Plant Pathology (2007) **56**, 729–742 Doi: 10.1111/j.1365-3059.2007.01653.x

Virus impact at the interface of an ancient ecosystem and a recent agroecosystem: studies on three legume-infecting potyviruses in the southwest Australian floristic region

C. G. Webster[a], B. A. Coutts[b], R. A. C. Jones[a,b,c]*, M. G. K. Jones[a,c] and S. J. Wylie[a,c]

[a]*Western Australian State Agricultural Biotechnology Centre, Murdoch University, Perth, WA 6150;* [b]*Agricultural Research Western Australia, Locked Bag No. 4, Bentley Delivery Centre, Perth, WA 6983; and* [c]*Centre for Legumes in Mediterranean Agriculture, University of Western Australia, Perth, WA 6009, Australia*

The southwest Australian floristic region (SWAFR) is an internationally recognized 'hot spot' of global biodiversity and has an endangered flora. It represents a unique interface between an ancient ecosystem and a recent agroecosystem, providing the opportunity to investigate encounters where the recipient of the virus is an introduced crop and the donor a native plant and vice versa. Phylogenetic analysis of the virus coat-protein genes was used to study isolates of three potyviruses representing different 'new encounter' scenarios at this interface. The incidence, symptomatology, host range, non-persistent aphid transmission and considerable genetic diversity of the first indigenous virus described from the SWAFR, where it infects the native legume *Hardenbergia comptoniana*, and its potential to damage lupin, a locally important, newly introduced cultivated grain legume, was studied. The name Hardenbergia mosaic virus is proposed for this virus. Two other examples of 'new encounter' scenarios involving other legume-infecting potyviruses studied were: *Passion fruit woodiness virus*, which has been found only in Australasia, where it damages recently introduced species of *Passiflora* and legumes; and *Bean yellow mosaic virus*, which is not indigenous to Australia and was introduced recently to the SWAFR, where it infects a number of introduced legumes, but also damages the local native legume *Kennedia prostrata*. Isolates of the former had considerable genetic diversity consistent with the virus being indigenous, while isolates of the latter virus from *K. prostrata* had a low genetic diversity consistent with recent arrival. This research illustrates how introduced viruses can damage indigenous plants and indigenous viruses can damage introduced cultivated plants within this unique ecosystem, and how human activities can facilitate damaging 'new encounters' between plants and viruses.

Keywords: biodiversity ecology, evolution, new encounter, phylogeography, *Potyviridae*

Introduction

Plant viruses are considered to have co-evolved with wild plants long before any were domesticated (Lovisolo *et al*., 2003). Damaging epidemics of indigenous viruses seem rare in native plant communities, but human activities have profound impacts in facilitating 'new encounters' between viruses and plants (Buddenhagen, 1977; Thresh, 1980, 1981, 2006; Bos, 1981; Fargette *et al*., 2006; Jones, 2006). Newly introduced viruses spreading from cultivated plants to wild plant communities can threaten biodiversity. A greater frequency of introduction of invasive viruses that threaten biodiversity is inevitable because of the continually expanding volume and rapidity of world trade in plants and plant products and the worldwide movement of cultivated plants away from their centres of origin. The relaxing of national plant-quarantine regulations to meet revised World Trade Organisation regulations also contributes to this trend. Conversely, because of the increase in plant introductions for commercial reasons, there is also an increasing threat from new encounters with 'emerging' viruses spreading from indigenous plants to introduced cultivated plants (Thresh, 1980; Anderson *et al*., 2004; Cooper & Jones, 2006; Fargette *et al*., 2006). Studying viruses in wild plant communities and at the interface between indigenous vegetation and cultivated areas has the potential to provide critical information, not only on threats to biodiversity and cultivated species, but also on virus evolution, both in very recent times and over millions of years (Lovisolo *et al*., 2003; Jeger *et al*., 2006).

The southwest Australian floristic region (SWAFR) occupies 302 627 km^2 and contains one of the world's oldest, weathered, infertile landscapes. It constitutes an

*E-mail: rjones@agric.wa.gov.au

Accepted 14 March 2007

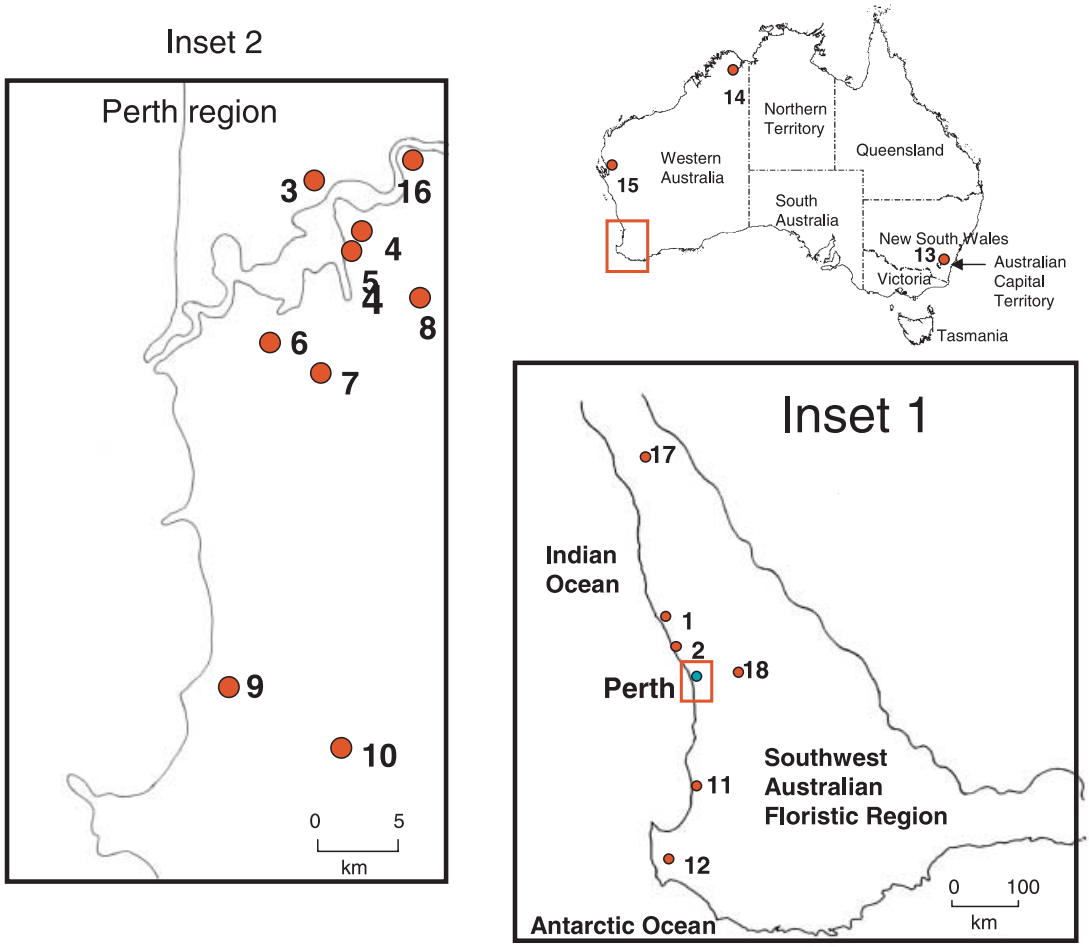

Figure 1 Sites sampled in Australia: map of the Australian continent showing the location of the southwest Australian floristic region (SWAFR). Inset 1, boundaries of the SWAFR; inset 2, southern Perth region where most virus isolates were collected (site names in Table 1).

isolated, relatively wet 'refuge', bordered by ocean to the south and west, and arid lands, including the Nullabor and Western deserts, to the east and north (Fig. 1) (Hopper, 1979). It is one of the world's 25 recognized 'hot spots' of global biodiversity and endemism (Myers *et al*., 2000; Brooks *et al*., 2002; Hopper & Gioia, 2004) and is unique topographically among the world's five regions with Mediterranean-type climates because of its prolonged period of isolation, its geological stability and the absence of disturbance by humans until very recently. It is species-rich, with 7380 indigenous vascular plant species, 49% of which are endemic and 2500 of conservation concern because of their much depleted distributions. There was no plant cultivation in the SWAFR before 1829, when European colonization began. All of the threats to the biota of the region are related directly or indirectly to its exploitation by humans, and include habitat loss, population fragmentation, rising saline groundwater tables from excessive land clearing, wood collection, too-frequent fires, and introduced weeds and pathogens (Brooks *et al*., 2002; Hopper & Gioia, 2004). The ravages caused by the introduced oomycete root pathogen *Phytophthora cinnamomi*, which causes 'dieback disease' and damages 2500 species, are well documented (e.g. Hopper & Gioia, 2004). However, the impacts of introduced pathogens with more subtle effects, such as viruses, have not been studied. In natural communities of plants and pasture swards containing mixtures of species, even virus diseases that cause only mild symptoms can still impair fitness, decreasing both competitive and reproductive ability, and so causing major changes in relative species predominance (Jones & Nicholas, 1998; Coutts & Jones, 2002; Malmstrom *et al*., 2005; Cooper & Jones, 2006).

This region provides an interface between an ancient ecosystem and a recent agroecosystem that is ideal for study of viruses from indigenous flora, their potential to damage nearby cultivated plants, and the consequences

when newly introduced viruses spread from cultivated plants into native flora. In Africa there are several well studied examples of introduced crops being damaged by viruses that originated in native plants (Thresh, 1980, 1981, 2006). However, in general, in most parts of the world such studies are difficult, as plants have been cultivated for so long that it is impossible to say whether the viruses found evolved locally or were introduced. In the study reported here, this subject was addressed through phylogenetic studies with three members of the genus *Poytvirus*, in the *Potyviridae*, the largest family of plant viruses and also one of the most important economically (Adams *et al*., 2005; Ward *et al*., 2005). A newly found potyvirus was described which causes a widespread mosaic disease in the perennial legume *Hardenbergia comptoniana* (native wisteria) and poses a potential threat to introduced cultivated legumes. *Hardenbergia comptoniana* is native to the region, its natural distribution extending from its northern edge along an 800-km coastal strip beside the Indian Ocean to its southern edge adjacent to the Antarctic Ocean (Paczkowska & Chapman, 2000). *Passion fruit woodiness virus* (PWV) was selected as an example of an indigenous virus that often causes a severe disease in an introduced cultivated plant (*Passiflora edulis*, passion fruit), an introduced rootstock commonly used for it (*P. caerulea*, hardy passion flower), and in the introduced wild plant *P. foetida* (stinking passion flower); *P. caerulea*, *P. edulis* and *P. foetida* all originate in South America. PWV also infects introduced grain legume species such as *Arachis hypogaea* (peanut), *Glycine max* (soybean) and *Phaseolus vulgaris* (common bean), and several introduced wild legume species that are now naturalized within Australia (Buchen-Osmond *et al*., 1988; Sokhandan *et al*., 1997). Moreover, 'passionfruit woodiness' was the first virus disease recorded in Australia (Cobb, 1901). It was also reported from New Zealand (Buchen-Osmond *et al*., 1988), but no PWV sequences are available from there. The 'PWV' sequences from outside Australia available on GenBank are incorrectly labelled as they are actually either *Cowpea aphid-borne mosaic virus* or East Asian passiflora virus (Adams *et al*., 2005; Nascimento *et al*., 2006). In contrast, sequences of PWV *sensu stricto* are reported only from Australia, suggesting that PWV is indigenous to the Australian island continent, where it infects the indigenous species *P. aurantia* (golden passion flower) (Buchen-Osmond *et al*., 1988). *Bean yellow mosaic virus* (BYMV) was selected as an example of a non-Australian potyvirus that was introduced in cultivated plants and has become widely established in pasture and grain legumes in the region (Jones & McLean, 1989; Jones, 1996; Latham & Jones, 2001). BYMV causes a damaging disease in the indigenous perennial legume *Kennedia prostrata* (scarlet runner). It also infects other native legumes, including *K. coccinia* (coral vine), *Hovea elliptica* (tree hovea) and *H. pungens* (devil's pins) (McKirdy *et al*., 1994).

Prior to this study, no indigenous plant viruses had been described from the SWAFR flora, and apart from limited surveys for alternative hosts of economically important viruses (e.g. McKirdy *et al*., 1994; Latham & Jones, 1997), there were no studies on introduced viruses isolated from native plants or indigenous viruses 'emerging' to cause epidemics in cultivated plants. Within the region, there are three small areas exceptionally rich in plant biodiversity (Marchant *et al*., 1987; Hopper & Gioia, 2004), one of which is the greater Perth city area, where most of the virus isolates in this study were collected. The principal aims of this study were (i) to study the biological properties and genetic diversity of potyvirus isolates obtained from diseased *H. comptoniana* plants, and to establish whether they belong to a virus indigenous to the region and might pose a threat to any introduced cultivated plants; (ii) to determine the genetic relationships between PWV isolates from Western Australia and those from elsewhere in the continent; and (iii) to identify the likely origin of BYMV isolates obtained from the indigenous plant species *K. prostrata*. The implications of the findings for the endangered indigenous flora of the SWAFR and its introduced cultivated plants are discussed.

Materials and methods

Plants, virus isolates, inoculations, ELISA and antibodies

Virus-indicator hosts and cultivated plants were grown in insect-proofed glasshouses. Potyvirus isolates came from leaf samples with symptoms obtained from naturally infected *H. comptoniana* plants growing at different sites in the SWAFR (Table 1). In addition, one isolate came from a leaf sample with symptoms from a garden plant of *H. comptoniana* growing in Canberra in eastern Australia and another from an affected leaf from a plant of the related introduced species *H. violacea* growing in a commercial plant nursery in Perth; *H. violacea* is a native to eastern Australia. Similarly, isolates of PWV were obtained from samples with symptoms from naturally infected *P. edulis*, *P. caerulea* or *P. foetida* plants from Western Australia, and those of BYMV from naturally infected samples of *K. prostrata* showing symptoms from the SWAFR. For sap inoculations, infected leaf tissue was ground in 0·05 M potassium phosphate buffer (pH 7·2) in a mortar and pestle and the sap mixed with the diatomaceous earth 'celite' before being rubbed onto leaves. For aphid inoculations, apterous *Myzus persicae* were (i) starved for 2 h and then given 10-min acquisition-access feeds on infected *H. comptoniana* or *Nicotiana benthamiana* leaves, followed by 2-h inoculation-access feeds on *N. benthamiana* plants (five aphids per plant), or (ii) starved for 12 h and then given 6-h acquisition-access feeds on infected leaves of *N. benthamiana*, followed by 18-h inoculation-access feeds on *H. comptoniana* plants (10 aphids per plant). Cultures of isolates from *Hardenbergia* spp. and PWV were maintained in *N. benthamiana*, and those of BYMV in *Trifolium subterraneum*. These cultures were used for subsequent inoculations and to provide positive control samples for ELISA and RT-PCR. Leaf samples

Table 1 Site names, symptoms, incidences and virus isolates sequenced in this study

Virus and site no.[a]	Site name	Site type	Predominant symptoms[b]	Percentage of plants with symptoms[c]	Virus isolates sequenced[d]
HarMV					
1	Seabird	Coastal dune vegetation	M, LD, YS, YB, YR	16 (167)	**Sb-3**, Sb-5, Sb-6, Sb-15, **Sb19-1**, Sb19-4
2	Burns Beach	Coastal dune vegetation	M, LD	ND (7)	BB-1, BB-2, **BB-6**
3	Kings Park	Nature reserve	M, LD	20 (40)	KiP-1
4	South Perth/Como	Roadside vegetation	M, LD	ND (15)	**Co-1**
5	State Herbarium	Research facility (grounds)	M, LD, YB	20 (50)	He-1, **He-2**
6	Wireless Hill Park	Nature reserve	M, LD	73 (110)	WHP-1, WHP-2
7	Murdoch University	Research facility (campus)	M, LD	28 (104)	MU-1A, **MU-1C**, **MU-2A**, **MU-3A**
8	Cannington	Plant nursery	M, LD	ND (1)	Cgt-1
9	Medina	Research facility (wild verge)	M, LD	52 (62)	Med-1, Med-7
10	Wellard	Roadside vegetation	M, LD	ND (1)	**Wel-1**
11	Bunbury	Roadside vegetation	M, LD	20 (50)	Bun-9
12	Margaret River	Roadside vegetation	M, LD	60 (62)	MR-3, MR-13, MR-21, MR-30
13	Scullin, Canberra	Garden	M, LD	ND (1)	Can1-1, Can1-2
PWV					
4	South Perth	Research facility (grounds)	SM, LD	100 (40)	Not sequenced
4	Como	Garden	M	ND (1)	CoP-1
14	Kununurra	Irrigation channel	M	ND (20)	Ku-1
15	Carnarvon	Wild reserve on market garden	SM, LD	ND (6)	Car-1
16	Guildford	Garden	M, LD	ND (5)	Gld-1
BYMV					
6	Wireless Hill Park	Nature reserve	LD, YB, St	5 (40)	WHPB-1, WHPB-2
7	Murdoch University	Research facility (campus)	M, LD	2 (50)	MUB-1
17	Badgingarra	Roadside wild vegetation	SM, LD, St, YB	40 (50)	BadgB-1
18	'The Lakes'	Nature reserve	SM, LD, St	ND (15)	KP (McKirdy *et al.*, 1994; Cheng & Jones, 2000)

[a]Virus names: HarMV (Hardenbergia mosaic virus), the name suggested for the potyvirus isolated from *Hardenbergia* spp.; PWV (*Passion fruit woodiness virus*); and BYMV (*Bean yellow mosaic virus*). PWV was isolated from *Passiflora edulis* (site 16), *P. foetida* (sites 14 and 15) and *P. caerulea* (site 4); and BYMV from *Kennedia prostrata*. Figure 1 shows geographical locations of site numbers.

[b]Coded symptom descriptions: M, mosaic; SM, severe mosaic; LD, leaf distortion; YS, yellow spotting; YB, yellow blotchy mosaic; YR, yellow rings; St, stunting.

[c]Incidences based on numbers of plants with symptoms out of 40 –167 inspected per site. ND, not determined (too few plants). Numbers in parentheses are total numbers of plants inspected for symptoms at each site.

[d]Isolates of HarMV used in host-range inoculations shown in bold. Isolate Cgt-1 from site 8 was from *H. violacea*, but all others of HarMV were from *H. comptoniana*.

were tested by DAS-ELISA (Clark & Adams, 1977) using a generic potyvirus monoclonal antibody (Agdia Inc) for isolates from *Hardenbergia* spp. and PWV, and virus-specific polyclonal antiserum for BYMV (BioRad Plantest).

Incidence, host-range and seed-transmission studies

Where there were sufficient plants present at a site, the incidence of virus affected plants in each natural population of *H. comptoniana* was estimated by counting numbers of plants with and without symptoms. Also, leaves with and without symptoms were sampled from different plants at these sites, and the samples tested individually by ELISA (samples with symptoms) or in groups of appropriate size (samples without symptoms) as described previously (McKirdy *et al.*, 1994). For test results from grouped samples, incidence was determined using the formula of Gibbs & Gower (1960).

In studies on host range and symptomatology in the glasshouse, plants (two or more per species) were inoculated with infective *N. benthamiana* sap. Any symptoms that developed were recorded and samples from inoculated and tip leaves tested for potyvirus infection by ELISA, 3 or 4–6 weeks after inoculation, respectively. In seed-transmission studies, seeds from *N. benthamiana* plants infected with seven different potyvirus isolates from *H. comptoniana* were germinated and the seedlings tested in groups of 10 by ELISA.

RT-PCR and sequence analysis

To extract viral RNA, infected leaf material was ground to a fine powder in liquid nitrogen and total RNA extracted with an RNeasy Plant Miniprep kit (Qiagen). Leaf material used came from: infected leaves of *Hardenbergia* spp. or cultures of isolates from *Hardenbergia* spp. in *N. benthamiana*; for PWV, infected *Passiflora* spp. leaves or

cultures of isolates in *N. benthamiana*; for BYMV, infected freeze-dried *T. subterraneum* culture leaves (isolate KP only) or directly from diseased *K. prostrata* leaves. With all three viruses, cDNA was synthesized using ThermoScript™ reverse transcriptase (Invitrogen) according to the manufacturer's instructions. For isolates from *Hardenbergia* spp. and PWV, primer T7T24 (5′-CCCTATAGTGAGTCGTATTATTTTTTTTTTTTTTTT-3′) was used to prime cDNA synthesis, whereas for BYMV, random hexamer primers (Invitrogen) were used. All PCRs were carried out with *Pfu* DNA polymerase (Promega) using the reaction buffer supplied. PCR conditions were as follows: one step of denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 10 s, 50°C for 30 s, and 72°C for 1 min. Amplification of isolates from *Hardenbergia* spp. and PWV was performed with primers LegPotyF (5′-GCWKCHATGATYGARGCHTGGG-3′) and T7 (5′-TAATACGACTCACTATAGGG-3′) to generate products of approximately 1·3 kb. LegPotyF was based on an alignment of sequences from six legume-infecting potyviruses (BYMV, *Bean common mosaic virus* (BCMV), *Clover yellow vein virus*, *Cowpea aphid-borne mosaic virus*, *Pea seed-borne mosaic virus* and *Soybean mosaic virus*) and corresponded to the 3′ portion of the *NIb* gene upstream of the coat-protein gene (*CP*). Amplification of PCR products of *c.* 900 bp from BYMV cDNA was performed with the primers LegPotyF and BYMVUTR-rev (5′-CTCGCTCTACAAAGATCAGGCTCACACG-3′). Primer BYMVUTR-rev was based on an alignment of seven BYMV sequences at the 3′ untranslated region. Both strands of the isolates from *Hardenbergia* spp. and PWV amplicons were sequenced (i) directly after purification with the primers used in their amplification or (ii) after first cloning into the PCR® Blunt-Topo® vector (Invitrogen), and then using primers M13F (5′-TCCCAGTCACGACGTCGT-3′), M13R (5′-GGAAACAGCTATGACCATG-3′), LegPotyF and T7. Both strands of BYMV amplicons were sequenced directly with the primers used previously for PCR amplification with this virus. Automated sequencing was carried out using an Applied Biosystems/Hitachi 3730 DNA Analyzer with BigDye terminator V3·1 chemistry. The sequences were submitted to GenBank (Table 2).

After trimming primer and non-*CP* gene sequences, nucleotides were compared with those in GenBank using BlastN (National Centre for Biotechnology Information, Bethesda, MD, USA) (Table 2). Sequences representing the full *CP*-coding regions of the different isolates from *Hardenbergia* spp., and of PWV and BYMV, were aligned using ClustalW (Thompson *et al.*, 1994) in mega 3·1 (Kumar *et al.*, 2004) and bootstrapped with 1000 resamplings of alignments to assess the robustness of the lineages in the trees. Genetic distances were calculated using OldDistances (GCG). Neighbour-joining trees were generated using mega 3·1. The phylogenetic comparisons with other potyviruses included all the potyviruses found in Australia for which sequence information was available.

Results

Naturally occurring symptoms

Potyvirus from Hardenbergia *spp.*

From April to November (autumn to spring), symptoms of virus infection were often apparent in newly emerged leaves of *H. comptoniana* plants growing in the region. At 11 different sites, natural populations of this species were inspected, incidences of diseased plants determined where possible and leaf samples collected (Fig. 1; Table 1). On most plants with symptoms, young leaves on affected shoot tips had mosaic and leaf distortion (Fig. 2a,b), but additional symptoms of bright yellow spots, rings and mosaic were present on occasional plants (Fig. 2c). In affected plants, normally only some shoots had symptoms. Presence of potyvirus infection was confirmed by testing leaf samples by ELISA and RT-PCR. Based on symptoms alone, which were recorded in 2005 during one visit per site at seven sites, the incidence of affected plants ranged from 16 to 73%. Based on ELISA tests using potyvirus antibody on randomly collected samples collected on one occasion per site from seven sites, the incidence of infection at individual sites in 2005 ranged from 13 to 72% (mean 45%) of *H. comptoniana* plants. In random samples from symptomless plants only, the corresponding incidence figures were 0–12% (range) and 5% (mean). Symptoms were identical in the *H. comptoniana* with symptoms from eastern Australia, and the *H. violacea* with symptoms from Perth.

PWV and BYMV

PWV and BYMV both often caused severe symptoms in naturally infected *Passiflora* spp. and *K. prostrata*, respectively (Table 1). Plants of *P. edulis* and *P. caerulea* in the SWAFR, and of *P. foetida* growing in warmer locations further north in Western Australia, often exhibited chlorotic spots, mosaic, leaf distortion and diminished plant growth (Fig. 2e). PWV was readily detected in leaf samples with symptoms using ELISA and RT-PCR. Plants of *K. prostrata* growing in native vegetation near walking or vehicle tracks, or other disturbed areas in the region, often showed generalized leaf distortion, vein clearing, chlorotic mosaic and severe plant dwarfing (Fig. 2f). Leaf samples with symptoms from four sites gave positive results for BYMV when tested by ELISA or RT-PCR and based on symptoms alone, BYMV incidence ranged from 2 to 40% of plants at these sites.

Aphid- and seed-transmission tests

Under glasshouse conditions, aphid inoculations using *M. persicae* and brief acquisition- and inoculation-access times transmitted potyvirus successfully from *H. comptoniana* leaves with symptoms (isolate Co-1; Table 1) to plants of the virus-indicator host *N. benthamiana*. Leaf mosaic or distortion resulted, and virus was detected in tip-leaf samples from the inoculated plants using ELISA with potyvirus antibody. Similar aphid inoculations

Table 2 Virus species and isolates used

Virus species[a]	Isolate name	Geographical origin[b]	GenBank accession no.	Reference
HarMV	Sb-3	Australia (WA)	DQ898204	This study
HarMV	Sb-5	Australia (WA)	DQ898205	This study
HarMV	Sb-6	Australia (WA)	DQ898214	This study
HarMV	Sb-15	Australia (WA)	DQ898213	This study
HarMV	Sb19-1	Australia (WA)	DQ898202	This study
HarMV	Sb19-4[c]	Australia (WA)	DQ898203	This study
HarMV	BB-1	Australia (WA)	DQ898189	This study
HarMV	BB-2	Australia (WA)	DQ898190	This study
HarMV	BB-6	Australia (WA)	DQ898188	This study
HarMV	KiP-1	Australia (WA)	DQ898210	This study
HarMV	Co-1	Australia (WA)	DQ898192	This study
HarMV	He-1	Australia (WA)	DQ898209	This study
HarMV	He-2	Australia (WA)	DQ898193	This study
HarMV	WHP-1	Australia (WA)	DQ898206	This study
HarMV	WHP-2	Australia (WA)	DQ898207	This study
HarMV	MU-1A	Australia (WA)	DQ898194	This study
HarMV	MU-1C	Australia (WA)	DQ898195	This study
HarMV	MU-2A	Australia (WA)	DQ898196	This study
HarMV	MU-3A	Australia (WA)	DQ898197	This study
HarMV	Cgt-1	Australia (WA)	EF375606	This study
HarMV	Med-1	Australia (WA)	DQ898200	This study
HarMV	Med-7	Australia (WA)	DQ898201	This study
HarMV	Wel-1	Australia (WA)	DQ898208	This study
HarMV	Bun-9	Australia (WA)	DQ898191	This study
HarMV	MR-3	Australia (WA)	DQ898211	This study
HarMV	MR-13	Australia (WA)	DQ898198	This study
HarMV	MR-21	Australia (WA)	DQ898199	This study
HarMV	MR-30	Australia (WA)	DQ898212	This study
HarMV	Can1-1	Australia (ACT)	EF375607	This study
HarMV	Can1-2[c]	Australia (ACT)	EF375608	This study
PWV	Ku-1	Australia (WA)	DQ898217	This study
PWV	Car-1	Australia (WA)	DQ898216	This study
PWV	Gld-1	Australia (WA)	DQ898215	This study
PWV	CoP-1	Australia (WA)	DQ898218	This study
PWV	CLI	Australia (NSW)	U67149	Sokhandan *et al.* (1997)
PWV	SD1	Australia (NSW)	U67150	Sokhandan *et al.* (1997)
PWV	NB5c5	Australia (NSW)	U67151	Sokhandan *et al.* (1997)
PWV	299	Australia (Qld)	AJ430527	unpublished
ApVY	Cm	Australia (NSW)	AY049716	Moran *et al.* (2002)
BCMV	R	China (Zhejiang)	NC_003397	Zheng *et al.* (2002)
BCMNV	NL-3	USA (Michigan)	NC_004047	Fang *et al.* (1995)
CarVY	–	Australia (Vic)	AF203537	Moran *et al.* (2002)
CerMV	1	Australia	AF022443	Mackenzie *et al.* (1998)
CliVY	–	Australia (Qld)	AF228515	unpublished
CABMV	Z	Zimbabwe	AF348210	Mlotshwa *et al.* (2002)
DsMV	M13	China (Zhejiang)	AJ298033	Chen *et al.* (2001)
DiVY	–	Australia	AF203527	Gibbs *et al.* (2000)
EAPV	AO	Japan (Kagoshima)	AB246773	Iwai *et al.* (2006)
EVY	–	Australia	DQ098904	unpublished
HiVY	Kiola	Australia (NSW)	AF228516	Kiratiya-Angul & Gibbs (1992); Gibbs & MacKenzie (1997)
PClV	–	USA (Florida)	DQ860147	Baker & Jones (2007)
PFVY	–	Australia	DQ112219	unpublished
PStV	Ts	Taiwan	AY968604	unpublished
PleVY	–	Australia	AF185958	Gibbs *et al.* (2000)
PtVY	Pterostylis	Australia	AF185964	Gibbs *et al.* (2000)
RhoVY	–	Australia	AF185956	Gibbs *et al.* (2000)
SarVY	–	Australia	AF185957	Gibbs *et al.* (2000)
S1VY	–	Australia	DQ098900	unpublished

Tabel 2 *Continued*

Virus species[a]	Isolate name	Geographical origin[b]	GenBank accession no.	Reference
S2VY	–	Australia	DQ098901	unpublished
SMV	G7	USA	AY216010	Hajimorad *et al.* (2003)
WMV	WMV-Fr	France	NC_006262	Desbiez & Lecoq (2004)
WVMV	Beijing	China	AY656816	Liang *et al.* (2006)
ZYMV	TW-TN3	Taiwan (Tainan)	AF127929	Lin *et al.* (2001)
BYMV	BadjB-1	Australia (WA)	DQ901433	This study
BYMV	WHPB-1	Australia (WA)	DQ901435	This study
BYMV	WHPB-2	Australia (WA)	DQ901434	This study
BYMV	MUB-1	Australia (WA)	DQ901431	This study
BYMV	KP	Australia (WA)	DQ901432	McKirdy *et al.* (1994); Cheng & Jones (2000); sequenced this study
BYMV	Diuris	Australia (ACT)	AF185962	Gibbs *et al.* (2000)
BYMV	Pterostylis	Australia (ACT)	AF185960	Gibbs *et al.* (2000)
BYMV	E441	Japan	AJ844916	unpublished
BYMV	BH8	Japan	AB041972	unpublished
BYMV	Danish	Denmark	X53684	Boye *et al.* (1990)
BYMV	LutKP	Australia (WA)	AF192781	unpublished
BYMV	LKoj1-NN	Australia (WA)	AF192782	unpublished

[a]ApVY, *Apium virus Y*; BCMV, *Bean common mosaic virus*; BCMNV, *Bean common mosaic necrosis virus*; BYMV, *Bean yellow mosaic virus*; CarVY, *Carrot virus Y*; CABMV, *Cowpea aphid-borne mosaic virus*; CerMV, *Ceratobium mosaic virus*; CliVY, *Clitoria virus Y*; DsMV, *Dasheen mosaic virus*; DiVY, *Diuris virus Y*; EAPV, East Asian passiflora virus; EVY, Eustrephus virus Y; HarMV (Hardenbergia mosaic virus) = the name suggested for the potyvirus isolated from *Hardenbergia spp.*; HiVY, *Hibbertia virus Y*; PWV, *Passionfruit woodiness virus*; PClV, Passiflora chlorosis virus; PFVY, Passiflora foetida virus Y; PleVY, *Pleione virus Y*; PStV, Peanut stripe virus; PtVY, Pterostylis virus Y; RhoVY, *Rhopalanthe virus Y*; SarVY, *Sarcochilus virus Y*; S1VY, Siratro 1 virus Y; S2VY, Siratro 2 virus Y; SMV, *Soybean mosaic virus*; WMV, *Watermelon mosaic virus*; WVMV, *Wisteria vein mosaic virus*; ZYMV, *Zucchini yellow mosaic virus*.

[b]In parentheses: ACT, Australian Capital Territory; NSW, New South Wales; Qld, Queensland; WA, Western Australia.

[c]Two clones each of isolates Sb19 and Can1 were sequenced.

Figure 2 Symptoms caused by the newly described potyvirus found in *Hardenbergia* spp., PWV (*Passion fruit woodiness virus*) and BYMV (*Bean yellow mosaic virus*). Natural infection of the new potyvirus in *H. comptoniana*: (a) leaves showing chlorotic mosaic; (b) shoot showing mild mosaic and leaf distortion; and (c) leaf showing bright yellow spots and blotches. (d) *Lupinus angustifolius* plant showing tip necrosis, leaflet downcurling and stunting following sap inoculation with the new potyvirus from *H. comptoniana*. (e) *Passiflora caerulea* leaf showing severe mosaic and leaf distortion caused by natural infection with PWV. (f) *Kennedia prostrata* shoot showing severe leaf mosaic and distortion caused by natural infection with BYMV.

transmitted isolates MU-2A and Co-1 from infected to healthy *N. benthamiana* plants. When seeds harvested from infected *N. benthamiana* plants were sown and samples from the 720 resulting seedlings tested by ELISA, no virus was detected. Thus, although no seed transmission was found, as with most other potyviruses, the virus from *H. comptoniana* was transmitted non-persistently by aphids.

Host reactions

Potyvirus from Hardenbergia *spp*.

Koch's postulates were satisfied under glasshouse conditions when healthy *H. comptoniana* plants inoculated with isolate MU-2A using aphids developed the characteristic mosaic and leaf-distortion symptoms in young leaves and potyvirus was detected in tip-leaf samples by ELISA. Sap from leaf samples with symptoms from *H. comptoniana* from 11 different locations in the region and from *H. violacae* infected plants of *N. benthamiana*. When virus-indicator plants and cultivated plants from eight different families were inoculated with infective *N. benthamiana* sap from each of nine isolates, few became infected, indicating a narrow host range (Table 3). *Nicotiana benthamiana* was the only solanaceous host and the only host species that all isolates infected systemically. In general, all isolates induced similar types of symptoms, except with isolates BB-6, MU-3A and, especially, Sb-3, with which symptoms tended to be milder. Apart from Sb-3, all isolates infected inoculated leaves of members of the Amaranthaceae (*Gomphrena globosa*) and Chenopodiaceae (*Chenopodium amaranticolor* and *C. quinoa*) and, apart from one instance with BB-6, induced systemic infection in the legumes *Lupinus angustifolius* (narrow-leafed lupin), *L. cosentinii* (sandplain lupin) and *L. luteus* (yellow lupin). These lupin species developed severe symptoms mostly consisting of mosaic, leaf bunching, curling or distortion, and plant stunting, associated with minimal seed production (Fig. 2d). Other legume species became infected by some of the isolates (*L. mutabilis*, *Pisum sativum*), one isolate (*Medicago polymorpha*, *T. subterraneum*, *Vigna unguiculata*) or none of them (*Vicia faba*). Isolate Sb-3 was less virulent than the others, only invading *N. benthamiana* and *L. cosentinii* systemically and infecting three other species locally. Non-hosts included *Apium graveolens*, *Daucus carota* (Apiaceae), *Brassica napus* (Brassicaceae), *Cucumis sativus, Cucurbita pepo* (Cucurbitaceae) and *Capsicum annuum*, *Lycopersicon esculentum*, *N. glutinosa* and *N. tabacum* (Solanaceae).

PWV

Sap from leaf samples of *Passiflora* spp. with symptoms infected with PWV from four sites (Table 1) caused mosaic, leaf distortion and stunting when inoculated onto *N. benthamiana*, and leaf samples gave positive results when tested by ELISA and RT-PCR.

Sequencing and phylogenetic analyses

Potyvirus from Hardenbergia *spp*.

The nucleotide sequences of the *CP* genes of 27 potyvirus isolates from *H. comptoniana* and one from *H. violacea* were determined (Table 2). These nucleotide sequences showed considerable diversity, clustering into eight distinct clades (Fig. 3). Divergence between isolates within clades was 0·0–5·3% and that between clades was 5·4–21·1%. Clades III, IV and VII each contained only one isolate: Cgt-1 from *H. violacea* (III), MU-2A (IV) and Sb19 (VII). Isolate Can1 from eastern Australia fitted into clade

Table 3 Plant responses to inoculation with nine isolates of the newly described potyvirus from *Hardenbergia* spp.

	Isolate and clade[a]								
	MU-1C	Wel-1	He-2	MU-2A	BB-6	Co-1	MU-3A	Sb-3	Sb19-1
Species	I	I	II	IV	V	V	V	VI	VII
Amaranthaceae									
Gomphrena globosa	RS[b]	RS	RS	RS	RS	RS	RS	–	RS
Chenopodiaceae									
Chenopodium amaranticolor	NS	SI	CS	CS	CS	NS	CS	SI	NS
C. quinoa	SI	SI	CS	CS	SI	CS	CS	–	SI
Fabaceae									
Lupinus angustifolius	M, LD, LC, S	LC, B, S	M, LC, B, S	M, B, S	M, B, S	B, LC, S	M, B, S	–	M, B, LD, S
L. cosentinii	M, LC	M, LD, D	LD, S	M, LD, S, D	M, S	M, LD, S, D	M, LD	M, S	LD, S
L. luteus	M, LD, S	M, LD, S	M, LD	M, LD, S	SI	M, LD, S	M, B, LD	–	M, LD, S
L. mutabilis	MM	–	–	MM	–	MM	MM	SI	–
Pisum sativum	M	M	SI	–	–	–	–	SI	–
Solanaceae									
Nicotiana benthamiana	M, LD	M, LD	M	M	M, LD	M, LD	M	M, LD	M, LD

[a]Leaves were inoculated with infective *N. benthamiana* sap. Samples from inoculated and tip leaves of all plants were tested for potyvirus infection by ELISA after 2–3 and 4–6 weeks, respectively.

[b]Coded symptom descriptions: B, bunching of young leaves; CS, chlorotic spots in inoculated leaves; D, plant death; LC, leaf downcurling; LD, leaf distortion; M, mosaic; MM, mild mosaic; NS, necrotic spots in inoculated leaves; SI, symptomless infection in inoculated leaves only; S, stunting; RS, red spots in inoculated leaves; –, no virus detected by ELISA in inoculated or tip-leaf samples.

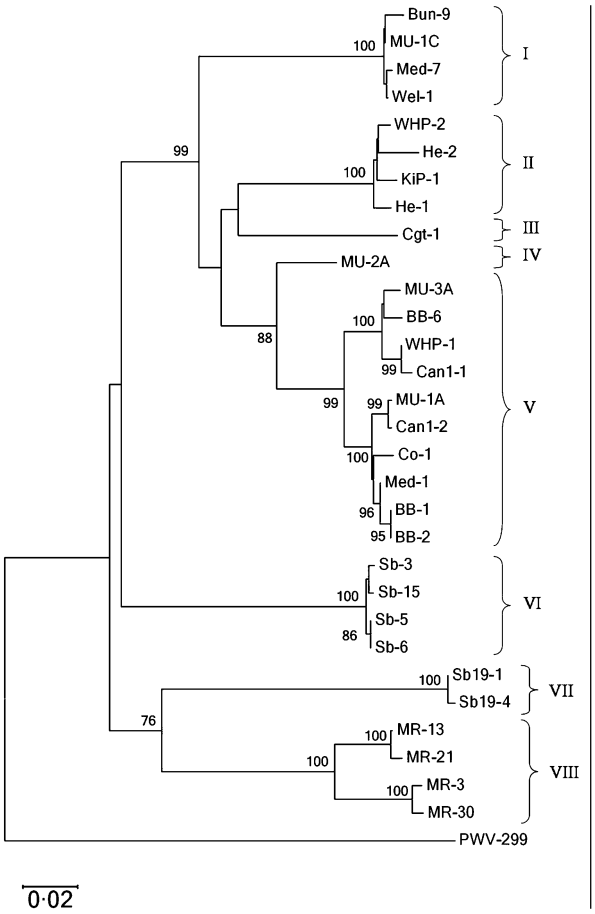

Figure 3 Neighbour-joining relationship dendrogram of the coat-protein gene nucleotide sequences of 28 potyvirus isolates from *Hardenbergia* spp., using the closest known relative PWV-299 to provide the outgroup sequence. Clades are numbered I to VIII. Trees were generated using MEGA 3·1 using the default parameters. Tree branches were bootstrapped with 1000 replications. Numbers at nodes indicate bootstrap scores > 75%. The scale bar represents a genetic distance of 0·02 for horizontal branch lengths. For isolate designations see Table 2.

V, which was the largest, containing nine isolates. The most widely separated isolates were Sb19 (of which two clones were sequenced) and He-2 (21·1% nucleotide diversity). There was often considerable sequence diversity between isolates collected from the same locality (Table 1), e.g. at site 1 isolates from two clades (VI and VII) were found and there was up to 18·6% nucleotide difference between them. When the *CP* sequences of these isolates were compared with those of other potyviruses, they were closest to five species known only from Australia: PWV, Siratro 1 virus Y (S1VY), Siratro 2 virus Y (S2VY), *Clitoria virus Y* (CliVY) and *Hibbertia virus Y* (HiVY) (Kiratiya-Angul & Gibbs, 1992; Sokhandan *et al*., 1997), with which they constituted an Australian subgroup within the broader BCMV group of potyviruses (Fig 4; Table 2). PWV diverged from the isolates from *Hardenbergia* spp. by 24·1–28·7%. The corresponding figures were for S1VY, 26·4–28·8%; HiVY, 26·6–30·6%; CliVY, 27·2–29·8%; and S2VY, 27·5–29·8%.

PWV

The four PWV isolates from Western Australia exhibited relatively low genetic diversity (0·9–6·2% nucleotide divergence). They were most similar to a previously sequenced isolate PWV-299 (2·3–6·5% nucleotide divergence; Fig. 4) obtained from the naturalized tropical American species *P. suberosa* (corky stem passion flower) growing in Queensland. However, the nucleotide sequences of these five isolates differed by 23·1–25·0% from those of three others from New South Wales (Sokhandan *et al*., 1997), revealing that there are two very distinct PWV clades.

BYMV

In contrast to the potyvirus isolates from *Hardenbergia* spp., the five BYMV isolates from *K. prostrata* showed far less diversity (1·1–4·4% nucleotide divergence). They fitted into a grouping which also contained six other BYMV isolates: two from lupins from Western Australia (Cheng & Jones, 1999, 2000), one each from the orchids *Pterostylis curta* and *Diuris maculata* which are indigenous to eastern Australia (Gibbs *et al*., 2000), and three from *Gladiolus* spp. from Denmark (Boye *et al*., 1990) and Japan (Fig. 4; Table 2).

Discussion

In this study, the biological and genetic properties of a newly discovered potyvirus from the unique native vegetation of the SWAFR is described. This virus caused a widespread mosaic disease in the native legume *H. comptoniana*, so the name Hardenbergia mosaic virus (HarMV) is proposed. It had a narrow host range and was transmitted non-persistently by the aphid *M. persicae*. Information on the symptoms induced by nine isolates in different hosts and *CP* sequence data of 28 isolates were obtained. New sequence data for four isolates of PWV from *Passiflora* spp. and five of BYMV from *K. prostrata* were also obtained.

When the nucleotide sequences of the *CP* genes of the 28 HarMV isolates were determined, they clustered into eight distinct clades that diverged by 5·4–21·1% in nucleotide identity. This was slightly below the species demarcation borderline of 23–24% at the nucleotide level for potyviruses (Adams *et al*., 2005; Fauquet *et al*., 2005; Ward *et al*., 2005), showing that only one species was involved. However, the genetic divergence between some clades was still considerable, notably isolate Sb19 in clade VII, to which no other isolates were closer than MR-3 and Sb-3 in clades VI and VIII, respectively, with 16·9% nucleotide difference between them. HarMV was most closely related genetically to five other potyvirus species found only in Australia: CliVY, HiVY, S1VY, S2VY and PWV, the first four of which are reported only from the east of the continent (Gibbs & Mackenzie 1997; Mackenzie *et al*., 1998). Along with HarMV, these constitute an

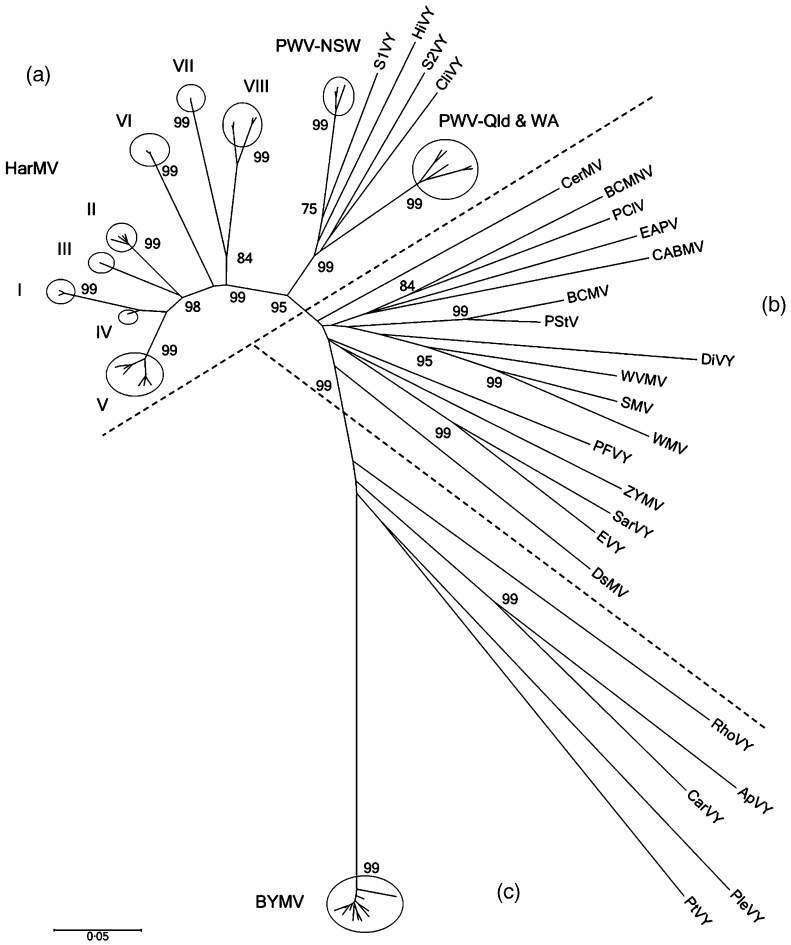

Figure 4 Unrooted neighbour-joining tree obtained from the alignment of coat protein nucleotide sequences using the CLUSTALW and MEGA 3·1 programmes and their default parameters. (a) 'Australian-only' subgroup of the BCMV group; clades of the potyvirus isolates from *Hardenbergia* spp. are numbered I to VIII; (b) remainder of BCMV group (one isolate per virus) containing members of the genus *Potyvirus* known only from Australia and others; (c) non-BCMV group members of the genus *Potyvirus* known only from Australia (one isolate per virus), and the BYMV clade containing the *Kennedia prostrata* isolates. NSW, New South Wales; Qld, Queensland, WA, Western Australia. Tree branches were bootstrapped with 1000 replications. Numbers at nodes indicate bootstrap scores > 75%. Scale bar represents a genetic distance of 0·05 for branch lengths. For isolate designations see Table 2.

exclusively Australian subgroup within the overall BCMV potyvirus grouping.

Genomic divergence is roughly proportional to the evolutionary distance from a common ancestor (Van Regenmortel, 2000), and a high degree of sequence diversity over a small geographic range is typical for potyvirus species that co-evolved with indigenous wild plants over a long period of time (e.g. Spetz *et al.*, 2003). Thus, the occurrence of widely divergent isolates and clades suggests that HarMV co-evolved within one or more indigenous plants in the SWAFR, and was not introduced from elsewhere during the short period of cultivation since 1829. Occurrence of HarMV infecting the eastern Australian species *H. violacea* within the region and *H. comptoniana* in a garden in eastern Australia do not necessarily alter this conclusion. The former presumably became infected during propagation after it arrived in the SWAFR, while the latter may have been infected before it was sent to eastern Australia, where it is not indigenous; *H. comptoniana* is now grown in gardens throughout Australia because of its attractive blue flowers.

More information on genes other than the *CP* gene studied here would help by providing more sensitive discrimination between distinct HarMV isolates. Choi *et al.* (2001), Stenger *et al.* (2002) and French & Stenger (2005) studied factors affecting the rate of increase of diversity of the natural North American population of *Wheat streak mosaic virus*, another member of the *Potyviridae*. They derived an estimate of the long-term rate of nucleotide sequence change for this population. It would be instructive to apply such an approach to HarMV to provide additional evidence of its co-evolution with one or more indigenous plants in the region.

Natural infection with HarMV in *H. comptoniana* caused mosaic and leaf distortion, occasionally associated with bright yellowing, but symptomless infection was also found. Aphid inoculation of healthy *H. comptoniana* with isolate MU-2A reproduced the characteristic mosaic and leaf-distortion symptoms under glasshouse conditions. Host range and symptomatology in test plants have often been used in the past to differentiate strains and pathotypes of viruses. Sap inoculation of nine selected isolates representing six distinct HarMV clades onto a range of test plants revealed differences in symptoms and host range. *Nicotiana benthamiana*, which is native to Western Australia (Paczkowska & Chapman, 2000), was the only solanaceous host found and the only host species that all

isolates infected systemically. In general, all isolates induced similar types of symptoms, except that BB-6, MU-3A and especially Sb-3 tended to induce milder symptoms. Apart from Sb-3, all isolates infected inoculated leaves of members of the Amaranthaceae and Chenopodiaceae, and induced systemic infection in most of the legume species they infected. Isolate Sb-3 had a narrower host range than the others. It invaded only *N. benthamiana* and the legume *L. cosentinii* systemically, infected only three other species locally, did not infect the species of Amaranthaceae tested, and caused milder symptoms. This isolate was the only one inoculated that represented clade VI. However, further studies would be needed to determine whether all clade-VI isolates behave in this way and whether differences in the *CP* nucleotide sequence might account for symptom and host-range differences between it and the other HarMV isolates. Apart from Sb-3 and BB-6, all of the isolates induced severe systemic symptoms associated with minimal seed production in experimentally infected plants of each of three lupin species, *L. angustifolius*, *L. cosentinii* and *L. luteus*.

There was no evidence that any other virus in addition to HarMV was also present in leaves of *H. comptoniana* with symptoms. No unusual symptoms that might have indicated presence of another virus developed upon inoculation of sap from affected leaf tissue into indicator hosts, and Koch's postulates were satisfied when healthy *H. comptoniana* plants were inoculated with an isolate of HarMV using aphids. Also, extracts from leaf samples with symptoms were always positive when tested by ELISA and RT-PCR using generic potyvirus antibody and primers, respectively.

Induction by HarMV of severe systemic symptoms in *L. angustifolius*, *L. cosentinii* and *L. luteus* is an important finding, as the nutrient-deficient, predominantly sandy soils of southwest Australia support the world's largest lupin industry, exporting its grain to many other parts of the world, predominantly for stock feed. *Lupinus angustifolius* is the main lupin species planted and is grown on over 0·5–1·1 million ha annually in the region. Some *L. luteus* is also grown and *L. cosentinii* is widely established in sheep pastures. Lupins were first introduced in the 20th century, and the *L. angustifolius* industry is only 30 years old (Gladstones *et al*., 1998). The widespread occurrence of HarMV in populations of *H. comptoniana* and the severe systemic disease that greatly diminishes seed set that it induced in the three lupin species, indicate that the virus may be a cause for concern, especially with *L. angustifolius*. *Hardenbergia comptoniana* populations frequently occur next to lupin fields in roadside verges that also contain naturalized wild lupins and in native bush remnants, so the opportunity often exists for naturally occurring aphid vectors to transfer the virus to lupins. Moreover, the symptoms caused by non-necrotic strains of BYMV in *L. angustifolius* resemble those caused by HarMV. Past surveys for BYMV in wild populations and crops of this species were based mainly on recording plants with symptoms (Cheng & Jones, 1999). Inadvertently, such surveys may have attributed symptoms of HarMV in lupin to non-necrotic BYMV strains. Other locally important introduced cultivated plant hosts in the region that became infected with some isolates of HarMV were *P. sativum* (field pea) and the important annual pasture legume species *M. polymorpha* (burr medic) and *T. subterraneum* (subterranean clover).

PWV, the Australian potyvirus to which HarMV is most closely related, provides a local example of a 'new encounter' scenario where an indigenous virus has apparently emerged to damage recently introduced *Passiflora* spp. and legume species, both cultivated and wild. Possibly, it spread to these introduced plants from the indigenous PWV host *P. aurantia*. Its *CP* gene sequences showed great diversity, suggesting that it co-evolved with native Australian vegetation. As with HarMV, such diversity is consistent with that expected for a virus in its centre of origin (Thresh, 1980; Jones, 1981). Indeed, the two distinct PWV clades found diverged sufficiently to be considered candidates for splitting into two distinct species, as previously suggested (Adams *et al*., 2005). Since its members were described first (Shukla *et al*., 1988), it is proposed that the name PWV be retained for the clade containing the Queensland isolate and the four new Western Australian isolates, and it is tentatively suggested that the name Passiflora mosaic virus be used for the clade containing the three New South Wales isolates. Previously, Sokhandan *et al.* (1997) studied sequences from 13 PWV isolates from New South Wales and reported that they formed two very distinct clades. However, they only lodged sequence data for the three isolates in the latter clade.

BYMV, a potyvirus recently introduced to Australia, now damages the native legume species *K. prostrata*, providing an example of the consequences of 'new encounter' scenarios where introduced invasive viruses invade vulnerable populations of indigenous plants. In contrast to the situation with HarMV and PWV, there was low genetic diversity among BYMV isolates from *K. prostrata*. This seems consistent with BYMV having originated from a genetic bottleneck typical of a founder virus population. The five isolates from *K. prostrata* fitted into a phylogenetic grouping which also contained six other BYMV isolates: two from lupin from Western Australia, two from orchids indigenous to eastern Australia, and three from *Gladiolus* spp. from Denmark or Japan. Possibly, the suggested founder population of BYMV that gave rise to the *K. prostrata* isolates was originally introduced to the Australian island continent in gladiolus bulbs. To quantify the impacts of introduced viruses such as BYMV on biodiversity, future studies with infected indigenous species will need to measure the dynamics of plant population structure before and after virus challenge, including recruitment rates and genetic diversity.

Apart from *Wild potato mosaic virus* (Jones & Fribourg, 1979) and *Pokeweed mosaic virus* (Shepherd *et al*., 1969), few potyviruses described to date are considered sufficiently distinct from cultivated plant potyviruses to fit

the definition of being 'wild-plant-adapted' (Harrison 1981; Cooper & Jones, 2006). However, HarMV seems to be an example and is also clearly a 'specialist' virus (Thresh, 1981) because of its narrow natural host range and close association with its principal host. PWV infects several *Passiflora* spp. and legume species naturally, and so would not be classed as a 'specialist' virus (Buchen-Osmond *et al*., 1988; Sokhandan *et al*., 1997). Neither can it be considered 'wild-plant-adapted' as it spreads readily in introduced cultivated *Passiflora* and legume species. Similarly, BYMV infects both wild and cultivated leguminous and non-leguminous species naturally, so it too is neither 'wild-plant-adapted' nor 'specialist'.

Plant virus ecology has been neglected as a discipline and urgently needs to address questions related to virus movement between natural and managed ecosystems, and the relative threats posed by introduced and native viruses. This need is becoming increasingly critical because of the anticipated impacts of rapid climate change on vectors of viruses, host plants and the viruses themselves. It is likely that global warming will not only exacerbate the damage caused by virus epidemics in known pathosystems, but also accelerate the emergence of new plant virus disease problems. Thresh (1980, 1981) provided earlier examples of the damage that viruses cause when crops are introduced to new continents. These included the spread of *Cocoa swollen shoot virus* (family *Caulimoviridae*; genus *Badnavirus*) from indigenous tree hosts into cocoa plantations after this crop was introduced to West Africa at the end of the 19th century. His examples also included spread of *Rice yellow mottle virus* (family unassigned; genus *Sobemovirus*) to rice and *Maize streak virus* (family *Geminviridae*; genus *Mastrevirus*) to maize after these crops were introduced to Africa. Fargette *et al*. (2006) reviewed information on the molecular virology of some these pathosystems and discussed the factors responsible for their emergence. The present study on three potyviruses in the SWAFR describes examples of such 'new encounters' from another continent. It also provides insights into the role played by viruses at the crucial interface between indigenous vegetation and cultivated areas, and offers a model system for future studies of such interfaces elsewhere. It illustrates how human activities facilitate the increasing frequency of 'new encounters' between viruses and plants, which has the potential to threaten biodiversity and introduced cultivated plants alike. The threat posed by introduced viral pathogens to plant biodiversity is underrated in areas of the world with rich but endangered floras (see Cooper & Jones, 2006). The studies with BYMV isolates from *K. prostrata* also illustrate why there is a need for greater awareness about the consequences of relaxing national plant quarantine controls to facilitate trade, as has occurred in Australia. These controls play an important role in restricting the possibility of invasive viruses, like BYMV, entering inadvertently with imported plant materials. There is also an increasing need to study viruses in natural ecosystems to provide critical information on virus evolution and facilitate the development of effective strategies to conserve endangered populations of indigenous plants. Moreover, such studies also help to identify potential threats posed by indigenous viruses, such as PWV, which damage introduced cultivated plants and subsequently may be distributed worldwide through the burgeoning world trade in plants and plant products.

Acknowledgements

Murdoch University provided CW with a research scholarship. Bill Kenworthy assisted with bioinformatics. Eva Gajda and Monica Kehoe helped with plant maintenance and ELISA.

References

Adams MJ, Antoniw JF, Fauquet CM, 2005. Molecular criteria for genus and species discrimination within the family *Potyviridae*. *Archives of Virology* **150**, 459–79.

Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P, 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecological Evolution* **19**, 535–44.

Baker CA, Jones L, 2007. A new potyvirus found in *Passiflora incence* in Florida. *Plant Disease* **91**, 227.

Bos L, 1981. Wild plants in the ecology of virus diseases. In: Maramarosch K, Harris KF, eds. *Plant Diseases and Vectors: Ecology and Epidemiology*. New York, USA: Academic Press, 1–28.

Boye K, Jensen PE, Stummann BM, Henningsen KW, 1990. Nucleotide sequence of cDNA encoding the BYMV coat protein gene. *Nucleic Acids Research* **18**, 4926.

Brooks TM, Mittermeier RA, Mittermeier CG *et al*., 2002. Habitat loss and extinction in the hotspots of biodiversity. *Conservation Biology* **16**, 909–23.

Buchen-Osmond C, Crabtree K, Gibbs A, McLean G, 1988. *Viruses of Plants in Australia*. Canberra, Australia: Australian National University Printing Service.

Buddenhagen IW, 1977. Resistance and vulnerability of tropical crops in relation to their evolution and breeding. *Annals of the New York Academy of Sciences* **287**, 309–26.

Chen J, Chen J, Chen J, Adams MJ, 2001. Molecular characterisation of an isolate of *Dasheen mosaic virus* from *Zantedeschia aethiopica* in China and comparisons in the genus *Potyvirus*. *Archives of Virology* **146**, 1821–9.

Cheng Y, Jones RAC, 1999. Distribution and incidence of necrotic and non-necrotic strains of bean yellow mosaic virus in wild and crop lupins. *Australian Journal of Agricultural Research* **50**, 589–99.

Cheng Y, Jones RAC, 2000. Biological properties of necrotic and non-necrotic strains of bean yellow mosaic virus in cool season grain legumes. *Annals of Applied Biology* **136**, 215–27.

Choi IR, Hall JS, Henry M *et al*., 2001. Contributions of genetic drift and negative selection on the evolution of three strains of wheat streak mosaic tritimovirus. *Archives of Virology* **146**, 619–28.

Clark MF, Adams AN, 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* **34**, 475–83.

Cobb NA, 1901. Woodiness of the passion fruit. *Agricultural Gazette of New South Wales* **12**, 407–18.
Cooper JI, Jones RAC, 2006. Wild plants and viruses: under-investigated ecosystems. *Advances in Virus Research* **67**, 1–47.
Coutts BA, Jones RAC, 2002. Temporal dynamics of spread of four viruses within mixed species perennial pastures. *Annals of Applied Biology* **140**, 37–52.
Desbiez C, Lecoq H, 2004. The nucleotide sequence of *Watermelon mosaic virus* (WMV, *Potyvirus*) reveals interspecific recombination between two related potyviruses in the 5' part of the genome. *Archives of Virology* **149**, 1619–32.
Fang GW, Allison RF, Zambolim EM, Maxwell DP, Gilbertson RL, 1995. The complete nucleotide sequence and genome organization of bean common mosaic virus (NL3 strain). *Virus Research* **39**, 13–23.
Fargette D, Konate G, Fauquet C, Muller E, Peterscmitt M, Thresh JM, 2006. Molecular ecology and emergence of tropical plant viruses. *Annual Review of Phytopathology* **44**, 235–260.
Fauquet CM, Mayo MA, Maniloff J, Desselburger U, Ball LA, 2005. *Virus Taxonomy. Eighth Report of the International Committee for the Nomenclature of Viruses*. San Diego, CA, USA: Elsevier.
French R, Stenger DC, 2005. Population structure within lineages of *Wheat streak mosaic virus* derived from a common founding event exhibits stochastic variation inconsistent with the deterministic quasi-species model. *Virology* **343**, 179–89.
Gibbs A, Mackenzie A, Blanchfield A *et al*., 2000. Viruses of orchids in Australia: their identification, biology and control. *Australian Orchid Review* **65**, 10–21.
Gibbs A, Mackenzie A, 1997. A primer pair for amplifying part of the genome of all potyvirids by RT-PCR. *Journal of Virological Methods* **63**, 9–16.
Gibbs AJ, Gower JC, 1960. The use of a multiple transfer method in plant virus transmission studies and some statistical points arising from the analysis of results. *Annals of Applied Biology* **48**, 75–83.
Gladstones JS, Atkins CA, Hamblin J, 1998. *Lupins as Crop Plants: Biology, Production and Utilization*. Wallingford, UK: CAB International.
Hajimorad MR, Eggenberger AL, Hill JH, 2003. Evolution of *Soybean mosaic virus*-G7 molecularly cloned genome in Rsv1-genotype soybean results in emergence of a mutant capable of evading Rsv1-mediated recognition. *Virology* **314**, 497–509.
Harrison BD, 1981. Plant virus ecology: ingredients, interactions and environmental influences. *Annals of Applied Biology* **99**, 195–209.
Hopper SD, 1979. Biogeographical aspects of speciation in the southwest Australian flora. *Annual Review of Ecology and Systematics* **10**, 399–422.
Hopper SD, Gioia P, 2004. The southwest Australian floristic region: evolution and conservation of a global hot spot of biodiversity. *Annual Review of Ecology, Evolution and Systematics* **35**, 623–50.
Iwai H, Terahara Y, Yamashita Y, Ueda S, Nakamura M, 2006. Complete nucleotide sequence of the genomic RNA of an Amami-O-shima strain of East Asian passiflora potyvirus. *Archives of Virology* **151**, 1457–60.
Jeger MJ, Seal SE, Van den bosch F, 2006. Evolutionary epidemiology of plant virus disease. *Advances in Virus Research* **67**, 163–204.
Jones RAC, 1981. The ecology of viruses infecting wild and cultivated potatoes in the Andean Region of South America. In: Thresh JM, ed. *Pests, Pathogens and Vegetation*. London, UK: Pitman, 89–107.
Jones RAC, 1996. Virus diseases of Australian pastures. In: Chakraborty S, Leath KT, Skipp RA *et al*., eds. *Pasture and Forage Crop Pathology*. Madison, WI, USA: American Society of Agronomy, 303–22.
Jones RAC, 2006. Control of plant virus diseases. *Advances in Virus Research* **67**, 205–44.
Jones RAC, Fribourg CE, 1979. Host plant reactions, some properties and serology of wild potato mosaic virus. *Phytopathology* **69**, 446–9.
Jones RAC, McLean GD, 1989. Virus diseases of lupins. *Annals of Applied Biology* **114**, 609–37.
Jones RAC, Nicholas DA, 1998. Impact of an insidious virus disease in the legume component on the species balance within self-regenerating annual pasture. *Journal of Agricultural Science* **131**, 155–70.
Kiratiya-Angul S, Gibbs A, 1992. Group-specific antiserum detects potyviruses in several wild plant species in south-eastern Australia. *Australasian Plant Pathology* **21**, 118–9.
Kumar S, Tamura K, Nei M, 2004. MEGA 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* **5**, 150–63.
Latham LJ, Jones RAC, 1997. Occurrence of tomato spotted wilt tospovirus in native flora, weeds, and horticultural crops. *Australian Journal of Agricultural Research* **48**, 359–69.
Latham LJ, Jones RAC, 2001. Distribution and incidence of virus infection in experimental plots, commercial crops and seed stocks of cool season crop legumes. *Australian Journal of Agricultural Research* **52**, 397–413.
Liang WX, Song LM, Tian GZ, Li HF, Fan ZF, 2006. The genomic sequence of *Wisteria vein mosaic virus* and its similarities with other potyviruses. *Archives of Virology* **151**, 2311–9.
Lin S-S, Hou RF, Yeh S-D, 2001. Complete genome sequence and genetic organization of a Taiwan isolate of *Zucchini yellow mosaic virus*. *Botanical Bulletin of Academia Sinica* **42**, 243–50.
Lovisolo O, Hull R, Rosler O, 2003. Coevolution of viruses with hosts and vectors and possible paleontology. *Advances in Virus Research* **62**, 325–79.
Mackenzie AM, Nolan M, Wei KJ *et al*., 1998. Ceratobium mosaic potyvirus: another virus from orchids. *Archives of Virology* **143**, 903–14.
Malmstrom CM, Hughes CC, Newton LA, Stoner CL, 2005. Virus infection in remnant native bunchgrasses from invaded California grasslands. *New Phytologist* **168**, 217–30.
Marchant NG, Wheeler JR, Rye BL, Bennett EM, Lander NS, MacFarlane TD, 1987. *Flora of the Perth Region*. Perth, Australia: Western Australian Herbarium.
McKirdy SJ, Coutts BA, Jones RAC, 1994. Occurrence of bean yellow mosaic virus in subterranean clover pastures and perennial native legumes. *Australian Journal of Agricultural Research* **45**, 183–94.
Mlotshwa S, Verver J, Sithole-Niang I, Van Kampen T, Van Kammen A, Wellink J, 2002. The genomic sequence of *Cowpea aphid-borne mosaic virus* and its similarities with other potyviruses. *Archives of Virology* **147**, 1043–52.

Moran J, van Rijswijk B, Traicevski V, Kitajima EW, Mackenzie AM, Gibbs AJ, 2002. Potyviruses, novel and known, in cultivated and wild species of the family Apiaceae in Australia. *Archives of Virology* **147**, 1855–67.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J, 2000. Biodiversity hot spots for conservation priorities. *Nature* **403**, 853–8.

Nascimento AVS, Santana EN, Braz ASK *et al*., 2006. *Cowpea aphid-borne mosaic virus* (CABMV) is widespread in passionfruit in Brazil and causes passionfruit woodiness disease. *Archives of Virology* **151**, 1797–809.

Paczkowska G, Chapman AR, 2000. *The Western Australian Flora. A Descriptive Catalogue*. Perth, Australia: Wildflower Society of Western Australia, Western Australian Conservation and Land Management, and Botanic Gardens and Parks Authorities.

Shepherd RJ, Fulton JP, Wakeman RJ, 1969. Properties of a virus causing pokeweed mosaic. *Phytopathology* **59**, 219–22.

Shukla DD, McKern NM, Gough KH, Tracey SL, Letho SG, 1988. Differentiation of potyviruses and their strains by high performance liquid chromatographic peptide profiling of coat proteins. *Journal of General Virology* **69**, 493–502.

Sokhandan N, Gillings MR, Bowyer JW, 1997. Polymerase chain reaction detection and assessment of genetic variation in New South Wales isolates of passionfruit woodiness potyvirus. *Australasian Plant Pathology* **26**, 155–64.

Spetz C, Taboada AM, Darwich S, Ramsell J, Salazar LF, Valkonen JPT, 2003. Molecular resolution of a complex of potyviruses infecting solanaceous crops at the centre of origin in Peru. *Journal of General Virology* **84**, 2565–78.

Stenger DC, Seifers DL, French R. 2002. Patterns of polymorphism in *Wheat streak mosaic virus*: sequence space explored by a clade of closely related viral genotypes rivals that between the most divergent strains. *Virology* **302**, 58–70.

Thompson JD, Higgins DG, Gibson TJ, 1994. CLUSTAL W: improving sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22**, 4673–80.

Thresh JM, 1980. The origins and epidemiolology of some important plant virus diseases. *Applied Biology* **5**, 1–65.

Thresh JM, ed. 1981. *Pests, Pathogens and Vegetation*. London, UK: Pitman.

Thresh JM, 2006. Plant virus epidemiology: the concept of host genetic vulnerability. *Advances in Virus Research* **67**, 89–125.

Van Regenmortel MHV, 2000. Introduction to the species concept in virus taxonomy. In: Van Regenmortel *et al*., eds. *Virus Taxonomy. Seventh Report of the International Committee for Taxonomy of Viruses*. San Diego, CA, USA: Academic Press, 3–16.

Ward CW, Weiller GF, Shukla DD, Gibbs AJ, 2005. Molecular systematics of the *Potyviridae*, the largest plant virus family. In: Gibbs AJ, Calisher CH, Garcia-Arenal F, eds. *Molecular Basis of Virus Evolution*. Cambridge, UK: Cambridge University Press, 477–500.

Zheng H, Chen J, Chen J, Adams MJ, Hou M, 2002. *Bean common mosaic virus* isolates causing different symptoms in asparagus bean in China differ greatly in the 5′-parts of their genomes. *Archives of Virology* **147**, 1257–62.