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Supplemental host range of *Araujia mosaic virus*, a potential biological control agent of moth plant in New Zealand

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Abstract. *Araujia mosaic virus* (ArjMV) has previously been confirmed to have a narrow host range and is considered a potential biological control agent for moth plant (*Araujia hortorum*), a troublesome environmental weed in northern regions of New Zealand. In this study, the virus was tested on 14 plant species, including species of importance to New Zealand to explore its possible use as a biocontrol agent for moth plant. Mosaic symptoms and occasional distortion were observed on leaves of the three *Araujia* species tested. Mosaic symptoms were seen on *Morrenia odorata* and *Oxypetalum caeruleum*, while mosaic symptoms, chlorotic spots and leaf distortion were observed on all three of the *Gomphocarpus* species tested. No symptoms were observed on *Asclepias curassavica*, *Catharanthus roseus*, *Hoya carnosa*, *Mandevilla* × *amabilis*, *Nerium oleander* and *Vinca major*. Because ArjMV infected the three *Araujia* species causing stunting, this virus would significantly reduce flowering and seed production in these species. The monarch butterfly (*Danaus plexippus*) relies on *Gomphocarpus fruticosus* for successful development and is highly valued in New Zealand. It may be possible to genetically modify ArjMV to prevent transmission by insects but it is not an option because genetically modified organisms are not widely supported by the New Zealand public. The research efforts are now focusing on other potential agents for biocontrol of moth plant in New Zealand.

Additional keywords: *Araujia mosaic virus*, *Araujia sericifera*/*hortorum*, moth plant, biocontrol.

Introduction

The taxonomy of moth plant is uncertain and still under debate. After examining herbarium specimens, Forster and Bruyns (1992) reported that *Araujia sericifera* and *A. hortorum* are synonymous and that the species name *sericifera* takes precedence. They also reported that in Australia moth plant is considered *A. hortorum* Fourn, while in North America it is often considered *A. sericifera* Brot. Dr Carlos Villamil surveyed four *Araujia* spp. (*A. hortorum*, *A. sericifera*, *A. angustifolia* and *A. megapotamica*) in Argentina and concluded that moth plant in New Zealand is probably the same species as the Argentinean *A. hortorum* rather than *A. sericifera* as it is currently known (Waipara 2006). Biogeography and climate data also support Villamil's morphology work, as the Argentinean *A. hortorum* occurs at the same temperate latitudes in Argentina as moth plant in New Zealand, while *A. sericifera* only grows in the tropical part of northern Argentina and is not found in the cooler southern parts where *A. hortorum* grows. Hereafter, the *Araujia* grown from seeds obtained from New Zealand will be referred to as *A. hortorum.*

The genus *Araujia* is a member of the family Apocynaceae, which is known for ornamental plants of the genera *Asclepias* and *Hoya*. The subfamily Asclepiadoideae comprises ~348 genera and 2900 species, mostly in South American and African tropics (Endress and Bruyns 2000). There are no native species of Asclepiadoideae in New Zealand. Moth plant was introduced to New Zealand as an ornamental species during the 1880s. In addition to *A. hortorum*, other species in the Asclepiadoideae in New Zealand include swan plant (*Gomphocarpus fruticosus*), giant swan plant (*G. physocarpus*) and milkweeds (*Asclepias curassavica* and *A. incarnata*), which are all cultivated as host plants for monarch butterfly caterpillar (*Danaus plexippus*) (Winks and Fowler 2000; Monarch Butterfly NZ Trust 2009), and another ornamental species *Oxypetalum caeruleum*. Monarch caterpillars can complete development on *A. hortorum*, but are rarely seen on the plants in New Zealand, as the adults prefer to lay their eggs on other hosts (Winks and Fowler 2000). Other ornamental species in the Asclepiadoideae cultivated in New Zealand include *Hoya* spp.

Moth plant is a vigorous, climbing, and perennial vine. Each vine produces a large number of seedpods and each pod produces a large number of wind-borne seeds, each of which is carried on the wind by a coma of long silky hairs. In commercial and

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industrial areas, vines climb over fences and spread across vacant land. In natural areas, the moth plant is a serious threat to biodiversity. At present, moth plant is a troublesome environmental weed in northern regions of New Zealand.

A survey of plant pathogenic fungi and invertebrates associated with moth plant in New Zealand carried out from 2002 to 2004 determined that few pathogens were present that potentially could be used for biocontrol (C. J. Winks, N. W. Waipara, A. F. Gianotti, 2004 Landcare Research, unpubl. data). However, a survey in South America showed that *Araujia mosaic virus* (ArjMV) was widespread throughout most of the plant's range and that populations of *Aphis nerii*, a known vector of *Araujia* viruses (Charudattan *et al*. 1980; N. Waipara, 2004 Landcare Research, unpubl. data), were frequently observed on plants exhibiting mosaic symptoms (Waipara *et al*. 2006). ArjMV was first reported in 1980 as a pathogen of *Araujia* spp., and *Morrenia* spp. found in Argentina (Charudattan *et al*. 1980) and it has been since characterised by *in vitro* translation analyses (Hiebert and Charudattan 1984) and partial genomic sequencing (Massey *et al*. 2007). To study the potential use of ArjMV as an agent for biocontrol of moth plant in New Zealand, a collaborative project was initiated in 2005 between Landcare Research and the University of Florida. In this study, the host range of ArjMV concentrated on several non-target plants that are of interest to New Zealand but were not included in the study conducted by Charudattan *et al*. (1980).

Methods

Test plants and plant cultivation

Plants of *Hoya carnosa*, *Mandevilla* × *amabilis* 'Alice Dupont', *Nerium oleander* and *Vinca major* were vegetatively propagated. *A. angustifolia*, an unidentified *Araujia* species collected in Resistencia, Argentina, *A. hortorum*, *Asclepias curassavica*, *Catharanthus roseus*, *Oxypetalum caeruleum* and three *Gomphocarpus* spp. were started from seeds and grown, 1 per 10-cm pot in Metro-Mix 300 (SunGro Horticulture Canada CM Ltd, Bellevue, WA, US) soilless medium. *A. hortorum* seeds were imported from New Zealand under USDA-Animal and Plant Health Inspection Service (APHIS) permit # P37–08–00163.

Inoculation and evaluation of ArjMV on test plants

Dried ArjMV-infected *Morrenia odorata* leaves were supplied by R. Charudattan (Charudattan *et al*. 1980). Inoculum was prepared by triturating dry leaf tissue 1 : 1 (w : v) in 0.02 M sodium phosphate buffer, pH 7.2. Plants were dusted with 0.22-μm-mesh carborundum and inoculated using sterile cheesecloth wetted with the triturated tissue-buffer mixture. Five plants were inoculated with ArjMV and one plant was inoculated with buffer only as a control on each of the 14 plant species (Tables 1 and 2). Plants were observed for hypersensitive reactions on inoculated leaves and systemic symptoms on subsequent new growth. Infectivity of the

Table 1. Symptoms and enzyme-linked immunosorbent assay (I-ELISA) results of *Araujia* species 4 weeks after inoculation with *Araujia mosaic virus*

Species	Study 1		Study 2	
	Symptoms	I-ELISA	Symptoms	I-ELISA
Araujia sp. Resistencia, Chaco, Argentina	Mosaic, distortion (4/5)[A]	4/5[B]	Mosaic, distortion (5/5)	3/5
Araujia angustifolia	Mosaic, distortion (5/5)	5/5	Mosaic, distortion (0/5)	5/5
A. hortorum (New Zealand)	Mosaic, possible stunting (4/5)	1/5[C]	Mosaic, possible stunting (3/5)	3/5[C]

[A]No. of plants with symptoms/No. of plants inoculated.
[B]No. of plants positive in I-ELISA/No. of plants inoculated.
[C]A_{405} values greater than 2 × but less than 3 × the healthy values and considered marginally positive.

Table 2. Symptoms and enzyme-linked immunosorbent assay (I-ELISA) results of non-target species 4 weeks after inoculation with *Araujia mosaic virus*

Species	Study 1		Study 2	
	Symptoms	I-ELISA	Symptoms	I-ELISA
Asclepias curassavica	(0/5)[A]	0/5[B]	(0/5)	0/5
Catharanthus roseus	(0/5)	0/5	(0/5)	0/5
Gomphocarpus cancellatus	Mild mosaic (4/5)	5/5	Mosaic, distortion or stunting (5/5)	2/5
G. fruticosus	Mosaic (5/5)	5/5	Mosaic (2/5)	2/5
G. glaucophylla	Chlorotic spots, mild mosaic (5/5)	1/5	Mosaic, distortion or stunting (3/5)	2/5
Hoya carnosa[C]	(0/5)	0/5	(0/5)	0/5
Mandevilla × *amabilis*[C]	(0/5)	0/5	(0/5)	0/5
Morrenia odorata	Mosaic, distortion (5/5)	5/5	Mosaic, distortion (1/1)[D]	1/1
Nerium oleander	(0/5)	0/5	(0/5)	0/5
Oxypetalum caeruleum	Mosaic, distortion (5/5)	5/5	Mosaic (5/5)	5/5
Vinca major	(0/5)	0/5	(0/5)	0/5

[A]No. of plants with symptoms/No. of plants inoculated.
[B]No. of plants positive in I-ELISA/No. of plants inoculated.
[C]Some plants produced no new growth and inoculated leaf was sampled.
[D]One plant inoculated as a positive control.

inoculum was confirmed by including five *M. odorata* plants in Study 1 and one in Study 2, respectively (Tables 1 and 2). One plant was used as a healthy control. All inoculations were conducted in a Biosafety-Level-2 quarantine greenhouse in Florida, USA, and the inoculation series was repeated once.

Indirect enzyme-linked immunosorbent assay (*I-ELISA*)

After a minimum of two new leaves were produced following inoculation, the newest leaf tissue was collected and tested for ArjMV using antigen-trapped indirect enzyme-linked immunosorbent assay (I-ELISA) (Elliott *et al*. 1996; Siegmann *et al*. 1998). Antiserum to ArjMV was provided by R. Charudattan (Hiebert and Charudattan 1984).

Samples were triturated 1 : 10 (w : v) in extraction buffer (0.01 M Na_2SO_3, 2% polyvinyl pyrolidone-40, 0.2% bovine serum albumin, phosphate-buffered saline and Tween-20 (PBST), pH 9.6). Antiserum against ArjMV was diluted 1 : 1000 in conjugate buffer (0.2% bovine serum albumin, 2% polyvinyl pyrrolidone-40, PBST, pH 7.4). Goat anti-rabbit IgG (whole-molecule) alkaline phosphatase conjugate (Sigma, St Louis, MO, US) was diluted 1 : 30 000 in conjugate buffer. Plates were given three 3-min washes between 1-h incubation periods at 37°C. Each sample was replicated in three wells and absorbance readings at 405 nm recorded at 15-min intervals using an EL 309 Universal Microplate Reader (Bio-Tek Instruments, Winooski, VT, US). Only the 60-min values are reported. Absorbance values greater than three times the healthy values were considered positive (Sutula *et al*. 1986).

Results

Symptoms

In Study 1, mosaic, leaf distortion and stunting were observed in 5/5 *A. angustifolia* plants, 4/5 *Araujia* sp. plants from Resistencia, 4/5 *A. hortorum* plants (Table 1), 5/5 *Oxypetalum* plants, 5/5 plants of both *Gomphocarpus fruticosus* and *G. glaucophylla* and 4/5 plants of *G. cancellatus* (Table 2). In Study 2, symptoms were observed in 0/5 *A. angustifolia* plants, 5/5 *Araujia* sp. plants from Resistencia, 3/5 *A. hortorum* plants (Table 1), 5/5 *Oxypetalum* plants, 2/5 *G. fruticosus* plants and 3/5 and 5/5 *G. glaucophylla* and *G. cancellatus* plants, respectively (Table 2). Mosaic and distortion symptoms were observed on all 5/5 *M. odorata* plants in Study 1 and the single *M. odorata* plant in Study 2 inoculated as positive controls (Table 2). No symptoms were observed in *Asclepias*, *Catharanthus*, *Hoya*, *Mandevilla*, *Nerium*, or *Vinca* plants in Studies 1 and 2 (Table 2). While the *Hoya* plants did not produce new leaves during the 4 weeks of the studies, no symptoms (hypersensitive) were observed on the inoculated leaves. No symptoms were observed in the healthy control plants.

I-ELISA

In Studies 1 and 2, none of the absorbance values (A_{405}) of the *Asclepias*, *Catharanthus*, *Hoya*, *Mandevilla*, *Nerium* or *Vinca* leaf samples were greater than or equal to 3 times (3×) the control A_{405} values (Table 2). Although the *Hoya* plants did not produce new leaves, virus replication in inoculated leaves would have been detected by I-ELISA. In Study 1, A_{405} values greater than the 3× threshold were observed in 5/5 *A. angustifolia* plants, 4/5 *Araujia* sp. plants from Resistencia (Table 1), 1/5 of *G. glaucophylla* and 5/5 *Oxypetalum*, *G. fruticosus* and *G. cancellatus* (Table 2). Positive A_{405} values were observed on all the five *M. odorata* plants inoculated as positive controls. No *A. hortorum* plants reached the 3× threshold; however, the A_{405} value of one plant was greater than twice the healthy value and considered positive (Table 1).

In Study 2, A_{405} values greater than the 3× threshold were observed in 5/5 *A. angustifolia* plants, 3/5 *Araujia* sp. plants from Resistencia (Table 1), 5/5 *Oxypetalum* plants, 2/5 plants of each of the three *Gomphocarpus* spp. (Table 2). Positive A_{405} value was observed on the *M. odorata* plant inoculated as a positive control. No *A. hortorum* plants reached the 3× threshold; however, the A_{405} values of three plants were greater than 2× the healthy value and considered positive (Table 1).

Discussion

ArjMV was characterised as a distinct member of the *Potyvirus* genus (Charudattan *et al*. 1976, 1980; Hiebert and Charudattan 1984). This characterisation was confirmed by protein and sequencing analyses (Hiebert and Charudattan 1984; Massey *et al*. 2007). This virus is naturally transmitted by *Aphis nerii*, *A. spiraecola* and *Myzus persicae*, or by mechanical inoculation (Charudattan *et al*. 1980). ArjMV has previously been well studied, when it was assessed for use for biocontrol of the milkweed vine *M. odorata*, a non-native weed in Florida, USA (Charudattan *et al*. 1980; Hiebert and Charudattan 1984). ArjMV was tested on 121 plant species belonging to 25 families by mechanical inoculation and on 37 plant species through aphid transmission (Charudattan *et al*. 1980). Host testing results indicated that it has a restricted host range, infecting only six genera, all vines in the Asclepiadoideae (*Araujia*, *Cynanchum*, *Hoya*, *Matelea*, *Morrenia*, and *Sarcostemma*), when inoculated manually (Charudattan *et al*. 1980). In the present study, ArjMV infected several species within the Asclepiadeae tribe including the three *Araujia* spp., *M. odorata*, *O. caeruleum* and three *Gomphocarpus* spp. but not *Asclepias curassavica* that is in the same subtribe (Asclepiadinae) as *Gomphocarpus*, or *Hoya carnosa* from the closely related Marsdenieae tribe. It also did not infect the two plants from the Apocynoideae subfamily (*N. oleander* and *Mandevilla* × *amabilis*) or the Rauvolfioideae subfamily (*C. roseus* and *V. major*). This is consistent with the previous study by Charudattan *et al*. (1980), who reported that ArjMV did not infect any of the species they tested that were not in the Asclepiadaceae (= Asclepiadoideae in the Endress *et al*. classification system (Endress *et al*. 2007)). Whereas no virus was detected in the *Hoya* plants in this study, Charudattan *et al*. (1980) reported infection in 1/3 inoculated *H. carnosa* and 1/3 *H. coronaria* plants.

The discrepancies between symptoms observed and I-ELISA results in this study might be due to the threshold used for the I-ELISA. Reverse transcription polymerase chain reaction (RT–PCR) and hybridisation techniques would be more sensitive for detecting the low titre viruses in the infected plants (Kawakami *et al*. 2007). A less stringent threshold, such as 2× (also commonly used) would reduce some of the I-ELISA-symptom discrepancies. The latex, which is characteristic of the

Apocynaceae, contributes to the lack of sensitivity of the I-ELISA system and may be responsible for the discrepancies.

In South America more than 90% of moth plant populations are infected by several diseases, which cause widespread leaf, stem, flower, and sometimes even fruit damage. The most widespread pathogen is a leaf spot fungus (*Pseudocercospora* spp.) (Waipara 2006). Given that a mixed infection by different pathogens in plants can enhance the pathogenicity of individual pathogens, an effective biological control of moth plant in New Zealand may require the application of a mixture of several pathogens including fungi, bacteria and/or viruses. While ArjMV did not kill the three *Araujia* spp. in this study, it caused stunting that would significantly reduce flowering and seed production. Stunting was characterised by mosaic, distortion and reduction of the size of leaves and stems and substantially reduced growth. Charudattan *et al*. (1980) reported that ArjMV-infected *M. odorata* plants grew less rapidly than their healthy counterparts in a greenhouse study and remained stunted.

Biological weed control can be an environmentally friendly method of utilising host-specific pathogens to control targeted weeds without damage to non-target crops or native plants. Therefore, a crucial factor for using any biocontrol agent is its host specificity and host range stability (Anderson 1983). Screening procedures for the host specificity of biocontrol agents and country-based recognition of genera at risk are essential to limit the possibility of agents affecting non-target species (Medal *et al*. 2002).

As ArjMV is aphid-transmitted and the oleander aphid *Aphis nerii* is present in New Zealand, it would be expected to naturally spread throughout the *Araujia* populations in New Zealand, if introduced. To our knowledge, there are no studies on ArjMV transmission to *Gomphocarpus* spp. by *Aphis nerii*. However, *Aphis nerii*, *A. spiraecola* and *Myzus persicae* are known vectors of ArjMV (Charudattan *et al*. 1980). This suggests that the virus might also be spread by aphids to swan plant, *G. fruticosus*, and giant swan plant (*G. physocarpus*) that are introduced adventive species valued as hosts for monarch butterfly caterpillars (Winks and Fowler 2000; Monarch Butterfly NZ Trust 2009). Although the monarch butterfly *D. plexippus*, is exotic in New Zealand, it is an iconic species. Exotic milkweeds (*Asclepias* spp.) are also cultivated less commonly for the monarch butterfly in New Zealand and do not appear to be at risk from ArjMV. While *O. caeruleum* is also susceptible, it is also an introduced adventive species with minor economic value, other than being an occasional ornamental plant and an alternative food source for monarch butterfly larvae (Wise 1963; Ramsay 1964).

Since naturally occurring *Potyvirus* isolates that are not aphid-transmissible have a mutation in one of the amino acids in the DAG motif (Atreya *et al*. 1990; Gal-On *et al*. 1992) and this mutation potentially eliminates aphid transmissibility but not virus infectivity (Flasinski and Cassidy 1998), it is possible that some DAG motif mutations could make ArjMV a more suitable candidate for biocontrol by reducing the risk to *Gomphocarpus* spp.

If the DAG motif for aphid transmission of ArjMV was mutated using genetic engineering technology, the modified ArjMVs would need to be tested for infectivity of moth plant and other non-target plants like swan plant, within Biosafety-Level-2 quarantine conditions. Mechanical inoculation of moth plant with the modified ArjMV would significantly reduce the natural transmission to non-target plants. While it may be possible to manipulate ArjMV to make it more suitable for biocontrol purposes, mechanical inoculation of each plant of *Araujia* would likely be required. However, attempting to genetically modify ArjMV is unlikely to be practical in the near future in New Zealand due to the current low acceptability by the general public of genetically modified organisms being released into the environment (Cook *et al*. 2004; Amin *et al*. 2007). Research efforts in New Zealand are now focusing on assessing the host-range and suitability of several fungal pathogens and insects found during surveys of *Araujia* in Argentina for biocontrol of *A. hortorum* (L. Hayes, pers. comm.).

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