Past Victorian work on *Emex australis* Stenheil and *Tribulus terrestris* L.

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Summary

The host specificity of *Microthrix inconspicuella* and *Rhodometra sacraria*, potential biological control agents for *Emex australis*, was investigated in South Africa and later under quarantine conditions in Australia. Neither insect was subsequently released in Australia, due to possible conflicts of interest. A literature review of *Tribulus terrestris* showed that the taxonomy of the plant required further investigation before proceeding with host specificity tests of potential biological control agents.

Introduction

Emex australis Stenheil is a weed of South African origin for which two Curculionids, *Perapion antiquum* (Gyllenhal) and *Lixus cribicollis* Boheman, have been introduced into Australia as biological control agents (Harley and Kassulke 1975, Julien and Bourne 1983, Julien *et al.* 1982). Establishment of *P. antiquum* only has been reported and no control of the plant has been observed (Julien 1981).

Host specificity testing of a South African moth, *Microthrix inconspicuella* Ragonot, was carried out in Brisbane by CSIRO and it was observed that the larvae fed on young apple leaves of the variety Tropical Beauty (Harley *et al.* 1979). It was decided that this feeding was probably a laboratory phenomenon, but no request was made to have M. *inconspicuella* introduced as a biological control agent for *E. australis.*

The Dried Fruits Research Council helped fund a project aimed at re-testing the host specificity of *M. inconspicuella* to apples in South Africa. Host specificity tests on the two polygonaceous plants of economic value, buckwheat and rhubarb, were also suggested but no further testing of native Australian Polygonaceae was considered necessary. The presence of other *Emex* feeding insects was investigated and host specificity tests carried out (Shepherd 1989, 1990a, 1990b).

Tribulus terrestris L. is probably a weed of Saharan origin which is now spread throughout the Mediterranean region. It is also thought to be native in southern Africa and maybe in Australia (Squires 1979, Holm *et al.* 1979). In Australia, *T. terrestris* probably has two forms, one that is introduced and one that is native. There are morphological differences between these forms, however it is possible that it is an aggregate species which contains components which are indigenous to Australia (Eichler personal communications). The taxonomy of this species is therefore not clear and the two spineless forms of *T. terrestris* should possibly be designated *T. micrococcus* and *T. minutus* (Wilson personal communications).

T. terrestris is considered a serious weed in many parts of Australia. It causes damage to grazing animals, by physical damage via seeds (Parsons 1973) and physiological damage caused by ingestion of toxic substances (Everist 1981). Regular feeding on the plant can lead to the disease chronic *Tribulus* staggers, a locomotary disturbance of sheep which occurs occasionally (Everist 1981). Nitrate poisoning is also a problem with sheep feeding on green growth (Holm *et al.* 1977).

Seeds also have a nuisance value to people as seeds can puncture bicycle tyres, contaminate wool and lower the value of the fleece. The problem to the Dried Fruits Industry is contamination of the drying areas and of the dried fruit by the spiny seed.

Because of the previous use of biological control agents in parts of the USA (Angalet and Andres 1963, Andres 1978), a literature survey was carried out for the Dried Fruits Research Council and a submission put to Australian Weeds Council to have *T. terrestris* declared a target weed for biological control.

Materials and methods

Host specificity of Microthrix inconspicuella

The apple varieties tested did not include Tropical Beauty as this was unavailable in South Africa. Apple varieties common to Australia were tested, and included Jonathan, Starking (Red Delicious), Granny Smith and Golden Delicious. Mature and immature trees were tested, from flowering to fruit fall.

Mature apple trees grew in an unsprayed commercial orchards and the branch being tested was encased in a sleeve cage. Immature trees were planted in walk in cages and insects were therefore free to move freely within the cage. All stages of the life cycle of *M. inconspicuella* were tested. All these experiments were repeated, but without the branches being caged.

Regularly sprayed mature orchards of Starking, Topred and Golden Delicious varieties, sprayed to control codling moth, snout beetle and red spider mite, were used to see if *M. inconspicuella* larvae feeding on *Emex* plants growing in the orchard were killed by these sprays.

All stages of the four apples varieties were tested in the laboratory to see whether larval feeding would occur when larvae were confined with their food source.

Further laboratory tests were carried out on buckwheat and rhubarb using adults and all larval instars to see whether these plants were attractive to *M. inconspicuella.* Ovipositing adults were caged with plants and all larval instars placed on plants to see whether they fed.

Olfactometer tests were carried out in the laboratory using day old *M. inconspicuella* adults, and *E. australis,* buckwheat and rhubarb leaves. An empty tube acted as a control. The attractiveness of *M. inconspicuella* to each species was observed.

Host Specificity testing of other E. australis insects

Larvae of the geometrid moth, *Rhodometra sacraria* L., were found feeding on the foliage of *E. australis* in the Cape region of South Africa. This insect has been recorded from polygonaceous and non-polygonaceous plants (Laithwaite *et al.* 1975, Pinhey 1975). The latter plants were so botanically diverse it was considered possible that the records were inaccurate. Host specificity tests were carried out as *R. sacraria* larvae appeared capable of defoliating *E. australis* plants and could possibly be a useful biological control agent for *E. australis.* Sixty-seven plant species belonging to 21 families were tested using choice and non-choice, oviposition and larval starvation tests (Shepherd 1989).

Tribulus terrestris

The literature survey included all known literature on the taxonomy and biological control of T. *terrestris* and related species.

Results and discussion

Investigation of the host specificity of M. inconspicuella in South African orchards.

Experiment using sleeve cages in unsprayed apple orchards showed that eggs were only laid on leaves and fruit of Granny Smith apples. No first or second instar larvae fed, and only one third instar larva fed on a Golden Delicious leaf and one on a Granny Smith leaf. All other larvae died without feeding. Feeding by fourth and fifth instar larvae was mainly restricted to petals and fruit, especially to young fruit which fell into the base of the sleeves, also to leaves and/or fruit which touched each other, or to previously insect damaged fruit. Some feeding was also observed where the cages touched the fruit, forming a shelter from which larvae could feed. Feeding was slight and death usually occurred within 7 days. Some pupation occurred after fifth instar larvae fed on petals, leaves, young or damaged fruit (Shepherd 1990a).

Fewer than 40% of older larvae pupated, few adults developed and mated and none of their progeny survived or fed on apples. Larval damage to stamens, flowers and young fruit was minimal and unlikely to contribute to loss in fruit production. Even if Granny Smith fruit are slightly attractive to *M. inconspicuella* larvae, no second generation resulted from any feeding and damage to the plant was minimal.

When all experiments were repeated on the same trees under the same conditions but without cages, a considerable amount of larval wandering was observed. Larvae fell off the branches and were preyed upon by Argentine ants. No second generation was observed from these experiments.

M. inconspicuella did not survive a regular apple orchard spraying regime, therefore apple trees would be protected from larval attack for several weeks. Routine use of sprays in an apple orchard would appear to be sufficient protection for the apple crop if E. *australis* and *M. inconspicuella* are found in orchards.

Under confined conditions in the laboratory eggs were laid on all varieties of apples but there were no significant differences in the number of eggs laid on apple fruit under choice and non-choice conditions. No feeding by newly emerged, first, second or third instar larvae occurred. All larvae died without feeding. Again, final instar larvae were capable of feeding on apples, preferably the smaller sized more immature fruit of Granny Smith and Jonathan varieties, and the larger mature fruit of Golden Delicious. Few larvae pupated and fewer adults emerged. No second generation resulted. It appears that the apple skin forms a barrier through which the younger instars cannot bite. If damage occurs to the skin some feeding on the apple flesh will result (Shepherd 1990b).

The results from all these tests did not agree with the findings of the original host specificity tests, that *M. inconspicuella* could feed on apple leaves and maintain a culture for three generations. The apple orchard results showed that apples would have been safe if *M. inconspicuella* had been introduced for control of *E. australis.*

Polygonaceous plants of economic importance

None of these tests proved conclusively that *M. inconspicuella* larvae could not feed on rhubarb and buckwheat. Although feeding occurred on rhubarb, few pupae were produced and fewer adults emerged. A second generation could be maintained on rhubarb but no third generation was produced. The amount of larval damage was minimal, and only leaves were fed upon. Adults released near a small patch of rhubarb did not appear to be attracted to it, no oviposition or larval feeding was observed.

Some oviposition was observed on buckwheat but very little feeding occurred. Most larvae died without pupating and a second generation was not observed.

With the olfactometer tests no significant differences were found between the attractiveness of the plants and the empty tube. It was not possible to show that *M. inconspicuella* was only attracted to *E. australis*.

Because of the possible conflict of interest raised with *M. inconspicuella* permission was not sought to introduce it into Australia. Some State Departments considered it should be put through the Biological Control Act before permission was granted for introduction to quarantine for rearing and release.

Host Specificity testing of other E. australis insects

Host specificity tests indicated that *R. sacraria* was family specific rather than species specific, as no non-polygonaceous plants were fed on. Eggs were frequently laid on some of these plant species but no feeding by any larvae resulted. A number of the plants fed on were Australian natives belonging to Polygonaceae, some of which would never come in contact with *E. australis* because of their different growth habits and location. These plants included *Polygonum attenuata,* which grows in North Queensland, *Polygonum prostrata* which grows on creek and lagoon banks and *Rumex bidens* which forms floating mats in creeks, lagoons and rivers. Eggs laid on these plants hatched and the larvae fed, developed and another generation could develop on these plants.

Because of the likely problems with conflicts of interest with conservationalists and botanists, as *R. sacraria* is family rather than species specific, permission was not sought to introduce this insect into Australia as a biological control agent against E. *australis.*

Prospects for the biological control of T. terrestris

T. terrestris belongs to the family Zygophyllaceae which has five genera in Australia and three introduced species, including *T. terrestris* and *T. cistoides.*

There are taxonomic problems with *T. terrestris*, and it is possible that it is an aggregate species of native and introduced species. Consequently a biological control agent might be introduced to control an Australian native plant. This immediately raises thoughts of conflicts of interest.

Two of the Australian genera of Zygophyllaceae, *Zygophyllum* and *Tribulus,* are semi-arid plants of a spreading prostrate habit found in most states. *Tribulopis* is also prostrate, but common in tropical Australia. The other genera present in Australia are *Peganum* which is introduced and the native genus *Nitraria. P. harmala* L., African Rue, was introduced from the Mediterranean region and there is one species of *Nitraria, N. billardierei* DC. Dillon Bush, commonly found in Australia.

Biological control agents

Two weevils, *Microlarinus lypriformis* (Wollaston) a stem weevil and *M. lareynii* (Jacquelin du Val) a seed weevil, were introduced from Sicily to the USA as biological control agents.

These insects are dormant in winter and feed in spring on the green leaves of the plant. Only then can the weevils reproduce. Oviposition usually occurs on the newly germinated plants or on the newly developed seeds. Given the right temperatures, 1-3 generations can occur during summer. Adults that emerge in late summer go into a reproductive diapause and do not develop eggs until the plant germinates in spring.

Host specificity testing

Host specificity tests were carried out on both weevil species in USA before they were introduced into the field. Thirty-nine plants belonging to 21 families were tested. Egg development and oviposition occurred only after adults fed on *T. terrestris* and closely related plants belonging to Zygophyllaceae. In non-choice tests both weevils fed on plants unrelated to *T. terrestris* and survived for varying lengths of time. If ovipositing females were removed from *T. terrestris* their ovaries regressed and they ceased egg laying. During starvation tests nearly all plant species were fed on, however the damage was minimal in comparison to that on *T. terrestris.* Most feeding was insufficient to support growth and the insects died.

Microlarinus spp. are therefore Zygophyllaceae specific rather than *Tribulus* specific, although there is a preference for *Tribulus* species. They have however developed to maturity on some *Kallstroemia* spp., a species not present in Australia.

If *Microlarinus* spp. are to be introduced into Australia a host specificity test list has been devised (Shepherd 1990c). This represents 80 plant species from 43 families and covers the categories:-

1. Species from the same sub-family
2. Species from the same family
3. Species from the sub-class Rosidae
4. Species of Economic importance in *Tribulus* areas of the world
5. Species of economic importance in Australia which have had inadequate exposure in countries of origin and country of introduction
6. Plants fed on by *Microlarinus* spp. in previous host specificity tests

Although *T. terrestris* is considered a weed of importance in Australia, it would probably be controversial and need to be considered under the Biological Control Act.

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