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Alfalfa mosaic virus infects the tropical legume *Desmanthus virgatus* in Australia and the potential role of the cowpea aphid (*Aphis craccivora*) as the virus vector

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Abstract
Severe yellowing and stunting of plant growth was observed in experimental plots of *Desmanthus virgatus* (desmanthus) at the Tamworth Agricultural Institute during the 2015/16 summer season. Both symptomatic and non-symptomatic plants were tested for the presence of a range of viruses by Tissue blot immunoassay and symptomatic plants consistently reacted positive to *Alfalfa mosaic virus* (AMV), while non-symptomatic plants were negative to all viruses tested. AMV was not detected in seedlings grown from seed collected from AMV-positive desmanthus plants. AMV was readily transmitted from AMV-positive desmanthus plants by mechanical inoculation to faba bean, but attempts to transmit the virus from AMV-positive *Medicago sativa* (lucerne) plants to desmanthus by mechanical inoculation were unsuccessful. However, AMV was successfully transmitted from lucerne to desmanthus by *Aphis craccivora* (cowpea aphid). Aphid feeding studies showed that the cowpea aphid, but not *Acyrthosiphon pisum* (pea aphid), could colonise and multiply on desmanthus. AMV could become a limiting factor for the adoption of desmanthus as a pasture legume in NSW, particularly as AMV has been reported to be seed transmitted in desmanthus.

Keywords Pasture legumes · AMV · Virus vectors

Desmanthus species (desmanthus) are a group of summer growing legumes adapted to neutral to alkaline soils of medium to heavy clay texture in the drier subtropical environment. Desmanthus is a drought-tolerant perennial legume, commonly found in regions with annual rainfall 500–1000 mm (Cook et al. 2005). It is palatable to livestock with high nutritive value (Gardiner and Rangel 1994; Cook et al. 2005), but does not cause bloat in cattle due to presence of condensed tannins (Adjei et al. 1993). Desmanthus plants are defoliated by heavy frosts, but regrow from plant crowns in early spring. Plants can set large quantities of seed which have high levels of hardseededness, but desmanthus readily recruits from seed following summer rainfall (Cook et al. 2005).

Desmanthus has been identified as a productive component of sown and native pastures in tropical and subtropical regions in Australia. Two commercial cultivars are currently available that performed well in experiments (e.g.; Pengelly and Conway 2000; Boschma et al. 2012), but adoption has been slow and its potential has not yet been realised. A number of *Desmanthus* spp. are represented in the Australian commercial cultivars: cv. Marc (*D. virgatus*) and cv. Progardes (composite of 5 cultivars from 3 species: *D. virgatus*, *D. bicornutus* and *D. leptophyllus*). Over the last 8 years, desmanthus has also been evaluated in northern NSW (e.g. Boschma and Harris 2009) and has shown potential as a companion legume in sown tropical pastures. These findings extend the boundaries of desmanthus adaptation beyond tropical environments, thereby potentially exposing it to different abiotic (higher frequency of frost and lower proportion of summer rainfall) and biotic (insect pests and diseases) stresses.

Severe leaf yellowing (Fig. 1) and plant stunting was observed in 12 month old *D. virgatus* cv. Marc plants in experimental plots at the Tamworth Agricultural Research Institute (Latitude −31.1456°, Longitude 150.9680°) in November 2015. Symptomatic plants were randomly distributed through the plots and did not form clear foci (Fig. 2). No similar symptoms were observed in other legume species co-located in the

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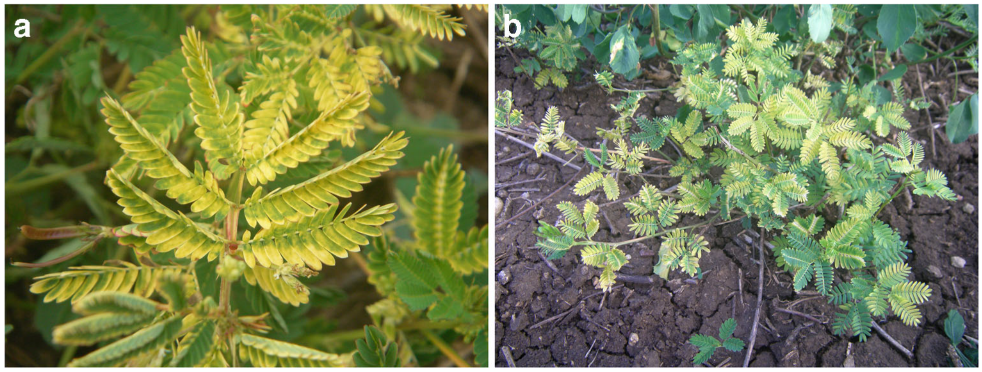

Fig. 1 An example of (**a**) typical leaf symptoms and (**b**) extensive yellowing and plant stunting of *Desmanthus virgatus* cv Marc in an experimental plot at Tamworth, New South Wales

paddock, but *Cicer arietinum* (chickpea) grown in neighbouring paddocks showed a high incidence of severe virus symptoms in October. Virus infection was suspected and Tissue blot immunoassay (TBIA), a reliable, fast and cost efficient methodology to detect viruses in large numbers of individual plants (Freeman et al. 2013) was used to test plants for the presence of viruses known to occur on legume crops in the northern Australian grain growing region. In November 2015, 18 symptomatic and 28 non-symptomatic desmanthus plants were sampled by taking 5–10 cm long shoot tips. Random samples of other legumes trialled in the same paddock were also taken: *Medicago sativa* (lucerne), *Leucaena leucocephala* (leucaena), *Biserrula pelecinus* (biserrula) and *Trifolium vesiculosum* (arrowleaf clover). None of these other legumes showed any symptoms indicative of virus infection. The stem of each sample was blotted on nitrocellulose membranes (Amersham Protran, 0.45 μm pore size) using 6 replicates. The blotting position of each individual stem sample was kept the same on each replicate membrane, so an individual plant's reaction to different antibodies could be compared. The membranes were processed with polyclonal antibodies specific for *Alfalfa mosaic virus* (AMV), *Bean yellow mosaic virus* (BYMV) and *Cucumber mosaic virus* (CMV) and with a monoclonal antibody (5G4) that reacts to luteo and poleroviruses, including *Bean leafroll virus* (BLRV), *Turnip yellows virus* (TuYV), *Soybean dwarf virus* (SbDV) and *Phasey bean mild yellow virus* (PBMYV). All antibodies were obtained from DSMZ (Braunschweig, Germany); catalogue codes AS-0779 (AMV), AS-0717 (BYMV), AS-0929 (CMV) and AS-0227/1 (general luteovirus). TBIA processing protocols described by Freeman et al. (2013) were followed. Symptomatic and non-symptomatic chickpea plants were separately sampled from a neighbouring paddock in October 2015 and analysed using the same procedure. All 18 symptomatic desmanthus plants reacted positive to AMV, but not to any of the other antibodies used. All 28 non-symptomatic desmanthus plants reacted negative to all of the antibodies tested. Five out of 10 lucerne plants, 3 out of 16 biserrula, and 1 out of 13 arrowleaf clover plants were AMV positive, but none reacted to the other antibodies. AMV was readily transmitted from AMV-positive desmanthus plants to greenhouse-grown faba bean plants by mechanical inoculation, using inoculation procedures described below. AMV was also the predominant virus in the symptomatic chickpea plants; of the 40 symptomatic chickpea plants sampled, 34 were AMV positive and 8 reacted with the general luteovirus monoclonal antibody, while only 4 of the 40 non-symptomatic chickpea plants sampled were AMV positive and none reacted with the luteovirus antibody. Similarly high incidences of severe symptoms associated with AMV infection were found in chickpea and faba bean (*Vicia faba*) crops at several locations in northern NSW during the end of the 2015 growing season

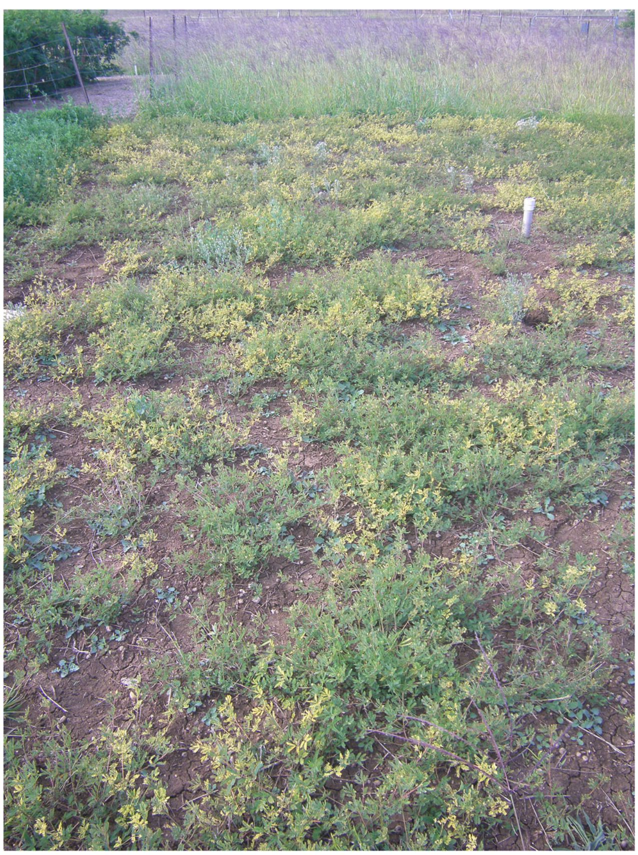

Fig. 2 Symptomatic desmanthus plants were randomly distributed through the experimental plots and did not form clear foci

(J. van Leur, pers. comm.). The high incidence of AMV was unusual compared to earlier surveys done in the same region (van Leur et al. 2013) and likely related to a peak in migrating aphids during September (data not presented).

AMV is a non-persistently transmitted virus with a very wide host range and has been reported to occur naturally in 47 species in 12 families (Hull 1969), including commonly grown pasture and crop legumes in Australia. Lucerne is widely grown in northern NSW and can harbour very high levels of AMV infection without showing obvious symptoms (van Leur and Kumari 2011). As a perennial legume it is a likely source of AMV inoculum for other legume species. Inoculum for mechanical transmission (Hull 2009) was prepared by grinding AMV positive lucerne in a pH neutral phosphate buffer using a chilled mortar and pestle. Healthy desmanthus plants, grown in pots inside a greenhouse, were inoculated by dusting young leaves with carborundum powder and gently rubbing the inoculum into the leaves. The same inoculum was applied to 3-week old greenhouse grown faba bean plants (var. Fiesta). Inoculated plants were observed for symptom development and tested for AMV presence with TBIA. Several attempts to mechanically inoculate desmanthus with AMV did not result in symptoms or in positive TBIA tests, while faba bean plants showed typical symptoms (leaf yellowing, stem necrosis) within 3 weeks of inoculation. Failure to mechanically inoculate plants with AMV have also been reported for lucerne (Hull 1969) and *Cullen australasicum* (Nair et al. 2009).

Aphis craccivora Koch (Hemiptera, Aphididae) (cowpea aphid) is considered a serious pest and one of the most common species on legume crops in Australia (Kamphuis et al. 2012). It is a cosmopolitan species that is highly polyphagous and feeds on a wide range of pasture and crop legumes including lucerne (Blackman and Eastop 2000; Ilse 2000). Currently it is also the only aphid species reported to feed on desmanthus (Blackman and Eastop 2006). Cowpea aphids can reach high numbers on individual plants and cause damage directly by feeding. It is also a highly effective vector of over 50 virus species (Chan et al. 1991). The potential of cowpea aphids to transmit AMV from lucerne to desmanthus was studied in a greenhouse experiment. Morphological characters of the starting colony of laboratory raised cowpea aphid were examined under stereomicroscope and identification confirmed using descriptions in Blackman and Eastop (2000). Mass reproduction of cowpea aphids was performed on a mixture of faba bean, *Pisum sativum* (common pea) and *V. sativa* (common vetch) plants in entomological cages. The plants were regularly watered and examined for aphid colonisation. After the population reached a density sufficient for inoculation the cowpea aphids were placed on healthy desmanthus cv. Marc plants and colonised the plants within 2 weeks (Fig. 3). The same protocol was followed for *Acyrthosiphon pisum* Harris (pea aphid), however, after four trial repetitions pea aphids did not feed or multiply on desmanthus. To determine if cowpea aphid was a suitable vector for AMV transmission to healthy desmanthus plants, an aphid colony was placed on AMV positive lucerne plants in entomological cages. After 7 days, during which time the aphids fed and multiplied, virus-free desmanthus plants were placed in the cages with the infected lucerne. AMV was detected by TBIA in the desmanthus plants after 4 weeks, but no clear yellowing symptoms were observed.

Fig. 3 Colonisations of desmanthus by cowpea aphids in the greenhouse

This is the first report of *D. virgatus* as a host of AMV in Australia. While its suitability as a host for cowpea aphids increases its vulnerability to AMV and other viruses, cowpea aphids are not necessarily the only vector to infect desmanthus with AMV: AMV is a non-persistent virus that can be transmitted by short probing periods of a range of aphids, not only species that are feeding on the host plant. Our field observations suggest that desmanthus productivity is greatly affected by AMV infection, but this warrants quantification. AMV is reported to be seed transmitted in *Desmanthus virgatus* (Mih and Hanson 1998), which would have implications for seed multiplication and distribution. We tested over 500 seedlings emerged from seed harvested from AMV positive plants for AMV presence, but did not find any positives.

Seed transmission of and susceptibility to AMV could differ between *Desmanthus* species. There are over 300 accessions of *Desmanthus* spp. held in the Australian Pastures Genebank (Pengelly and Liu 2001). Further investigations on the susceptibility of these accessions and other commercially available *Desmanthus* spp. is warranted.

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