

Vector species, pasture legume host range, and impact on grain legumes of an Australian soybean dwarf virus isolate

Benjamin Steven Congdon (✉ benjamin.congdon@dpird.wa.gov.au)

Western Australia Department of Primary Industries and Regional Development <https://orcid.org/0000-0001-7242-7765>

J.R. Baulch

Western Australia Department of Primary Industries and Regional Development

K.J. Foster

University of Western Australia Faculty of Natural and Agricultural Sciences: The University of Western Australia Faculty of Science

Research Article

Keywords:

Posted Date: August 1st, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1891688/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Archives of Virology on January 3rd, 2023. See the published version at <https://doi.org/10.1007/s00705-022-05664-8>.

Abstract

Soybean dwarf virus (SbDV; family *Tombusviridae*, genus *Luteovirus*, species *Soybean dwarf virus*) can cause damaging disease epidemics in cultivated Fabaceae. The biological characteristics of SbDV isolate WA-8, including its vector species, host range and potential impact on Australian grain legume cultivars, were investigated in a series of glasshouse experiments. WA-8 was determined to be a YP strain isolate as it was transmitted by *Acyrtosiphon pisum* (pea aphid) and *Myzus persicae* (green peach aphid) and infected known strain indicator species. Of the 18 pasture legume species inoculated with SbDV, 12 were SbDV hosts including eight previously unrecorded as hosts. When inoculated with SbDV, field pea (*Pisum sativum*), faba bean (*Vicia faba*), lentil (*Lens culinaris*) and narrow-leaved lupin cv. Jurien were most susceptible (70 to 100% plant infection rates), and albus lupin (*Lupinus albus*), chickpea (*Cicer arietinum*) and narrow-leaved lupin cv. Mandelup were less susceptible (20 to 70%). Over the course of three experiments, chickpea was the most sensitive to infection, with > 97% reductions in dry above ground biomass (AGB) and 100% reductions in seed yield. Field pea cv. Gunyah, faba bean and lentil were also sensitive with 36 to 61% reductions in AGB. Field pea cv. Kaspera was tolerant with no significant reduction in AGB or seed yield. The information generated in this study provides important clues in understanding SbDV epidemiology and suggests it has potential to threaten to grain legume production in Australia, especially if the impacts of climate change promote its spread.

Introduction

Soybean dwarf virus (SbDV; family *Tombusviridae*, genus *Luteovirus*, species *Soybean dwarf virus*) is a plant virus that primarily infects the Fabaceae and is transmitted solely by aphids in a persistent, circulative and non-propagative manner [47]. SbDV isolates are classified into four strains YP, YS, DP and DS which differ in important biological characteristics including their predominant vector species, host range and symptomatology. For example, YP and DP strains are transmitted primarily by *Acyrtosiphon pisum* Harris (pea aphid) [2, 33, 51], and YS and DS strains transmitted primarily by *Aulacorthum solani* Kaltentbach (foxglove aphid) [49]. *Myzus persicae* Sulzer (green peach aphid) has been shown to transmit P strains only [11, 16, 46]. Furthermore, only the Y strain readily infects white clover (*Trifolium repens* L.), albus lupin (*Lupinus albus* L.) and common bean (*Phaseolus vulgaris* L.) and only D strain isolates readily infect red clover (*Trifolium pratense* L.) [9, 22, 40, 48]. When analysed at the molecular level, these strains form separate phylogenetic groups [49, 50]. Many SbDV isolates collected from Australian grain-growing regions that underwent whole genome sequencing and phylogenetic analysis grouped with known YP strain isolates suggesting this is a common strain in Australia [6]. However, there is no biological data from these isolates to confirm their strain classification.

Legumes form an integral component of the pasture feed base of Australia's \$12.3 billion wool, dairy and red meat production industries [38]. A vast range of annual and perennial legume pasture species are utilised including clovers (*Trifolium* sp.), annual medics (*Medicago* spp.) and lucerne (*Medicago sativa* L.), serradellas (*Ornithopus* spp.) and biserrula (*Biserrula pellicinus* L.), with subterranean clover (*T. subterraneum* L.) being the most widely utilised [42]. Studies on SbDV in mainland Australia have primarily focussed on the substantial damage it causes to subterranean clover pastures [19, 20, 33]. Since its first report causing a serious 'red leaf disease' in northern Victoria, SbDV (initially known as 'subterranean clover red leaf virus') has caused sporadic disease epidemics across the country occasionally resulting in total pasture collapse [20, 29, 31, 33, 45]. Host range studies and field surveys have shown SbDV can infect a wide range of other pasture legume species including red clover, white clover, strawberry clover (*T. fragiferum* L.), annual medics, lucerne and vetch (*Vicia* spp.) [2, 23, 27, 34, 37, 47]. However, it is unknown whether several other species (e.g., serradellas and biserrula) that are grown in pasture swards or act as weeds in grain focussed systems are SbDV hosts. To better understand its epidemiology, the host range of SbDV requires further examination as these hosts could act as infection reservoirs for epidemics in more economically important legume pastures and grain legume crops.

As both cash and break crops, grain legumes are a vital component of Australia's broadacre cropping industry worth >\$1.5 billion to its economy. The predominant grain legume crops sown are narrow-leaved lupin (*L. angustifolius* L.), field pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* L.) and faba bean (*Vicia faba* L.) [1]. SbDV can infect all these species [2, 14, 23, 24, 27, 35]. A review of the literature suggests there is just one study examining the impact of SbDV on grain legumes; field trials conducted in Syria Makkouk and Kumari [36] demonstrated that SbDV could cause up to 56% yield loss in faba bean and > 90% in lentil. Apart from these, a couple of studies demonstrate a significant impact on the yield of vegetable varieties of some of these species [24, 25]. Two important characteristics of a host species that greatly determines its potential for virus-induced losses in the field are its (i) susceptibility/resistance i.e. the degree to which the virus can infect and multiply in the host and (ii) sensitivity/tolerance i.e. the disease reaction of the host once infected with a virus [7]. No studies have assessed these characteristics following SbDV inoculation in Australian grain legume cultivars. This would provide important baseline data to assess the potential impact of SbDV in Australian grain legume crops.

This study (i) determines the strain classification of SbDV isolate WA-8 by investigating vector species and inoculating indicator plant species, (ii) assesses the host range of WA-8 among 18 annual and perennial pasture legume species, and (iii) assesses the reaction of two cultivars each of field pea, faba bean, lentil, chickpea, narrow-leaved lupin and albus lupin to SbDV inoculation.

Materials And Methods

Glasshouse details, aphid colonies, SbDV cultures and inoculation. All plants were grown in commercial premium potting mix (Baileys, Australia) inside a naturally lit air-conditioned glasshouse kept at 15°C to 25°C located at South Perth, Australia (31°59'22"S, 115°53'09"E). For aphid colonies and SbDV infected source material, faba bean cv. Fiord and subterranean clover cv. Dalkeith were used, respectively. SbDV isolate WA-8 was obtained from subterranean clover in 2018 at Torbay, Western Australia (35°01'36"S, 117°38'57"E). Leaf samples from plants infected with SbDV were used as positive controls, and uninfected plants as negative controls in quantitative reverse transcription polymerase chain reaction (qRT-PCR) and reverse transcription loop-mediated isothermal amplification (RT-LAMP). Colonies of *A. pisum* (Supplementary Figure S1), *A. kondoi* Shinji (blue green aphid) and *M. persicae* were maintained inside aphid rearing cages (Bugdorm, Australia) kept in an air-conditioned controlled environment room (maintained at 20°C with a 16 h photoperiod). For inoculations, aphids were given a 48 h acquisition access period (AAP) on a SbDV culture plant before being transferred to test plants using a fine-tipped paintbrush and given a 48 h inoculation access period (IAP). These time periods are deemed optimal for SbDV transmission [8]. All plants were then treated with an imidacloprid soil drench (0.125g/litre) to eliminate aphids.

RNA extraction, qPCR and LAMP.

All RNA extractions of test plants in experiments were done using the QIAGEN RNeasy plant mini-kit according to manufacturer instructions (QIAGEN, Australia). Based on previous research indicating SbDV reaches highest concentration in younger tissue [21], only the younger leaves of plants were sampled for testing in this study.

The set of qPCR primers and probe and LAMP primers developed and used to test for presence of SbDV infection in this study were derived from the ORF1 gene nt sequence of SbDV isolate WA-8 (Table 1).

Table 1
Soybean dwarf virus (SbDV) reverse transcription quantitative polymerase chain reaction (RT-qPCR) and loop-mediated isothermal amplification (RT-LAMP) primers and probes derived from ORF1 nt sequence of SbDV isolate WA-8 developed for this study.

Assay	Primer	Sequence 5' to 3'
qPCR-WA-8	Fwd	CATGTGCTACGACGCTATGT
	Rev	CAAATTCGTGCTTGGCGATTAG
	Probe	TEX615/ACAGTTCTCAAGTGCCGAACACGA/BHQ_2
LAMP-WA-8	F3	GAGTTTGAGTGGGAAGTCA
	B3	GGAGGGTCTGGGTCCATT
	LF2	GCGCTCAAATTCGTGCTTGG
	LB2	GCAGTTTCCGGTCGTCTGA
	FIP	ATGGGGCGCCATCCCTTAACCCGAACACGACGTGCTAA
	BIP	AGACATGGCCACAACCATGTGCGCTTCTTGAGCTGCTACCTG

All RT-qPCR reactions were done using the QIAGEN Rotor-Gene multiplex RT-PCR kit on the QIAGEN Rotor-Gene Q (QIAGEN, Australia). In a total volume of 25 µL, the reaction mixture contained 1 µL of total RNA extraction template, 12.5 µL of 2X QIAGEN Rotor-Gene Multiplex PCR Master Mix, 1 µL of Rotor-Gene Reverse Transcriptase Mix, 0.5 µL of each primer and 1.25 µL of probe 10 µM stock, and 5 µL of RNase free water. The SbDV assay was run on the orange channel, and an internal control assay which amplified the NADH-ubiquinone oxidoreductase chain 5 (*nad5*) mitochondrial gene run on the yellow channel [4, 39]. The cycling conditions were initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15 s, and annealing and elongation at 60°C for 15s. The threshold was manually set at 0.5 and a positive result was indicated by a Ct value of ≤ 30, an indeterminate result if 31 to 35 and negative if > 35 or no Ct value given. Indeterminate samples were retested using RT-LAMP.

All RT-LAMP reactions were done using a dual-block (eight reaction wells per block) Genie® II instrument (Optigene, United Kingdom). In a total volume of 25 µL, the reaction mixture contained 1 µL of total RNA extraction template, 15 µL of ISO-004RT master mix (Optigene, United Kingdom), 0.5 µL each of F3 and B3, 2 µL FIP and BIP and 1 µL LF2 and LB2 10 µM primer stock, and 2 µL of RNase free water. The reaction mixture was incubated at 65°C for 40 minutes followed by an annealing step for 10 minutes. Results were analysed in real-time *via* amplification and annealing graphs. A sample was considered positive if fluorescence exceeded 10,000 and peaked within the incubation time, and annealing temperatures within 1°C of those of the positive controls.

Vector species of SbDV isolate WA-8.

In repeat experiments 1a and 1b, ten *A. pisum*, *A. kondoi* and *M. persicae* per plant were used to inoculate ten subterranean clover cv. Dalkeith plants and five plants were left uninoculated as negative controls. At 21 and 35 days after inoculation (DAI), all plants were checked for leaf reddening symptoms and leaf samples were tested for SbDV infection by RT-qPCR.

Experimental host range of SbDV in pasture legumes.

In experiment 2a, seedlings of 29 cultivars representing 18 pasture legume species (Table 3) were grown in small pots (160 mm high by 130 mm diameter). At the two leaf growth stage, plants were inoculated with SbDV using five aphids per plant. Just one cultivar per species was used except for subterranean clover (nine cultivars), French serradella (*O. sativus*, three cultivars) and red clover (two cultivars). Due to seed availability and germination rate differences, the number of plants inoculated varied between cultivars. In experiment 2b, cultivars with ≤ 2 plants infected in experiment 2a were inoculated with 10 aphids per plant. In both experiments, each set of plants was split into two and inoculated at separate times as repeats. Two plants per cultivar were left uninoculated as negative controls. At 21 and 35 DAI, tip leaf samples from all plants were tested for SbDV infection by RT-LAMP or RT-qPCR.

Susceptibility of grain legumes and common bean.

In experiments 3a, seedlings of field pea cvs Kaspera and Gunyah, faba bean cvs Fiord and Samira, chickpea cvs Genesis and Hattrick, lentils cvs Hurricane and Jumbo2, narrow-leaved lupin cvs Mandelup and Jurien and albus lupin cvs Luxor and Amira, and common bean vegetable cvs Borlotti and Simba were grown in small pots (160 mm high by 130 mm diameter). Ten plants of each cultivar were inoculated with SbDV using five aphids per plant at the two to three-leaf growth stage. A further five plants of each cultivar were left uninoculated as negative controls. At 21 and 35 DAI, all plants were tested for SbDV infection by RT-qPCR and RT-LAMP. Experiment 3b was a repeat of experiment 3a.

Sensitivity of grain legumes.

In experiments 4a to 4c, seedlings of grain legume cultivars were grown in large pots (230 mm high by 270 mm diameter) and organised into a factorial randomized block design. In experiment 4a, field pea cv. Kaspera, faba bean cv. Fiord, lentil cv. Hurricane, chickpea cv. Hattrick and subterranean-clover cv. Dalkeith were tested. In experiments 4b and 4c, field pea cv. Gunyah, faba bean cv. Samira, lentil cv. Jumbo2 and chickpea cv. Genesis were tested in addition to those tested in experiment 4a. Subterranean-clover was not tested in experiment 4c. The appropriate rhizobium (ALOSCA Technologies, Australia) for each species was applied immediately after sowing to the soil surface. Up to 10 plants of each cultivar were inoculated with SbDV at the two to three-leaf growth stage, and an equal number of plants were left uninoculated as controls. At 21 and 35 DAI, all plants were tested for SbDV infection by RT-LAMP and RT-qPCR. Plants that were inoculated but did not become infected were treated as uninfected plants in statistical analysis. Plants were observed for symptoms throughout their life. Once plants senesced, they were harvested to measure growth variables. In all experiments, pods were removed, and the remaining oven dried above ground biomass (AGB) was weighed. Furthermore, depending on the cultivar and the experiment, the pods were counted, and for each plant ten pods were randomly selected to measure their length and the number of seeds in each. Pods were threshed to obtain seed, and the weight of 20 randomly selected seeds and total seed yield were measured for each plant. Pod and seed data were not available or incomplete for every cultivar in every experiment because of rodent damage to pods (field pea, lentil and chickpea in experiment 4b), poor growth and podding in some cultivars (lentil in experiment 4c) or because AGB was the primary focus (subterranean clover).

A paired two-sample T-test was used to analyse differences in individual variables between infected and uninfected plants of each cultivar. Multiple linear regression was used to examine relationships between growth variables for each species. To enable comparison across cultivars and experiments, the difference in AGB between the infected and uninfected plant in each block for all three experiments was calculated. Statistical analysis on mean AGB reduction across all cultivars was performed using analysis of variance (ANOVA) and Tukey's honest significant difference (HSD). All statistical analyses were conducted in RStudio 1.4.1717 (RStudio PBC, USA).

Results

Vector species of SbDV isolate WA-8.

In experiments 1a and 1b, SbDV was detected in all plants inoculated using *A. pisum* and in 20% and 30% of plants, respectively, inoculated using *M. persicae*. SbDV was not detected in plants inoculated using *A. kondoi* (Table 2).

Table 2

Subterranean clover (*Trifolium subterraneum*) plants infected with soybean dwarf virus (SbDV) following inoculation with ten aphids per plant^a.

Species	Expt 1a	Expt 1b
<i>Acyrtosiphon pisum</i>	10/10 ^a	10/10
<i>Myzus persicae</i>	2/10	3/10
<i>Acyrtosiphon kondoi</i>	0/10	0/10
^a Number of plants infected/number of plants inoculated. Aphids given a 48 h acquisition access period on a SbDV-infected subterranean clover plant before being transferred to test plants for a 48 h inoculation access period. Plants tested 21 and 35 days after inoculation by RT-qPCR.		

Experimental host range of SbDV in pasture legumes.

Following inoculation using five aphids per plant, SbDV was detected in 30 to 100% of plants of the eight subterranean clover cultivars, and in 71 to 100% plants of the three French serradella cultivars (Table 3). SbDV was detected in 20 to 83% of plants of strand medic (*M. littoralis*), Moroccan clover (*T. isthmocarpum*), barrell medic (*M. trunculata*), balansa clover (*T. michelianum*), strawberry clover, sulla (*H. coronarium*) and Persian clover (*T. resupinatum*). Following inoculation using five aphids per plant, SbDV was detected in 4 to 15% of plants of biserulla, arrowleaf clover (*T. vesiculosum*) and white clover, and 20 to 100% of plants of these species when they were inoculated with ten aphids per plant. Following inoculation using either five or ten aphids per plant, SbDV was not detected in plants of the three red clover cultivars, or running postman (*Kennedia prostrata*), lucerne, tiger-snake vine (*K. nigricans*), sainfoin (*Onobrychis viciifolia*) and bladder clover (*T. spumosum*).

Table 3
Soybean dwarf virus (SbDV) infection in pasture legume cultivars following aphid-inoculation

Species	Common name	Cultivar/line	Plants infected ^a	
			5 aphids	10 aphids
<i>Biserrula pelecinus</i>	Biserulla	Casbah	2/13	2/10
<i>Hedysarum coronarium</i>	Sulla	Flamenco	3/6	
<i>Kennedia nigricans</i>	Tiger-snake vine		0/6	0/8
<i>Kennedia prostrata</i>	Running postman		0/21	0/15
<i>Medicago littoralis</i>	Strand medic	Angel	24/29	
<i>Medicago sativa</i>	Lucerne	SARDI 10	0/29	0/17
<i>Medicago truncatula</i>	Barrell medic	Caliph	14/20	
<i>Onobrychis viciifolia</i>	Sainfoin	Othello	0/5	0/8
<i>Ornithopus sativus</i>	French serradella	Cadiz	25/25	
<i>Ornithopus sativus</i>	French serradella	Erica	19/20	
<i>Ornithopus sativus</i>	French serradella	Margurita	15/21	
<i>Trifolium fragiferum</i>	Strawberry clover	Palestine	11/20	
<i>Trifolium isthmocarpum</i>	Moroccan clover	APG 18	13/18	
<i>Trifolium michelianum</i>	Balansa clover	Bartolo	7/10	
<i>Trifolium pratense</i>	Red clover	NSE	0/19	0/19
<i>Trifolium pratense</i>	Red clover	NFE	0/11	0/10
<i>Trifolium repens</i>	White clover	Haifa	1/23	4/4
<i>Trifolium resupinatum</i>	Persian clover	Prolific	4/20	
<i>Trifolium spumosum</i>	Bladder clover	Bartolo	0/20	0/7
<i>Trifolium subterraneum</i>	Subterranean clover	Dalkeith	15/20	
<i>Trifolium subterraneum</i>	Subterranean clover	Dinninup	16/20	
<i>Trifolium subterraneum</i>	Subterranean clover	Leura	6/6	
<i>Trifolium subterraneum</i>	Subterranean clover	MCD 60	12/20	
<i>Trifolium subterraneum</i>	Subterranean clover	Riverina	12/20	
<i>Trifolium subterraneum</i>	Subterranean clover	Seaton Park	6/20	
<i>Trifolium subterraneum</i>	Subterranean clover	Trikkala	10/20	
<i>Trifolium subterraneum</i>	Subterranean clover	UniWager	14/15	
<i>Trifolium subterraneum</i>	Subterranean clover	Woogenellup	8/20	
<i>Trifolium vesiculosum</i>	Arrowleaf clover	Cefalu	2/21	1/3

^aNumber of plants infected/number of plants inoculated pooled over two repeat inoculations. *Acyrtosiphon pisum* given a 48 h acquisition access period on a SbDV-infected subterranean clover plant before being transferred to test plants for a 48 h inoculation access period. Plants tested 21 and 35 days after inoculation by RT-LAMP or RT-qPCR.

Susceptibility of grain legume cultivars.

In both experiments 3a and 3b, 74 to 100% of aphids remained alive at the end of the 48h IAP on field pea and lentil plants and 32 to 74% on faba bean plants (Table 4). By 35 DAI, SbDV was detected in 80 to 100% of plants of field pea, lentil and faba bean. For chickpea, 2 to 24% aphids remained on plants and SbDV was detected in 20 to 40% of plants. For narrow-leaved lupin, 16 to 52% of aphids remained on plants. SbDV was detected in 70 to 90% of cv. Jurien plants and 20 to 70% cv. Mandelup plants. For albus lupin, 1 to 3% aphids remained on plants and SbDV was detected in 40 to 60% of plants. SbDV was detected in 20 to 40% of common bean plants.

Table 4
Aphids remaining following SbDV-inoculation and subsequent SbDV infection on cultivated legume species

Species	Common name	Cultivar	Experiment 2a		Experiment 2b	
			% aphids ^a	Plants infected ^b	% aphids	Plants infected
<i>Pisum sativum</i>	Field pea	Kaspa	100	10/10	100	10/10
<i>Pisum sativum</i>	Field pea	Gunyah	74	10/10	100	10/10
<i>Vicia faba</i>	Faba bean	Fiord	74	10/10	32	7/10
<i>Vicia faba</i>	Faba bean	Samira	66	8/10	38	9/10
<i>Lens culinaris</i>	Lentil	Hurricane	100	10/10	90	10/10
<i>Lens culinaris</i>	Lentil	Jumbo2	88	10/10	90	10/10
<i>Cicer arietinum</i>	Chickpea	Hatrick	24	3/10	2	2/10
<i>Cicer arietinum</i>	Chickpea	Genesis	22	2/10	8	4/10
<i>Lupinus angustifolius</i>	Narrow-leafed lupin	Mandelup	52	2/10	20	7/10
<i>Lupinus angustifolius</i>	Narrow-leafed lupin	Jurien	40	7/10	16	10/10
<i>Lupinus albus</i>	Albus lupin	Amira	3	5/10	2	4/10
<i>Lupinus albus</i>	Albus lupin	Luxor	2	6/10	2	5/10
<i>Phaseolus vulgaris</i>	Common bean	Borlotti	nr	2/10	nr	3/10
<i>Phaseolus vulgaris</i>	Common bean	Simba	26	4/10	28	4/10
^a <i>Acyrtosiphon pisum</i> given a 48 h acquisition access period on a SbDV-infected subterranean clover plant before five aphids per plant being transferred to test plants for a 48 h inoculation access period. Aphids remaining on the plant alive at end of 48 h IAP recorded for each plant, nr = not recorded.						
^b Number of plants infected/number of plants inoculated. Plants tested 21 and 35 days after inoculation by RT-LAMP or RT-qPCR.						

Sensitivity of grain legume cultivars.

Symptoms

Symptoms associated with SbDV infection were consistent across experiments 4a to 4c. Infected plants of subterranean clover were stunted with chlorotic foliage and developed margin reddening of lower leaves which progressed inward until leaves senesced (Figs. 1a and 1b). Infected plants of field pea cvs Kaspa and Gunyah commonly developed interveinal chlorosis of lower leaves (Fig. 2a). Often infected plants of cv. Gunyah, but not cv. Kaspa, were moderately stunted and chlorotic with distorted leaves (Fig. 3d). Infected plants of both faba bean cultivars developed interveinal chlorosis of lower leaves which, more commonly in cv. Samira, developed into interveinal reddening (Fig. 2c), and were often moderately stunted (Fig. 3b). Infected plants of both lentil cultivars commonly developed interveinal yellowing and distortion of leaves (Fig. 2b) and were almost always stunted (Fig. 3c). Infected plants of both chickpea cultivars developed distorted, chlorotic and reddened foliage (Fig. 2d) and were always extremely stunted and never produced pods, and most eventually died (Fig. 3a).

Experiment 4a.

AGB ($P = .17$), seed weight ($P = .34$) and seed yield ($P = .31$) of ten SbDV-infected plants of field pea cv. Kaspa did not differ significantly from ten uninfected plants, although infected plants had 11% shorter pods ($P < .001$) and 20% less seeds per pod ($P < .001$) than uninfected plants (Tables 5 and 6). Eight SbDV-infected plants of faba bean cv. Fiord had 27% lower AGB ($P = .05$), 12% shorter pods ($P < .001$), 16% lower seed weight ($P = .048$) and 33% lower seed yield ($P = .01$) than 12 uninfected plants. Seeds per pod did not differ significantly ($P = .29$) between infected and uninfected plants. Ten SbDV-infected plants of lentil cv. Hurricane had 60% lower AGB ($P < .001$), 60% lower seed weight ($P < .001$), 20% shorter pods ($P < .001$) and 91% lower seed yield ($P < .001$) than ten uninfected plants. Five SbDV-infected plants of chickpea cv. Hatrick had 97% lower AGB ($P < .001$) than 15 uninfected plants and produced no pods. Ten SbDV-infected plants of subterranean clover cv. Dalkeith had 80% lower AGB ($P < .001$) than ten uninfected plants.

Table 5
Growth variables of soybean dwarf virus-infected (+) and uninfected (-) plants

Growth variables of soybean dwarf virus infected (+) and uninfected (-) plants										
			Number of plants ^a		Dry above-ground biomass (g)			Seed yield (g)		
Species	Cultivar	Expt	+	-	+	-	Ratio ^b	+	-	Ratio
Field pea	Kaspa	4a	10	10	32.6	29.2	0.12	26	27.7	-0.06
		4b	5	5	25.2	24.7	0.02			
		4c	5	5	13.5	18.5	-0.27**	7.6	9.5	-0.20
	Gunyah	4b	5	5	13.4	31.7	-0.58**			
		4c	5	5	6.5	12.5	-0.48**	4.1	11.1	-0.63***
Faba bean	Fiord	4a	8	12	35.4	48.8	-0.27*	25.4	38	-0.33**
		4b	5	5	18.1	48.4	-0.63**	8.1	24.8	-0.67**
		4c	7	8	8.3	12.5	-0.34**	11.3	16.3	-0.31*
	Samira	4b	5	5	19.3	59.0	-0.67**	12.7	36.1	-0.65***
		4c	13	7	7.1	13.3	-0.47*	10.8	17.4	-0.38*
Lentil	Hurricane	4a	10	10	8.2	20.6	-0.60***	0.6	6.87	-0.91***
		4b	5	5	3.6	19.7	-0.82***			
		4c	10	10	0.6	2.0	-0.70***			
	Jumbo2	4b	5	5	3.3	17.2	-0.81***			
		4c	9	8	0.9	1.9	-0.53**			
Chickpea	Hattrick	4a	5	15	0.9	33.6	-0.97***	0	11	-1***
		4b	2	8	0.4	32.0	-0.99***	0	2.4	-1***
		4c	5	6	0.6	33.0	-0.98***	0	5.3	-1***
	Genesis	4b	2	8	0.3	36.3	-0.99***	0	2.0	-1***
		4c	9	6	0.4	45.4	-0.99***	0	9.12	-1***
^a Plants inoculated with SbDV using five to 20 <i>Acyrtosiphon pisum</i> per plant and tested for SbDV infection 21 and 35 days after inoculation. Up to 10 plants inoculated per cultivar per experiment. Plants that were inoculated but did not become infected were used as uninfected plants in statistical analysis.										
^b Ratio of growth variable metric in infected plants to uninfected plants. Asterisks indicate significance at * α = 0.05, ** α = 0.01, and *** α = 0.001 from paired two-sample T-test.										

Table 6
Growth variables of soybean dwarf virus-infected (+) and uninfected (-) plants

Growth variables of soybean dwarf virus infected (+) and uninfected (-) plants																
			Number of plants ^a		Number of pods			Pod length (cm)			Seeds per pod			Seed weight (mg)		
Species	Cultivar	Expt	+	-	+	-	Ratio ^b	+	-	Ratio	+	-	Ratio	+	-	Ratio
Field pea	Kaspa	3a	10	10				4.21	4.72	-0.11**	4.07	5.11	-0.20**	221	225	-0.02
		3b	5	5												
		3c	5	5	11.2	12.8	-0.13									
	Gunyah	3c	5	5	6.4	14.6	-0.56***									
Faba bean	Fiord	3a	8	12	-	-	-	4.48	5.08	-0.12***	1.84	1.91	-0.04	519	619	-0.16*
		3b	5	5	13.2	28.8	-0.54*	4.45	5.02	-0.11***	1.8	1.92	-0.06	416	547	-0.24**
		3c	7	8	14.3	17.6	-0.19	4.39	5.03	-0.13***	1.69	1.88	-0.10	537	530	0.01
	Samira	3b	5	5	9	25.2	-0.64**	5.34	5.88	-0.09**	1.78	1.86	-0.04	765	909	-0.16*
		3c	13	7	7.1	12.1	-0.41*	5.51	5.49	0.004	1.94	1.83	0.06	743	807	-0.08
Lentil	Hurricane	3a	10	10				0.82	1.02	0.20***				11	27	-0.60***
^a Plants inoculated with SbDV using five to 20 <i>Acyrtosiphon pisum</i> per plant and tested for SbDV infection 21 and 35 days after inoculation. Up to 10 plants inoculated per cultivar per experiment. Plants that were inoculated but did not become infected were used as uninfected plants in statistical analysis.																
^b Ratio of growth variable metric in infected plants to uninfected plants. Asterisks indicate significance at * α = 0.05, ** α = 0.01, and *** α = 0.001 from paired two-sample T-test.																

Experiment 4b

AGB of five SbDV-infected plants of field pea cv. Kaspa did not differ significantly ($P = .16$) from five uninfected plants. Five SbDV-infected plants of field pea cv. Gunyah had 58% lower AGB ($P = .01$) than five uninfected plants. Five SbDV-infected plants of faba bean cv. Fiord had 63% lower AGB ($P = .002$), 54% less pods ($P = .04$), 11% shorter pods ($P < .001$), 24% lower seed weight ($P = .01$) and 67% lower seed yield ($P = .01$) than 5 uninfected plants. Seeds per pod did not differ significantly ($P = .78$) between infected and uninfected plants. Five SbDV-infected plants of faba bean cv. Samira had 67% lower AGB ($P < .01$), 64% less pods ($P < .01$), 9% shorter pods ($P < .01$), 16% lower seed weight ($P = .03$) and 65% lower seed yield ($P < .001$) than 5 uninfected plants. Seeds per pod did not differ significantly ($P = .51$) between infected and uninfected plants. Five SbDV-infected plants of lentil cvs Hurricane and Jumbo2 had 82% and 81% lower AGB, respectively, than 5 uninfected plants ($P < .001$). Two infected plants each of chickpea cvs Hatrick and Genesis both had 99% lower AGB than eight uninfected plants and produced no pods. Five SbDV-infected plants of subterranean clover cv. Dalkeith had 62% lower AGB than five uninfected plants ($P < .001$).

Experiment 4c

Five SbDV-infected plants of field pea cv. Kaspa had 27% lower AGB than five uninfected plants ($P = .005$). Number of pods ($P = .25$) and seed yield ($P = .20$) did not differ significantly between infected and uninfected plants. Five SbDV-infected plants of field pea cv. Gunyah had 48% lower AGB ($P = .006$), 56% less pods ($P = .001$) and 63% lower seed yield ($P = .001$) than five uninfected plants. Seven SbDV-infected plants of faba bean cv. Fiord had 34% lower AGB ($P = .004$), 13% shorter pods ($P < .001$) and 31% lower seed yield ($P = .02$) than eight uninfected plants. The number of pods ($P = .12$), seeds per pod ($P = .07$) and seed weight ($P = .42$) did not differ significantly between infected and uninfected plants. 13 SbDV-infected plants of faba bean cv. Samira had 47% lower AGB ($P = .02$), 41% less pods ($P = .04$) and 38% lower seed yield ($P = .049$) than seven uninfected plants. Pod length ($P = .47$), seeds per pod ($P = .22$) and seed weight ($P = .17$) did not differ significantly between infected and uninfected plants. Ten SbDV-infected plants of lentil cv. Hurricane had 70% lower AGB than ten uninfected plants ($P < .001$). Nine infected plants of Jumbo2 had 53% lower AGB than eight uninfected plants ($P = .001$). Five SbDV-infected plants of chickpea cv. Hatrick and nine of cv. Genesis had 98% and 99% lower AGB (both $P < .001$), respectively, than six uninfected plants of each cultivar, and produced no pods.

Relationships between growth variables

In regression analysis, field pea seed yield was predicted by AGB ($R^2 = 0.73$, $P < .001$, Fig. 4a). In lentil cv. Hurricane plants in experiment 4a, seed yield was predicted by AGB ($R^2 = 0.86$, $P < .001$, Fig. 4b), seed weight ($R^2 = 0.54$, $P < .001$), pod length ($R^2 = 0.55$, $P < .001$) and R^2 was increased to

0.81 ($P < .001$) when interactions between these variables were included. Lentil seed weight was predicted by pod length ($R^2 = 0.77$, $P < .001$) and AGB ($R^2 = 0.55$, $P < .001$), and pod length was predicted by AGB ($R^2 = 0.55$, $P < .001$).

In faba bean plants, seed yield was predicted by AGB ($R^2 = 0.44$, $P < .001$, Fig. 4c). The predictive strength of AGB significantly differed between experiments ($P = .047$) but not between cultivars. Seed yield was predicted by the number of pods ($R^2 = 0.64$, $P < .001$) but not by seed weight ($P = .53$). Seed yield was predicted by pod length in experiments 4a and b ($R^2 = 0.15$, $P = .01$) but not in 4c ($P = .69$). Seed weight was predicted by pod length ($R^2 = 0.4$, $P < .001$). There was no relationship between seed weight or pod length, and AGB ($P = .29$ and $.87$, respectively).

Comparative analysis of AGB reduction

Following ANOVA and Tukey's HSD of AGB reduction across all cultivars and experiments, Hattrick and Genesis had significantly higher reductions (98 and 99%, respectively, $P < .003$) than Gonyah (49%), Fiord (36%), Samira (46%), Hurricane (61%) and Jumbo2 (58%), which in turn, had higher reductions ($P < .002$) than Kaspera which did not have reduction significantly different from zero (2.7%, Fig. 5).

Discussion

This study classifies SbDV isolate WA-8 as a YP strain as it was transmitted efficiently by *A. pisum* infects known indicator species white clover, albus lupin and common bean, but not red clover. Using *A. pisum* to inoculate plants of 18 pasture legume species with this isolate, eight previously unknown SbDV host species were identified. These were balansa clover, biserulla, barrell medic, Persian clover, Moroccan clover, arrowleaf clover, sulla and French serradella. Furthermore, by inoculating grain legume species, susceptibility and sensitivity data for cultivars frequently sown in Australia is provided for the first time. Field pea, faba bean and lentil cvs were highly susceptible to infection, and albus lupin and chickpea were less susceptible. Narrow-leaved lupin cv. Jurien was more susceptible than cv. Mandelup. The sensitivity of these cultivars to SbDV infection was also assessed by measuring growth variables, primarily AGB, in infected and uninfected plants across three experiments. Field pea cv. Kaspera was tolerant, field pea cv. Gonyah, faba bean cvs Fiord and Samira and lentil cvs Hurricane and Jumbo2 sensitive, and chickpea cvs Hattrick and Genesis extremely sensitive.

The spread of P-type SbDV strains such as WA-8 is likely to significantly depend on the local abundance and movement patterns of *A. pisum*. However, considering its abundance in several important Australian agricultural regions, *M. persicae*, confirmed to be a vector in this study, could be epidemiologically important and therefore field research investigating its role is required. *A. kondoi*, a common aphid species in Australian grain and pasture legume crops, was not a vector of SbDV WA-8 and, in an earlier study, was not a vector of an S strain isolate [27]. Other aphid species that frequently occur in these systems such as *Aphis craccivora* (cowpea aphid), previously shown to transmit P strain isolates [46], and *Megoura crassicauda* (faba bean aphid) should also be considered. Although results from this study support the established strain classification system, exceptions apparently can occur. Such anomalies could be due to presence of other viruses or SbDV strains which facilitate the transmission of a strain into a non-host or by a non-vector [46, 51]. Furthermore, such mixed infections could facilitate generation of recombinant isolates with unique biological characteristics [46]. Biological characterisation of more isolates coupled with genomic studies on SbDV in Australia is required to establish the relevance of the classification system in Australia and identify any important exceptions.

Of the pasture legume cultivars tested, 22 of 29 were hosts of SbDV WA-8. They represented 12 of the 18 species tested, exemplifying the wide host range of SbDV among the *Fabaceae*. Four of the eight new host species identified were from the genus *Trifolium*, a common host genus in the literature [2, 9, 13, 47]. SbDV is often asymptomatic or produces mild symptoms on many important host species including red clover, white clover, strawberry clover, annual medics and lucerne [2, 10, 17, 27]. A similar lack of obvious symptoms was observed in several of the host species tested in this study. Therefore, any significant yet subtle impact in these hosts becomes difficult to diagnose, and moreover, they could all serve as concealed sources of infection to more economically important hosts. French serradella is a particularly well-suited candidate as a reservoir host as it was highly susceptible yet asymptomatic to SbDV infection and is relatively common as a summer sown pasture or early-season germinating weed [12]. Climate change could exacerbate the risk of SbDV epidemics if the frequency of summer rainfall events increases thus increasing the abundance of virus reservoirs. On the other hand, an increase in summer temperatures may decrease aphid survival and decrease epidemic risk [32]. As non-*Fabaceae* plant families are also potential SbDV hosts [2, 27], other regionally common weed and pasture species should be investigated as potential sources of SbDV infection. No plants of running postman, tiger-snake vine, lucerne, sainfoin, red clover and bladder clover became infected with SbDV in this study. Possibly they are (i) non-hosts of SbDV, (ii) non-hosts of the strain used i.e., red clover, or (iii) resistant and would have become infected with a higher inoculum load. Lucerne has been reported infecting south-west Australian lucerne pastures at low incidences concurrently with *A. pisum* infestation [30]. Furthermore, lucerne was a non-host in some studies with some isolates [27, 33, 47] and therefore should be investigated as an indicator species for strain differences. Although some subterranean clover cultivars had lower infection rates than others e.g., Seaton Park (30%) and Woogenellup (40%), strong resistance was not identified. This reflects results from more extensive resistance screening studies done in the past which did not uncover any promising resistance accessions [26, 28, 33]. Tolerance to SbDV infection was reported in some cultivars including Woogenellup but this might have been resistance to virus accumulation [29]. Both traits would be worthwhile assessing in future resistance screening efforts.

The susceptibility and sensitivity data provided shows SbDV has the potential to be as damaging in many grain legumes as in subterranean clover. SbDV had a significant impact on most growth variables measured in this study, including consistently severe impacts on AGB and seed yield, and in most cases when measured, the number and size of pods and seed weight. SbDV also has severe impact on some of these growth variables in other hosts [3, 15, 24, 25]. Regression analysis indicated that the reduction in AGB causes reductions in seed yield. In this study, SbDV infection symptoms and associated losses in faba bean, field pea, lentil and subterranean clover resembled those described in previous studies. Makkouk and Kumari [36] demonstrated up to 56% seed yield losses in faba bean, and Johnstone [24] and Johnstone and Rapley [25] demonstrated seed yield losses of >80% in broad bean when infected early. In this study, AGB and seed yield reductions in SbDV-infected faba bean plants ranged from 27–67% depending on the experiment. Johnstone [24] also found vast differences in SbDV infection tolerance of green pea varieties, which was also observed between the two field pea cultivars tested in this study. In the one experiment in which it was measured, SbDV reduced seed yield in lentil cv. Hurricane by 91%, similar to those described by Makkouk and Kumari [36], who also showed using resistant lentil cultivars reduced yield losses to ~20%. This is the first study to demonstrate the extreme sensitivity of chickpea to SbDV infection. The sensitivity of the narrow-leaved and albus lupin cultivars was not tested but should be in future research as there are reports that SbDV causes disease in both [2]. The 60 to 80% reduction in AGB of SbDV-infected subterranean clover plants in this study is consistent with field reports and previous glasshouse studies in which fresh AGB was reduced by 50 to 90% [19, 26]. Future research should generate susceptibility and sensitivity data for other potentially vulnerable and economically important cultivated legume species grown in Australia such common bean, peanut (*Arachis hypogaea*) and vetches (*Vicia* spp.).

There are many factors that would influence the likelihood of SbDV damage under field conditions that were not considered in the glasshouse experiments. Firstly, environmental factors such as temperature and light intensity greatly impact symptoms, virus accumulation and infection severity in subterranean clover [18, 19]. Whilst these factors could have contributed to differences in host susceptibility and sensitivity between experiments in this study, they vary to a far greater degree under field conditions and are therefore likely to play a significant role in SbDV epidemiology and impact. Evidently, multiple SbDV strains are circulating in Australian grain-growing regions and susceptibility and sensitivity is likely to differ between them [9]. Furthermore, plants were inoculated at single early growth stage only, possibly when most vulnerable, whilst in field crops virus epidemics progress over multiple growth stages. The inoculation method used was artificial in that it circumvented any alighting preferences that aphids may have in the free choice environment of the field. The differences in aphids remaining on plants following inoculation e.g. 2–24% on chickpea and albus lupin compared to 74–100% on field pea and lentil likely reflect differences host suitability i.e., physical and biochemical traits that have antixenotic or antibiotic effects on the aphid [43, 44]. Due to the nature of luteovirus transmission, crop colonisation by the aphid vector is vital in optimising their spread [41] so unpalatable host crops would be less susceptible to widespread infection than palatable hosts. Host plant palatability may have influenced SbDV infection rates in this study and may be considered as a component of susceptibility and resistance [7]. Therefore, field-based research is now required to understand the epidemiology and impact of SbDV in Australian grain legume crops. Such research should include surveys of grain legume crops, especially during epidemics in pastures such as subterranean clover, and field experiments repeated under a range of conditions to examine biomass, and seed yield and quality impacts.

With the economic importance of grain legumes likely to increase with rising N input costs, reductions in biomass, seed weight and yield from virus infection have significant implications. Firstly, impacts on seed yield and quality directly diminish the value of the grain and secondly, reductions in biomass would likely result in reduced N deposition and weed competitiveness [5], greatly compromising their value as break crops. If the epidemiology and impact field studies proposed above determine the risk of future SbDV epidemics to be high, research should then focus on identifying durable resistance and tolerance and incorporate it into commercial cultivars, together with other integrated management strategies.

Declarations

Acknowledgments

This study was funded by the Department of Primary Industries and Regional Development (DPIRD) and Grains Research and Development Corporation project DAN00202 led by Dr. Joop van Leur (New South Wales Department of Primary Industries). We thank Jenny Reidy-Crofts (Commonwealth Scientific and Industrial Research Organisation) for provision of pea aphid clone Flor-1 and Paul Sanford (DPIRD) for original collection of SbDV isolate WA-8.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

Design of experiments, data analysis and writing of manuscript was done by B. Congdon. Glasshouse experiments, virus testing, and data collection was done by B. Congdon and J. Baulch. K. Foster provided seed of pasture legume species in experiment 2 and editing of manuscript.

Data availability

References

1. Australian Export Grains Innovation Centre (AEGIC) (2020) What grows where? Australian grain production – a snapshot. Australian Grain Note. AEGIC, Sydney and Perth, Australia
2. Ashby JW, Teh PB, Close RC (1979) Symptomatology of subterranean clover red leaf virus and its incidence in some legume crops, weed hosts, and certain alate aphids in Canterbury, New Zealand. *NZ J Agric Res* 22:361–365
3. Banba H, Tanimura Y, Matuskawa I (1986) Breeding for resistance to soybean dwarf virus in soybeans. *Trop Agric Res Ser* 19:236–246
4. Botermans M, van de Vossen BTLH, Verhoeven JJJ, Roenhorst JW, Hooftman M, Dekter R, Meekes ETM (2013) Development and validation of a real-time RT-PCR assay for generic detection of pospiviroids. *J Virol Methods* 187:43–50
5. Chen C, Lawes R, Fletcher A, Oliver Y, Robertson M, Bell M, Wang E (2016) How well can APSIM simulate nitrogen uptake and nitrogen fixation of legume crops? *Field Crops Res* 187:35–48
6. Congdon BS (2021) Soybean dwarf virus in Australia (oral presentation). 2021 Australasian Plant Pathology Society Biennial Conference 2021, Online event, 23–26 November 2021
7. Cooper JI, Jones AT (1983) Responses of plant viruses: proposals for the use of terms. *Phytopathology* 73:127–128
8. Damsteegt VD, Hewings AD (1987) Relationships between *Aulacorthum solani* and soybean dwarf virus: effect of temperature on transmission. *Phytopathology* 77:515–518
9. Damsteegt VD (1990) Soybean dwarf virus: experimental host range, soybean germplasm reactions, and assessment of potential threat to U.S. soybean production. *Plant Dis* 74:992–995
10. Damsteegt VD, Stone AL, Hewings AD (1995) Soybean dwarf, bean leaf roll, and beet western yellows luteoviruses in southeastern U.S. white clover. *Plant Dis* 79:48–50
11. Damsteegt VD, Stone AL, Kuhlmann M, Gildow FE, Domier LL, Sherman DJ, Tian B, Schneider WL (2011) Acquisition and transmissibility of U.S. soybean dwarf virus isolates by the soybean aphid, *Aphis glycines*. *Plant Dis* 95:945–950
12. Western Australian Department of Primary Industries and Regional Development (2018) Using summer sowing to introduce serradella into subtropical perennial pastures 2013 trial report. Available at: <https://www.agric.wa.gov.au/pasture-establishment/using-summer-sowing-introduce-serradella-subtropical-perennial-pastures-2013>. Accessed 16 July 2022
13. Fletcher JD (2001) New hosts of alfalfa mosaic virus, cucumber mosaic virus, potato virus Y, soybean dwarf virus, and tomato spotted wilt virus in New Zealand. *NZ J Crop Hort Sci* 29:213–217
14. Fowkes AR, McGreig S, Pufal H, Duffy S, Howard B, Adams IP, Macarthur R, Weekes R, Fox A (2021) Integrating high throughput sequencing into survey design reveals turnip yellows virus and soybean dwarf virus in pea (*Pisum sativum*) in the United Kingdom. *Viruses* 13:2530
15. Ghosh N, Gotoh K, Nakaseko K, Mori Y (1976) Influence of soybean dwarf virus on growth and yield. *Jpn J Crop Sci* 45:624–629
16. Gildow FE, Damsteegt VD, Stone AL, Smith OP, Gray SM (2000) Virus-vector cell interactions regulating transmission specificity of soybean dwarf luteoviruses. *J Phytopathol* 148:333–342
17. Harrison B, Steinlage TA, Domier LL, D'Arcy CJ (2005) Incidence of soybean dwarf virus and identification of potential vectors in Illinois. *Plant Dis* 89:28–32
18. Helms K, Waterhouse PM, Muller WJ (1985) Subterranean clover red leaf virus disease: effects of temperature on plant symptoms, growth, and virus content. *Phytopathology* 75:337–341
19. Helms K, Müller WJ, Waterhouse PM (1987) Subterranean clover red leaf virus disease: effects of light intensity on plant symptoms, growth, and virus content. *Phytopathology* 77:1297–1300
20. Helms K, Muller W, Waterhouse P (1993) National survey of viruses in pastures of subterranean clover. I. Incidence of four viruses assessed by ELISA. *Aust J Agric Res* 44:1837–1862
21. Hewings AD, Damsteegt VD, Sindermann AE, Tolin SA (1990) Variations in serologically detectable antigen of soybean dwarf virus in soybean leaflets as a function of time after inoculation and plant age. *Plant Dis* 74:844–848
22. Honda K, Kanematsu S, Mikoshiba Y (1999) Dwarfing strain of soybean dwarf luteovirus transmitted by *Nearctaphis bakeri* and *Acyrtosiphon pisum*. *Ann Phytopathol Soc Jpn* 65:387
23. Johnstone G, Duffus J (1984) Some luteovirus diseases in Tasmania caused by beet western yellows and subterranean clover red leaf viruses. *Aust J Agric Res* 35:821–830
24. Johnstone GR (1978) Diseases of broad bean (*Vicia faba* L. major) and green pea (*Pisum sativum* L.) in Tasmania caused by subterranean clover red leaf virus. *Aust J Agric Res* 29:1003–1010
25. Johnstone GR, Rapley PEL (1979) The effect of time of sowing on the incidence of subterranean clover red leaf virus infection in broad bean (*Vicia faba*). *Ann Appl Biol* 91:345–351

26. Johnstone GR(1983) The reactions of subterranean clover to inoculation with two persistent aphid transmitted viruses. In: Proceedings of the International Working Group on Legume Viruses, Binu Burra, Queensland. Queensland Department of Primary Industries, Brisbane, pp 7–8
27. Johnstone GR, Ashby JW, Gibbs AJ, Duffus JE, Thottappilly G, Fletcher JD (1984) The host ranges, classification and identification of eight persistent aphid-transmitted viruses causing diseases in legumes. *Neth J Plant Pathol* 90:225–245
28. Johnstone GR, Barbeti MJ (1987) Impact of fungal and virus diseases on pasture. In: Wheeler JL, Pearson CJ, Roberts GE (eds) *Temperate pastures: their production, use and management*. Australian Wool Corporation/CSIRO, Melbourne, Australia, pp 235–248
29. Johnstone GR, McLean GD (1987) Virus diseases of subterranean clover. *Ann Appl Biol* 110:421–440
30. Jones RAC (2004) Occurrence of virus infection in seed stocks and 3-year-old pastures of lucerne (*Medicago sativa*). *Aust J Agric Res* 55:757–764
31. Jones RAC (2012) Virus diseases of annual pasture legumes: incidences, losses, epidemiology, and management. *Crop Pasture Sci* 63:399–418
32. Jones RAC, Barbeti MJ (2012) Influence of climate change on plant disease infections and epidemics caused by viruses and bacteria. *CAB Rev* 7:1–33
33. Kellock A (1971) Red-leaf virus - a newly recognized virus disease of subterranean clover (*Trifolium subterraneum* L.). *Aust J Agric Res* 22:615–624
34. Makkouk K (2001) Viruses on cultivated forage legumes in Syria. *Arab J Plant Prot* 19:73–79
35. Makkouk K, Kumari S, Shahraeen N, Fazlali Y, Farzadfar S, Ghotbi T, Mansouri A (2003) Identification and seasonal variation of viral diseases of chickpea and lentil in Iran. *J Plant Dis Prot* 110:157–169
36. Makkouk KM, Kumari SG (2001) Reduction of incidence of three persistently transmitted aphid-borne viruses affecting legume crops by seed-treatment with the insecticide imidacloprid (Gaucho®). *Crop Prot* 20:433–437
37. McKirdy S, Jones R (1995) Occurrence of alfalfa mosaic and subterranean clover red leaf viruses in legume pastures in Western Australia. *Aust J Agric Res* 46:763–774
38. Meat and Livestock Australia (2020) The economic significance of Australia's red meat and livestock industry. Available at: <https://www.mla.com.au/prices-markets/market-news/2020/the-economic-significance-of-australias-red-meat-and-livestock-industry/>. Accessed 10 February 2022
39. Menzel W, Jelkmann W, Maiss E (2002) Detection of four apple viruses by multiplex RT-PCR assays with coamplification of plant mRNA as internal control. *J Virol Methods* 99:81–92
40. Mikoshiba Y, Fujisawa I, Honda K (1991) A new strain of soybean dwarf virus transmitted by *Acyrtosiphon pisum* in Japan. *Ann Phytopathol Soc Jpn* 57:448
41. Ng JC, Perry KL (2004) Transmission of plant viruses by aphid vectors. *Mol Plant Pathol* 5:505–511
42. Nichols PGH, Revell CK, Humphries AW, Howie JH, Hall EJ, Sandral GA, Ghamkhar K, Harris CA (2012) Temperate pasture legumes in Australia –their history, current use, and future prospects. *Crop Pasture Sci* 63:691–725
43. Peter A, Shanower T, Romeis J (1995) The role of plant trichomes in insect resistance: a selective review. *Phytophaga* 7:41–64
44. Philippi J, Schliephake E, Jürgens H-U, Jansen G, Ordon F (2015) Feeding behavior of aphids on narrow-leaved lupin (*Lupinus angustifolius*) genotypes varying in the content of quinolizidine alkaloids. *Entomol Exp Appl* 156:37–51
45. Sanford P, Congdon BS, Foster KJ(2021) Identifying the cause of recent outbreaks of subterranean clover red leaf syndrome in Western Australia. Australian Grassland Association Virtual Symposium 2021, 10–31 March 2021
46. Schneider WL, Damsteegt VD, Stone AL, Kuhlmann M, Bunyard BA, Sherman DJ, Graves MV, Smythers G, Smith OP, Hatziloukas E (2011) Molecular analysis of soybean dwarf virus isolates in the eastern United States confirms the presence of both D and Y strains and provides evidence of mixed infections and recombination. *Virology* 412:46–54
47. Tamada T (1970) Aphid transmission and host range of soybean dwarf virus. *Ann Phytopath Soc Japan* 36:266–274
48. Tamada T (1975) Studies on the soybean dwarf disease. *Rep Hokkaido Prefect Agric Exp Stn* 25:1–144
49. Terauchi H, Kanematsu S, Honda K, Mikoshiba Y, Ishiguro K, Hidaka S (2001) Comparison of complete nucleotide sequences of genomic RNAs of four soybean dwarf virus strains that differ in their vector specificity and symptom production. *Arch Virol* 146:1885–1898
50. Terauchi H, Honda K-I, Yamagishi N, Kanematsu S, Ishiguro K, Hidaka S (2004) The N-terminal tegion of the readthrough domain is closely related to aphid vector specificity of soybean dwarf virus. *Phytopathology* 93:1560–1564
51. Wilson J, Close RC (1973) Subterranean clover red leaf virus and other legume viruses in Canterbury. *NZ J Agric Res* 16:305–311

Figures

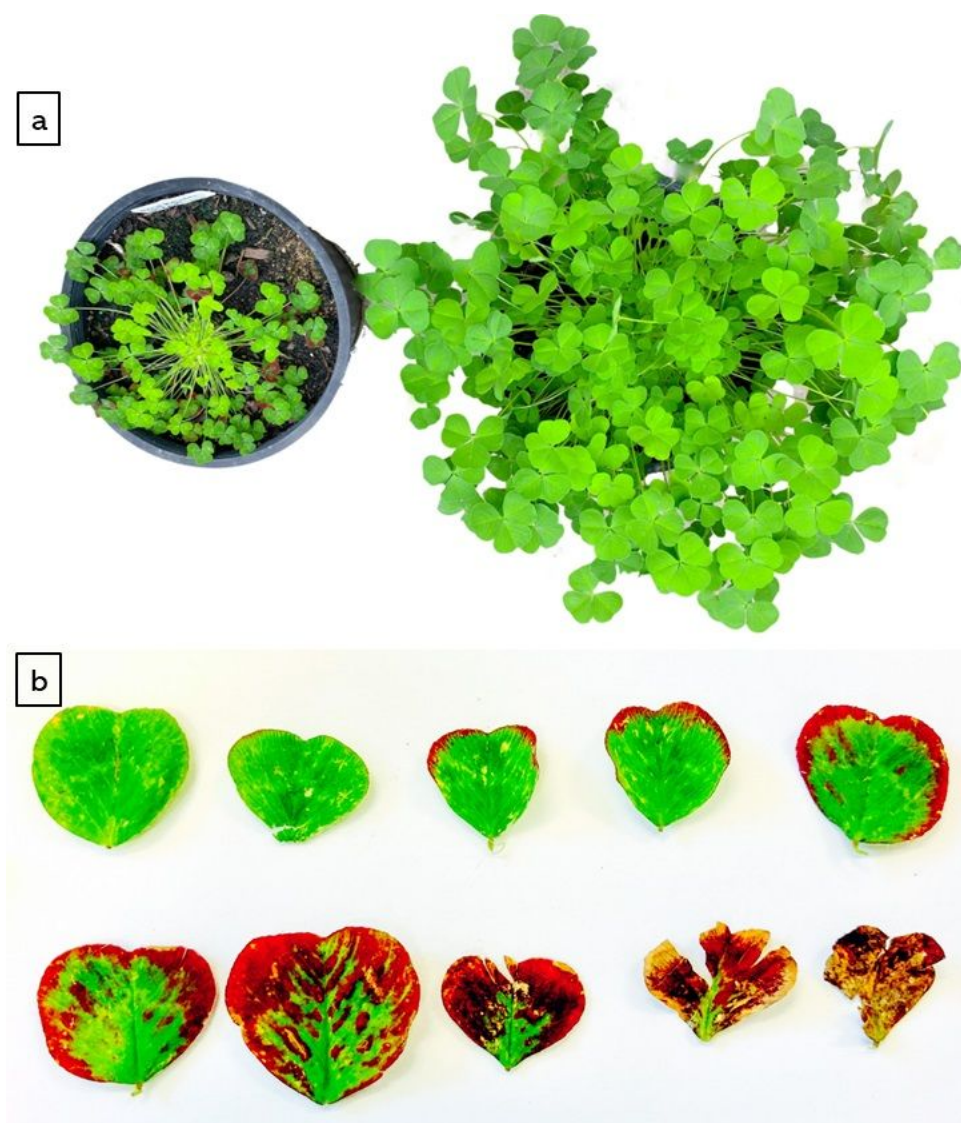


Figure 1

Typical symptoms of soybean dwarf virus (SbDV) infection in subterranean clover (*Trifolium subterraneum*) observed in this study; **[a]** comparison of SbDV-infected (left) and uninfected (right) cv. Dalkeith plants and **[b]** symptom progression in lower leaves of an infected plant

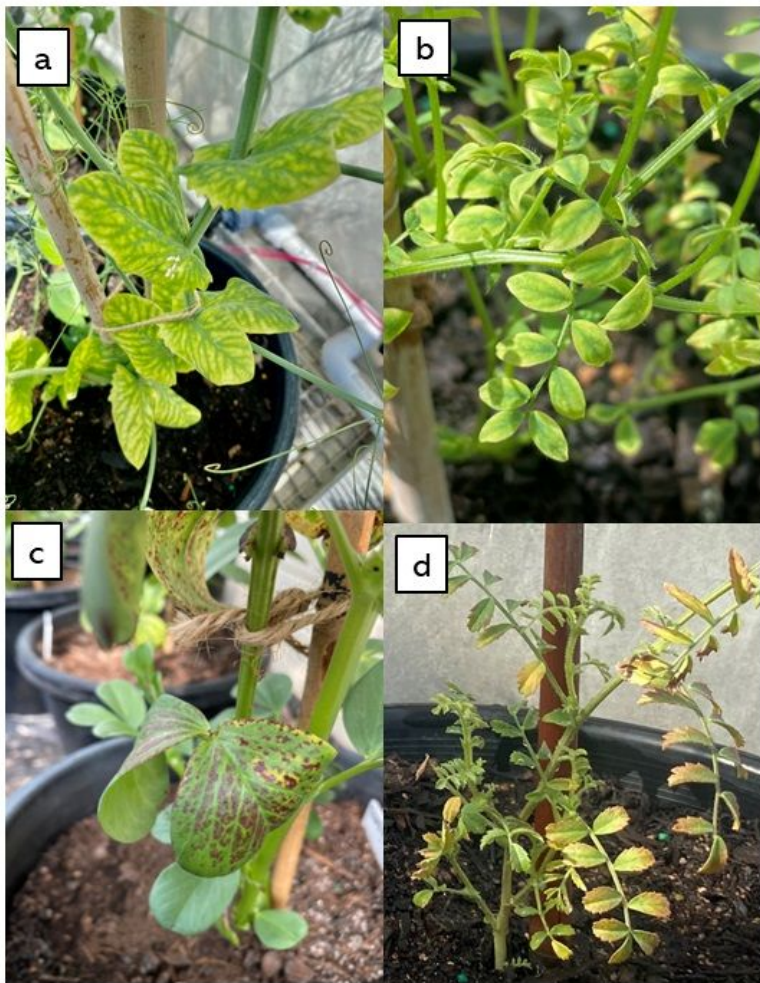


Figure 2

Foliar symptoms of soybean dwarf virus infection in **[a]** field pea (*Pisum sativum*), **[b]** lentil (*Lens culinaris*), **[c]** faba bean (*Vicia faba*), and **[d]** chickpea (*Cicer arietinum*)

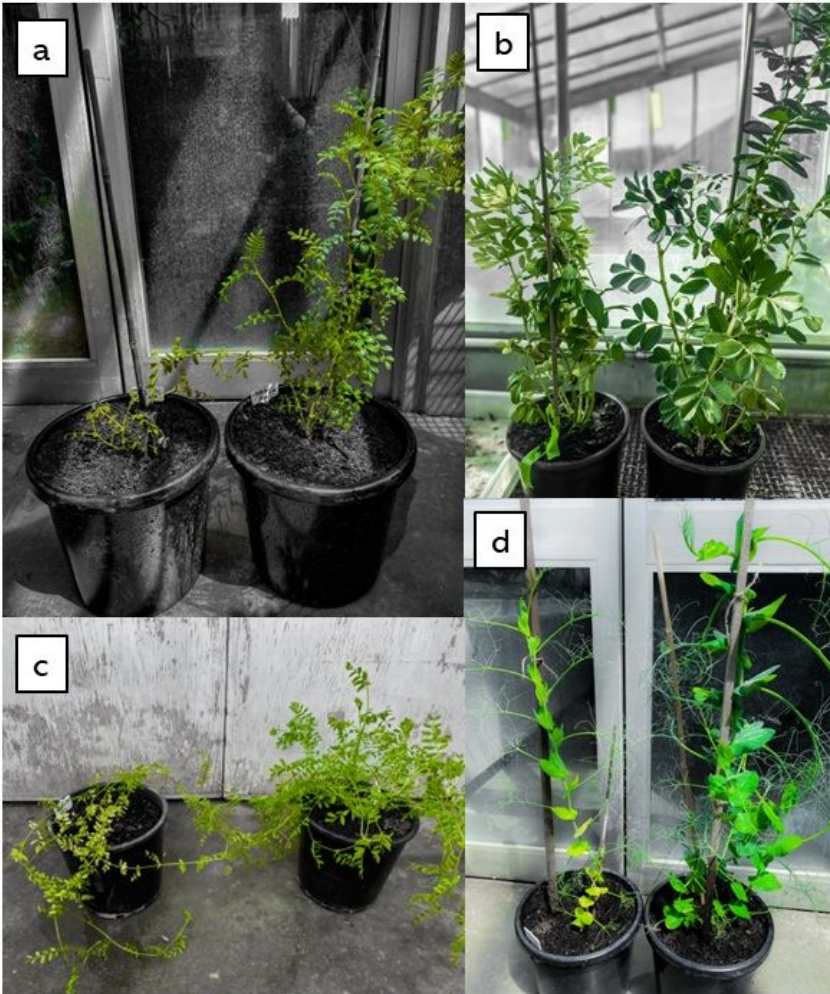


Figure 3

Comparison of soybean dwarf virus-infected (left) and uninfected (right) plants of **[a]** chickpea (*Cicer arietinum*), **[b]** faba bean (*Vicia faba*), **[c]** lentil (*Lens culinaris*) and **[d]** field pea (*Pisum sativum*)

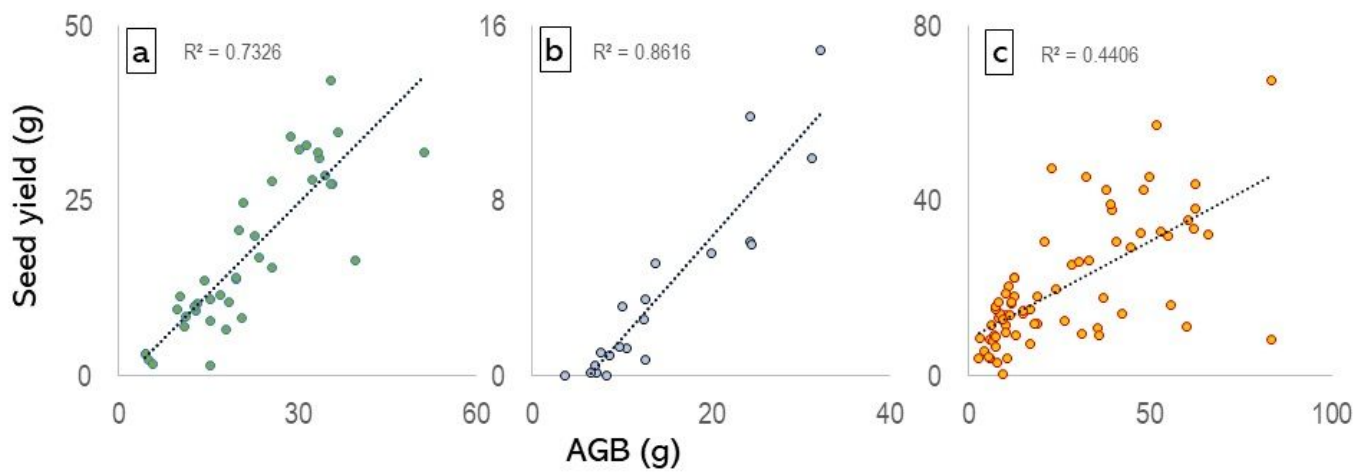


Figure 4

Relationship between dry above-ground biomass (AGB) and seed yield of all **[a]** field pea (*Pisum sativum*), **[b]** lentil (*Lens culinaris*) and **[c]** faba bean (*Vicia faba*) plants tested in this study

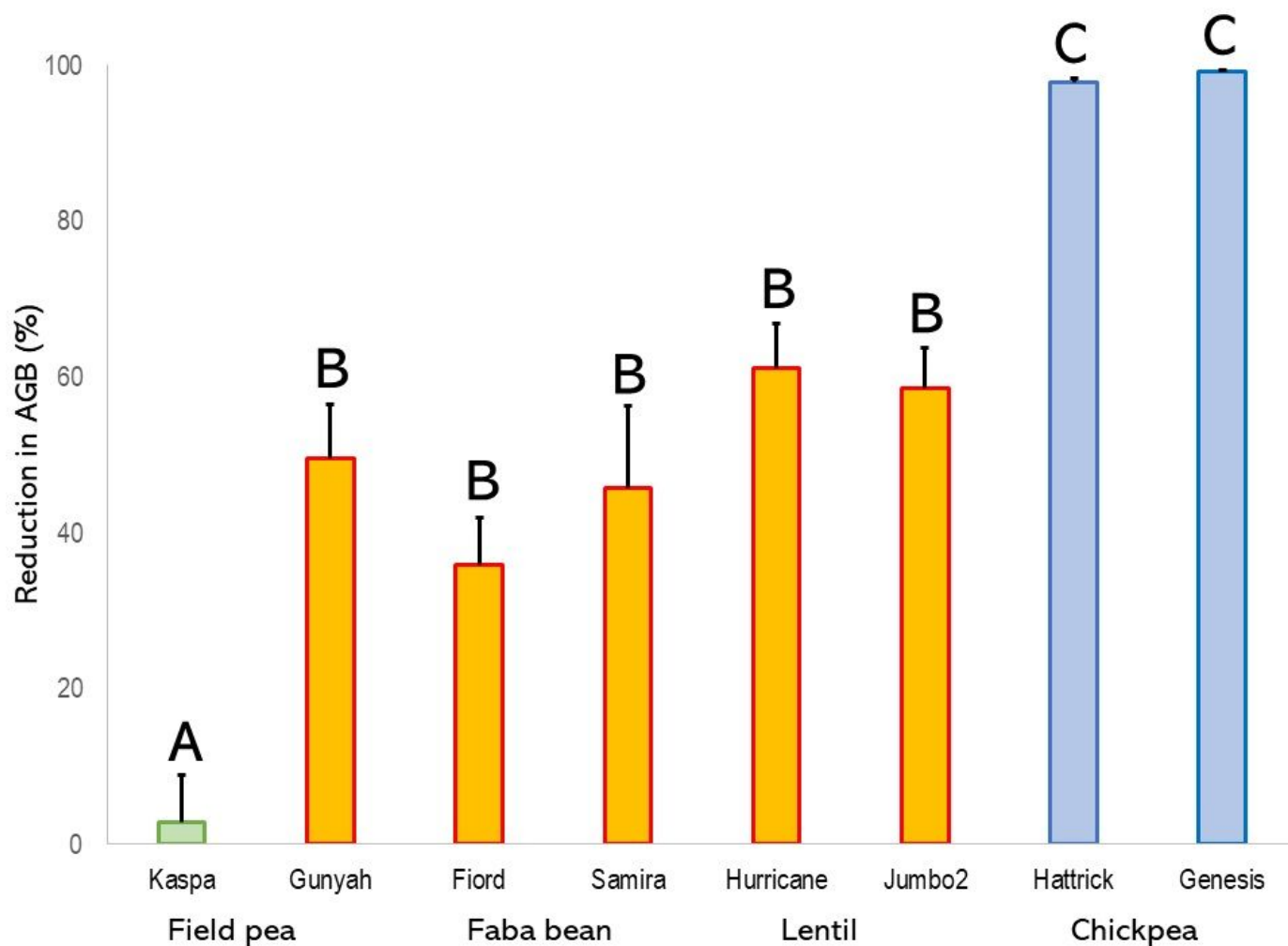


Figure 5

Mean reduction in dry above-ground biomass (AGB)^a from soybean dwarf virus infection across all field pea (*Pisum sativum*), faba bean (*Vicia faba*), lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*) plants tested in experiments 4a to 4c. Letters A to C and bar colours indicate significant difference at $\alpha=0.05$ in analysis of variance and Tukey's honest significant difference.

^aDifference in AGB between the infected and uninfected plant in each block for all three experiments.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigure1.tif](#)