

Opinion piece

Spread of introduced viruses to new plants in natural ecosystems and the threat this poses to plant biodiversity

ROGER A. C. JONES* AND BRENDA A. COUTTS

School of Plant Biology and Institute of Agriculture, Faculty of Science, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia and Department of Agriculture and Food Western Australia, Locked Bag no. 4, Bentley Delivery Centre, Perth, WA 6983, Australia

Biodiversity is endangered in many regions of the world, with loss of native plant species a growing concern requiring a major focus on conservation measures. However, the threat posed by introduced viral pathogens to native plant biodiversity has been ignored almost completely. What occurs when weed seeds and vegetative propagules or seeds of cultivated plants unknowingly infected with viruses are introduced to other regions of the world and the viruses introduced with them then invade indigenous plants for the first time? To what extent are introduced viruses capable of causing damaging diseases which then threaten native plant communities and their species biodiversity, and in what ways can they do this? What can be done about it? In this Opinion piece, our intent is to answer these questions by awakening worldwide interest to undertake research activities that provide a comprehensive understanding of the threat posed by introduced viruses to natural plant ecosystems and biodiversity. Development of such an understanding requires research activities capable of providing in-depth information at both biological and molecular levels. Without such knowledge, effective solutions are unlikely to emerge.

New encounters involving viruses and plant species are becoming increasingly common at the agro-ecological interface between managed and natural vegetation. This is because of rapidly increasing human activity, such as agricultural extensification, diversification and intensification practices to increase food production and to address food insecurity, encroaching urbanization and ever increasing international trade in plants and plant products (e.g. Alexander *et al.*, 2014; Jones, 2009, 2014; Roossinck and Garcia-Arenal, 2015). Moreover, inadvertent introduction of new, more efficient virus vectors often exacerbates spread of viruses to previously uninvaded plant species at vegetation interfaces. In the future, the frequency of new encounters between viruses and plant species is likely to increase even more rapidly because of the major alterations in cultivated plant distributions anticipated from climate change (Jones and Barbetti, 2012).

Genomic divergence is roughly proportional to the evolutionary distance from a common ancestor, and a high degree of nucleotide

sequence diversity over a small geographical range is typical of viruses that have co-evolved locally with native plants (e.g. Coutts *et al.*, 2011; Webster *et al.*, 2007). These viruses are referred to as 'indigenous' to distinguish them from others that have arrived from elsewhere and therefore show much less sequence diversity, for which the term 'introduced' is used (e.g. Coutts *et al.*, 2011; Jones, 2009; Webster *et al.*, 2007). The threat posed by introduced fungal pathogens to native plant communities and their biodiversity has received considerable attention (e.g. Burdon *et al.*, 2006), and the consequences of virus infection seem likely to resemble those of fungi, including a reduced ability of infected plants to compete with other plants and produce sufficient seed for the next generation (e.g. Cooper and Jones, 2006). However, the threat posed by introduced viral pathogens has received much less attention (Vincent *et al.*, 2014). This is so despite the considerable research activity aimed at understanding how emerging viruses spread in the opposite direction, i.e. from native plants to damage introduced cultivated plant species, especially in the tropics. Such studies normally involve investigation of new encounter scenarios at the agro-ecological interface (e.g. Alexander *et al.*, 2014; Jones, 2009; Roossinck and Garcia-Arenal, 2015).

Native plants do not grow as stands of genetically identical plants of the same species exhibiting uniform virus susceptibilities, but as mixed species communities exhibiting both within- and between-species diversity. Natural control measures which serve to decrease virus spread in undisturbed native plant communities, such as mixture with non-hosts, isolation, host resistance/tolerance and the presence of predators and parasites of their vectors, tend to be disrupted when such communities are disturbed, as occurs at the agro-ecological interface and in otherwise disturbed natural vegetation (e.g. Cooper and Jones, 2006; Jones 2009).

Co-evolution of viruses with their plant hosts and vectors is thought to have been underway since plants first appeared (Fraile and Garcia-Arenal, 2010; Lovisolo *et al.*, 2003). Thus, long before plants were first domesticated by former hunter gatherers when agriculture began 10 000–15 000 years ago, plants were co-evolving with native plants growing in different world regions. This co-evolutionary process moulded both viruses and native plants (e.g. Vincent *et al.*, 2014). In undisturbed native plant

*Correspondence: Email: roger.jones@uwa.edu.au

communities, virus infections resulting from this process mostly cause little in the way of disease symptoms and therefore may seem benign (e.g. Cooper and Jones, 2006; Roossinck and Garcia-Arenal, 2015). However, obvious virus disease does sometimes develop in plants growing in such vegetation, and the widespread occurrence of virus resistance genes in wild ancestors of cultivated plants provides clear evidence of past battles with viruses (e.g. Cooper and Jones, 2006; Jones 2009; Vincent *et al.*, 2014).

Generalist plant viruses are thought to have evolved first in species-rich native plant communities where they infected a wide range of hosts belonging to different plant families (Jones, 2009). In contrast, specialist viruses are thought to have evolved first in native plant communities with few species, and so relied on infection of just a few natural hosts (e.g. Vincent *et al.*, 2014). Virus epidemics in cultivated plants are caused by generalist and specialist viruses. Moreover, the process of wild plant domestication in different regions of the world has favoured selection of specialist viruses and specialist host-adapted strains within the same generalist virus (e.g. Jones, 2009; Jones and Barbetti, 2012). A decision over whether a virus is a generalist or specialist is based on its known natural host range and not on the extent of its host range when plants are inoculated under glasshouse conditions.

RNA viruses are known to host shift more easily than all other pathogen types (Longdon *et al.*, 2014) and almost 50% of all emerging plant pathogens are DNA or RNA viruses (Anderson *et al.*, 2004). Thus, viruses adapt to new hosts more readily than do plant pathogens with more complex genomes. In response to the altered circumstances when they invade new species, viruses undergo genomic changes that allow them to jump to, and survive within, their new hosts (e.g. Jones, 2009; Longdon *et al.*, 2014; Roossinck and Garcia-Arenal, 2015). Newly introduced generalist viruses therefore pose a potential threat to communities of native plants they invade for the first time. In addition, if new virus variants are generated at the agro-ecological interface as a result of different evolutionary pressures encountered whilst replicating in newly acquired native plant hosts, these might be important when deploying control measures involving single gene resistance in nearby cultivated plants.

WHAT EVIDENCE IS THERE THAT NEWLY INTRODUCED GENERALIST VIRUSES ARE INVADING COMMUNITIES OF NATIVE PLANTS?

Many studies in different parts of the world have examined wild plants to establish whether they can act as reservoirs of virus infection for spread to cultivated plants. Often the wild species involved are naturalized weeds, but sometimes they are native plants (e.g. Cooper and Jones, 2006). However, although it may be known whether viruses found infecting alternative hosts are generalists or specialists, making the distinction between indigenous and introduced viruses often tends to be difficult or

impossible in parts of the world in which plants have been cultivated for many years. In contrast, where cultivated plants from other continents have been recently introduced or plant cultivation is a recent phenomenon, it is often possible to make this distinction. An ideal example of the latter is provided by the vegetation interfaces of the South West Australian Floristic Region (SWAFR) which are ideally suited for study of the impact of newly introduced viruses on communities of native plants (Vincent *et al.*, 2014; Webster *et al.*, 2007). The SWAFR is unique geographically because of its isolation, geological stability and absence of human disturbance before European colonization in 1829. It is a species-rich global biodiversity hotspot with c. 8000 native plant species, 49% of which are endemic. It occupies 302 672 m², is isolated from the rest of Australia by deserts and has a harsh Mediterranean-type climate. Virus-like foliage symptoms, which are sometimes severe, have been observed for more than 50 years occurring commonly in native plants growing at the ancient ecosystem–recent agro-ecosystem interface in the SWAFR (Fig. S1, see Supporting Information). However, the significance of these virus-like symptoms was misunderstood because they were attributed to other causes, especially nutritional deficiencies (Vincent *et al.*, 2014).

When samples from native plants with leaf symptoms growing at the agro-ecological interface in the SWAFR were first tested for viruses, the introduced generalist virus *Bean yellow mosaic virus* (BYMV; genus *Potyvirus*) was found at several sites, infecting plants of *Kennedia prostrata*, *K. coccinea*, *Hovea elliptica* and/or *H. pungens*. Subsequently, both BYMV and the additional introduced generalists *Cucumber mosaic virus* (CMV; genus *Cucumovirus*), *Tomato spotted wilt virus* (TSWV; genus *Tospovirus*) and *Turnip yellows virus* (TuYV; genus *Polerovirus*) were identified infecting native plants at interface or disturbed natural vegetation sites distributed widely in the SWAFR. The native species infected were *K. eximia*, *K. prostrata*, *Drosera* sp. and *Triglochin* sp. (BYMV), *Anigozanthos manglesii* (CMV), *Calectasia cyanea* (TSWV), and *Bossiaea ornata*, *Daviesia nudiflora* and *Damperia* sp. (TuYV). In addition, BYMV, TuMV and the introduced generalist *Ornithogalum mosaic virus* (OrMV; genus *Potyvirus*) were found infecting a collection of native orchid plants. Examples of introduced specialist viruses found infecting native plants in SWAFR include *Barley yellow dwarf virus* (BYDV; genus *Luteovirus*), *Cereal yellow dwarf virus* (CYDV; genus *Polerovirus*) and *Wheat streak mosaic virus* (WSMV; genus *Tritimovirus*). The original small-scale studies mentioned above and performed over the last 25 years by different researchers were recently summarized by Vincent *et al.* (2014). An example of a virus indigenous to the SWAFR, as evidenced by the very wide nucleotide diversity between its isolates, is *Hardenbergia mosaic virus* (HarMV; genus *Potyvirus*), which is often found infecting the native species *Hardenbergia comptoniana* (Coutts *et al.*, 2011; Webster *et al.*, 2007). Further information on its natural host

range is required before it can be assigned to a specialist or generalist category. Several other viruses recently found infecting native plants using deep sequencing methodologies are also likely to be indigenous. Thus, evidence has been obtained that introduced generalist and specialist viruses are invading communities of native plants at the agro-ecological interface and in otherwise disturbed native vegetation in the SWAFR. In addition, evidence is accumulating that local indigenous viruses occur in native plants.

In addition to spread from nearby infected cultivated plants, another pathway by which introduced generalist viruses can spread to native plant communities in the SWAFR was identified when native orchids being propagated before being returned to the wild were found to be infected with BYMV. They were being grown centrally, infected plants often showed obvious virus symptoms and some infected species were highly endangered. The BYMV-infected native orchid infected species found belonged to four different genera: *Caladenia*, *Diuris*, *Microtis* and *Thelymitra* (e.g. Vincent *et al.*, 2014).

HOW COULD NEWLY INTRODUCED GENERALIST VIRUSES DAMAGE MIXED SPECIES COMMUNITIES OF NATIVE PLANTS?

Evidence of what viruses are capable of doing when they invade mixed species populations of plants comes from studies on: (i) mixed sown and wild plant species populations growing in commercial pastures; and (ii) natural pasture situations involving mixtures of wild plant species. In these studies, virus infections altered the species composition and dynamics of plant communities composed of species mixtures by decreasing the fitness of individual species within the plant population. Here, relative fitness refers to survivorship arising from ability to compete with plants of other species, reproduce sufficiently and produce the next generation of seedlings (Alexander *et al.*, 2014; Cooper and Jones, 2006). For example, an Australian study examined the effects of infection with *Alfalfa mosaic virus* (AMV, genus *Alfamovirus*) in annually self-regenerating pasture swards containing a mixed plant species population consisting of a sown pasture host species (*Medicago polymorpha*) and naturalized weed species that were poor or non-hosts. Although AMV infection caused only mild symptoms, it still diminished the fitness of the pasture species sufficiently to alter the species composition drastically in favour of the other species. Another Australian study examined the effects of infection with AMV, BYDV and *Ryegrass mosaic virus* (RyMV, genus *Rymovirus*) in perennial pastures consisting of the pasture species *Trifolium repens* and *Lolium perenne*, and the naturalized wild grass *Pennisetum clandestinum*. AMV infection of *T. repens* plants markedly decreased their invasiveness, competitive ability and survival, and so the proportion of both grasses increased or decreased when AMV incidences were high or low, respectively. In addition, RyMV-infected *L. perenne* and BYDV-infected

P. clandestinum plants were less able than healthy plants of the same species to survive competition with the other two species (Jones, 2009, 2013, and references cited therein). In an example from the USA, the effects of infection with BYDV were examined in natural pastures consisting of the naturalized annual grass *Avena fatua* and remnant native perennial bunch grass species. Widespread BYDV infection causing mild symptoms in *A. fatua* provided a potent reservoir for virus spread to the more sensitive native grass species and, once infected, they declined markedly because of a lack of fitness. Introduced annual grasses amplify both BYDV and its vector populations in such systems, but perennial grasses are critically important for sustaining virus populations in USA grasslands largely composed of introduced species (Alexander *et al.*, 2014, and references cited therein).

Vincent *et al.* (2014) determined the potential of six introduced generalist viruses (AMV, BYMV, CMV, TSWV, TuYV and *Turnip mosaic virus*, genus *Potyvirus*) to cause severe or mild systemic symptoms in 14 native plant species from the SWAFR belonging to seven different families (two to six viruses inoculated per species). The symptoms caused by infection with seven introduced specialist viruses (which included BYDV and WSMV) and four Australian indigenous viruses (which included HarMV) were also examined in the same study. Losses in biomass and reproductive ability were determined for some host–virus combinations. They found that: (i) the introduced generalist viruses caused severe systemic symptoms and growth reductions when they infected some native plant species; (ii) the specialist viruses caused only mild or symptomless systemic infection and no significant growth reduction; and (iii) the surprisingly severe systemic symptoms and growth reductions induced by indigenous viruses in some native plant species did not support the suggestion that indigenous viruses are mostly benign (see above), indicating instead that some have the potential to cause serious damage when they make host species jumps in populations of disturbed native flora. This research highlighted the potential for serious damage to plant biodiversity from virus disease epidemics that arise from new encounters between introduced generalist viruses and native plants. Moreover, some viruses (especially CMV and TSWV) infected plants in more families than others and so posed a greater potential threat. The study also revealed the potential for serious damage to plant biodiversity from indigenous viruses in natural vegetation when they encounter hosts to which they are poorly adapted. However, the introduced specialist viruses studied seemed to be less cause for concern (Vincent *et al.*, 2014).

WHAT CAN BE DONE ABOUT IT?

In this article, we have demonstrated an urgent need to study introduced generalist viruses in native plants at the agro-ecological interface and in otherwise disturbed or undisturbed natural ecosystems in regions of the world with rich but

endangered floras. We can suggest ideas about what research is needed, but currently these can only be generic and untested. In addition, such research requires a multidisciplinary approach involving collaboration between traditional and molecular virologists, vector entomologists and ecologists.

First, we need to understand: (i) which introduced generalist viruses are invading disturbed and undisturbed native plant communities in each region and which occur often enough to be potential causes for concern; (ii) the likely impacts of introduced generalist virus epidemics on the species dynamics of native plant communities; (iii) the roles of arthropod and other vectors in assisting virus spread within these communities; and (iv) the biological and molecular basis of virus evolution associated with the ability to make host species jumps from cultivated to wild plants. To address point (i), it would be necessary to undertake large-scale surveys involving biological, serological and/or molecular virus testing of symptomatic and randomly collected samples of native plants. The objective would be to find which introduced viruses have invaded native plants at the agro-ecological interface and in otherwise disturbed natural vegetation, which have already spread to undisturbed natural vegetation, and which are sufficiently widespread to be cause for concern. To address point (ii), two approaches could be adopted to study the impact of infection with the most widespread viruses on important native plants: first, by simulating simple, mixed species native plant communities in small replicated field plots at research station sites and introducing native plants infected with generalist plant viruses, and second, by using existing native plant communities at locations at which introduced generalist plant viruses are already present. The objective would be to quantify the effects of common introduced generalist viruses on fitness, competitiveness and survivorship in the different native plant species, and to determine their effects on the species composition and dynamics of native plant communities. To address point (iii), the approach would be to use the simulated, mixed native plant species field plots and natural infection sites to count the numbers of potential vector species visiting and/or colonizing native plant species, and to determine through biological and molecular testing whether they have been exposed to virus-infected plants. Glasshouse experiments would investigate preferential feeding behaviour and virus transmissibility of the vector species found. The objective would be to identify vector species able to transmit introduced generalist viruses to and between native plant species, and to understand the process by which they transmit viruses within mixed species native plant communities. To address point (iv), next-generation sequencing techniques would be employed to sequence the virus isolates introduced into the simulated mixed species native plant field plots. When a host species jump occurred, isolates from the invaded native plants would be sequenced again to compare the before and after sequences and so identify genomic changes. The viruses isolated from new hosts would be inoculated to their

original and new hosts and a selection of indicator plant species to assess the biological significance of the genomic changes found. The objective would be to understand the viral genomic alterations required to make a successful host species jump to a native plant host and the biological consequences of such changes that might pose a threat to the species dynamics of mixed species native plant communities.

Other important issues about which information is required include establishing whether introduced generalist viruses are seed borne in native plant species and whether vegetatively propagated stocks of planting material being prepared for return to the wild have already become contaminated. Once sufficient information has been accumulated about such factors, and also others, such as vector incidence, host susceptibilities in native species and environmental influences, models can be devised to predict the risk of generalist virus invasion of native plant communities. There is also a need to accumulate a sound understanding of the likely impacts of climate change on the magnitude of virus epidemics and virus-induced damage likely to occur in such communities. When considering future research needs in the broader context of both sides of the agro-ecological interface and associated landscapes, Alexander *et al.* (2014) suggested that future research requires: (i) an increased effort to describe plant virus diversity and distribution across agricultural and ecological boundaries; (ii) multi-scale studies on virus transmission to develop predictive power in estimating propagation across landscapes; and (iii) quantitative evaluation of virus influence on plant fitness and populations in environmental contexts beyond cultivated fields (summarized in Jones, 2014).

There is an urgent need for enhanced awareness by conservation authorities, and wild flower and tourist industries, about the importance of protecting native plants from invasive virus diseases, and to ensure that international quarantine authorities are kept informed over the possible entry of viruses capable of damaging natural vegetation. Vincent *et al.* (2014) emphasized the need to consider the establishment of worldwide conservation policies and management approaches that protect endangered plant species and biodiversity from virus invasion. Such policies and approaches require the management of virus disease in situations in which new encounters occur at agro-ecological interfaces or natural vegetation is otherwise disturbed, and the avoidance of virus spread to undisturbed natural vegetation. Vincent *et al.* (2014) also emphasized the need to tighten quarantine regulations when commercial planting material is moved from one part of the world to another to avoid introducing viruses that might damage natural vegetation. Avoidance of entry of plant materials containing viruses or new virus vectors at frontiers, sea ports and airports will require the re-examination and modification of existing quarantine protocols for cultivated plants to accommodate threats to native plant populations and biodiversity.

REFERENCES

- Alexander, H.M., Mauck, K.E., Whitfield, A.E., Garrett, K.A. and Malmstrom, C.M. (2014) Plant virus interactions and the agro-ecological interface. *Eur. J. Plant Pathol.* **138**, 529–547.
- Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R. and Daszak, P. (2004) Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends. Ecol. Evol.* **19**, 535–544.
- Burdon, J.J., Thrall, P.H. and Ericson, L. (2006) The current and future dynamics of disease in plant communities. *Annu. Rev. Phytopathol.* **44**, 19–39.
- Cooper, J.I. and Jones, R.A.C. (2006) Wild plants and viruses: under-investigated ecosystems. *Adv. Virus Res.* **67**, 1–47.
- Coutts, B.A., Kehoe, M.A., Webster, G.C., Wylie, S.J. and Jones, R.A.C. (2011) Indigenous and introduced potyviruses of legumes and *Passiflora* spp. from Australia: biological properties and phylogenetic placement. *Arch. Virol.* **156**, 1757–1774.
- Fraile, A. and Garcia-Arenal, F. (2010) The coevolution of plants and viruses: resistance and pathogenicity. *Adv. Virus Res.* **76**, 1–31.
- Jones, R.A.C. (2009) Plant virus emergence and evolution: origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. *Virus Res.* **141**, 113–130.
- Jones, R.A.C. (2013) Virus diseases of pasture grasses in Australia: incidences, losses, epidemiology and management. *Crop and Pasture Science*, **64**, 216–233.
- Jones, R.A.C. (2014) Plant virus ecology and epidemiology: historical perspectives, recent progress and future prospects. *Ann. Appl. Biol.* **164**, 320–347.
- Jones, R.A.C. and Barbetti, M.J. (2012) Influence of climate change on plant disease infections and epidemics caused by viruses and bacteria. *CAB Rev.* **7**, 1–32. <http://www.cabi.org/cabreviews>.
- Longdon, B., Blockhourst, M.A., Russell, C.A., Welch, J.J. and Jiggins, F.M. (2014) The evolution and genetics of virus host shifts. *PLoS Pathogens*, **10**(11), e1004395.
- Lovisolo, O., Hull, R. and Rosler, O. (2003) Coevolution of viruses with hosts and vectors and possible paleontology. *Adv. Virus Res.* **62**, 325–379.
- Roossinck, M.J. and Garcia-Arenal, F. (2015) Ecosystem simplification, biodiversity loss and plant emergence. *Curr. Opin. Virol.* **10**, 56–62.
- Vincent, S.J., Coutts, B.A. and Jones, R.A.C. (2014) Effects of introduced and indigenous viruses on native plants: exploring their disease causing potential at the agro-ecological interface. *PLoS One*, **9**(3), e91224.
- Webster, C.G., Coutts, B.A., Jones, R.A.C., Jones, M.G.K. and Wylie, S.J. (2007) Virus impact at the interface of an ancient ecosystem and a recent agroecosystem: studies on three legume-infecting potyviruses in the South West Australian Floristic Region. *Plant Pathol.* **56**, 729–742.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1 Native plants showing obvious virus symptoms growing in the South West Australian Floristic Region. (A) Severe chlorotic mottle and leaf deformation caused by infection with an introduced generalist virus (*Bean yellow mosaic virus*, BYMV) in plants of *Kennedia prostrata* growing near Badgingarra. (B) Mild mosaic caused by infection with an unidentified virus in young leaflets (right) of *K. coccinea* plants growing near Wooroloo. (C) Bright yellow leaf mottle caused by infection with an unidentified virus in young leaves of a *Bossiaea* sp. plant growing at The Lakes. (D) Chlorotic leaf mottle, leaf deformation and severe plant stunting (plants with arrows) caused by infection with an unidentified virus in plants of *K. prostrata* growing near Wooroloo compared with vigorously growing healthy *K. prostrata* plants (top left and right). (E) Leaf mosaic caused by infection with BYMV in a leaf of *Caladenia paludosa* growing in an orchid collection at Kings Park, Perth (Vincent *et al.*, 2014).