6). The seedlings were inoculated at two weeks of age and tested a month after inoculation by TBIA.

Table 6. Results from challenging lentil varieties with CMV transmission in the glasshouse using green peach aphid

Variety	Number of plants inoculated	Number of positive plants	% transmission
Aldinga	81	9	11
Boomer	80	20	25
Cassab	48	1	2
CIPAL0714	90	16	18
CIPAL2122	96	1	1
Cobber	46	2	5
Commando	82	20	24
Digger	65	18	28
Eston	75	9	12
Giant	72	1	1
Greenfield	72	3	4
Hack	39	4	10
Hallmark	82	34	42
Herald	100	0	0
Highland	103	0	0
Hurricane	84	5	6
Indianhead	85	48	57
Jumbo	81	26	32
Jumbo2	90	64	71
Kelpie	94	57	61
Matilda	69	3	5
Northfield	90	42	47
Nipper	96	0	0
Nugget	72	0	0

Twenty cultivars were infected with variable rates of infection (1-71%). The highest rate of aphid transmission of CMV happened in cv. Jumbo (71%) while the cultivars Herald, Highland, Nipper and Nugget were not infected (0% transmission) (Table 6). The rate of CMV transmission was 28% in cultivar Digger, 5% in the cultivars Cobber and Matilda, 4% in PBA Greenfield and 1% in PBA Giant and CIPAL2122. To validate the TBIA testing three healthy and three CMV-infected plants of PBA Greenfield and one healthy and one CMV-infected plant of PBA Giant were confirmed by PCR.

Comparison of seed numbers and seed weight from healthy and CMV-infected lentil plants in the glasshouse.

Seeds were collected from aphid-inoculated CMV positive and negative lentil plants belonging to six cultivars: Aldinga, Boomer, Cassab, Commando, Eston and Hack (Table 7). The plants were inoculated at the 2-week stage in October 2022 and the plants harvested in January 2023. The number of seeds produced by healthy and CMV-infected lentil plants were counted and seed weights were compared.

In the glasshouse experiment, the number of seeds and the weight of the seeds produced by CMV-infected plants were less than those produced by healthy plants in all cultivars except Commando, where there was no difference in number in seed number or seed weight (Table 7). For example, an average of 26 seeds were harvested from each healthy Aldinga plant, with each seed weighing 0.043 grams, compared to only 12 seeds harvested per CMV-infected Aldinga plant with each seed weighing just 0.033 grams. Across the 6 cultivars, when comparing the average seed weight between CMV infected vs healthy seed, the greatest reduction in seed weight was 93% in Cassab and lowest reduction in seed weight was 10% in Commando (Table 7).

Table 7. Comparison of seed number and weight between CMV-infected and healthy plants of 6 lentil cultivars.

Variety name	No of plants		No of seeds		No of seeds per plant		Weight (g) of seeds		Weight (g) of seeds per plant		Reduction (%) in weight of seeds due to CMV infection
	H	I	H	I	H	I	H	I	H	I	
Aldinga	4	4	105	48	26	12	4.5	1.6	1.13	0.4	64
Boomer	16	16	227	112	14	7	12	5.3	0.75	0.33	56
Cassab	1	1	30	3	30	3	1.4	0.1	1.4	0.1	93
Commando	20	20	301	308	15	15	10.4	9.4	0.52	0.47	10
Eston	7	7	204	156	29	22	6.3	4.6	0.9	0.66	27
Hack	4	4	130	64	33	16	3.7	1.6	0.93	0.4	57

H = Healthy; I = Infected

Testing of CMV transmission in seedlings germinated from seeds collected from glasshouse maintained, aphid-inoculated CMV positive and negative plants

A variable number of seeds harvested from the glasshouse-grown, aphid inoculated CMV positive and negative plants were collected and sown in the glasshouse, and the germinated seedlings were tested for CMV using TBIA. These cultivars included Aldinga, Boomer, Cassab, Commando, CIPAL0714, Eston, Hack, Hallmark and Indianhead. CMV was only detected in seedlings germinated from seeds harvested from glasshouse maintained CMV-infected plants in 2 of 9 cultivars tested. A 2% CMV transmission rate was detected in CMV-infected Hallmark and a 7% CMV transmission rate was detected in CMV-infected Aldinga. No virus was detected in seedlings germinated from seeds collected from CMV-infected plants of CIPAL0714, Indianhead, Hack, Cassab, Commando, Eston and Boomer (Table 8).

Table 8. Testing of seeds collected from aphid inoculated CMV positive and negative plants.

Variety	Number of seedlings tested	Number of seedlings positive to CMV by TBIA	% CMV transmission
PBA Hallmark XT infected	87	2	2
CIPAL0714 infected	62	0	0
Indianhead infected	20	0	0
Hack Healthy	68	0	0
Hack Infected	26	0	0
Cassab Healthy	19	0	0
Cassab Infected	3	0	0
Commando healthy	34	0	0
Commando infected	41	0	0
Eston healthy	49	0	0
Eston infected	31	0	0
Boomer healthy	117	0	0
Boomer infected	37	0	0
Aldinga healthy	56	0	0
Aldinga infected	14	1	7

Bulk up of seed lots of lentil cultivars with known levels of CMV-infected seed.

Seven of the 13 Hallmark buffer plots in the 2022 Horsham trial that were challenged with CMV via aphid transmission had a CMV incidence of less than 15% and six of the 13 Hallmark plots had CMV infection incidences ranging from 20-50%. The seed from these high CMV incidence plots were harvested and screened to estimate the levels of CMV infection in the seed. Only 1% of the seedlings germinated from the harvested seed tested positive for CMV by TBIA, while > 2.9% of the hallmark seed was CMV positive when using PCR.

To generate seed from additional lentil cultivars with known levels of CMV, the seed from the CMV infected trial plots of Eston, Indianhead, SP1333 and Hallmark was harvested and tested by germinating some seed from each batch and testing for the presence of CMV using TBIA. Using this approach, CMV was detected in SP1333 at a rate of 2.6% CMV infected seed and in Hallmark seed at a rate of 0.5% CMV infected seed (Table 9).

Table 9. CMV-infected and healthy bulked up lentil seed

Cultivar	% CMV-infected seed	Weight of available seeds (g)	Number of available seeds (estimated)	200 grain weight (g)
Hallmark	Healthy	1382	34550	8
Hallmark	1.5	8257	206425	8
Hallmark	0.5	2525	66447	7.6
SP1333	Healthy	N/A	N/A	N/A
SP1333	2.6	1564	24438	12.8

These three CMV-infected seed lots SP1333 (2.9%), Hallmark (1.5%) and Hallmark (0.5%), along with virus-free Hallmark and SP1333 seed lots, are now available for future CMV lentil trials.

A Risk Matrix Guide

Introduction

Effective risk management is a core strategy farmers and businesses in general use to maximise both sustainability and profitability of their businesses. Critical in understanding risk and identifying appropriate management strategies is to identify, understand, and assess the metrics that contribute to "risk".

A risk assessment matrix identifies and captures the likelihood of a risk/s occurring and evaluates the potential damage or interruption caused by those risks. In general terms, the risk matrix assesses the level of risk by comparing the likelihood (low, medium high) of a risk metric occurring against the consequence of that metric occurring (e.g. minor, moderate, severe). This is a simple mechanism to increase visibility of risks and assist management decision making. Depending on likelihood and severity, risks can be categorized as high, moderate, or low.

A risk assessment matrix, also known as a Probability and Severity or Likelihood and Impact risk matrix, is a visual tool depicting potential risks affecting a business. The risk matrix is based on two intersecting factors: the *likelihood* the risk event will occur and the potential *impact* the risk event will have.

Currently, there is a data gap relating to the plant health impacts and actual yield risk from sowing lentil seeds infected with known levels of cucumber mosaic virus (CMV). As the yield risk is unknown, growers are not seed testing and are unable to make informed choices on planting virus infected lentil seeds. To address this gap, the project team has generated a risk matrix for the impact of sowing CMV-infected lentil seed on yield.

In this context, the risk matrix must take into consideration the likely variables that may be present in the lentil production system and the direct impact on yield that CMV infection can cause. There are many variables that remain unknown in this biological system, the CMV-lentil pathosystem, including: variation in virulence between strains of the pathogen (CMV); variation in the mode and efficiency of CMV transmission (high or low rates of seed transmission); efficient or inefficient aphid transmission of CMV; host tolerance to CMV infection (e.g. some lentil cultivars are more susceptible to CMV infection than others); and the impact these variables have on yield loss within a plant and within the crop.

To better understand these variables, following is some basic information on the biology of this pathosystem, including parameters that are not fully understood.

Cucumber mosaic virus (CMV)

Cucumber mosaic virus (CMV) causes severe disease in lentils, chickpeas and lupins. Symptoms include: reduced internodes, bunchy appearance and plant stunting. If the virus is carried in the seed the entire plant may be stunted and may die before maturity. A plant infected late in the season may have normal sized leaves at the bottom but those near the top, above the point of infection, can be less than half the normal size. Infected plants may occur randomly throughout paddocks, or appear in small patches 1-2 metres in diameter.

CMV is transmitted by aphids, including green peach aphid (*Myzus persicae*), in a non-persistent manner. With this mode of virus transmission, the aphids acquire the CMV virus particles within seconds to minutes of feeding on the CMV-infected plant (either CMV-infected crop plants or weeds) and can then transmit CMV to a healthy plant within seconds, minutes or hours of feeding on the healthy plant. Aphids lose infectivity after a few probes because non-persistently transmitted viruses such as CMV are not kept long on an aphid's mouth parts and aphids do not remain infective throughout their life. Insecticide sprays are generally much less effective against non- persistent than persistent viruses because a non- persistent virus would typically have already been transmitted in the time that it takes an aphid to be affected by the insecticide, due to the short transmission time.

CMV does not go through a lifecycle like many other pathogens: It cannot survive for very long outside the plant and during the growing season it relies solely on aphids for spread to other live plants. CMV can survive for long periods of time in seed but does not have stages which allow it to survive in the soil or stubble as sources of infection for future crops.

A primary source of infection for CMV is infected lentil seed. If the seed infection level is one per cent, one plant in every 100 will be virus-infected, therefore the virus will be randomly distributed across the field. Alternate non-crop hosts, such as weeds, are also an important primary source of inoculum for CMV. For the broad acre cropping system for lentils, volunteer crop hosts that survive in the green bridge between harvest and sowing each year are also an important primary source of inoculum. The secondary spread of CMV by aphids is from either CMV-infected seedlings (germinated from CMV infected seed) or from CMV infected weeds or volunteer crop hosts located in nearby green bridge.

Yield loss in lentils as a result of CMV infection

CMV is of economic importance in a range of crops worldwide. In areas where large aphid populations occur, crop losses can be high due to reduced herbage production and grain yield. However, accurate data on yield losses associated with CMV infection in lentils, and pulses in general, is not well understood. In Western Australia, high levels of CMV infection in lupin crops have resulted in subsequent yield losses of up to 60 per cent, leading to the development of integrated management packages to control the disease (Jones, 2000). In Western Australian field experiments, CMV infection in Matilda lentils was shown to diminish shoot dry weight by 72 to 81 per cent, seed yield by 80 to 90 per cent and individual seed weight by 17 to 25 per cent (Latham et al., 2004). Virus infection typically causes more severe yield losses when plants are infected in the early growth stages.

Yield losses associated with pulse viruses are often crop- and virus- specific and season- dependent. Based on survey results (Victorian Surveillance Project - DJP1907-004RTX), turnip yellows virus (TuYV, previously known as beet western yellows virus) and cucumber mosaic virus (CMV) are the most commonly detected and important viruses affecting pulse crops in Victoria and considerable year-to-year variation in virus prevalence and incidence is observed. Although this variation can be complex and difficult to define, it is influenced by differences in environmental conditions, availability of virus and vector reservoirs, aphid populations, aphid activity, long-distance migration of aphids, the abundance of natural enemies and interactions between these factors.

Management of pulse viruses, including CMV

The following guidelines for growers are recommended for the control of CMV and other seed-borne viruses of pulses:

- Growing virus resistant varieties is the best long-term option, but the number of resistant varieties is limited. Breeding is underway to develop a faba bean variety with BLRV resistance and field pea variety with PSbMV and BLRV resistance.
- Sowing virus tested seed which has a virus level less than 0.1-0.5 per cent is recommended to control seed-borne viruses, which can only be spread short distances in the field by their aphid vectors (non-persistent transmission).
- Seed treatment with neonicotinoid (e.g. imidacloprid) insecticide reduces aphid feeding and virus spread. It is especially beneficial for persistently transmitted viruses.
- Eliminating weeds and self-sown pulses at least two weeks prior to sowing. These are a source of virus and aphids, and this is usually where they survive between crop growing seasons.
- Monitoring aphids, particularly at the early growth stages of the crop. If aphids develop heavy colonies at this early stage, then it may be economical to spray the crop with an aphicide.
- Good plant stands with minimal bare ground may reduce aphid colonisation.
- Rotation of pulses with cereals and reduce volunteer hosts and weeds.
- Removal of plants with symptoms will reduce the amount of infected seed harvested (appropriate for some seed crops). The amount of virus infection in the harvested seed (per cent) depends not only on the number of infected plants, but also on the rate of seed transmission of the virus.

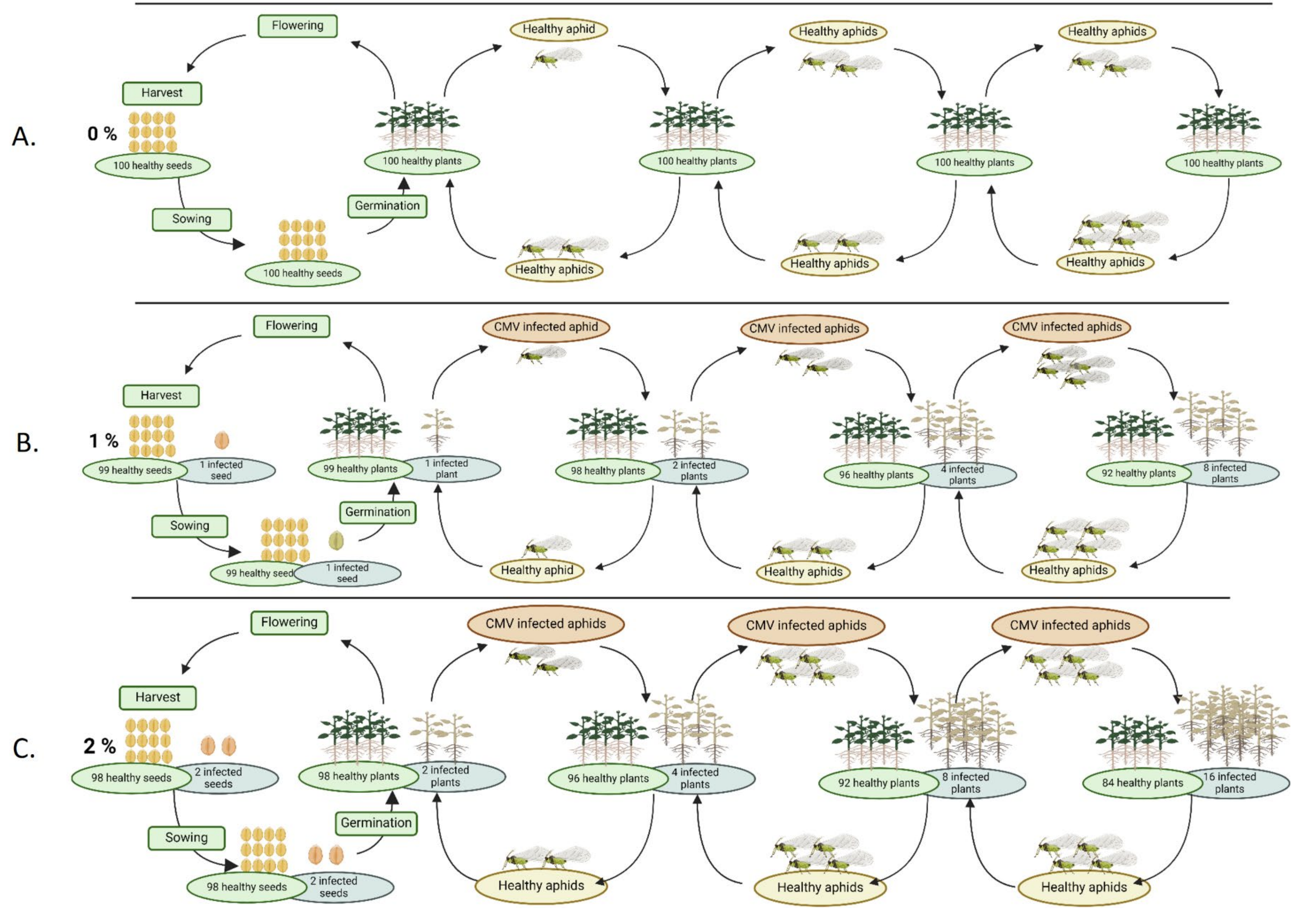
Parameters that impact the Risk Matrix for the CMV-Lentil Pathosystem

In the simplest form, with all biological variables equal, the impact of planting CMV infected seeds can be easily described (Figure 4). In the first scenario (Figure 4A), the assumption is that when planting 100% healthy seed, there is no CMV inoculum and the impact of aphids feeding on these lentil plants is negligible as there is no CMV inoculum to transmit to healthy plants. This scenario assumes that there is no external inoculum of CMV in alternate hosts surrounding the lentil crop and therefore aphids are not introducing CMV inoculum into the crop. The influence of lentil cultivar susceptibility/resistance, timing and abundance of aphids feeding on the lentil crop, the efficacy of the aphids to acquire and transmit CMV, and the seasonal conditions on the resulting CMV epidemic and subsequent yield reduction at harvest is negligible.

In the second scenario (Figure 4B), lentil seed that was 1% CMV infected was sown. The assumption in this scenario is that the lentil cultivar is susceptible, that when the aphid feeds on the CMV-infected lentil plant it acquires the virus, and when it feeds on a healthy plant, it infects that plant with CMV. This scenario also assumes that the lentil plants are only exposed to three transmission events by aphids during the growing season. In this scenario the CMV incidence increases from 1% CMV infection at sowing to 8% CMV infection in the crop after 3 aphid feeding events.

In the third scenario (Figure 4C), lentil seed that was 2% infected was sown. The assumptions are the same as for scenario 2 in that the lentil cultivar is susceptible to CMV infection, the acquisition and transmission rate of the aphids is 100% efficient and that the lentil plants in the crop are only exposed to three aphid transmission events during the growing season. In this scenario, the incidence of CMV infection increases from 2% at sowing to 16% CMV infection in the crop after 3 aphid feeding events.

Figure 4. A hypothetical schematic demonstrating the impact of CMV infected seed, coupled with the presence of aphid vectors, on the incidence of CMV in a lentil crop. The three scenarios are based on a 100 seed lot with A) 0%, B) 1% and C) 2% CMV infected seed, with vectors present to acquire and transit three times through the growing season.



In this most simple CMV-Lentil disease model, the impact of sowing CMV infected seed on disease incidence in the lentil crop is clear. Additionally, this simple model helps highlight how the many biological variables including the virus, lentil host, aphid vectors, as well as the seasonal and climate conditions, influence both disease incidence and severity.

Some of these variables include:

CMV strain variability

CMV, like all viruses, has many strains with subtly different biological parameters. Variations include:

- Strain virulence
    - Disease impact /severity on host (symptoms and yield)
- Seed transmissibility
- Aphid transmissibility
- Host range

CMV strain variation associated with pulse crops in south eastern Australia has been reported but is not well understood. During 2019, two isolates of CMV were collected from different hosts (lentil and lupin) in the Wimmera district in Victoria and glasshouse experiments investigated the reaction of different varieties belonging to the *Fabaceae* and *Solanaceae* families to these two isolates of CMV, along with a third isolate of CMV from tobacco. Test plants were grown in the glasshouse and were mechanically inoculated with infected sap at the 3-4 leaf stage. After 3 weeks, the seedlings were tested for the presence or absence of CMV using tissue blot immunoassay (TBIA).

Susceptibility to infection with the three CMV isolates varied between host families, between varieties of the same species and between virus isolates. The lentil and lupin CMV isolates were most readily transmissible to field peas. However, the lentil isolate was only able to infect 1 of 5 cultivars of faba bean, while the lupin CMV isolate was not transmitted to any of the faba bean varieties challenged in this experiment. In contrast, the tobacco isolate of CMV was most readily transmissible to faba bean varieties and least readily transmissible to field pea varieties. All 3 isolates exhibited a severe mosaic reaction on *Nicotiana tabacum* and *N. glutinosa*.

These results demonstrate the importance of screening different CMV strains for host susceptibility, particularly for new varieties, while also identifying some potential sources of CMV resistance for further investigation.

Aphids

CMV is transmitted in a non-persistent manner by more than 80 aphid species and the following aphid vectors of CMV have been detected in the Wimmera region in Victoria in recent years:

- lucerne blue green aphid (*Acyrthosiphon kondoi*)
- cowpea aphid (*Aphis craccivora*)
- foxglove aphid (*Aulacorthum solani*)

- ornate aphid (*Myzus ornatus*)
- green peach aphid (*Myzus persicae*)
- cabbage aphid (*Brevicoryne brassicae*)
- sowthistle green aphid (*Hypermyzus lactucae*)
- sowthistle brown aphid (*Uroleocon sonchi*).

The prevalence, abundance and timing of feeding of these aphid species can have a significant impact on the CMV incidence in a lentil crop. Each aphid species will have preferred host plants to colonise and show varying degrees of tolerance to climatic and environmental factors, which together result in population peaks at varying times during the lentil growing season. Additionally, for each aphid species, the acquisition and transmission rates of CMV (and its strains) will vary and these transmission efficiencies will also impact the prevalence of CMV infection in the lentil crop.

Host range of CMV

CMV has a host range of more than 1,200 species. Significant vegetable hosts include carrot, celery, cucurbits, legumes, lettuce, onion, pepper, spinach, tomato, and rarely potato. Many woody and non-woody ornamentals are also hosts. All pulse crops grown in southern Australia are hosts of CMV. This includes major pulses such as chickpeas, lentils, lupins, field peas and faba beans. Many pasture and forage legumes grown in the cropping regions, such as lucerne, vetch, medic, annual and perennial clover, are also hosts. Surveys in Victoria and South Australia identified a number of weed hosts including bifora (*Bifora testiculata*), prickly lettuce (*Lactuca serriola*), common sowthistle (*Sonchus oleraceus*), Indian hedge mustard (*Sisymbrium orientale*), burr medic (*Medicago polymorpha*), snail medic (*Medicago scutellata*) and barrel medic (*Medicago truncatula*).

This broad host range of CMV can significantly influence a CMV epidemic in a susceptible crop host. Specifically for lentils, surveys during the 2000's detected CMV in 7 – 50 % of lentil crops sampled with a "within crop" CMV % incidence ranging from 1 – to 100%. Higher levels of virus prevalence and incidence were recorded in South Australia with CMV detected in 50 – 100% lentil crops sampled with a "within crop" CMV % incidence ranging from 1 to 100%.

Within lentils, there will be variation in host response to CMV infection. Indeed, a significant management strategy is to breed lentil cultivars which are either resistant to CMV infection (i.e. not infected by CMV) or tolerant to CMV infection (i.e. lentil cultivars that can be infected with CMV, but do not show symptoms or a reduction in yield as a result of CMV infection, when compared to a susceptible cultivar). Tolerant cultivars are likely to have lower rates of seed transmission of CMV.

CMV infection levels in seed

CMV is transmitted via seed in a number of host species, including lentils. A recent survey of pulse seed-stock in Victoria found that 7% of lentil seed lots, and 9% of faba bean seed lots, had up to 0.2 per cent and 0.1 per cent CMV infection, respectively. In Western Australia, seed transmission experiments showed seed transmission rates for CMV of up to 1 per cent in lentils (Latham and Jones, 2001).

There is natural variation in the CMV seed transmission rates between lentil cultivars and this is influenced by both the susceptibility and tolerance of the lentil cultivar to CMV infection, as well as the strain of CMV – some strains of CMV will be more seed transmissible than others.

Climate and location impact the CMV-Lentil pathosystem

A number of factors interact to determine whether or not a particular year is conducive to virus spread and lentil infection. The main factors which influence virus spread are those that affect the aphid population (such as the weather) and the availability of sources of virus for the aphids to spread (seed, weeds, pasture hosts, etc.).

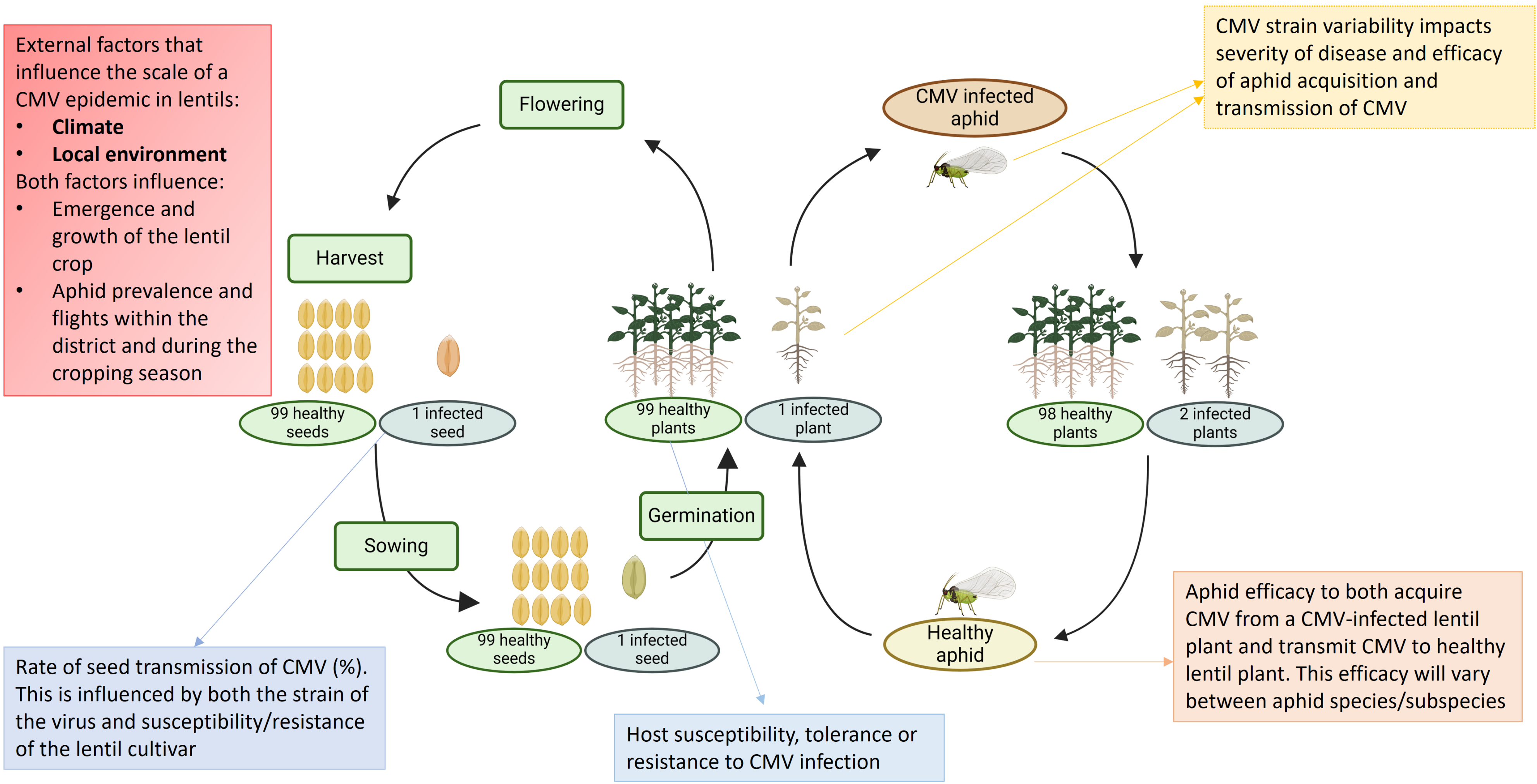
Viruses cause a variety of symptoms in plants they infect and can be present in plants without showing any symptoms. Common virus symptoms are local lesions, mosaic patterns, yellowing, leaf rolling or curling, ringspots, necrosis and total plant death. Some viruses are seed-borne, others are mechanically transmitted, however, most viruses are transmitted by insect vectors.

To summarise (Figure 5), the three key biological parameters that influence the extent and severity of a CMV epidemic are:

- The CMV strain which influences the severity of disease infection, the rate of seed transmissibility and the efficacy of aphid transmission of CMV.
- The lentil cultivar which may be susceptible or resistant to CMV infection, and if susceptible, the host plant may be more tolerant to CMV infection and display limited symptoms and/or limited yield loss and/or low rates of seed transmission.
- Aphid species, efficacy of CMV transmission (acquisition and transmission) and the prevalence of aphid vectors of CMV within a geographic region and their abundance during the growing season.

Local environmental and climatic conditions are important factors that change from year to year and during the growing season, and can greatly impact the emergence and severity of a CMV epidemic in a lentil crop (Figure 5).

Figure 2: Biological variables that impact on the incidence, prevalence and severity of CMV infection in lentils



The CMV-Lentil Risk Matrix

Informed management strategies driven by a Risk Matrix guide will have a long-term outcome of reducing the levels of, and yield losses resulting from, the viral disease caused by CMV in the lentil cropping system. Based on the field and controlled environment research results from Years 1 and 2 of this project (DJP211-001RTX "Quantifying the yield risk of planting CMV infected seed in the Southern Region"), the drafted risk matrix aims to define the yield risk associated with planting lentil seeds infected with known levels of CMV. The 'Risk Matrix Guide' encompasses information regarding different levels of virus infection in a seed lot for CMV, based on low, medium and high infection levels; preliminary data on the yield penalties as a consequence of sowing seeds with those levels of virus infection was generated.

Several biological factors that impact directly on lentil yield as a result of CMV infection include the levels of CMV infection in the seed sown and the tolerance/susceptibility levels of the lentil cultivar to CMV infection. A series of field and glasshouse trials were conducted and have generated data on lentil cultivar tolerance/susceptibility to CMV, seed transmissibility rates of CMV, and the impact of CMV on yield in several lentil cultivars. Following is a summary of those key findings.

CMV seed infection rates at sowing can have an impact on lentil yield

Field trials located in four different geographic regions (Wimmera, Victoria; Mallee, Victoria; North Central district, SA; York Peninsula, SA) and across two growing seasons (2022 and 2023) using the same source of CMV infected seed of three lentil cultivars at sowing have generated data indicating that certain lentil cultivars are more tolerant to CMV infection, while yield is impacted by the level of CMV infected seed that is sown (Table 10).

For example, when the cultivar Eston was grown in four regions (Wimmera, Mallee, Mid-north SA, York Peninsula), there was no impact on seed germination, plant height or incidence of CMV detected in the mature crop when comparing healthy (0% CMV infected) seed to 2% CMV infected seed at sowing. However, there was a reduction in grain weight of seed harvested for Eston sown with 2% CMV infected seed in the Mallee field trial.

In contrast, for the cultivar SP1333, there was a difference in seed germination rate, plant height and grain weight of harvested seed when comparing healthy (0% CMV infected) seed to 3% CMV infected seed at sowing at three (Wimmera, Mid North – SA, York Peninsula) of the four regions.

Together, these two results demonstrate that there is:

a) a range of susceptibility to CMV infection between lentil cultivars, with SP1333 being more susceptible to CMV infection when compared to Eston, and

b) a geographic effect on the impact of CMV on lentils, as we observed variation of CMV infection within a lentil cultivar and between regions.

The data summarised in Table 10 identifies trends only. Due to the requirement for this project to build up a supply of lentil seed stocks from the same cultivar with known levels of seed infection, seed lots from a single lentil cultivar with varying levels of seed infection of a single isolate of CMV could not be included in the field trials (for details see the project final report). However, it is clear that there is variation in the tolerance/susceptibility levels of different lentil cultivars to CMV infection, and in general, the higher the level of CMV infected seed sown, the greater the yield loss, with 3% CMV infection having a larger impact on yield than 0 or 1% CMV infection levels in seed.

Table 10: An estimate of the impacts of CMV infection on seed germination, plant height, incidence of CMV infection in the crop prior to harvest and the impact of CMV infection on grain weight harvested from experimental plots.

Region	**Cultivar**	**% Virus infected seed**	**Yield risk**		**Incidence of CMV (%) in crop**	**Grain wt. of seed harvested from plots (g)**
			Plant germination count	**Plant height (cm)**		
Wimmera	Eston	0 vs 2%	No negative impact[a]	No negative impact	No negative impact	No negative impact
Wimmera	Indianhead	0 vs 3%	No negative impact	No negative impact	No negative impact	No negative impact
Wimmera	SP1333	1 vs 3%	Negative impact observed[b]	Negative impact observed	No negative impact	Negative impact observed
Mallee	Eston	0 vs 2%	No negative impact	No negative impact	No negative impact	Negative impact observed
Mallee	Indianhead	0 vs 3%	No negative impact	No negative impact	No negative impact	Slight negative impact observed[c]
Mallee	SP1333	1 vs 3%	Negative impact observed	Negative impact observed t	Negative impact observed	No negative impact
Mid North	Eston	0 vs 2%	No negative impact	No negative impact	No negative impact	No negative impact
Mid North	Indianhead	0 vs 3%	Negative impact observed	No negative impact	No negative impact	No negative impact
Mid North	SP1333	1 vs 3%	No negative impact	Negative impact observed	Negative impact observed	Negative impact observed

York Peninsula	Eston	0 vs 2%	No negative impact	No negative impact	Negative impact observed	No negative impact
York Peninsula	Indianhead	0 vs 3%	Negative impact observed	No negative impact	No negative impact	No negative impact
York Peninsula	SP1333	1 vs 3%	Negative impact observed	Negative impact observed	No negative impact	Negative impact observed

[a]No negative impact: No to minimal difference in data recorded between the two treatments.
[b]Negative impact observed: A negative impact was recorded between the two treatments,
[c]Slight negative impact observed: Only a slight negative was recorded between the two treatments.

To correlate this further into a risk matrix, we assume that a non-significant outcome represents a low risk for a grower, a slightly significant outcome is considered a medium risk, and a significant outcome, particularly with respect to yield, is considered a high risk. Using this logic, the risk for a grower planting three cultivars (Eston, Indianhead, SP1333) in each of the four locations with either 0, 1, 2 or 3% CMV infection in seed at sowing is provided in Table 11:

Table 11: An estimate of risk for sowing 0, 1, 2 or 3% CMV infected seed in 3 lentil cultivars located at four geographic locations.

Region	Cultivar	Virus level in the seed	Yield risk			
			Plant germination count	Plant height	CMV incidence	Affect on Yield
Wimmera	Eston	0%	Low	Low	Low	Low
Wimmera	Eston	2%	Low	Medium	Medium	Medium
Wimmera	Indianhead	0%	Low	Low	Low	Low
Wimmera	Indianhead	3%	Low	Medium	Medium	Medium
Wimmera	SP1333	1%	Low	Medium	Medium	Medium
Wimmera	SP1333	3%	Low	High	High	High
Mallee	Eston	0%	Low	Low	Low	Low
Mallee	Eston	2%	Low	Medium	Medium	Medium
Mallee	Indianhead	0%	Low	Low	Low	Low
Mallee	Indianhead	3%	Low	Medium	Medium	Medium
Mallee	SP1333	1%	Low	Medium	Medium	Medium
Mallee	SP1333	3%	Low	High	High	High
Mid North	Eston	0%	Low	Low	Low	Low
Mid North	Eston	2%	Low	Medium	Medium	Medium
Mid North	Indianhead	0%	Low	Low	Low	Low
Mid North	Indianhead	3%	Low	Medium	Medium	Medium
Mid North	SP1333	1%	Low	Medium	Medium	Medium
Mid North	SP1333	3%	Low	High	High	High
York Peninsula	Eston	0%	Low	Low	Low	Low
York Peninsula	Eston	2%	Low	Medium	Medium	Medium
York Peninsula	Indianhead	0%	Low	Low	Low	Low
York Peninsula	Indianhead	3%	Low	Medium	Medium	Medium
York Peninsula	SP1333	1%	Low	Medium	Medium	Medium
York Peninsula	SP1333	3%	Low	High	High	High

Risk scale: no negative impact = Low; Slight negative impact between treatments observed = Medium; Negative impact observed between treatments = High.

Aphid transmission of CMV in lentils is influenced by the lentil cultivar

As part of this project (DJP211-001RTX "Quantifying the yield risk of planting CMV infected seed in the Southern Region"), a glasshouse trial was conducted to determine the susceptibility of different lentil cultivars to aphid transmission of CMV. Ten aphids from a colony of GPA feeding on a CMV infected plant were placed on a lentil plant and left to feed for several days, then the inoculated lentil plants were later tested for the presence of CMV (Table 6). A total of 24 lentil cultivars were tested and the percentage of CMV positive plants ranged from 0 (four cultivars: Herald, Highland, Nipper Nugget) to 71% (Jumbo2).

This dataset clearly shows variability in the susceptibility of lentil germplasm to CMV infection, with four cultivars suggesting resistance to aphid transmission of CMV.

CMV infection has a variable impact on lentil yield

Glasshouse trials to determine the impact of CMV infected lentils on yield were also conducted. Yield, measured as the number seeds produced per plant and weight of seed harvested per plant, from healthy and CMV infected lentil plants was compared for six lentil cultivars. Yield reduction in CMV-infected plants, measured as a reduction in weight of harvested seed of infected plants vs healthy plants, ranged from 10 – 93% reduction in yield (noting that only 1 plant of Cassab was CMV infected and therefore needs further investigation) (Table 7). However, there is a clear range of yield impacts for the remaining five cultivars and there is evidence that there is a range of tolerance to CMV infection between lentil cultivars (Table 7).

The biological and environmental parameters that contribute to the risk matrix for the CMV-Lentil pathosystem

A holistic view of the CMV- lentil pathosystem is needed when considering "risk" associated with sowing CMV-infected seed. The preliminary data presented to date with this project clearly identifies a range of host susceptibility to CMV infection between lentil cultivars with respect to rates of seed transmission, aphid transmission and direct impact on yield. When determining risk, it is important to take into account all potential biological and environmental parameters. For example:

- The biological variables of CMV, the pathogen include strain, virulence, seed transmission rates, aphid transmission rates, host range.
- The biological variables of the lentil host plant include susceptibility, tolerance or resistance to CMV infection, rate of seed transmission of CMV, impact on yield.

- The biological variables of the aphid vectors of CMV include aphid species, CMV acquisition rates, CMV transmission rates, plant host preference for feeding – within lentil cultivars as well as alternate feeding hosts within the cropping system.
- The environmental factors including, seasonal conditions, (temperature, frost, rainfall) time of sowing, the local cropping system and surrounding environment (alternate hosts for both CMV and aphids).

Possible scenarios that will impact the level of risk associated with the CMV-Lentil pathosystem

In combining these factors together, Table 12 presents an estimate of the likely severity of CMV disease in lentils and the subsequent risk/consequence for a range of scenarios.

Table 12: Possible scenarios associated with the CMV-lentil pathosystem.

Parameters	Scenarios	Severity Score	Risk/ consequences
CMV			
Strain	virulent, susceptible variety, vectors present	3	High
	virulent, resistant variety	1	Low
	virulent, no vectors	1	Low
	non-virulent, susceptible variety, vectors present	2	Medium
	non-virulent, resistant variety	1	Low
	non-virulent, no vectors	1	Low
Seed transmission	seed transmitted, no vectors	1	low
	seed transmitted and vectors present	3	High
Aphid transmission	susceptible variety, vectors present	3	High
	susceptible variety, vectors absent	1	Low
	resistant variety, vectors present	1	Low
Presence of virus alternate hosts	hosts present, variety resistant, no vectors	1	Low
	hosts present, susceptible variety, no vectors	2	Medium
	no hosts present, variety susceptible, no vectors	1	Low
	no hosts present, variety resistant, vectors present	1	Low
Incidence	incidence high in the crop, early season, not seed transmitted	2	Medium
	Incidence high in the crop, late season, not seed transmitted	1	Low
	incidence high in crop, seed transmitted	2	Medium
	incidence high in crop, seed transmitted, vectors present	3	High
Lentil			
Variety	resistant, virus present, vectors present	1	Low
	tolerant, virus present, vectors present	2	Medium
	susceptible, virus present, no vectors	1	Low
	susceptible, vectors present	3	High
Symptoms	symptoms present, no virus detected	1	Low
	symptoms present, virus detected	2	Medium
	symptoms absent, virus detected	2	Medium
	symptoms present, no yield loss	1	Low
	symptoms absent, yield loss	3	High
Aphid Vectors			
	vectors present, no virus detected in the crop	1	Low
	vectors present, virus detected in crop	2	Medium
	vectors arrived early, susceptible variety	3	Medium
	vectors arrived late in the crop	1	Low
	vectors abundant, variety resistant	1	Low
	vectors abundant, variety susceptible	3	High

Environment			
Temperature	mild, susceptible variety, vectors abundant	3	High
	mild, resistant variety, vectors abundant	1	Low
	harsh, susceptible variety, low vector abundance	2	Medium
Rainfall	wet season, susceptible variety, no vectors	1	Low
Frost	aphids present, virus source present, heavy frost	1	Low
	no regular frosting, mild winter, virus present, aphid present	2	High
Sowing	early sowing, susceptible variety, less vectors	2	Medium
	early sowing, susceptible variety, abundant vectors	3	High
	early sowing, resistant variety, abundant vectors	1	Low
	late sowing, abundant vectors, susceptible variety	3	High
	late sowing, abundant vectors, resistant variety	1	Low

Disease Severity score: 1- Low; 2- Medium; 3 -High.
Potential Risk: 1= Low; 2= Medium; 3= High

This table highlights the complexity of the CMV-Lentil pathosystem and the many biological parameters that are currently poorly understood. Critical gaps in our knowledge of this pathosystem include:

- A disease resistance score for CMV infection in lentil germplasm.
- The variance in seed transmission of CMV within the lentil germplasm.
- The variance in virulence of CMV strains circulating in south east Australia that infect lentil crops.
- An understanding of which aphid species infect lentils with CMV and contribute to their spread.
- Optional seasonal conditions that influence aphid vector abundance and critical infection periods that result in significant yield reduction in the lentil crop.
- Precise data on expected yield reductions as a result of sowing CMV infected lentil seed.

A risk matrix guide for the CMV-Lentil pathosystem

Following is the proposed risk matrix guide for the CMV-Lentil pathosystem that considers two key biological parameters:

1. Percentage CMV infected seed at sowing (0, 1%, 2%, 3%, >3% CMV-infected seed)
2. Susceptibility – tolerance of lentil cultivars to CMV infection (susceptible, moderately susceptible, tolerant, moderately resistant, resistant)

The risk matrix guide for the CMV-Lentil pathosystem considering the impact of lentil host susceptibility/resistance to CMV infection versus % CMV infection levels in lentil seed at sowing.

Resistant					
Moderately resistant					
tolerant					
Moderately susceptible					
Susceptible					
Level of CMV infection	**0%**	**1%**	**2%**	**3%**	**>3%**

*Scale

Red	Major risk
Yellow	Moderate risk
Green	Minimal risk

Following are some generalisations that can be used by growers based on the risk matrix:

1. An effective control strategy for growers is to plant lentil cultivars that are resistant to CMV infection.
2. An effective control strategy for growers is to sow lentil seeds with zero levels of CMV infected seed. In this scenario the only source of CMV inoculum is from migratory aphids moving from CMV infected alternate hosts onto the lentil crop, and for this infection event to happen early in the growing season to have a negative impact on lentil yield.
3. There is a moderate risk to growers to plant lentil seed with 1% CMV infection for moderately resistant, tolerant or moderately susceptible lentil cultivars, while susceptible cultivars will have a measurable reduction in yield. These impacts will be influenced by the abundance of aphid vectors in the crop, particularly early in the season.
4. There is a major risk to growers to sow tolerant, moderately susceptible or susceptible cultivars of lentil with 2-3 % infection levels of CMV in seed at sowing.

5. There is a major risk to growers to sow moderately resistant, tolerant, moderately susceptible or susceptible cultivars of lentil with greater than 3% infection levels of CMV in seed at sowing.

Conclusions

As part of project "DJP211-001RTX Quantifying the yield risk of planting CMV infected seed in the Southern Region" the project team was tasked to generate a risk matrix guide for the CMV-Lentil pathosystem that informs growers of the risk to yield of planting CMV infected lentil seed in the southern growing region of Australia. Based on the field and controlled environment research results from Years 1 and 2 of this project, together with our knowledge of CMV, the pathogen, lentils as the plant host, and seasonal conditions for lentil production in southern Australia, including the presence of aphid vectors of CMV, a risk matrix has been generated (Table 6).

This risk matrix focused on two key biological variables in the CMV-Lentil pathosystem: lentil cultivar susceptibility to CMV infection; and the levels of CMV infected seed planted at sowing. The project team has observed significant variation in lentil cultivar tolerance to CMV infection as well as negative impacts on lentil yield due to increased levels of CMV infection at sowing (Tables 2, 3, 4). Our findings indicate that sowing lentil seed with 1% CMV infection is a moderate risk for the grower if lentil cultivars with moderate resistance, tolerance or moderate susceptibility to CMV infection are planted. Planting lentil seed with less than 1% CMV infection for most lentil cultivars will provide a minimal risk to lentil yield reduction for growers, while planting lentil cultivars that are resistant to CMV infection is the most effective management strategy. Conversely, sowing lentil seed with 2-3% CMV infection is a moderate to major risk of yield reduction due to disease impacts associated with CMV infection.

It is important to acknowledge that the risk matrix presented is based on observed trends and not based on statistically significant data. Experiments have been conducted to determine the impact of CMV infection yield, and clear trends were observed between lentil cultivars and levels of CMV infected seed at sowing. However, the project team did not have access to volumes of seed from a lentil cultivar with known levels of CMV infection for planting in the experimental plots. The project team has bulked up seed batches for two cultivars with known levels of CMV infection for future field experiments. It is also clear from the data presented that lentil cultivars have variable degrees of susceptibility to CMV infection, with some cultivars clearly more resistant to infection than other cultivars. However, there is no disease resistance score for CMV infection in lentil germplasm. It is a recommendation from this project that the lentil industry consider generating CMV disease resistance data and tools that are available for breeding programs to phenotype lentil germplasm for CMV resistance.

There are many other variables that need to be considered relating to the CMV-Lentil pathosystem (Figure 2) to improve management of CMV in lentils and further work is required to further optimize the disease risk matrix presented here. However, based on the findings of this project, we recommend that sowing lentil seed with less than 1% CMV infection and/or sowing lentil cultivars that are moderately resistant to resistant to CMV infection are two important management strategies for growers.

DISCUSSION OF RESULTS

CMV in lentil is a major problem in south-eastern Australia and the primary source of infection has been identified as CMV-infected seed. There is a data gap relating to the plant health impacts and actual yield risk from sowing lentil seeds with known levels of infected CMV. To address this gap this project investigated the impact of sowing varying levels of CMV infected seed on yield, the susceptibility of a range of lentil cultivars to CMV infection and the generation of a disease risk matrix to provide guidelines to growers on managing and minimising the impact of CMV on yield of lentil crops.

As a first step, commercial seed lots of lentil seed were screened for CMV infection to determine the level of CMV infection in these seed lots. In all, 143 seed lots from 69 lentil varieties were screened for CMV infection and only three seed lots, Indianhead, Eston, and SP1333 were identified as having 3%, 2% and 1% levels of CMV-infected seeds, respectively. Unfortunately, the research team had access to only 1 seed lot each of these three cultivars (Table 1) and therefore the team did not have access to healthy or "virus-free" seed lots of Indianhead, Eston and SP1333. To address this limitation the research team has, during the course of this project, bulked up quantities of seed lots of known levels of CMV-infected seed and healthy seed lots of SP1333 and Hallmark (Table 9). These seedstocks are a valuable asset that can form the basis for future validated field trials to actively determine yield losses associated with sowing CMV-infected lentil seed.

Despite the absence of "virus-free" seed lots of SP1333, Eston and Indianhead for inclusion in the field trials, some definite trends on the impact of CMV-infected seed levels at sowing on CMV incidence in lentil crops and on the subsequent reduction in yield were observed in the eight field trials conducted across two growing seasons. Overall, across all sites during 2022, the mean incidence of CMV in the treatment plots was highest in SP1333 (26%) followed by Indianhead (8%) and Eston (15%), while the mean incidence in control plots (PBA Hallmark, PBA Hurricane and PBA Jumbo 2) was only 1% (Figure 3A).

Similarly in 2023 across all sites, the mean incidence of CMV in the treatment plots was highest in SP1333 (20%) followed by Indianhead (13%) and Eston (10%), while the mean incidence in control plots (CIPAL 2122, PBA Highland XT and PBA Jumbo 2) was 3% (Figure 3B). At three of the four sites the incidence of CMV in Indianhead and Eston treatment plots was much higher than the control plots. These results suggest that either the TBIA test did not detect CMV in the seedlings tested, or enough seedlings were not grown out to detect a low CMV seed transmission rate such as 0.5 % (e.g. 200 seeds), 0.1 % (e.g. 1000 seeds) or lower. Collectively these results further demonstrate the risk associated with sowing CMV-infected lentil seed.

The mean incidence of CMV infection in trial plots was highest in SP1333, which had 1% and 3% CMV seed infection in 2022 and 2023, respectively, despite SP1333 seed infection being only 1% in 2022 compared to Eston (2% CMV infected seed) and Indianhead (3% CMV infected seed). This suggests that SP1333 is more susceptible to CMV infection and highlights a key factor in understanding the impact of CMV on lentil yield: there is variability in the tolerance and/or resistance to CMV infection between lentil cultivars.

There is also a clear trend showing that CMV infection in lentils has reduced yield in diseased plants and that the higher the percentage of CMV infected seed that is sown results in a higher incidence of CMV in the lentil crop. For example, in the 2022 field trials, the mean yield across all trial sites in control plots was significantly higher than the CMV-infected treatment plots. At the Horsham site the yield for the control plots for Hallmark, Hurricane and Jumbo2 was 3380, 3035 and 3663 grams per plot, compared to the treatment plots for SP1333 (1% CMV infected seed), Eston (2% infected seed) and Indianhead (3% infected seed) of 1635, 1589 and 2163 grams per plot, respectively.

Overall, the mean yield across all trial sites was significantly higher in the three control plots that were sown with seed with 0% CMV-infection levels when compared to the treatment plots that had been sown with CMV-infected seed levels ranging from 0% to 3% (Figure 3C, 3D). However, it is difficult to estimate the impact of CMV in lentil seed due to the absence of a true control plot of 0% seed sown of the corresponding lentil cultivar. For example, the mean yield of the three CMV-infected treatment plots was only significantly lower than two of the three control treatment plots in 2022 (Figure 3C). In glasshouse experiments seed number and weight metrics were compared between CMV-infected and healthy plants for six lentil cultivars (Table 7). In this experiment, the reduction in yield between healthy and infected seed ranged from 10% for Commando to 93% for Cassab, with CMV infected Eston plants (which was one of the CMV-infected cultivars used in both the 2022 and 2023 field trials) yielding 27% less weight of seeds harvested when compared to healthy Eston plants. Together these results demonstrate the negative impact of CMV infection on lentil seed yield.

During the course of this project, while testing field plants and lentil seedlings to detect CMV using TBIA, it became apparent that the level of sensitivity of this assay was not sufficient to detect low titre CMV infections. For example, the TBIA test did not detect CMV in the seeds collected from the aphid inoculated control Hallmark plots in the 2022 field trials while a CMV PCR assay (conducted by a commercial diagnostic lab: Crop Health Services) detected CMV in two Hallmark seed lots with virus levels of 0.5% and 1.5% respectively. In total, over 150 PCR assays were conducted on lentil samples and while the results were mostly equivalent between the two assays, the PCR assay did detect CMV in several samples that tested negative by TBIA. This result is not unexpected as PCR is widely reported to be more sensitive than TBIA for detecting many plant viruses. We recommend that the TBIA assay is used for broad scale surveillance activities where large numbers of plants are sampled, while for detailed biological analyses on the CMV-Lentil pathosystem, the PCR test is used for detection.

Twenty cultivars were infected with CMV by aphid transmission with variable rates of transmission ranging from 1-71% being recorded (Table 6). Four of the twenty cultivars Herald, Highland, Nipper and Nugget) were not infected with CMV via aphids. Importantly these zero transmission rates were generated in the same experiment where CMV viruliferous aphids infected 71% of lentil seedlings of the cultivar Jumbo. These results clearly demonstrate variation in the tolerance of lentil cultivars to CMV infection with four of the cultivars potentially showing resistance to CMV infection by aphids.

Harvested seed from CMV-positive lentil plants from nine lentil cultivars was germinated and the resultant seedlings were tested by TBIA to determine the rate of seed transmission of CMV. CMV was detected in seedlings from only two of the nine cultivars and the transmission rate was 7% and 2% respectively. These preliminary results further demonstrate varying cultivar responses to CMV infection. It is recommended that a more detailed analysis for potential disease resistance to CMV in lentil germplasm is conducted with the aim to generate a disease resistance score that can be used by both growers as a management strategy and for breeders to screen lentil germplasm for CMV resistance.

There are many other variables that need to be considered relating to the CMV-lentil pathosystem (Figure 12) to improve management of CMV in lentils. To help growers manage disease levels in lentil associated with CMV infection, a Risk Matrix Guide was developed as a part of this project. Several biological factors that impact directly on lentil yield as a result of CMV infection were taken into consideration, including the levels of CMV infection in the seed sown and the tolerance/susceptibility levels of the lentil cultivar to CMV infection.

Following are some generalisations that can be used by growers based on the risk matrix:

1. An effective control strategy for growers is to plant lentil cultivars that are resistant to CMV infection.

2. An effective control strategy for growers is to sow lentil seeds with zero levels of CMV infected seed. In this scenario the only source of CMV inoculum is from migratory aphids moving from CMV infected alternate hosts onto the lentil crop, and for this infection event to happen early in the growing season to have a negative impact on lentil yield.

3. There is a moderate risk to growers to plant lentil seed with 1% CMV infection for moderately resistant, tolerant or moderately susceptible lentil cultivars, while susceptible cultivars will have a measurable reduction in yield. These impacts will be influenced by the abundance of aphid vectors in the crop, particularly early in the season.

4. There is a major risk to growers to sow tolerant, moderately susceptible or susceptible cultivars of lentil with 2-3 % infection levels of CMV in seed at sowing.

5. There is a major risk to growers to sow moderately resistant, tolerant, moderately susceptible or susceptible cultivars of lentil with greater than 3% infection levels of CMV in seed at sowing.

Informed management strategies driven by the Risk Matrix guide will have a long-term outcome of reducing the levels of, and yield losses resulting from, the viral disease caused by CMV in the lentil cropping system.

CONCLUSION

This project has shown that there is significant variation in lentil cultivar tolerance to CMV infection as well as negative impacts on lentil yield due to increased levels of CMV infection at sowing. Our field and glasshouse trials have demonstrated that planting CMV-infected seed is a yield risk to lentil crop. Farmers should sow virus-free or virus-tested seed.

As a result of these findings a Risk Matrix Guide has been generated and is based on the degree of risk of sowing varying levels of CMV-infected seed, the degree of lentil cultivar susceptibility, tolerance and/or resistance to CMV infection and the rate of aphid and/or seed transmission. Our findings indicate that sowing lentil seed with 1% CMV infection is a moderate risk for the grower if lentil cultivars with moderate resistance, tolerance or moderate susceptibility to CMV infection are planted. Planting lentil seed with less than 1% CMV infection for most lentil cultivars will likely provide a minimal risk to lentil yield reduction for growers (e.g. 0.5% CMV infection for lupins is recommended in low risk areas), while planting lentil cultivars that are resistant to CMV infection is the most effective management strategy.

Recommendations from the risk matrix for growers to consider include:

- Test seed for CMV before sowing and sow seeds with less than 0.1% CMV infection OR avoid sowing seed infected with CMV.
- Grow lentil varieties that are resistant to CMV infection, if available.
- Utilise crop rotation to avoid CMV-infected volunteer plants growing from CMV-infected seed from previous crops throughout the crop.
- Before sowing, remove weeds and volunteer hosts (which can be reservoirs for CMV and the aphids that transmit it) from within and around the crops.
- Monitor for aphids and virus symptoms throughout the season.
- Apply insecticides to try to control or repel incoming aphids, if required, noting that insecticides are less effective against non-persistent viruses.

It is recommended that further research on the CMV-Lentil pathosystem is required, particularly in accurately quantifying yield loss associated with CMV infection, the development of a disease resistance score for lentil germplasm, the development CMV disease resistance screening tools to be provided to support breeding programs and an assessment of potential CMV resistant lentil germplasm that can be used by growers to effectively manage this disease.

RECOMMENDATIONS

For farmers/agronomists:

Recommendation 1: We recommend that growers consult the CMV-Lentil Risk Matrix Guide to support management decisions for controlling CMV disease in lentils.

Recommendation 2: We recommend that sowing lentil seed with less than 1% CMV infection in low risk areas and/or sowing lentil cultivars that are moderately resistant to resistant to CMV infection are two important management strategies for growers.

For future research:

Recommendation 1: Develop disease resistance scores for CMV infection of lentil germplasm. Lentil germplasm should be challenged with CMV infection and the level of susceptibility, tolerance or resistance to infection should be measured using as many different CMV isolates as possible. Biological variables to be considered in developing the disease resistant scores include CMV seed transmission, CMV aphid transmission, symptomatology, yield reduction.

Recommendation 2: Develop diagnostic tools and phenotyping protocols for CMV infection in lentils to be used by breeding programs to assess lentil germplasm for CMV resistance.

Recommendation 3: Investigate the virulence and severity of CMV strains that infect lentils in the southern region of Australia. This data is essential to establish effective phenotyping protocols for the lentil breeding programs.

Recommendation 4: Develop optimal diagnostic tools and protocols, including sampling, for the detection of CMV in lentils (plants and seed) and identify when and how to use these protocols (e.g. TBIA for broadscale surveillance activities; PCR assays for biological assessments of CMV within the CMV-lentil pathosystem).

Recommendation 5: Investigate the efficacy of aphid species to acquire and transmit CMV to and from lentils. Presence and abundance of aphid vectors in the lentil cropping system will greatly influence the incidence of CMV within a lentil crop. Understanding which aphids effectively transmit CMV will inform management practices for growers.

Recommendation 6: Examine the optimal seasonal conditions that influence aphid vector abundance and critical infection periods that result in significant grain yield reduction in the lentil crop.

Recommendation 7: Obtain more precise data on yield reductions resulting from sowing CMV-infected lentil seed. Using the healthy and CMV-infected seed stocks generated in this study, conduct field trials to accurately determine the impact of CMV infection on lentil yield.

Communication and extension activities

Aftab M, **Trebicki P, Shunmugam A, Nancarrow N and Rodoni B** (2023) Impact of sowing CMV-infected seed om growth and yield of lentil (Abstract) in 24th Australian Plant Pathology Conference 20-24 November 2023 National Vine Centre Adelaide.

Aftab M, **Trebicki P, Nancarrow N, and Rodoni B** (2023) Identification of flying aphid species in Victorian grain crops (Abstract) in 54th Australian Entomology Conference 12- 15 November 2023, The University of Western Australia Albany campus.

Aftab M, Nancarrow N, Shunmugam A, Trebicki P and Rodoni B (2023). Effect of sowing CMV-infected lentil seed on growth components and yield. Poster presented in 12thInternational Congress of Plant Pathology 20-25 August 2023 Lyon, France.

Aftab M, Nancarrow N and Trebicki P (2022) Tropical dry beans, new hosts for seedborne viruses. 14th Australian Virology Workshop 5-7 December 2022 Melbourne.

Aftab M, Nancarrow N and Trebicki P (2022) Presence of seed-borne viruses in tropical dry beans (Abstract) submitted in "International Advances in Plant Virology" 5-7 October in Ljubljana, Slovenia.

Aftab M, Nancarrow N and Trebicki P (2022) Aphid population dynamics and diversity in southern Australia. Hemipteran-plant interaction symposium 7-9 December 2022 Melbourne.

Nancarrow N, Aftab M, Hollaway G, Rodoni B and Trebicki P (2022) The quantification of yield losses caused by non-symptomatic turnip yellows virus infection in lentil and field pea in south-eastern Australia (Abstract) submitted in "International Advances in Plant Virology" 5-7 October in Ljubljana, Slovenia.

Trebicki P, Aftab M and Nancarrow N (2022) Agronomist training workshop for plant disease identification. October 4-6 at Grains Innovation Park 110 Natimuk Road Horsham.

Nancarrow N, Aftab M (2023) Viral diseases- incidence and reducing their impact. Elders Rural Broadacre Agronomy Conference. The Horsham Golf Club, Horsham, Victoria, Australia: 31st July-2nd August 2023.

Nancarrow N, Aftab M (2023) Viruses (display and brief talk). Horsham Field Crops Pathology Early Season Field Day/ Disease Update. Grains Innovation Park, Horsham, Victoria, Australia: 19th July 2023.

Podcasts

A GRDC PodCast was presented in October 2022 on "Minimising the impacts of CMV particularly in lentil". This PodCast can be found on the GRDC website: 0DKDXIBR/Podcasts%20-%20GRDC.html.

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