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Problems which Arise with Host-specificity Testing of Insects

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Abstract

Some of the problems which arise with host-specificity testing are discussed using as examples the two moths *Microthrix inconspicuella* and *Rhodometra sacraria*, which were investigated as potential biological control agents against the weed *Emex australis*. Difficulties can arise with the agent and test plant species, but probably one of the most serious problems is the introduction of disease. Host-specificity testing can frequently highlight unexpected problems, tests may indicate that an insect will feed on a test plant species unrelated to the target plant. This may only be a laboratory aberration but results in either the rejection of the agent or the transfer of research work to the country of origin of the agent.

Introduction

Host-specificity tests are an important component of classical biological control of weeds, and without knowledge of these no biological control agent should be released. Organisms imported for the biological control of weeds should cause no significant damage to plants of economic or ecological importance.

Most host-specificity tests, particularly those carried out in Australia, follow the centrifugal phylogenetic method developed by Wapshere (1974), and concentrate on the feeding and oviposition behaviour of the agent on a series of test plants. In Australia there are adequate safeguards to ensure that no agent is released without first carrying out extensive tests, and guidelines have also been developed for the implementation of the biological control of weeds in the United States (Klingman and Coulson 1983).

Frequently host-specificity data indicate that plants both related and unrelated to the target species can be fed on (Table 1), but conflict of interest cases do not always arise as the insects may not be released, for example *Rayieria* sp. (Hemiptera: Miridae) (Donnelly 1986), or if released may not reproduce unless they feed on the host plant, as in the case of *Microlarinus* spp. (Coleoptera: Curculionidae) feeding on oranges (Andres and Angalet 1963).

Host-specificity testing in Australia of *Microthrix inconspicuella* Ragonot (Lepidoptera: Pyralidae), a possible biological control agent for *Emex australis* Steinheil (Polygonaceae), indicated that larvae were capable of feeding on young apple leaves for three generations (Harley *et al.* 1979). These findings resulted in the abandonment of the project in Australia. However the project was later resumed in South Africa under non-quarantine conditions to determine whether the observations of Harley *et al.* (1979) were laboratory aberrations or whether apples were an alternative food plant of *M. inconspicuella*.

Problems with host-specificity tests are considered in two sections, those related to the agent and those concerned with test plant species. Some problems encountered when conducting host-specificity tests in Australia and South Africa, using *M. inconspicuella* and *Rhodometra sacraria* L. (Lepidoptera: Geometridae), are discussed here.

Problems which Arise with Agent Organisms

Problems with the agent fall into two groups, those arising with culturing of insects and those

Table 1. Some examples of agents known to feed on plants other than target species during host-specificity tests.

Agent	Target species	Damaged species	Reference
Stobaera concinna (Stal) (Homoptera: Delphacidae)	*Parthenium hysterophorus* L. (Asteraceae)	*Ambrosia confertiflora* A. Cunn. ex D. Donn (Asteraceae)	McClay (1983)
Rayieria sp. (Hemiptera: Miridae)	*Acacia longifolia* (Andr.) Willd. (Fabaceae) *A. implexa* Benth. *A. melanoxylon* R. Br. *A. mearnsii* De Wild	*Acacia cyclops* (Mimosaceae) *A. saligna* (Labill.) H. Wendl.	Donnelly (1986)
Microlarinus lypriformis (Wollaston) (Coleoptera: Curculionidae)	*Tribulus terrestris* L. (Zygophyllaceae)	*Citrus* sp. (Rutaceae)	Andres (1978)
M. lareynii (Jacquelin du Val)		*Lactuca sativa* L. (Asteraceae) *Spinacia oleracea* L. (Chenopodiaceae) *Cucumis melo* L. var. *cantalupensis* Naud. (Cucurbitaceae) *Vitis vinifera* (L.) (Vitaceae) *Medicago sativa* L. (Papilionaceae) *Phaseolus vulgaris* L. (Papilionaceae)	Andres and Angelet (1963)
Rhodometra sacraria L. (Lepidoptera: Geometridae)	*Emex australis* Steinheil (Polygonaceae) *Persicaria* spp. (Polygonaceae)	*Polygonum aviculare* L. (Polygonaceae) *Rumex* spp. (Polygonaceae)	Shepherd unpub. data
Microthrix inconspicuella Ragonot (Lepidoptera: Pyralidae)	*E. australis* auct. non L.	*Rheum raponticum* L.	Shepherd, unpub. data
Acigona infusella Walker (Lepidoptera: Pyralidae)	*Eichhornia crassipes* (Mart.) Solms-Laubach (Pontederiaceae) *Musa* sp. (Musaceae)	*Zingiber officinale* Posc. (Zingiberaceae) *L. sativa*	Sands and Kassulke (1983)
Stenocarus umbrinus (Gylh.) (*fuliginosus* [Marsham]) (Coleoptera: Curculionidae)	*Papaver somniferum* L. (Papaveraceae)	*Cichorium intybus* L. (Asteraceae)	Buckingham *et al.* (1983)
Paulinia acuminata (De Geer) (Orthoptera: Pauliniidae)	*Salvinia molesta* (D.S. Mitchell) (Salviniaceae)	*Fragaria* x *ananassa* Duch. (Rosaceae)	Sands and Kassulke (1986)
Bembecia chrysidiformis (Lepidoptera: Sesiidae)	*Rumex pulcher* L. (Polygonaceae) *R. crispus* (Esper)	*R. raponticum*	J.K. Scott, unpub. data

encountered when carrying out tests (Table 2). To be able to perform host-specificity tests it is essential to rear large numbers of healthy, fecund insects, free of parasites and disease. Some of the problems listed in Table 2 are discussed below.

Table 2. Some problems encountered when testing an agent for host-specificity.

Problems with: The agent organism	Problems with: The test plant species
1. Mass Rearing	Classification of target and relatives
Disease - Microsporidian, viral, fungal, bacterial	Availability of plants
Low or non-fertile insects	Size of plants - use of part *vs.* whole
Predators	Availability of required plant stage
Egg laying - containers *vs.* plants	Correct plant biotype
Container size	Taxonomy
Food - fresh *vs.* artificial diet	Feeding on non-related plants
Diapausing insects	Feeding on related plants
Contamination of rearing plants	Contamination of test plants by insects
Development of correct breeding regime	Cage size
2. Testing of plants	
Container/cage size	
Parasites	
Predators	
Egg laying cages *vs.* plants	
Olfactometer tests	
3. Development of correct breeding regime for field release	

Introduction of Correct Agent Biotypes

Culturing

Disease. Diseases have been frequently introduced with imported agents (Table 3) and occasionally released (Dunn and Andres 1981). Disease was introduced with *M. inconspicuella* and *R. sacraria* from South Africa, but *R. sacraria* had been successfully reared there free of disease. After four generations in Australia disease was observed and identified as a cytoplasmic polyhedrosis virus, and presumably this was introduced with *R. sacraria* (K. Reinganum, pers. comm., 1988). This culture lasted six generations before it died out, and the mean number of eggs per female fell from 127.4 (SD = ± 43.2) to a mean of 47.3 (SD = ± 21.4) in the fifth generation, a decrease of about 65%. The mechanism of transmission, whether transovarially or through ingestion, is unknown (K. Reinganum pers. comm.), however up to 50% of larvae died per generation once the virus became established.

A pathogenic microsporidian, *Thelohania* sp., was found in the ovaries of *M. inconspicuella* in South Africa. Mass rearing was difficult, and larvae had to be reared in individual tubes, using selected lines known to be parasite free. This parasite was introduced into Australia and reduced egg laying in *M. inconspicuella* by up to 68% and larval mortality by a further 18-40%, with larvae dying in the third or fourth instars. The mean number of eggs laid per unparasitized female was 206.7 (SD = ± 66.5) and parasitized females, 56.6 (SD = ± 24.7).

Fertility. Insects sometimes fail to mate irrespective of light, temperature or humidity regimes, containers or plants/flowers introduced to induce mating. For *M. inconspicuella* no mating occurred in 38% of single pair matings and in 39% of these, fewer than 50 egg/female were laid in the first two generations. This was not due to the effect of *Thelohania* sp. as the same trend was observed in South Africa in a parasite-free culture.

Table 3. Some agents introduced for host-specificity tests or released with pathogens.

Insect species	Pathogen	Target Weed	Reference
Anaitis efformata Gueneé (Lepidoptera: Geometridae)	*Pleistophora*	*Hypericum perforatum* L. (Clusiaceae)	Milner and Briese (1986)
Rhinocyllus conicus (Froel.) (Coleoptera: Curculionidae)	*Nosema* sp.	*Carduus pycnocephalus* L. (Asteraceae)	Dunn and Andres (1981)
Ceutorhynchus litura (F.) (Coleoptera: Curculionidae)	*Nosema* sp.	*Cirsium vulgare* L. (Asteraceae)	Dunn and Andres (1981)
C. trimaculatus (F.)	*Nosema* sp.	*Carduus nutans* L.	Dunn and Andres (1981)
Longitarsus jacobaea (Waterhouse) (Coleoptera: Chrysomelidae)	Cephalogregarines	*Senecio jacobaea* L. (Asteraceae)	Dunn and Andres (1981)
Microthrix inconspicuella Ragonot (Lepidoptera: Pyralidae)	*Thelohania* sp.	*Emex australis* Steinheil (Polygonaceae)	Shepherd, unpubl. data
Rhodometra sacraria L. (Lepidoptera: Geometridae)	Cytoplasmic polyhedrosis virus	*E. australis*	Shepherd, unpubl. data

Predators. Host-specificity testing in the country of origin has some advantages, cultures do not need to be raised under strict quarantine conditions, however rearing a culture in the laboratory may also have disadvantages. Argentine ants, *Iridomyrmex humilis* (Mayr) (Hymenoptera: Formicidae), invaded cultures of *M. inconspicuella* housed in the glasshouse in South Africa and reduced the culture by up to 50% overnight. A well camouflaged and hungry chameleon introduced on foliage also reduced the population by about 50%.

Egg laying. M. inconspicuella preferred to lay eggs on gauze lids and paper inserts rather than on its host plant. The mean number of eggs laid per female on gauze lids was 50.3 (SD = ± 47.9) and on paper inserts was 62.2 (SD = ± 51.6), on *E. australis*, 13.3 (SD = ± 5.5) and on cage walls 80.7 (SD = ± 69.9). The low number of eggs laid per plant during host-specificity tests could be an adversion of females to lay on leaves and could result in incorrect information concerning host-specificity.

Testing of Plants

Cages. Host-specificity tests for *M. inconspicuella* were carried out in orchards, using four apple varieties and sleeve cages of varying diameter. Below a diameter of 30 cm the sleeves acted as small cages and some feeding by late instar larvae occurred. Small sleeves enabled leaves and apples to touch, thus providing a niche from which larvae could feed on leaves, flower petals and young fruit.

Predators. Some host-specificity tests were conducted in apple orchards without cages. Larvae could wander freely, however they were usually preyed upon by *I. humilis* before they had time to feed on any part of the plant.

Parasites. Parasitism was a problem with outdoor experiments. At least 11 species of parasitoids belonging to six families were collected from *M. inconspicuella*, and *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) was collected from eggs laid during experiments carried out in walk-in cages.

Egg laying. E. australis is a low growing plant and if apples are an alternative host plant for *M. inconspicuella*, it is important to know if oviposition will occur on plants up to 2 m tall. Walk-in cages were used to test the ability of *M. inconspicuella* to oviposit at varying heights. Branches were placed at ground level and at 1 and 2 m above, but egg laying was exceedingly low. For *E. australis* at ground level the mean number of eggs was 15.5 (SD = ± 4.1), 1 m above ground it was 3.6 (SD = ± 3.8) and 2 m above ground it was 12.9 (SD = ± 5.7), for apple branches the number of eggs laid was 5.2 (SD = ± 7.2), 0.8 (SD = ± 2.2) and 3.1 (SD = ± 3.6) respectively. *M. inconspicuella* adults were observed to remain either at ground level or to fly to the top of the cage and lay eggs there, thus giving an erroneous result concerning a preferred oviposition site.

Olfactometer tests. Larval olfactometer tests were performed in the open. *M. inconspicuella* larvae wandered freely to indicate host plant preference. Younger instars were preyed upon by *I. humilis* before the completion of the tests, and 10% of larvae returned to the laboratory and actively feeding on the preferred plants were parasitized and did not complete their development. Results from these tests were therefore limited.

Adult olfactometer tests were carried out under quarantine conditions to test if *E. australis* was more attractive to *M. inconspicuella* than rhubarb, *Rheum rhaponticum* auct. non L. (Polygonaceae), for oviposition. Cages and Y-shaped olfactometer tubes were used.

Adults were released singly or in pairs, and plants and their positions were changed regularly. A total of 80 adults were released in the cage experiments, and there was no significant difference ($P = 0.05$) with respect to direction of flight, to either *E. australis* or rhubarb. The Y-shaped olfactometers were constructed of transparent or opaque plastic piping. Leaves rather than plants were used as attractants and were placed at the end of the 0.5 m long arms. A total of 68 adults were tested and no significant differences were observed ($P = 0.01$), irrespective of olfactometer, species of plant, position of leaves and sex of moth. When one arm was left empty moths frequently flew there irrespective of plant species present in the other arm. *M. inconspicuella* adults generally flew to the right arm irrespective of light position or plant source. These experimental moths behaved in such a random manner that results were inconclusive.

Problems which Arise with the Test Plants

Taxonomy

Most host-specificity test lists are usually constructed using biological, botanical and economic rational. However problems may occur if the taxonomy of the target or any test species is unsure and require revision. This is the case with *Tribulus terrestris* L. (Zygophyllaceae) in Australia where the taxonomy of the introduced and endemic species is confused and biological control of a native species may be considered undesirable.

Availability of Plants

Some plants may be relatively rare and difficult to collect; e.g., *Tribulopis* spp. which are Australian polygonaceous plants related to *T. terrestris*, and found in relatively inaccessible arid regions of the country. Some aquatic plants may also be difficult to locate and frequently plants were difficult to keep healthy and free of unwanted insects.

Size of Plants

Many plants tested are trees too large for specificity testing in cages. This presents problems when seed-feeding insects are tested; i.e., when flowers and fruit are necessary. When individual leaves or branches rather than whole plants are tested oviposition behaviour could be affected. Is the odour of one leaf sufficient to attract an ovipositing moth? Does the continual changing to fresh leaves upset a possible feeding pattern, or does the presence of stale food mean that larvae will not attempt to feed whereas they may have otherwise?

Feeding on Non-target Plants

Laboratory aberrations are frequently observed in host-specificity tests. Plants which are unlikely to be fed on in the field act as hosts when plants and agents are confined together (Table 1). Because *M. inconspicuella* fed on apples during host-specificity tests (Harley *et al.* 1979) research on this insect was completed in South Africa.

Plant Biotypes

Plant biotypes have influenced skeleton weed, *Chondrilla juncea* L. (Asteraceae), research in Australia since the first introduction of the biological control agents *Aceria chondrillae* Can. (Acari: Eriophyidae) and *Puccinia chondrillina* Bubak and Sydenham (Uredinales) (Cullen 1978). These agents indicated that *C. juncea* was not a homogeneous plant species, but had at least three biotypes. Several specialized methods of identification have been developed; e.g., starch gel electrophoresis (Burdon *et al.* 1980) and polyacrylamide gel electrophoresis (Reinganum 1986), which have replaced the visual technique of Hull and Groves (1973). The existence of numerous biotypes in target plant species may explain why some biological control agents do not establish, or why some plants appear to be controlled but reappear several years later at densities not vastly different to the original population, e.g. *C. juncea* in the Mallee region of Victoria.

Suitable Plant Growth Stage for Agent

Growing plants for defoliating agents, *M. inconspicuella* or *R. sacraria*, presents few problems in comparison to those faced when growing plants for seed-eating insects, *Rhinocyllus conicus* Froelich (Coleoptera: Curculionidae) on *Carduus* spp. (Asteraceae), *Microlarinus lareynii* Jacquelin du Val (Coleoptera: Curculionidae) on *T. terrestris*; or for stem gallers, *A. chondrillae* on *C. juncea*; or stem borers, *Perapion antiquum* (Gyllenhal) (Coleoptera: Curculionidae) on *E. australis* and *Phytoecia coerulescens* (Scopoli) (Coleoptera: Cerambycidae) on *Echium plantagineum* L. (Boraginaceae); and crown borers, *Cochylis atricapitana* (Stephens) on *Senecio jacobaea* L. (Asteraceae).

Discussion

Some host-specificity tests have shown "false" results as in the case of *M. inconspicuella* and apples, or of *Uroplata lantana* Pic (Coleoptera: Chrysomelidae), which fed on *Lantana camara* L. in the laboratory, but which did not feed on this species in the field (Wapshere 1985).

When disease is present, culturing is time consuming and not cost effective. Disease-free material is essential for host-specificity tests and subsequent release (Briese and Milner 1986), and Dunn and Andres (1981) concluded that only insects which were free of pathogens should be released as disease could negate the usefulness of an expensive introduced agent.

To eradicate or even check for disease means that ideally every individual imported into the country must be screened before being cultured for host-specificity tests. The alternate is to screen agent in the country of origin and only the progeny of those adults found free of disease should be imported. This is more desirable but leads to the problem of who will clean up the culture prior to importation? Many countries/organisations are opportunists when it comes to biological agents, as they do not have facilities outside their own country. Therefore, importation of disease is common, and has been known to eradicate a colony of insects; e.g., *Tyria jacobaeae* L. (Lepidoptera: Arctiidae) (Bornemissa 1965). Does the insect feed, or not feed, on a particular plant because it does not like the plant or because of a disrupted metabolism caused by disease?

The parameters which should be considered when assessing whether an agent will damage a plant species are: (1) its ability; (2) opportunity; (3) advantage in doing so (Harris 1981). If an insect feeds upon a plant during host-specificity tests it does not mean that it will do so in the field unless the other two parameters exist and are positive. Thus if host finding ability is strong and the temporal and spatial distribution of a secondary plant which was attacked in host-specificity tests was limited, these results could be invalid with respect to field conditions. A potential conflict of interest may not exist. Most of the potential agents for the polygonaceous weeds, *Rumex* spp. and *Emex* spp., also attack rhubarb, a minor crop plant in Australia. However opportunity of attack is limited as distribution of crop and plant do not greatly overlap and ability to find rhubarb appears to be limited. It is therefore possible that rhubarb attack is more of a laboratory aberration than an actuality, and the probability of attack in the field is likely to be very low.

Occasionally it would appear that host-specificity tests raise as many problems as they solve. The stopping of these tests is not advocated but problems raised with *E. australis* and the two insects *M. inconspicuella* and *R. sacraria* are examples of problems that are probably encountered by all research workers.

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