Host-specificity testing of the boneseed (*Chrysanthemoides monilifera* ssp. *monilifera*) leaf buckle mite (*Aceria neseri*)

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Summary

The eriophyid mite *Aceria neseri* is a candidate agent for biological control of *Chrysanthemoides monilifera* ssp. *monilifera* (boneseed), one of two weedy *Chrysanthemoides* taxa in Australia. Based on testing carried out in a shadehouse at the Agricultural Research Council's Plant Protection Research Institute premises in Stellenbosch, South Africa, the host range of *A. neseri* was found to be restricted to *Chrysanthemoides.* Sixty-two plant species were tested including a few *Chrysanthemoides* taxa other than boneseed, and three species (*Osteospermum fruticosum, Calendula officinalis* and *Dimorphotheca sinuata*) in the same tribe as boneseed (Calenduleae). No signs of feeding or damage attributable to *A. neseri* were observed on species other than *Chrysanthemoides*. *A. neseri* would be safe to release in Australia since the only representatives of *Chrysanthemoides* in Australia are pests.

Keywords: *Aceria neseri*, *Chrysanthemoides monilifera*, erineum, Eriophyiidae, host specificity.

Introduction

After two decades of research and development, it has so far proven difficult to develop effective biological control for *Chrysanthemoides monilifera* (L.) Norl., two of whose taxa, *C. m.* ssp. *monilifera* (boneseed) and *C. m.* ssp. *rotundata* (DC.) Norl. (bitou bush), are serious weeds in Australia (Weiss *et al*. 1998). The first eight agents released in Australia have either failed or achieved only limited success to date. They include two foliage-feeding moths, four foliage-feeding chrysomelid beetles and two seed-feeding flies. Two further organisms are currently under development as biological control agents: an eriophyid mite *Aceria neseri* Meyer and a rust fungus, *Endophyllum osteospermi* (Doidge) comb. nov. This paper reports on the host-specificity testing of *A. neseri*.

A. neseri is a small whitish, worm-like mite up to 175 microns long and about 50 microns wide. Feeding by *A. neseri* on developing boneseed leaves induces the formation of *erinea* (patches of densely packed hair-like outgrowths) that are initially white but turn brown with age and are associated with distorted leaf growth. Erinea are composed of non-photosynthetic tissue, reduce photosynthetic efficiency and provide shelter and substrate in which *A. neseri* colonies grow. Heavily infested *C. monilifera* plants in the native range, South Africa, are unthrifty and appear to have lower reproductive outputs and less vigorous growth than uninfested plants.

Following several unsuccessful attempts to establish a culture of *A. neseri* in quarantine at the Keith Turnbull Research Institute (KTRI) in Australia operations were transferred to the Agricultural Research Council's Plant Protection Research Institute (PPRI) premises in Stellenbosch , South Africa, where tests could be conducted in an outdoor shadehouse. This removed the constraints of quarantine on test plant propagation and *A. neseri* colony maintenance, and allowed easy access to local populations of *A. neseri* for inoculum.

Materials and methods

A host-specificity test list was compiled by Adair (1999) along the lines of the method described by Wapshere (1974) (i.e. centrifugal phylogenetic method plus safeguard criteria) and approved in accordance

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with the protocol described on the web site of the Department of Agriculture, Fisheries and Forestry – Australia (Anon.). In addition, a selection of opportunistically available *C. monilifera* and *C. incana* accessions, whose subspecific identity was uncertain, were also tested as hosts for *A. neseri*. Taxa tested are listed in Table 1. Test plants were acquired from commercial nurseries or propagated from seed and grown in 20 cm diameter plastic pots. *C. m.* ssp. *monilifera* plants were collected as seedlings from roadsides and other waste places and transplanted into 20 cm pots for use as controls.

Most of the host-specificity tests were conducted between December 2001 and May 2002 in a shadehouse at PPRI in batches of one to several test taxa. The remainder were done in a PPRI quarantine glasshouse (two batches, March to May 2002) or in a KTRI quarantine controlled environment room (one batch, June/July 2002) depending on quarantine status and availability of test plants. (Sufficient experience with *A. neseri* had been gained by June 2002 to enable its successful use in host-specificity tests in quarantine at KTRI.) Test batches were inoculated with *A. neseri* collected from populations residing in natural stands of *C. m.* ssp. *monilifera* in Cape Town and environs, South Africa. A vegetative, actively growing shoot tip on each of three to eight (usually five or six) replicate test taxon plants was inoculated with two to three healthy erinea-bearing live *A. neseri*. Erinea were nestled in the shoot tip by gravity or by anchoring them in such a way that when the mites exited the drying inoculum they were likely to encounter the growing tip of the test plant.

Tests were controlled in three ways: 1) An uninoculated plant of each test taxon was used as a control for test plant inoculation effects. 2) In order to check that inoculum was infective, for each test plant batch, five replicate *C. m.* ssp. *monilifera* plants (positive controls) were inoculated with the same batch of inoculum as was used for that test plant batch. 3) In order to check that erineum development on positive controls was due to inoculation and not to *A. neseri* from ambient sources (e.g. wind-borne), for each test plant batch, five replicate uninoculated *C. m.* ssp. *monilifera* plants (negative controls) were incubated under the same conditions as test and positive control plants. Tests were considered valid only if the inoculated shoot tip continued to grow throughout the test, erinea developed normally on four out of the five positive control plants for that batch and no erinea developed on the negative control plants.

Test and control plants were inspected daily for development of erinea and other abnormalities that might be attributable to *A. neseri*. Erinea were counted and their surface area was estimated three weeks after they appeared on a majority on positive controls. Test plants were examined microscopically for *A. neseri* four to five weeks after inoculation.

Results

Results are presented in Table 1. The only genus affected by *A. neseri* was *Chrysanthemoides*. Normal erinea and *A. neseri* colonies were routinely induced on the inoculated shoot tip of positive control plants. Erinea usually appeared on these plants six to ten days after inoculation. Erinea did not develop on uninoculated shoot tips. No taxa in any other genera developed erinea or any other galls or sustained damage that could be attributed to or showed signs of infestation with *A. neseri*, nor were any *A. neseri* found on any of those taxa at the conclusion of tests.

Erinea developed on one of the unidentified *C. monilifera* and three of the unidentified *C. incana* accessions, although these responses were generally weaker than those that occurred on the positive controls.

Discussion

The tests described here indicate that *A. neseri* is restricted to the genus *Chrysanthemoides*, a favourable result in terms of its potential as a biological control agent, and that it would be safe to release in Australia, since the only representatives of *Chrysanthemoides* in Australia are pests and are accepted as biological control targets.

The results also give some indication that the laboratory host range of *A. neseri* accessions from *C. m.* ssp. *monilifera* includes taxa from *C. incana* as well as *C. monilifera*. Whether these laboratory hosts would be suitable as hosts in the field was not determined. However, given the generally weaker response of *A. neseri* in these tests to *Chrysanthemoides* taxa other than *C. m.* ssp. *monilifera* it would appear that *A. neseri* accessions from *C. m.* ssp. *monilifera* prefer that taxon.

A. neseri has also been observed on *C. m.* ssp. *rotundata* and *C. m.* ssp. *pisifera* (L.) Norl. (Adair 1999) and these mite populations are probably distinct biological races. As an adjunct to the tests described above, an *A. neseri* accession from *C. m.* ssp. *pisifera* was tested for its ability to induce erineum formation on *C. m.* ssp. *monilifera*. The response of *C. m.* ssp. *monilifera* to this accession was much weaker than that of *C. m.* ssp. *monilifera* to *A. neseri* accessions from *C. m.* ssp. *monilifera*. I also observed an erineum-forming eriophyid (probably another race of *A. neseri*) infesting an unidentified *C. incana* taxon in Cape Town and was able to induce erineum formation with it on *C. m.* ssp. *monilifera*.

Providing accessions of *A. neseri* that cause severe leaf distortion and abundant erineum formation on Australian forms of *Chrysanthemoides* can be located, the potential for suppression of Australian infestations is good.

Table 1. Taxa tested as hosts for *A. neseri* and erineum development on inoculated shoot tips three weeks after erinea appeared on the majority of test batch *C. m.* ssp. *monilifera* positive controls.

Taxon	Proportion of replicates that developed erinea	Mean number of erinea (*s*) per replicate	Mean total area of erinea (mm^2) (*s*) per replicate
C. monilifera ssp. *monilifera*	80/90[a]	10.0 (10.5)	57.6 (81.6)
C. monilifera unidentified taxon 1[b]	0/3	0	0
C. monilifera unidentified taxon 2[c]	2/4	1.8 (2.1)	2.3 (2.6)
C. monilifera unidentified taxon 3[d]	0/3	0	0
C. monilifera unidentified taxon 4[e]	0/5	0	0
C. incana unidentified taxon 1[f]	4/8	0.5 (0.5)	1.3 (1.4)
C. incana unidentified taxon 2[f]	3/5	5.8 (7.4)	12.8 (14.7)
C. incana unidentified taxon 3[g]	2/4	1.3 (1.5)	5.0 (5.8)
C. incana unidentified taxon 4[h]	0/6	0	0
Calendula officinalis	0/6	0	0
Dimorphotheca sinuata	0/5	0	0
Osteospermum fruticosum	0/5	0	0
Actites megalocarpa	0/6	0	0
Lactuca sativa	0/5	0	0
Cichorium intybus	0/6	0	0
Cichorium endivia	0/3	0	0
Tragopogon porrifolius	0/6	0	0
Arctotheca calendula	0/6	0	0
Cymbonotus preissianus	0/5	0	0
Gazania rigens	0/5	0	0
Artemisia dracunculus	0/6	0	0
Chamaemelum nobile	0/5	0	0
Cotula turbinata	0/6	0	0
Cotula coronopifolia	0/6	0	0
Chrysanthemum morifolium	0/5	0	0
Tanacetum cinerariifolium	0/5	0	0
Callistephus chinensis	0/6	0	0
Olearia axillaris	0/6	0	0
Cynara scolymus	0/5	0	0
Carthamus tinctorius	0/6	0	0
Stemmacantha australis	0/6	0	0
Ageratum houstonianum	0/6	0	0
Dahlia pinnata	0/5	0	0
Helianthus annuus	0/5	0	0
Helianthus tuberosus	0/6	0	0
Melanthera biflora	0/6	0	0
Tagetes patula	0/6	0	0
Bracteantha bracteata	0/6	0	0
Cassinia aculeata	0/6	0	0
Leucophyta brownii	0/6	0	0
Ozothamnus turbinatus	0/4	0	0
Gerbera jamesonii	0/5	0	0
Senecio odoratus	0/6	0	0
Senecio hybridus	0/6	0	0
Tussilago farfara	0/6	0	0
Allocasuarina verticilliata	0/3	0	0
Acacia sophorae	0/6	0	0
Eucalyptus grandis	0/6	0	0
Banksia integrifolia	0/6	0	0
Actinidia chinensis	0/6	0	0
Mangifera indica	0/5	0	0
Annona reticulata	0/6	0	0
Campanula medium	0/6	0	0
Humulus lupulus	0/6	0	0
Carica papaya	0/6	0	0
Ipomoea batatas	0/5	0	0
Beta vulgaris	0/6	0	0
Brassica napus	0/6	0	0
Vaccinium corymbosum	0/6	0	0
Persea americana	0/4	0	0
Pisum sativum	0/5	0	0
Trifolium repens	0/6	0	0
Allium cepa	0/6	0	0
Asparagus officinalis	0/6	0	0
Linum usitatissimum	0/6	0	0
Musa sapientum	0/4	0	0
Lolium perenne	0/6	0	0
Oryza sativa	0/6	0	0
Protea burchellii	0/3	0	0
Capsicum annuum	0/5	0	0
Camellia sinensis	0/6	0	0
Apium graveolens	0/6	0	0
Daucus carota	0/6	0	0
Zingiber officinale	0/6	0	0

Table 1. (Continued) Taxa tested as hosts for *A. neseri* and erineum development on inoculated shoot tips three weeks after erinea appeared on the majority of test batch *C. m.* ssp. *monilifera* positive controls.

[a] Includes all *C. m.* ssp. *monilifera* positive control plants from all test batches.

[b] Suspected of being ssp. *pisifera.* Purchased from Sonderpry's Nursery, Somerset West, Cape Town.

[c] Suspected of being ssp. *pisifera*. Propagated by Mitchell's Nursery

[d] Suspected of being an intermediate between ssp. *rotundata* & ssp. *pisifera.* Purchased from Helderberg Nature Reserve Nursery, Somerset West, Cape Town.

[e] Suspected of being an intermediate between ssp. *rotundata* & ssp. *pisifera*. Source unknown.

[f] Purchased from Good Hope Nursery, Cape Peninsula.

[g] Purchased from Helderberg Nature Reserve Nursery, Somerset West, Cape Town.

[h] Purchased from Nursery on the West Coast, Melkboschplaas, Cape Town.

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