

Department of Primary Industries and Regional Development





milestone report

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Managing Subterranean Clover Red Leaf Syndrome in Western Australia Stage 1 Final Report

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Abstract

There has been concern amongst livestock producers regarding recent outbreaks of subterranean clover red leaf syndrome of which the most likely primary cause is infection by the Soybean dwarf virus (SbDV). To improve our understanding of the epidemiology of SbDV we undertook an autumn sampling program in 2018 to determine which plant species harbour SbDV between growing seasons. A total of 6749 live plant samples were collected and tested from 22 locations. SbDV was only found at one of the locations in strawberry (*Trifolium fragiferum* L.) and white clover (*T. repens*). Migrating aphids, which are the vector for the virus, were not found at any of the sites where sticky traps were deployed. Based on these results and previous investigations, if an outbreak occurs this year (2018) it is likely to be in spring when aphid populations build up. Further research under controlled glasshouse conditions is required to determine if common broadleaf weeds, and native and exotic *Fabaceae* plant species are either over summer or growing season hosts of SbDV.

In addition to the autumn 2018 survey, aphids trapped in 2017 were tested for SbDV and found to regularly have the SbDV virus particularly in September suggesting that virus is relatively common in aphids in spring.

The subterranean clover red leaf syndrome factsheet has been completed and is currently being disseminated to the livestock and seed industry. Monitoring of pastures for the syndrome will continue throughout the 2018 growing season.

There are still significant gaps in our understanding of the subterranean clover red leaf syndrome. We recommend that future research focuses on the tolerance of legume species and cultivars to the virus, identifying the over summer hosts of SbDV is a priority and the development of disease management tools.

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1 Milestone description

1.1 Milestone 2

Report to MLA and AWI on summer sampling program to identify which plant species harbour the SbDV virus outside the growing season.

2 Project objectives

- 1. A factsheet for producers providing background information on the SbDV virus, how to identify infected plants, methods to reduce infection and how to manage the sub clover red leaf syndrome **by March 2018**.
- 2. Report to MLA and AWI on summer sampling program to identify which plant species harbour the SbDV virus outside the growing season by April 2018.
- 3. As part of the extension and communication model there will be raised awareness of the risks of SbDV in subterranean clovers amongst producers and advisors by April 2018.

3 Success in meeting the milestone

3.1 Background

There has been concern amongst livestock producers regarding recent outbreaks of subterranean clover red leaf syndrome. Symptoms include red leaves; stunted plants and even premature plant death. The Department of Primary Industries and Regional Development (DPIRD) investigated the 2017 outbreak finding that of the subterranean clover plants tested 80% with obvious red leaves were infected with *Soybean dwarf virus* (SbDV; synonym -Subterranean clover red leaf virus) compared to just 2% without obvious symptoms. These findings strongly suggested SbDV as a causal agent of subterranean red leaf syndrome.

A critical step to improve our understanding of the epidemiology of SbDV in WA is to determine the plant species that may harbour SbDV between growing seasons. This information will greatly assist in improving management strategies for SbDV.

An autumn sampling program was developed that focused on collecting leaf samples from a range of plant genera and species from properties throughout the south west agricultural zone. The sites included, but were not limited to farms investigated by DPIRD and UWA in spring 2017 and selected sites from DPIRD's green bridge survey. Plant samples were collected and tested for Soybean dwarf virus and sticky traps were placed at selected locations to provide early information on aphid movement into the environment.

3.2 Field survey – autumn 2018

3.2.1 Plant survey

This survey was undertaken to determine if SbDV was present in plant species growing prior to autumn/winter pasture re-establishment. Due to the lack of summer rainfall in many areas, the survey

was delayed until autumn when a green bridge was established. Between March and May 2018, a range of plant genera and species were collected from paddocks throughout the south west of WA. This consisted of predominantly summer active broadleaf weeds, perennial legumes and some subterranean clover, serradella and medic plants that established following a large summer rainfall event. All plant samples were submitted to the DPIRD Diagnostic Laboratory Service (DDLS) and tested for SbDV using real time polymerase chain reaction technique (qPCR).

A total of 6749 samples were collected and tested from 22 locations (Table 1).

Soybean dwarf virus was detected at one location only in two clover species (strawberry and white clover at Torbay on the south coast). This site remains in green pasture year-round.

Although SbDV was only detected in these two legume species at one location, this does not exclude the potential for other plant species tested to be hosts of the virus. To eliminate these species would require them to be challenged by aphids with SbDV in a controlled environment.

3.2.2 Aphid survey

Yellow sticky traps were deployed to catch any winged aphids migrating from over-summer hosts at growers farms located at Mt Barker (3 sites), Narrikup (2 sites), Pingelly (1 site), Kendenup (2 sites), Manypeaks (1 site), South Stirling's (1 site), Torbay (1 site), Gairdner (1 site) and Brookton (1 site) (Table 2). Depending on location, traps were deployed and removed at 10-14 day intervals between 8th March and 22nd May 2018.

The results were that no migrating aphids were found on any of the traps at any site, reflecting the dry March and April period.

Family	Species	Common name	Number of locations	Total no. of samples tested	Number of locations at which SbDV detected
Amaranthaceae	Ptilotus sp.	Ptilotus	1	12	
Asteraceae	Arctothera calendula	Capeweed	1	20	
Asteraceae	Cardus tenuiflorus	Slender thistle	2	21	
Asteraceae	Cirsium vulgare	Spear thistle	2	40	
Asteraceae	<i>Conyza</i> spp.	Fleabane	6	137	
Asteraceae	Helichrysum spp.	Cudweed	4	90	
Asteraceae	Hypochaeris spp.	Flat weed	14	416	
Asteraceae	Lactuca serriola	Prickly lettuce	2	34	
Asteraceae	Silybum marianum	Scotch thistle	6	152	
Asteraceae	Sonchus asper	Prickly sow thistle	2	37	
Asteraceae	Sonchus oleraceus	Sow thistle	10	225	
Asteraceae	Vellereophyton dealbatum	None	1	40	
Boraginaceae	Heliotropium spp.	Heliotrope	1	20	
Brassicaceae	Brassica napus	Canola	3	82	
Brassicaceae	Lepidium africanum	Common pepper cress	1	20	
Caryophyllaceae	Polycarpon tetraphyllum	Four-leaf allseed	4	31	
Chenopodiaceae	Dysphania pumilio	Goosefoot	11	319	
Chenopodiaceae	Dysphania sp.	Crumbweed	1	10	

Table 1. Plant species collected from 22 locations (March-May 2018) through the south-west of Western Australia and tested for Soybean dwarf virus.

Cucurbitaceae	Citrullus lanatus	Afghan melon	11	240	
Cucurbitaceae	Cucumis myriocarpus	Paddy melon	7	146	
Euphorbiaceae	Euphorbia spp.	Spurge	1	1	
Fabaceae	Chamaecytisus palmensis	Tagastaste	1	12	
Fabaceae	Lotus angustissimus	Narrowleaf trefoil	2	25	
Fabaceae	Medicago polymorpha	Burr medic	1	40	
Fabaceae	Medicago sativa	Lucerne	2	24	
Fabaceae	Ornithopus sativus	Serradella	2	90	
Fabaceae	Trifolium repens	White clover	2	20	Detected (2)
Fabaceae	Trifolium fragiferum	Strawberry clover	1	50	Detected (1)
Fabaceae	Trifolium spp.	Clover	10	820	
Fabaceae	Trifolium subterraneum	Sub clover	22	653	
Geraniaceae	Erodium botryts	Stork's bill	1	30	
Geraniaceae	Erodium cicuiarium	Stork's bill	1	30	
Geraniaceae	Geranium spp.	Geranium	2	66	
Lamiaceae	Mentha pulegum	Penny royal	1	60	
Malvaceae	Malva parviflora	Marshmallow	4	140	
Phytolaccaceae	Phytolacca octandra	Inkweed	1	30	
Polygonaceae	Persicaria maculosa	Red shank	2	50	
Polygonaceae	Polygonum spp.	Wireweed	12	870	
Polygonaceae	<i>Rumex</i> spp.	Dock	16	710	
Polygonaceae	Polygonum patulum	Tree hogweed	1	110	
Polygonaceae	<i>Rumex</i> spp.	Sorrel	2	95	
Solanaceae	Solanum nigrum	Nightshade	12	420	
Unknown	Unknown	Mintweed	4	240	
Unknown	Unknown	Plantago	1	40	
Unknown	Unknown	Unknown	3	31	
Total		44	22	6749	3

Table 2. Sticky traps located at various pasture paddocks in the south-west of Western Australia in autumn 2018.

Location	Date 1 st trap set	First sticky trap collected	Aphids caught
Mt Barker 1	8 th March	22 nd May	Nil
Mt Barker 2	8 th March	22 nd May	Nil
Narrikup 1	8 th March	18 th May	Nil
Narrikup 2	8 th March	18 th May	Nil
Kendenup 1	15 th March	22 nd May	Nil
Manypeaks	15 th March	18 th May	Nil
Mt Barker 3	21 st March	22 nd May	Nil
Kendenup 2	21 st March	22 nd May	Nil
South Stirlings	28 th March	18 th May	Nil
Torbay	6 th April	1 st May	Nil
Gairdner	12 th April	18 th May	Nil
Brookton	7 th March	9 th April	Nil

3.3 Conclusions

In the autumn of 2018, SbDV was only found at one of the 22 locations sampled, and migrating aphids were not found at the sites where sticky traps were deployed.

Previous observations and anecdotal evidence suggests that the incidence of SbDV and red leaf clover syndrome varies from year to year. This also occurs with other viruses that are closely related to SbDV, such as Turnip yellow virus that infects canola. The yearly variation in virus prevalence due to the build-up and movement of aphids carrying the virus into pasture stands. Increases in aphid populations are favoured by years with summer and autumn rains, promoting plant growth and thus an increase in aphid numbers early in the growing season. However, in seasons in which this period is dry, it is likely that SbDV epidemics will be delayed to spring.

During the spring 2017 survey of red clover where the aim was to identify the causal agent of the red clover syndrome clover plants with typical 'red clover syndrome' symptoms and plants without obvious symptoms were tested for a number of viruses. A small number of clover plants were found to be infected with TuYV, but these plants did not have red clover syndrome symptoms.

Previous investigations (Coutts et al. 2006; Coutts et al. 2011) have shown virus epidemics can occur even when no or very little virus can be found in the local environment prior to crop emergence. It may be that virus reservoirs exist in native bushland and these plants were not included in the surveys. Alternatively the virus titre in the plants collected is not sufficient to be detectable by the testing methods used and the plants tested are hosts of SbDV.

Although results from this autumn 2018 survey show SbDV present at one location and in two perennial plant species, SbDV may still infect sub clover plants later in the 2018 season as aphid populations build up and carry the virus from external infected plants into pastures. Importantly, the lack of migrating aphids found on the sticky traps, combined with the low incidence of SbDV found highlights the relationship between summer rainfall and presence of green vegetation for aphid and SbDV to survive between growing seasons. However, without the aphid vector and disease being found in the species surveyed, we cannot exclude the plant species tested in this survey as potential hosts of SbDV.

Further research is needed to conclusively determine if the common broadleaf weed and native *Fabaceae* plant species present in pasture growing areas are hosts of SbDV as we cannot rule out that those sampled in this project simply were not exposed to aphids with SbDV. This work would need to be done under controlled glasshouse conditions in which these plant species could be exposed to aphids carrying the virus. An improved understanding of SbDV host range, will allow development of informed disease management tools that are effective and sustainable.

4 Overall progress of the project

4.1 Factsheet

A professionally designed factsheet has been produced and is currently available on the DPIRD and AWI website and has been inserted into this document below. Promotion of the factsheet is currently underway through DPIRD, UWA, MLA and AWI media.

https://www.agric.wa.gov.au/crop-diseases/subterranean-clover-red-leaf-syndrome-causedsoybean-dwarf-virus https://www.wool.com/globalassets/start/on-farm-research-and-development/production-systemseco/pastures/weed-and-pest-management/sibi-subterranean-clover-red-leaf-syndromev2.pdf?mkt_tok=eyJpljoiTXprNVpEZzROVFF4TldGaClsInQiOiJwWTYxajhzUkg2VXQ2TzVsSmRwNktJZjR MQVIEZHVWWVh1S2ZQRDEweUZ4NFVcLzIVTIImTUhOZ2dvb3R3Y2FOT0NLbnNQK1RjcFBcLzdVSkxTK 2F3WVdqc2VKeUNNUkdLMnFzSURZRnJLSEtGTytWYUpzWG13ZVZCazhqbUtMbWdqIn0%3D



Subterranean clover red leaf syndrome (SbDV)



4.2 Testing of 2017 aphid traps for SbDV

As part of a DPIRD project to investigate the use of new molecular diagnostic technology to detect virus in aphids, yellow sticky traps were deployed along fence lines to monitor the movement of aphids carrying a canola infecting virus *Turnip yellows virus*. Samples were recently tested retrospectively for SbDV and it was detected in migrating aphids around Mt Barker, Kendenup, Gairdner, Munglinup and Gibson, particularly in September 2017. These aphids were likely migrating from clover pastures in search for new hosts. This finding suggests that SbDV is relatively common in aphids in these locations in springtime and spreads to summer hosts during this period.

4.3 Extension

The following extension activities have been conducted or are planned in addition to those reported in milestone 1.

- A sub clover red leaf syndrome webpage has been posted on the DPIRD website. The page covers background, symptoms, biology and management as well as providing a links to the DPIRD Diagnostic Laboratory Services, factsheet and AWI and MLA online survey. https://www.agric.wa.gov.au/crop-diseases/subterranean-clover-red-leaf-syndrome-caused-soybean-dwarf-virus
- An article entitled 'Sampling program to investigate sub clover red leaf syndrome' was published in the April 2018 edition of The UWA Institute of Agriculture Newsletter, refer to page 11. <u>http://www.ioa.uwa.edu.au/______data/assets/pdf__file/0007/3102685/WEB-IOA-News-April-_______</u>

<u>http://www.ioa.uwa.edu.au/ data/assets/pdf_file/0007/3102685/WEB-IOA-News-April-</u> 2018 E.pdf

- A section in the upcoming issue of DPIRD's Protecting WA Crops Newsletter is dedicated to covering the sub clover red leaf syndrome. https://www.agric.wa.gov.au/newsletters/pwac/protecting-wa-crops-may-2018-issue-10
- Paul Sanford gave a presentation at the ASheep Annual General Meeting on the 21st of June 2018 that provided an update from DPIRD and UWA on the sub clover red leaf syndrome.
- Paul Sanford will be speaking to the AgVivo consultant training day about sub clover red leaf syndrome on the 19th July 2018 at Kojonup.
- Copies of the factsheet have been provided to consultants Paul Omodei and Phil Barrett-Lennard to circulate to their clients. Copies are in the process of being circulated to farmer groups.

The growing season commenced late this year and we are monitoring pastures for signs of the syndrome via the producer contacts we have established and have responded with advice and assessment accordingly. To date many producers have contacted us with suspected cases all of which have tested negative. In addition we are maintaining the network of sticky insect traps to provide an early warning of aphid movement which we can report via the media including the DPRID PestFax reporting service.

4.4 Recommendations/future work

Current and future work consists of;

- 1. Monitoring newly establishing sub clover based pastures and responding if producers report the symptoms of red leaf syndrome.
- 2. Undertake a pilot plot trial at the Torbay property within the strawberry and white clover pasture that tested positive to SbDV in our survey. The aim of the trial is to determine the reactions (sensitivity and susceptibility) of a range of annual and perennial legumes to SbDV infection, assess their potential as hosts for the virus and test the effectiveness of an antifeeding insecticide to control virus infection.
- 3. Continue to inform the livestock industry about how to identify and manage the syndrome as well as improving our current understanding of the causes.

There is still significant gaps in our understanding of the sub clover red leaf syndrome and over summer host and the susceptibilities of our pasture species and cultivars and therefore we recommend the following future work to better understand this issue;

- 1. Further research is needed to determine if the common broadleaf weed and native *Fabaceae* plant species present in pasture growing areas are hosts of SbDV. This would need to be done initially under controlled glasshouse conditions. An improved understanding SbDV host range, will allow development of informed disease management tools to target the disease at its source in the field.
- 2. Rigorous field testing of a range of management practices and insecticide options to prevent an outbreak of the syndrome for example monitoring aphids and effectiveness of anti-feeding insecticides.
- 3. Analysis of historical information on disease occurrence combined with climate data that may improve our ability to predict aphid movements prior to an outbreak.
- 4. Assessment of annual and perennial legumes species and cultivars tolerance to SbDV and their potential of these species as hosts for the virus.
- 5. How much additional pasture could be produced if producers controlled insect populations?

5 Conclusions

The objective for this milestone 2 has been achieved. The summer/autumn sampling program has being successfully completed and the majority of producers are aware of the issue and where to seek additional help including testing of leaf samples for possible infection with SbDV. In addition, the factsheet has been completed and is currently being distributed and promoted. The project teams advice to producers is to continue to monitor for aphids in their pastures however, with low aphid migration detected so far, the likelihood of the an outbreak is higher now in spring than in the winter of 2018.

6 Acknowledgements

We thank pasture growers for assisting with this survey, DPIRD staff for collecting samples and DDLS staff for assisting with processing and testing samples.

7 References

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