Host specificity of *Ditylenchus phyllobius*, a potential biological control agent of silver-leaf nightshade (*Solanum elaeagnifolium* Cav.) in Australia

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Abstract

The leaf-galling nematode, *Ditylenchus phyllobius* was screened against 118 taxa of plants in host specificity tests to determine its suitability as a biological control agent of silver-leaf nightshade, *Solanum elaeagnifolium* Cav. in Australia. Only species in the genus *Solanum* and from the Sections *Oliganthes* and *Melongena* appeared to be suitable hosts for the nematode. No representatives screened from the Sections *Solanum*, *Dulcamara*, *Jasminosolanum*, *Archaesolanum*, *Brevantherum*, *Pseudocapsica*, *Androceras*, *Graciliflorum* or *Petota* were galled. As the nematode produced galls on 13 native *Solanum* species and 13 of 15 *S. melongena* (eggplant) cultivars tested, the lack of adequate specificity of *D. phyllobius* makes this nematode an unsuitable agent for release in Australia.

Key words: *Ditylenchus phyllobius*, *Solanum elaeagnifolium*, biological control, eggplant.

Introduction

Silver-leaf nightshade, *Solanum elaeagnifolium* Cav., is native to Mexico and south-western United States of America but has become an important weed of crops and causes stock poisoning both in its native range and elsewhere in the world (Boyd *et al*. 1984). In Australia, the weed causes most problems in the winter rainfall cropping areas of the South Australian and Victorian Mallee and the Riverina of New South Wales (Cuthbertson *et al.* 1976, Wapshere 1988) where it reduces wheat yields (Anon. 1980). The weed is difficult and expensive to control chemically and cultivation tends to intensify infestations (Anon. 1980).

Biological control of *S. elaeagnifolium* has been investigated in Texas (Robinson *et al.* 1978, Orr 1980), California (Goeden 1971) and South Africa (Siebert 1975, 1977, Neser *et al.* 1989). In Australia, Wapshere (1988, 1989) examined the natural enemies in Mexico and Texas and determined their prospects for use as biological control agents based on the occurrence and biology of *S. elaeagnifolium* in Australia. The leaf and stem-galling nematode *Ditylenchus phyllobius* (Thorne) Filip'ev (=*Nothanguina phyllobia* (Thorne), *Orrina phyllobia* (Thorne) Brzeski) appeared to have the attributes to succeed as an agent for both classical (Orr 1980, Neser *et al.* 1989, Wapshere 1988) and augmentative (bioherbicidal) biological control (Robinson *et al.* 1978, 1979, Northam and Orr 1982, Parker 1986).

In 1985 *S. elaeagnifolium* was approved as a target for biological control in Australia and in 1987 *D. phyllobius* was imported into quarantine at the Keith Turnbull Research Institute for host specificity studies. The results of the host specificity tests are reported in this paper.

Materials and methods

Biology of D. phyllobius

Infective juveniles (J4) enter apical meristems of actively growing hosts (Orr 1980). Within a week, adults and eggs are found in the plant tissue resulting in hypertrophy and hyperplasia of the pallisade, pith, cortical and vascular parenchyma and the formation of leaf, petiole and stem galls (Skinner *et al*. 1980). Reproduction occurs rapidly over six to eight weeks with tens of thousands of nematodes being produced in galls that range from 3 up to 25 cm^{-3} (Skinner *et al.* 1980). These mature galls contain mainly J4 juveniles, this stage being resistant to desiccation (Robinson 1985). Juveniles may reinfest other parts of the same plant or, if the leaf drops, overwinter in the gall or enter the soil in search for another host plant (Orr 1980). Infection of meristematic areas requires the presence of a moisture film for 48 h to enable rehydration of dormant infective nematodes, which occurs within an hour, and the movement of the nematodes from the soil, up the plant to meristematic areas, and penetration of the tissue (Orr 1980, Robinson *et al.* 1984). In the absence of water, J4 juveniles can last indefinitely in a state of anhydrobiosis (Orr 1980) and large numbers of dried viable juveniles are often found in the upper few centimetres of soil in Texas (Robinson *et al.* 1978).

Test plant production

Table 1 lists the 118 taxa of plants screened against *D. phyllobius*. Where possible, plants were grown from seed in a peat-moss/sand mixture in 75 mm diameter plastic pots and fertilized with a water soluble complete fertilizer (Aquasol®) to force leaf growth. Some plants were field collected and grown in pots up to 200 mm diameter prior to testing. All tests were carried out in a Conviron® temperature regulated walk-in chamber operating at 22°C, 50% RH and under a 13 h L, 11 h D light regime. After inoculation the plants were watered twice weekly for six weeks with tap water applied to saucers under each pot.

Nematode inoculum preparation and application

Air-dried, galled leaves of *S. elaeagnifolium* were imported from Texas, USA and stored in a freezer (–5°C) in a high security quarantine laboratory until required for test plant inoculation. Nematodes were extracted by placing 5 g of frozen plant material in 500 mL of distilled water in a 1 L conical flask and aerated via a rubber tube connected to a compressor. After four hours the coarse leaf material was removed using a 250 µm sieve. The concentration of live nematodes was determined in 25, 0.1 mL random samples of the solution and aliquots of inoculum, averaging 12,787 nematodes (SD = 160), prepared. The aliquots, which averaged 13.8 mL (SD = 25.0), were pipetted onto the growing points of the potted test plants and the control plant *S. elaeagnifolium*. Up to 35 plants were inoculated from each batch of inoculum. The plants were then sprayed using a hand-held atomizer with distilled water and enclosed in a plastic bag for seven days.

Infection assessment

During the six weeks of each trial, all fallen leaves were collected for assessment along with, and at the end of six weeks, all leaf/bud material that had grown since applying the inoculum. This plant material was boiled for three minutes in a lactoglycerol solution (equal volumes of glycerol, lactic acid and distilled water) plus 0.05% acid fuchsin. The solution was allowed to cool, then the material was washed in water and cleared in an acidified (lactic acid) solution of equal volumes of glycerol and distilled water (Southey 1986). As the low humidity (50–60% RH) during the last five weeks of incubation would not be conducive to infection occurring, most galls were initiated during the first week when plastic bags ensured that the humidity was close to 100%. The cleared leaves were then examined for nematodes and galls, the nematodes being stained red.

Results and discussion

Table 1 shows that only taxa from the *Solanum* Sections *Oliganthes* and *Melongena* were suitable hosts for *D. phyllobius* under

Table 1. Host specificity test results on *Ditylenchus phyllobius*.

Test species	Replicates	% plants galled	No. galls per plant
Family Fabaceae			
Acacia pycnantha Benth. (Australian golden wattle)	6	0	0
Family Brassicaceae			
**Brassica oleracea* L. (cabbage)	8	0	0
Family Apiaceae			
**Daucus carota* L. (carrot)	12	0	0
Family Myrtaceae			
Eucalyptus dumosa A.Cunn. ex J.Oxley (Dumosa mallee)	7	0	0
Family Malvaceae			
**Gossypium hirsutum* L. (cotton)	8	0	0
Family Asteraceae			
**Helianthus annuus* L. (sunflower)	6	0	0
Family Poaceae			
**Lolium rigidum* Gaudin (Wimmera ryegrass)	11	0	0
**Phalaris aquatica* L. (Toowoomba canary grass)	8	0	0
**Triticum aestivum* L. (wheat)	10	0	0
Family Proteaceae			
Macadamia integrifolia Maiden & Betche (macadamia nut)	7	0	0
Family Musaceae			
**Musa acuminata* Colla (banana)	6	0	0
Family Pinaceae			
**Pinus radiata* D.Don (Monterey pine)	7	0	0
Family Fabaceae			
**Trifolium subterraneum* L. (subterranean clover)	7	0	0
Family Solanaceae			
Lycium australe F.Muell. (Australian boxthorn)	11	0	0
**Physalis peruviana* L. (cape gooseberry)	3	0	0
Solanum (Section *Solanum*)			
**S. americanum* Mill. (glossy nightshade)	1	0	0
**S. furcatum* Dunal ex Poir. (broad nightshade)	4	0	0
**S. nigrum* L. (blackberry nightshade)	6	0	0
Solanum (Section *Oliganthes*)			
S. brownii Dunal *s. lat.* (violet nightshade)	4	25	0.25
S. centrale J.M.Black (desert raisin)	3	0	0
S. cleistogamum Symon	9	33	1.0
S. coactiliferum J.M.Black (western nightshade)	1	100	10.0
S. dallachii Benth.	3	33	0.66
**S. dimidiatum* Raf. (western horse nettle)	6	67	1.5
S. echinatum R.Br.	6	17	0.4
**S. elaeagnifolium* Cav. (silver-leaf nightshade)	55	71	2.00
S. ellipticum R.Br. (velvet potato bush)	11	27	2.09
S. esuriale Lindl. (quena)	8	0	0
S. hoplopetalum Bitter & Summerh. (thorny solanum)	7	0	0
S. hystrix R.Br. (Afghan thistle)	2	0	0
S. lasiophyllum Dunal ex Poir. (flannel bush)	18	33	1.89
S. lucani F.Muell.	5	0	0
S. macoorai F.M.Bailey	10	0	0
S. sturtianum F.Muell. (Thargomindah nightshade)	1	0	0
S. quadriloculatum F.Muell. (tomato bush)	2	50	2.0
S. nummularium S.Moore (money-leaf solanum)	2	50	2.0
S. oligacanthum F.Muell.	7	0	0
S. orbiculatum Dunal ex Poir. (wild tomato)	11	44	1.36
S. petrophilum F.Muell. (rock nightshade)	1	0	0
S. prinophyllum Dunal (forest nightshade)	7	0	0

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Table 1 continued.

Test species	Replicates	% plants galled	No. galls per plant
Solanum (Section *Melongena*)			
**S. melongena* L. (eggplant)			
**S. melongena* 'New York purple	9	44	1.0
**S. melongena* 'Black pearl'	10	33	0.5
**S. melongena* 'Black magic'	5	20	0.4
**S. melongena* 'Large teardrop'	6	50	1.67
**S. melongena* 'New York purple'	6	33	1.17
**S. melongena* 'Ebony bell'	11	45	1.27
**S. melongena* 'Hybrid black club'	9	11	0.11
**S. melongena* 'Visuba'	6	0	0
**S. melongena* 'Bonica'	9	0	0
**S. melongena* 'Long purple'	4	50	0.5
**S. melongena* 'Market supreme'	10	33	0.7
**S. melongena* 'Superlong'	6	17	0.33
**S. melongena* 'Hybrid black bell'	8	50	1.0
**S. melongena* 'Long Tom'	8	25	0.63
**S. melongena* 'Supreme'	7	14	0.43
S. phlomoides A.Cunn. ex Benth.	1	100	1.0
S. beaugleholei Symon	16	31	0.44
S. cinereum R.Br. (Narrawa burr)	6	50	1.17
S. clarkiae Symon	4	75	1.50
S. chippendalei Symon	3	0	0
S. diversiflorum F.Muell.	4	0	0
**S. linnaeanum* Hepper & P.-M.L.Jaeger (apple of Sodom)	13	0	0
**S. marginatum* L.f. (white-edge nightshade)	9	0	0
**S. muricatum* Aiton (pepino)	10	0	0
Solanum (Section *Dulcamara*)			
**S. triflorum* Nutt. (three-flowered nightshade)	9	0	0
Solanum (Section *Jasminosolanum*)			
**S. laxum* Spreng. (jasmine nightshade)	5	0	0
Solanum (Section *Archaesolanum*)			
S. aviculare G.Forst. (kangaroo apple)	7	0	0
S. laciniatum Aiton (kangaroo apple)	5	0	0
S. linearifolium Geras. ex Symon (mountain kangaroo apple)	1	0	0
S. simile F.Muell. (oondoroo)	5	0	0
S. symonii H.Eichler	9	0	0
S. vescum F.Muell. (gunyang)	2	0	0
Solanum (Section *Brevantherum*)			
**S. mauritianum* Scop. (wild tobacco tree)	1	0	0
Solanum (Section *Pseudocapsica*)			
**S. pseudocapsicum* L. (Madeira winter cherry)	9	0	0
Solanum (Section *Androceras*)			
**S. rostratum* Dunal (buffalo burr)	4	0	0
Solanum (Section *Graciliflorum*)			
S. ferocissimum Lindl.	1	0	0
Solanum (Section *Petota*)			
**S. tuberosum* 'Kenebec' (potato)	5	0	0
**S. tuberosum* 'Pontiac' (potato)	9	0	0
**Salpichroa origanifolia* (Lam.) Thell. (pampas lily-of-the-valley)	9	0	0
**Datura ferox* L. (long-spine thornapple)	3	0	0
**D. inoxia* Mill. (downy thornapple)	8	0	0
**D. leichhardtii* F.Muell. ex Benth. (native thornapple)	5	0	0
**D. stramonium* L. (common thornapple)	7	0	0
**Brugmansia* × *candida* Pers. (angel's trumpet)	4	0	0

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Table 1 continued.

Test species	Replicates	% plants galled	No. galls per plant
**Cestrum elegans* (Brongn. ex Neumann) Schltdl.	10	0	0
Nicotiana burbidgeae Symon	1	0	0
N. excelsior (J.M.Black) J.M.Black (mingkulpa)	8	0	0
**N. glauca* Graham (tree tobacco)	6	0	0
N. occidentalis H.-M.Wheeler (native tobacco)	7	0	0
N. rotundifolia Lindl.	2	0	0
N. velutina H.-M.Wheeler (velvet tobacco)	6	0	0
**N. tabacum* L. (tobacco)			
**N. tabacum* 'Hicks'	8	0	0
**N. tabacum* 'Ovens'	8	0	0
**N. tabacum* 'Smoko'	8	0	0
Anthocercis anisantha subsp. *collina* Haegi	3	0	0
A. genistoides Miers	3	0	0
A. ilicifolia Hook	6	0	0
A. littorea Labill. (yellow tailflower)	3	0	0
A. viscosa R.Br. subsp. *viscosa* (sticky tailflower)	7	0	0
A. viscosa subsp. *caudata* Haegi (sticky tailflower)	2	0	0
Cyphanthera albicans (A.Cunn.) Miers (grey rayflower)	9	0	0
C. anthocercidea (F.Muell) Haegi (large leaf rayflower)	1	0	0
C. microphylla Miers	1	0	0
C. myosotidea (F.Muell.) Haegi (small leaf rayflower)	3	0	0
C. scabrella (Benth.) Miers	11	0	0
Anthotroche pannosa Endl.	2	0	0
**Capsicum annuum* L. (capsicum)			
**Capsicum annuum* 'California wonder'	5	0	0
**Capsicum annuum* 'Big Jim pepper'	5	0	0
**C. frutescens* L. (ornamental chilli)	9	0	0
Duboisia myoporoides R.Br. (corkwood)	2	0	0
D. leichhardtii (F.Muell) F.Muell. (corkwood)	10	0	0
Duboisia leichhardtii (F.Muell.) F.Muell. × *Duboisia myoporoides* R.Br.	11	0	0
**Lycopersicon lycopersicum* (L.) H.Karst. ex Farw. (tomato)			
**L. lycopersicum* 'Grosse lisse	9	0	0
**L. lycopersicum* 'Quick pick egg tomato'	9	0	0
Symonanthus aromaticus (C.A.Gardner) Haegi	1	0	0
**Solanum betaceum* Cav. (tree tomato)	4	0	0
Family Polemoniaceae			
**Phlox drummondii* Hook (phlox)	4	0	0
Family Convolvulaceae			
**Ipomoea batatas* (L.) Lam. (sweet potato)	5	0	0

* Introduced species

the test conditions. None of the 16 *Solanum* species tested from the Sections *Solanum* (3 species), *Dulcamara* (1), *Jasminosolanum* (1), *Archaesolanum* (6), *Brevantherum* (1), *Pseudocapsica* (1), *Androceras* (1), *Graciliflorum* (1) or *Petota* (1) had gall formation. However, in the Section *Oliganthes*, the native species *S. brownii* Dunal, *S. cleistogamum* Symon, *S. coactiliferum* J.M.Black, *S. dallachii* Benth., *S. echinatum* R.Br., *S. ellipticum* R.Br., *S. lasiophyllum* Dunal ex Poir., *S. nummularium* S.Moore, *S. quadriloculatum* F.Muell. and *S. orbiculatum* Dunal ex Poir. and the introduced weedy species *S. elaeagnifolium* and *S. dimidiatum* Raf. were all galled, although ten native species in this Section were not. Of those species replicated at least five times, *S. elaeagnifolium* (71%), *S. dimidiatum* (67%), *S. orbiculatum* (44%), *S. cleistogamum* (33%), *S. lasiophyllum* (33%) and *S. ellipticum* var. *ellipticum* (27%) had at least 20% of the plants galled. *S. brownii*, *S. coactiliferum*, *S. dallachii*, *S. quadriloculatum* and *S nummularium* all had galling >20% but too few plants were tested to determine their relative susceptibility. In addition, *S. ellipticum*, *S. lasiophyllum*, *S. orbiculatum*, *S. dimidiatum* and *S. elaeagnifolium* all averaged more than one leaf being galled per replicate for species tested more than five times. In the Section *Melongena*, the native species *S. phlomoides* A.Cunn. ex Benth., *S. beaugleholei* Symon, *S. cinereum* R.Br. and *S. clarkiae* Symon were galled along with 13 of the 15 *S. melongena* L. (eggplant) cultivars screened. *S. cinereum* and three eggplant cultivars averaged more than one leaf per treatment being galled. In this Section there were also two native and three introduced species not galled.

It would appear that the host range of *D. phyllobius* is potentially quite broad within the *Solanum* Sections *Oliganthes* and *Melongena* with 48% and 50% of the

species galled respectively. The Section *Oliganthes* in Australia contains 50 species and sub-species of the 94 native *Solanum* species and the Section *Melongena* contains another 21 species (Purdie *et al.* 1982) and the majority of the eggplant cultivars are susceptible. Thus the lack of adequate host specificity of *D. phyllobius* makes this nematode an unsuitable agent for release in Australia.

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