FINAL REPORT

UM00041

Wheat curl mite, wheat streak mosaic and high plains virus: detection, transmission, epidemiology and management

PROJECT DETAILS

PROJECT CODE:	UM00041
PROJECT TITLE:	WHEAT CURL MITE, WHEAT STREAK MOSAIC AND HIGH PLAINS VIRUS: DETECTION, TRANSMISSION, EPIDEMIOLOGY AND MANAGEMENT
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Summary

Wheat streak mosaic virus (WSMV) and high plains virus (HPV) (Potyviridae - now wheat mosaic virus (WMV)) are major pathogens of wheat (*Triticum aestivum*) in cropping regions around the world. These diseases cause average yield losses of approx. 5% and complete losses occur annually in localised areas. Since 2005 significant outbreaks have led to yield losses annually in all wheat growing states and territories of Australia. The ability to devise effective control strategies hinges on the availability of information on the biology and ecology of the wheat curl mite (WCM)(the primary vector) and virus epidemiology. This information is currently limited. Project UM00041 was initiated to enhance our knowledge in these key areas.

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Conclusions

The outputs of project UM00041 add greatly to our understanding of (i) WCM biology and ecology and its role in the transmission of WSMV and HPV, (ii) how to address spread of WSMV and HPV seed transmission, and (iii) the *Polymyxa graminis* vector of soil-borne wheat viruses.

University of Melbourne (UoM):

* Genetic analyses confirm that WCM is a complex of 'host specific' species. This suggests that more targeted green-bridge management practices can potentially provide substantial economic and environmental benefits.

* Multiple mite species are likely to be responsible for the transmission of viruses in different cereal varieties such as wheat, barley and oats. These will require independent management consideration in the future.

* WCM on Australian wheat are identical to those in wheat crops in North America, South America and Europe. This international equivalence suggests opportunities to combine research efforts with international research groups to devise internationally effective management strategies.

* WCM1 appears to be a vector of WSMV and HPV and is likely to spread viruses to some extent. However, this threat is likely to be minimal compared to threats associated with WCM2. WCM2 is highly viruliferous and dominant across the Australian grainbelt, as shown by field surveys in 2011-2012. This suggests all wheat growing regions are at significant risk of virus outbreaks.

* WCM1 and WCM2 both appear to be carriers of HPV. However, WCM2 is likely to be the primary vector given it was the dominant and possibly only species of WCM present in HPV infected wheat crops.

* Varying degrees of WCM resistance were recorded in 42 wheat varieties. Levels of resistance ranged from complete and moderate resistance to susceptible. This provides growers with the opportunity to grow specific wheat varieties that minimise risks of mite infestations and yield losses from WSMV and HPV.

University of WA (UWA)/Department of Agriculture and Food, Western Australia (DAFWA):

* A real time polymerase chain reaction (RT-PCR) test to detect and quantify WSMV infection in bulk wheat seed samples was developed and validated. It establishes the percentage of WSMV infection in newly germinated seedlings grown from samples of growers' bulk seed wheat stocks and is suitable for use by national seed testing laboratories.

* RT-PCR protocols were developed for testing wheat samples to detect (i) HPV (now wheat mosaic virus) in wheat seed stocks, (ii) three soil-borne wheat viruses (wheat spindle streak mosaic virus

(WSSMV), soil-borne cereal mosaic virus (SBCMV), and soil-borne wheat mosaic virus (SBWMV)) in wheat leaf samples, and (iii) the soil-borne wheat virus vector *P. graminis* in wheat root samples. All these protocols are suitable for use by diagnostic testing laboratories in different Australian states.

*Accurate detection of WSMV and HPV in seed stocks allows growers to avoid sowing infected seed and so remove a major source of both viruses for their spread to and within wheat crops. Detection protocols for soil-borne viruses and their vector means the industry is ready to deal with future incursions of these damaging wheat viruses.

* Multiple introductions of *P. graminis* into Australia were shown to have occurred. The finding of four phylogenetically distinct temperate types in Western Australia (WA) and a subtropical type in Queensland (QLD) within just a small number of wheat samples collected at different locations indicates *P. graminis* is well established in cereal growing areas, especially in WA.

*While no wheat soil-borne viruses were found in surveys in WA, should these viruses arrive in Australia via imported seed or soil, they are likely to become established readily in wheat growing areas as their vector is already present.

Recommendations

Findings from phylogenetic analysis confirm WCM is a complex of host-specific species. Further research is needed to confirm the grass hosts of WCM found on cereals. Discovery of a species complex complicates control of WCM, WSMV and HPV. Mite species differ in biology and ecology so control strategies must take account of such differences. Presence of cryptic species has previously complicated management of the blue oat mite (BOM)/*Penthaleus* species complex. Species within this complex were treated identically in terms of pesticide control, leading to failures due to different species having different pesticide tolerances. WCM control will probably also require strategies that accommodate species differences.

Two WCM species co-occur on wheat hosts but only one is likely to be a major threat to the grains industry. Laboratory assays suggest both species are WSMV and HPV vectors but only WCM2 is a confirmed vector. Field surveys confirm only a weak association between WCM1 and viruses, so WCM1 poses a minimal threat as it is progressively being displaced by WCM2 across the entire wheatbelt. All wheat growing regions are at increased threat of virus outbreaks following WCM2 establishment. Future WCM control measures should be focused on WCM2.

WCM is an effective disperser with high colonisation potential (project UM00029), so options other than 'green-bridge' management may be needed for effective WCM control. Finding new sources of mite resistance in wheat provides a practical future control option. WCM resistant wheat varieties are important as WCM is implicated as the primary vector of at least five cereal pathogens. Resistance has the potential to provide a holistic management option for controlling mites and so several yield reducing viruses. Additional research is needed before this becomes a commercially viable management option.

Sowing infected wheat seed has resulted in wide dissemination of WSMV and HPV around Australia. Infected seed also provides the principal source of virus infection for WCM to acquire and spread WSMV (DAW00210 and DAW00229). This applies to spread to wheat volunteers pre-season and to crops during the growing season, with seed-infected wheat seedlings scattered throughout the crop. The most critical control measure to employ against any virus spread by a vector is removal of the local virus infection source; since spread of infection by vectors always declines rapidly over distance from such sources. This means that, with green bridge control one month before sowing seed, avoiding wheat grain being left in the paddock after harvest and seed testing of seed samples prior to sowing are the most important control measures to control seed-borne virus infection. Research with WSMV (DAW00210 and DAW00229) has shown this to be so with WSMV but similar epidemiological studies are urgently required to determine whether it also applies with HPV, especially in WA, where HPV is now occurring most often.

The wide distribution of the *P. graminis* vector in the WA wheatbelt is cause for considerable concern because of the likelihood of rapid spread of damaging soil-borne wheat viruses should they be introduced from overseas. Research is urgently needed to address this problem and to understand the epidemiology of *P. graminis* in WA wheatbelt conditions to enable development of strategies to avoid introduction of soil-borne viruses via contaminated seed or soil.

Outcomes

Economic benefits
This project presents significant economic benefits through providing a framework for effective, sustainable management strategies for minimising WSMV associated yield losses in Australia. Most notably this project (i) identifies new sources of mite resistance in wheat varieties that provide opportunities to minimise or eliminate risks of infection from yield reducing pathogens , (ii) delivers a RT-PCR wheat seed testing protocol that quantifies levels of WSMV in wheat seed stocks and is suitable for use by seed testing laboratories in different Australian states and (iii) demonstrates the presence of soil-borne wheat virus vector *P. graminis* at several sites in the WA wheatbelt.

This project has also clarified the taxonomy of WCM, finding it to be a complex of numerous host-limited species. This provides an important framework for future control strategies and suggests green-bridge management can be more targeted, leading to economic gain through reductions in the frequency of herbicide application. It has also delivered three other RT-PCR protocols for testing wheat samples to detect (i) HPV (now wheat mosaic virus) in wheat seed stocks, (ii) three soil-borne wheat viruses (WSSMV, SBCMV and SBWMV) in wheat leaf samples, and (iii) the soil-borne wheat virus vector *P. graminis* in wheat root samples. All these protocols are suitable for use by seed testing laboratories in different Australian states. Accurate detection of WSMV and HPV in seed stocks allows growers to remove a major source of both viruses for their introduction to and spread within wheat crops. Detection protocols for soil-borne viruses of wheat and their vector means the industry is now fore-armed to deal with incursions of any of these viruses.

Environmental Benefits
Finding WCM to be a complex of host-limited species suggests future green-bridge management can be more focused and the application of herbicides can be reduced. Herbicide use can be reduced further if mite resistant wheat varieties identified in this study are adopted for future cultivation.

Social Benefits
WSMV has the potential to devastate wheat crops. This can have social consequences. Effective management strategies arising from the findings of this and previous GRDC funded studies will benefit local farming communities in WSMV-prone districts indirectly via greater profitability that will result from greater reliability of wheat production. This will ensure continued viability and the possibility of future wheat industry expansion.

Soil-borne wheat viruses are a major cause for concern should they become established. Work done as part of this project has shown the vector for soil-borne wheat viruses is already present so there is a likelihood of rapid spread should they become introduced. The testing protocols developed will assist in eradication campaigns to eliminate them and so help ensure continued industry profitability

Achievements/Benefits

The increasing prevalence and spread of WSMV in Australia, coupled with recent confirmed cases of HPV, in Western and eastern Australia, have highlighted the necessity to fully understand the biology, ecology and genetics of the primary vector, WCM. The UoM and DAFWA have already contributed significantly to the understanding of WCM biology and WSMV epidemiology through previous GRDC projects (UM00022, UM00029, DAW00141, DAW00210). As identified by GRDC, continuation of the work conducted using previous grants has the potential to deliver substantial additional benefits to grain growers.

UoM: A number of important discoveries have significant implications for the management of WCM and associated viruses in Australian grains. In 2011-12 the UoM led a team of international mite experts to assess genetic variation and phylogenetic relationships among WCM from four continents and a wide range of host plants. WCM appears to be a complex of numerous 'host specific' species and a taxon in need of major taxonomic revision. These findings have significant implications for control of WCM in Australia and overseas, particularly within the context of identifying plants that form 'green bridge' refuges and vectors of cereal pathogens. This study indicates that green-bridge management practices can potentially be more targeted, providing substantial economic and environmental benefits. Patterns of international equivalence suggest that opportunities to combine research efforts with international research groups to devise internationally effective management strategies are now possible. These findings also indicate that multiple mite species are likely to be responsible for the transmission of viruses in cereals and to require independent management. Additional research will be needed to confirm the vector status of these species and their host ranges.

UoM: Previous results generated under project UM00029 identified two WCM lineages coexisting on wheat hosts. Laboratory experiments showed that only one of these, WCM2, was a likely vector of WSMV. This project also identified signs of regional dominance, with WCM1 and WCM2 dominating WA and eastern Australia respectively. Field and genetic surveys conducted in 2011 and 2012 under project UM00041 show that the viruliferous WCM2 has become dominant in WA, indicating increased risk of WSMV infection in this part of the country. Clarifying the vector status of WCM1 was a major objective of this study. WCM1 has been confirmed as capable of carrying the virus and contributing to the spread of WSMV in some capacity but was found to be weakly associated with virus presence in the field. This suggests WCM1 is a minor threat to the Australian grains industry. However, the prolific nature of WCM2 across the entire grain belt suggests all states and territories are at high risk of virus outbreaks.

UoM: In 2012-13 a comprehensive laboratory experiment was conducted in collaboration with CSIRO to test for mite resistance in 42 wheat varieties. Many of these varieties displayed resistance to WSMV. Rigorous laboratory trials identified significant differences in resistance, with some varieties exhibiting complete or moderate resistance and others highly susceptible to mite infestation. Given WCM is recognised as the primary vector of at least four cereal viruses, these trials are particularly important. This study provides growers with the opportunity to grow wheat varieties that minimise risks of mite infestations and yield losses from associated viruses. This has the potential to provide significant economic and environmental benefits to the grains industry.

UoM: Since 2010 only three incidences of HPV have been recorded in Australia. In each case these have involved dual infection with WSMV. Although infrequent, this is of concern because this combination of viruses has been shown to have devastating impacts on yields in the USA. The low incidence of HPV complicated efforts to obtain isolates for transmission experiments. To supplement these shortfalls, the project team conducted several experiments that have provided insight into the transmission of HPV in Australia. Field surveys confirm WCM2 as the dominant lineage at HPV infected sites, although ribonucleic acid (RNA) assay indicated both species are potential carriers of the virus. Experimental attempts to isolate the virus through seed found no evidence of seed transmission. These findings are preliminary and warrant more rigorous follow up research. However, this might suggest that mites are likely to be the primary vector of HPV.

UWA/DAFWA and UoM: Since 2010 six incidences of HPV have been officially recorded; one in northern Victoria (VIC), one in western VIC and, in 2012-2013 (GRDC project DAW00210), four in WA. In each case, these involved dual infection with WSMV. This is of concern as this combination of viruses has been shown to have devastating impacts on yields in the USA. In 2012, DAFWA successfully demonstrated WCM transmission by HPV (GRDC project DAW00210 - Coutts *et al*. 2014), and in 2014 its transmission through wheat seed (GRDC project DAW00229). Trials at UoM provide additional insight into HPV transmission: (i) WCM1 and WCM2 both occur at HPV infected sites, although WCM2 appears dominant, and (ii) an RNA assay which detects HPV in WCM indicates both species are potential HPV vectors. Seed transmission studies by UWA/DAFWA and UoM confirmed earlier published DAFWA data on seed transmission of WSMV. Seed transmission probably plays a much greater role in the spread of WSMV than currently realised, emphasising the importance of the DAFWA seed testing services for wheat growers.

UWA/DAFWA: To test bulk wheat seed for seed-borne WSMV infection, a representative seed sample must be germinated and leaf material extracted for virus testing. A previously developed WSMV bulk dry seed RT-PCR test for wheat was highly sensitive at detecting the presence of WSMV on the outside of the seeds (GRDC project DAW 00141), but this gave no information about the rate of WSMV seed-borne infection in the sample. Currently the most widely used method for testing wheat seedlings for WSMV is an enzyme linked immunosorbent assay (ELISA) test using up to 10 leaves grouped together in a single sample but this is a time consuming and labour intensive way to test large numbers of samples. Seed transmission rates of WSMV in bulk wheat seed can be very low, but any internal source of infection in a wheat crop can become significant if temperature and conditions are favourable for the WCM to spread the virus. 5,000-10,000 seedlings may need to be tested from a bulk wheat sample to determine if seed-borne WSMV is present.

UWA/DAFWA: PCR detection methods have far greater sensitivity than ELISA, which means far larger numbers of samples can be grouped together for testing, making the process faster and more efficient. RT-PCR uses fluorescently labelled DNA probes to further increase assay sensitivity and specificity, data are acquired during the reaction, reducing overall assay time, and multiple pathogens can be targeted in a single tube assay. A major aim of this project was to develop an RT-PCR test to detect and quantify WSMV infection in bulk wheat seed samples. Optimum conditions for growing seedlings for testing were established and a bulk RNA extraction method for processing up to 10,000 seedlings in one sample developed and optimised. Methods of decontaminating extraction equipment were evaluated and optimised to prevent RNA carry over contamination between samples and to ensure accuracy of WSMV detection. Consistent results were obtained for detection of a single WSMV infected seedling in 5,000 and 10,000 bulk seedling leaf samples and on a range of bulk samples simulating different levels of seedling infection up to 1%. RT-PCR primers and probes were designed for WSMV and tested for specificity and sensitivity. Standard curves were generated and used for reliable quantification of WSMV in 5,000 seedling samples (0.02-1%). Validation of assay was carried out by simultaneously testing bulk WSMV infected seed using ELISA and RT-PCR and comparable seed transmission results were obtained from the two methods.

UWA/DAFWA: To determine WSMV inherent seed transmission rates of different varieties seed must be obtained from plants with 100% infection. Ten wheat varieties were planted in field plots with WSMV infected plants to provide sources of WSMV infection. However, conditions were too cold for adequate spread of the virus by WCM. Wheat plants of seven varieties were then grown in pots in the glasshouse and all plants were inoculated with WSMV, tested to confirm infection and seed collected for grow out testing. Between 2,000 and 5,000 seedlings of each variety were tested but no seed-borne WSMV was found in any of the seed tested. Seed transmission had been known to occur in some of these varieties previously, so it is concluded that the conditions of the glasshouse experiment, possibly the temperature or age at which the plants were infected, were not favourable for seed transmission of WSMV to occur.

UWA/DAFWA: HPV was first found in wheat in WA at two sites in 2012 (Coutts et al. 2014). In 2013, it was found at two additional WA sites. HPV causes the same symptoms as WSMV, has the same WCM vector and the two viruses are often found in co-infection. Molecular diagnostic methods for HPV are limited because only a small section of the HPV genome had previously been sequenced. DAFWA sourced samples from four isolates of HPV in wheat (each from a different site), all of which had co-infection with WSMV. Sequencing was carried out using next generation sequencing technology. Full genome sequences were obtained for two HPV isolates and partial genome sequences for the other two. From this sequence information, primers and probes were developed for an RT-PCR test to detect HPV infection in bulk seed samples. The bulk leaf extraction method developed for the WSMV seed test was used and the assay was optimised so it could be combined with the WSMV test to detect both viruses in a single tube. Validation was carried out on samples simulating seed infection by spiking bulk leaf seedling samples with HPV infected dried leaf material, as no sources of known infected wheat seed were available at that time. The assay was shown to reliably detect all four HPV isolates found in WA and an imported HPV USA strain.

UWA/DAFWA: In 2010, *P.graminis* was reported infecting roots in a barley crop in QLD. *P. graminis* is a vector of several soil-borne cereal viruses including three viruses of wheat: SBCMV, SBWMV and WSSMV. These viruses have not been reported to occur in Australia but cause significant losses in wheat crops in many other parts of the world. This project was expanded to include a survey in WA for *P. graminis* and these three soil-borne wheat viruses and to develop diagnostic tests for them. Dried sources of *P. graminis* infected root and leaf samples of the soil-borne wheat viruses were obtained from QLD or overseas for use as positive controls. Assays for the detection of *P. graminis* in root samples and three soil-borne viruses in bulk leaf samples were evaluated. Surveys were conducted in the 2011 and 2012 growing seasons. Random 100 leaf samples were collected from 74 wheat crops in the WA wheatbelt and tested for the three soil-borne viruses using RT-PCR but none were found. Soil and root samples were also collected. *P. graminis* was detected by PCR in wheat root samples from Northam, Esperance and South Perth field plots. Results were confirmed by DNA sequencing and microscopic visual identification. This is the first detection of *P. graminis* in WA and the first outside QLD. Sequence analysis revealed that more than one type of *P. graminis* was present at each location. Five different *P. graminis* sequences were obtained from WA and QLD samples, with the WA samples including temperate type isolates similar to *P. graminis* sequences from Europe. The QLD sequence was significantly different, matching an isolate reported from sorghum in Africa. Soil bait experiments were carried out using soil from three WA sites where *P. graminis* was found to infect wheat roots and the presence of *P. graminis* in the roots was confirmed by PCR. RT-PCR methods for detection of *P. graminis* in root samples were developed and confirmed to detect the range of *P. graminis* types found in Australia.

UWA/DAFWA: To improve diagnostic methods for soil-borne wheat viruses, a multiplex RT-PCR assay to detect SBCMV, SBWMV and WSSMV in wheat leaf material was developed. The primers and probes were designed to detect a range of isolates of these viruses that occur in other parts of the world. The assay was tested on imported frozen dried leaf material and proved very reliable.

UWA/DAFWA: Laboratory protocols for the RT-PCR detection of WSMV, HPV and three soil-borne wheat viruses in bulk wheat leaf samples and for RT-PCR detection of *P. graminis* in wheat roots have been written and made available for use by virus seed testing laboratories across Australia.

Other research

Finding WCM to be a complex of multiple host-specific species has significant management implications. Species differ in their biology and ecology and these differences need to be considered when devising control strategies (refer to issues with the *Penthaleus* species complex). Different WSMV susceptible cereals such as wheat, barley and oats harbour unique mite species so additional research will be needed to confirm the vector status of these species and their host ranges. This will help focus management efforts, including the identification of hosts that act as green-bridge refuges.

New sources of WCM mite resistance in wheat have the potential to prevent the spread of yield-reducing pathogens. This is an area of research that needs much greater exploration. Sowing mite resistant wheat varieties offers a management approach. Previous work shows that WCM is an effective disperser and coloniser, so green-bridge management is likely to be largely ineffective in some cases. Providing growers with mite resistant wheat varieties is an effective method for mitigating risks of infection from multiple viruses and providing rapid economic and environmental benefits. Additional research that investigates methods of exploiting new sources of resistance by transfer onto homoeologous wheat chromosomes is needed in order to make this a commercially viable management option. Extending the resistance trials across more wheat varieties will provide greater opportunity for increasing the number of WCM resistances available to wheat breeders and potentially opening the way for resistance gene stacking to achieve protection that is greater and more ecologically durable.

Sowing infected wheat seed has resulted in wide dissemination of WSMV and HPV around Australia and provides the principal source of virus infection for WCM to acquire and spread WSMV (DAW00210 and DAW00229). This applies to spread to wheat volunteers pre-season and to crops during the growing season from seed-infected wheat seedlings scattered through the crop. As with all viruses spread by vectors, the most critical control measure to employ is removal of the local virus infection source, since spread of infection by vectors always declines rapidly over distance from an infection source. This means that, with green bridge control one month before sowing seed, controlling seed-borne virus infection through avoiding wheat grain being left in the paddock after harvest and seed testing of seed samples prior to sowing are the most important measures to control seed-borne virus infection. Research with WSMV (DAW00210 and DAW00229) has shown this to be so with WSMV, but similar epidemiological studies are urgently required to determine whether it also applies with HPV, especially in WA, where HPV is now occurring most often. Such information will greatly assist development of virus minimising control measures.

The wide distribution of the *P. graminis* vector in the WA wheatbelt is cause for considerable concern because of the likelihood of rapid spread of damaging soil-borne wheat viruses should they be introduced from overseas. Research is urgently needed to address this problem and to understand the epidemiology of *P. graminis* in WA wheatbelt conditions, to enable development of strategies to avoid introduction of soil-borne viruses via contaminated seed or soil.

Intellectual property summary

UoM research outputs from project UM00041 are intended for the public domain, consequently any IP is not envisaged.

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