SELECTION OF NON-TARGET SPECIES FOR 
HOST SPECIFICITY TESTING OF ENTOMOPHAGOUS 
BIOLOGICAL CONTROL AGENTS

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ABSTRACT

We present comprehensive recommendations for setting up test species lists for arthropod biological control programs that are scientifically based and ensure that all aspects of potential impacts are considered. It is proposed that a set of categories, including ecological similarities, phylogenetic/taxonomic affinities, and safeguard considerations are applied to ecological host range information to develop an initial test list. This list is then filtered to reduce the number of species to be tested by eliminating those with different spatial, temporal and morphological attributes and those species that are not readily obtained, thus unlikely to yield scientifically relevant data. The reduced test list is used for the actual testing but can (and should) be revised if new information obtained indicates that additional or more appropriate species should be included.

INTRODUCTION

The potential for non-target effects following the release of exotic species has raised concerns ever since biological control programmes were first set up. However, Howarth (1983; 1991) and Louda (1997) highlighted this issue of unwanted non-target effects in biological control and stimulated with these articles intense discussion even beyond the scientific community. Subsequently, a number of papers on non-target effects have been published within the last ten years (e.g., Follett et al. 2000a,b; Lockwood et al. 2001; Louda et al. 2003a; Lynch and Thomas 2000; Lynch et al. 2001; Simberloff and Stiling 1996; Stiling and Simberloff 2000; Thomas and Willis 1998). As host-specificity testing of entomophagous biological control agents has lagged behind that of phytophagous biological control agents, recent international efforts have been initiated. These efforts have been aimed at developing guidelines to provide a regulatory framework for the introduction of invertebrates for classical and inundative biological control of arthropods (e.g., OECD 2003). Generally, all these initiatives, research reviews
and guidelines, highlighted what should be done or what knowledge is required, but did not provide detailed methods on how tests should be conducted to assess potential non-target effects. As an exception, van Lenteren et al. (2003) recommended a risk assessment methodology for the evaluation of agents to be used in inundative biological control. Recently, Van Driesche and Reardon (2004) provided guidance to the best practice for assessing host ranges of parasitoids and predators used in classical biological control. Despite these valuable initiatives it is still important to provide standardized methods that can be universally applied for the assessment of potential non-target effects in arthropod biological control. Such methods are particularly relevant for parts of the guidelines where appropriate techniques are lacking to evaluate non-target effects (e.g., indirect impacts, interbreeding, establishment, dispersal and contaminants in agents). Selection of appropriate species for testing potential impacts of candidate biological control agents is the first critical step and although several independent arthropod biological control projects applied different approaches aiming the development of a test species list (e.g., Barratt 1997) a standardized method needs to be developed.

In this paper we review the approaches taken in some recent arthropod biological control programmes. Then we propose recommendations for setting up test species lists for arthropod biological control programmes that are scientifically based and ensure that all aspects of potential direct impacts are considered. Finally, we review the usefulness of selection criteria for setting up test species lists which will depend on the type of results that are generated by host-specificity tests, and the ease of their interpretation.

**SELECTION OF NON-TARGET SPECIES FOR TEST LIST: A REVIEW**

A review of some recent studies suggests that a variety of strategies have been used to select species for non-target host tests. As a general rule, test lists are based on knowledge from host records extracted from the literature (De Nardo and Hopper 2004; Sands and Van Driesche 2004). We concluded that although phylogenetic considerations were an underlying criterion (i.e., that a particular parasitoid group attacks certain host groups), ecological, biological and socio-economic information was very important for selecting non-target species for study. In addition, availability of test material was also critical for selection of non-target test species in most studies. Phylogenetic considerations were in reality based on taxonomic relatedness (e.g., same genus, same family, etc.) of test species to target host. Ecological features included overlap of geographic range, habitat preference, and feeding niche of species representing different components of the community. Biological characteristics included known host range, phenological overlap of the target and non-target species, dispersal capability of the candidate biological control agent (and parasitized host), morphological similarity, behavioural factors (e.g., feeding, oviposition, host location, etc.), and overlap of the physiological host range of biological control agents. Socio-economic factors included whether a potential test species was commercially important (e.g., a pollinator), beneficial (e.g., predator, weed biological control agent) or of conservation importance (e.g., rare or endangered). The availability of non-target material was considered, and sources included commercial or laboratory cultures, field collections, and progeny of field collected individuals. Many studies state the reasons behind selection of the test species, and all but three studies used at least two of the categories
in their selection. The numbers of non-target species tested in the laboratory ranged from one to 23. In Table 1, studies reviewed are compiled providing information about the selection criteria applied and the number of non-target species selected.

**RECOMMENDATIONS FOR COMPILING A NON-TARGET SPECIES TEST LIST FOR ARTHROPOD BIOLOGICAL CONTROL USING INVERTEBRATES**

It is widely believed that the criteria used to compile a suitable non-target test list in weed biological control projects are unlikely to provide such a reliable test list for entomophagous biological control agents. There are a number of arguments that support this claim; i) arthropods often outnumber plant species in communities by an order of magnitude (e.g., Kuhlmann et al. 2000; Messing 2001), ii) there is a significant lack of knowledge of arthropod phylogeny (e.g., Messing 2001; Sands and Van Driesche 2000), iii) natural enemies of arthropod pests respond to two trophic levels, i.e. the host and its host-plant(s) (e.g., Godfray 1994), iv) disjunct host-ranges appear to be the rule with parasitoids, rather than the exception as in herbivores (Messing 2001), and v) it is much more difficult and time-consuming to rear a large number of test arthropod species than test plant species (Kuhlmann et al. 1998; Sands and Van Driesche 2000).

One question that remains paramount with regards to the selection of non-target test species in arthropod biological control programmes is whether the host range of the parasitoid considered for use is restricted to one of a few closely related groups of herbivorous insects, or whether other factors such as phylogenetic disjunction in host range (a host range that includes phylogenetically unrelated species) are apparent. While it is commonly viewed by biological control scientists that initial predictions and assessments of parasitoid host range may be based on phylogeny, it is agreed that other highly relevant criteria, such as, ecological similarities shared between the target pest and other species in the field, should also be addressed as well as consideration of safeguard species selection. Thus, a more reductionist approach may be appropriate and selection of non-target test species is best carried out on a case-by-case basis.

At present, there is no standard protocol to refer to when compiling a species test list for assessment of an entomophagous biological control agent's host range. Numerous studies carried out in recent years illustrate that an array of criteria have been used to compile test species lists (Table 1).

In light of this, recommendations are proposed for developing a species list for host specificity testing of entomophagous arthropods (Fig. 1). The first step involves the collation of all recorded information on field hosts of not only the candidate biological control agent, but also of closely related species (see De Nardo and Hopper 2004). Literature reports and museum collections can provide valuable information relating to this but confirmation of the quality of the data must first be sought from a taxonomic expert as a precautionary measure. It must also be recognized that host records tend to be compiled using data from agricultural and forest habitats and often focus on more economically important species.
Table 1. Summary of selection criteria used in recent studies assessing host-specificity of entomophagous biological control agents.

<table>
<thead>
<tr>
<th>Agent and Target</th>
<th>Selection Criteria Used</th>
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</tr>
</thead>
</table>
| **Agent:** Cotesia erionotae Wilkinson  
[Hymenoptera: Braconidae]  
**Target:** Erionota thrax (L.)  
[Lepidoptera: Hesperiidae] | Phylogenetic: 1 sp. in the same family  
Socioeconomic: commercially important spp. | 4 Lepidoptera spp.: 1 Hesperiidae 3 Papilionidae | Sands et al. (1993) |
| **Agent:** Trichogramma nubilale Ertle and Davis  
[Hymenoptera: Trichogrammatidae]  
**Target:** Ostrinia nubilalis Hübner  
[Lepidoptera: Crambidae] | Socioeconomic: rare and endangered species  
Biological: wide host range of Trichogramma spp.; phonological overlap of target and non-target spp.; dispersal of agent and mortality during dispersal | 1 Lepidoptera sp.: 1 Lycaenidae | Andow et al. (1995) |
| **Agents:** Ageniaspis citricola (Logvinovskaya)  
[Hymenoptera: Encyrtidae]  
Citrostichus phyllocnistoides (Narayanan)  
[Cirrospilus quadristriatus Subba  
[Hymenoptera: Eulophidae]  
**Target:** Phyllocnistis citrella Stainton  
[Lepidoptera: Gracillariidae] | Ecological: leaf mining and gall forming flies; unrelated leafminers  
Phylogenetic: 1 sp. in same genus as target  
Socioeconomic: beneficial species (weed biocontrol agents) | 4 Diptera spp.: 1 Agromyzidae 1 Cecidomyiidae 2 Tephritidae  
1 Coleoptera sp.: Chrysomelidae 12 Lepidoptera spp.: 2 Bucculatricidae 1 Gelechiidae 5 Gracillariidae 1 Lyonetiidae 1 Pterophoridae 1 Pyralidae 1 Tortricidae | Neale et al. (1995) |
| **Agents:** Diachasmimiorpha longicaudata (Ashmead)  
Psyttalia fletcheri (Silvestri)  
[Hymenoptera: Braconidae]  
**Targets:** Ceratitis capitata (Wiedemann)  
Bactrocera dorsalis (Hendel)  
Bactrocera curbitae (Coquillet)  
[Diptera : Tephritidae] | Ecological: plant tissue of similar size and shape to that of target hosts; feeding niche  
Socioeconomic: weed biocontrol agent  
Biological: Morphology of parasitoid ovipositor, searching behaviour  
Availability: obtained from culture; field collected | 2 Diptera spp.: 2 Tephritidae | Duan and Messing (1996; 1997)  
Duan et al. (1997) |
| **Agents:** Cotesia rubecula (Marshall)  
Cotesia plutellae Kurdjumov  
[Hymenoptera: Braconidae]  
**Targets:** Pieris rapae L.  
Plutella xylostella (L.)  
[Lepidoptera : Pieridae]  
[Lepidoptera : Pluttellidae] | Ecological: taxa in geographic region and habitats where agent is abundant  
Biological: behaviour, attractiveness to host plant volatiles  
Availability: field collected material | 14 Lepidoptera spp.: 1 Pluttellidae 1 Tortricidae 1 Pyralidae 2 Nymphalidae 1 Arctiidae 8 Noctuidae | Cameron and Walker (1997) |
Table 1. Summary of selection criteria used in recent studies assessing host-specificity of entomophagous biological control agents (continued).

<table>
<thead>
<tr>
<th>Agent and Target</th>
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<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agent:</strong></td>
<td><strong>Microctonus aethiopoides Loan</strong> [Hymenoptera: Braconidae]</td>
<td></td>
<td>Barratt et al.</td>
</tr>
<tr>
<td><strong>Targets:</strong></td>
<td><em>Sitona discoideus</em> Gyllenhal</td>
<td></td>
<td>(1997; 1998; 2000; 2004)</td>
</tr>
<tr>
<td></td>
<td><em>Listronotus bonariensis</em> (Kuschel) [Coleoptera: Curculionidae]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>ecological: feeding niche; habitat overlap</td>
<td>11 Coleoptera spp.:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phylogenetic: taxa from subfamilies and tribes related to target</td>
<td>11 Curculionidae</td>
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<tr>
<td></td>
<td></td>
<td>socioeconomical: weed biological control agents</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>biological: phenology; diurnal activity, feeding and oviposition behaviour</td>
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<tr>
<td></td>
<td></td>
<td>availability: field collections</td>
<td></td>
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<tr>
<td><strong>Agent:</strong></td>
<td><strong>Aphidius rosae Haliday</strong> [Hymenoptera: Braconidae]</td>
<td></td>
<td>Kitt and Keller</td>
</tr>
<tr>
<td><strong>Target:</strong></td>
<td><em>Macrosiphum rosae</em> (L.) [Hemiptera: Aphidae]</td>
<td></td>
<td>(1998)</td>
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<tr>
<td></td>
<td></td>
<td>ecological: habitat where target occurred</td>
<td>7 Hemiptera spp.:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>biological: behaviour, attractiveness host plant volatiles</td>
<td>7 Aphidae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>availability: species from glass house and field collections</td>
<td></td>
</tr>
<tr>
<td><strong>Agents:</strong></td>
<td><strong>Cotesia flavipes Cameron</strong> <strong>Cotesia sesamiae</strong> (Cameron) [Hymenoptera: Braconidae]</td>
<td></td>
<td>Rutledge and Wiedenmann</td>
</tr>
<tr>
<td></td>
<td><strong>Cotesia chilonis</strong> (Matsumura) [Hymenoptera: Braconidae]</td>
<td></td>
<td>(1999)</td>
</tr>
<tr>
<td><strong>Target:</strong></td>
<td><em>Diatraea saccharalis</em> (F.) [Lepidoptera: Pyralidae]</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>ecological: habitat preference of agents</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>biological: physiological host range overlap of agents</td>
<td></td>
</tr>
<tr>
<td><strong>Agent:</strong></td>
<td><strong>Comsilura concinnata</strong> (Meigen) [Diptera: Tachinidae]</td>
<td></td>
<td>Boettner et al.</td>
</tr>
<tr>
<td><strong>Target:</strong></td>
<td><em>Lymantria dispar</em> (L.) [Lepidoptera: Lymantriidae]</td>
<td></td>
<td>(2000)</td>
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<tr>
<td></td>
<td></td>
<td>ecological: habitat overlap</td>
<td>3 Lepidoptera spp.:</td>
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<tr>
<td></td>
<td></td>
<td>biological: temporal overlap</td>
<td>3 Saturniidae</td>
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<tr>
<td></td>
<td></td>
<td>socioeconomical: threatened species</td>
<td></td>
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<tr>
<td><strong>Agent:</strong></td>
<td><strong>Trichogramma brassicae</strong> Bezenko [Hymenoptera: Trichogrammatidae]</td>
<td></td>
<td>Orr et al.</td>
</tr>
<tr>
<td><strong>Target:</strong></td>
<td><em>Ostrinia nubilalis</em> Hübner [Lepidoptera: Crambidae]</td>
<td></td>
<td>(2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>biological: temporal overlap</td>
<td>23 Lepidoptera spp.:</td>
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<tr>
<td></td>
<td></td>
<td>availability: collected by light trap, economically important pest</td>
<td>3 Arctiidae</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2 Geometridae</td>
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<td></td>
<td></td>
<td></td>
<td>1 Hesperidae</td>
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<td></td>
<td></td>
<td></td>
<td>1 Lycaenidae</td>
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<td></td>
<td></td>
<td></td>
<td>9 Noctuidae</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2 Pieridae</td>
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<td></td>
<td></td>
<td></td>
<td>1 Pyralidae</td>
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<td></td>
<td></td>
<td></td>
<td>1 Satyridae</td>
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<td></td>
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<td></td>
<td>1 Sphingidae</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1 Tortricidae</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 Yponomeutidae</td>
</tr>
<tr>
<td><strong>Agent:</strong></td>
<td><strong>Pseudacteon curvatus</strong> Borgmeier [Diptera: Phoridae]</td>
<td></td>
<td>Porter (2000)</td>
</tr>
<tr>
<td><strong>Targets:</strong></td>
<td><em>Solenopsis invicta</em> Buren</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Solenopsis richteri</em> Forei</td>
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<tr>
<td></td>
<td>[Hymenoptera: Formicidae]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>phylogenetic: taxonomically unrelated spp.</td>
<td>19 Hymenoptera spp.:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>biological: ovipositor morphology; similarity of non-targets to target species</td>
<td>19 Formicidae spp. (12 different genera)</td>
</tr>
</tbody>
</table>

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Table 1. Summary of selection criteria used in recent studies assessing host-specificity of entomophagous biological control agents (continued).

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<tbody>
<tr>
<td>Aphantorhaphopsis samarensis (Villeneuve) [Diptera: Tachinidae]</td>
<td>Eco: European spp. collected in wild in areas of target occurrence; NA species collected from field and reared</td>
<td>56 Lepidoptera spp.: 45 European spp.: 5 Arctiidae 1 Drepanidae 8 Geometridae 2 Lasiocampidae 1 Lycaenidae 5 Lymantriidae 1 Nemeobiidae 10 Noctuidae 2 Noctuidae 6 Nymphalidae 2 Saturniidae 1 Sphingidae 1 Thaumetopoeidae 11 North American spp.: 4 Arctiidae 1 Danaidae 1 Lymantriidae 2 Noctuidae 3 Saturniidae</td>
<td>Fuester et al. (2001)</td>
</tr>
<tr>
<td>Lymantria dispar (L.) [Lepidoptera: Lymantriidae]</td>
<td>Phy: Lepidoptera (known hosts) and non-Lepidoptera&lt;br&gt;Bi: host egg characteristics&lt;br&gt;A: 9 spp. from commercial cultures; 7 spp. from field-collected specimens reared in laboratory</td>
<td>2 Coleoptera spp.: 1 Cerambicidae 1 Chrysomelidae 1 Diptera sp.: 1 Muscidae 2 Hemiptera spp.: 1 Lygaeidae 1 Pentatomidae 11 Lepidoptera spp.: 1 Bombycidae 1 Danaidae 1 Gelechiidae 2 Noctuidae 1 Pyralidae 2 Saturniidae 1 Sphingidae 2 Tortricidae</td>
<td>Mansfield and Mills (2002)</td>
</tr>
<tr>
<td>Trichogramma platneri Nagarkatti [Hymenoptera: Trichogrammatidae]</td>
<td>Phy: Lepidoptera (known hosts) and non-Lepidoptera&lt;br&gt;Bi: host egg characteristics&lt;br&gt;A: 9 spp. from commercial cultures; 7 spp. from field-collected specimens reared in laboratory</td>
<td>2 Coleoptera spp.: 1 Cerambicidae 1 Chrysomelidae 1 Diptera sp.: 1 Muscidae 2 Hemiptera spp.: 1 Lygaeidae 1 Pentatomidae 11 Lepidoptera spp.: 1 Bombycidae 1 Danaidae 1 Gelechiidae 2 Noctuidae 1 Pyralidae 2 Saturniidae 1 Sphingidae 2 Tortricidae</td>
<td>Mansfield and Mills (2002)</td>
</tr>
<tr>
<td>Cydia pomonella (L.) [Lepidoptera: Tortricidae]</td>
<td>Ec: community interactions&lt;br&gt;A: field collections</td>
<td>14 Lepidoptera spp.: 12 Tortricidae 2 Oecophoridae</td>
<td>Munro and Henderson (2002)</td>
</tr>
<tr>
<td>Trigonospila brevifacies (Hardy) [Diptera: Tachinidae]</td>
<td>Eco: Community interactions&lt;br&gt;A: field collections</td>
<td>14 Lepidoptera spp.: 12 Tortricidae 2 Oecophoridae</td>
<td>Munro and Henderson (2002)</td>
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</tbody>
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</table>
| **Agent:** Laricobius nigrinus Fender  
[Coleoptera : Derontidae]  
**Target:** Adelges tsugae Annand  
[Hemiptera: Adelgidae] | Ecological: Habitat similarity/dissimilarity and vulnerable host stage occurs at same time as target  
Phylogenetic: Same genus, same family unrelated families  
Availability: Field collected in nearby ornamental trees or forest or from greenhouse colony | 6 Hemiptera spp.: 3 Adelgidae 2 Aphididae 1 Diaspididae | Zilahi-Balogh et al. 2002 |
| **Agent:** Trichogramma brassicae Bezenko  
[Hymenoptera: Trichogrammatidae]  
**Target:** Ostrinia nubilalis Hübner  
[Lepidoptera: Crambidae] | Ecological: Habitat and temporal overlap of hosts and released agent  
Socioeconomic: species at risk | 23 Lepidoptera spp.: 1 Hesperiidae 3 Lycaenidae 8 Nymphalidae 1 Papilionidae 1 Pieridae 6 Satyridae 2 Sphingidae 1 Zygaenidae | Babendreier et al. (2003a) Babendreier et al. (2003b) |
| **Agent:** Trichogramma brassicae Bezenko  
[Hymenoptera: Trichogrammatidae]  
**Target:** Ostrinia nubilalis Hübner  
Availability: laboratory culture, 2 Noctuidae collected from field | 6 Lepidoptera spp.: 3 Noctuidae 1 Plutellidae 2 Tortricidae | Babendreier et al. (2003c) |
| **Agent:** Trichogramma brassicae Bezenko  
[Hymenoptera: Trichogrammatidae]  
**Target:** Ostrinia nubilalis Hübner  
[Lepidoptera: Crambidae] | Ecological: predator groups represented in target (corn) ecosystem  
Biological: oviposition phenology, voltinism, overwintering stage, host-plant preferences, egg mass type and location  
Availability: Coleoptera and Diptera spp. commercially available, Neuroptera collected from field and reared | 2 Coleoptera spp.: (1 family) 1 Diptera sp. 1 Neuroptera sp. | Babendreier et al. (2003d) |
| **Agent:** Cotesia glomerata (L.)  
[Hymenoptera: Braconidae]  
**Target:** Pieris rapae L.  
| **Agent:** Trichogramma minutum Riley  
[Hymenoptera: Trichogrammatidae]  
**Target:** Choristoneura fumiferana (Clemens)  
[Lepidoptera: Tortricidae] | Ecological: geographic distribution  
Biological: oviposition phenology, voltinism, overwintering stage, host-plant preferences, egg mass type and location | 2 Lepidoptera spp.: 1 Lycaenidae 1 Nymphalidae 23 Lepidoptera spp.: in 4 families 14 Hesperiidae 5 Lycaenidae 7 Nymphalidae 1 Papilionidae | Bourchier (2003) |
<table>
<thead>
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<th>Agent and Target</th>
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</tr>
</thead>
</table>
| **Agent:** Peristenus digoneutis Loan [Hymenoptera: Braconidae]  
**Target:** Lygus lineolaris (Palisot de Beauvois) [Hemiptera: Miridae] | Ecological: Habitat and temporal overlap of hosts and released agent  
Phylogenetic: According to maximum fit cladogram of Lygus and its outgroup  
Biological: Geographical distribution; temporal pattern of occurrence  
Availability: Set up of culture and field collected | 9 Hemiptera spp.: 9 Miridae (different tribes) | Haye (2004) |
| **Agent:** Celatoria compressa Wulp [Diptera: Tachinidae]  
**Target:** Diabrotica v. virgifera LeConte [Coleoptera: Chrysomelidae] | Ecological: Habitat and temporal overlap of hosts and released agent  
Phylogenetic: One representative species from the sister genus of Diabrotica in the Old World; One representative not closely related Coleopteran  
Socioeconomic: Beneficial species including weed biocontrol agent  
Biological: Geographical distribution; temporal pattern of occurrence; similarity in host size  
Availability: obtained from culture and field collected | 9 Coleoptera spp.: 7 Chrysomelidae 1 Curculionidae 1 Coccinellidae | Kuhlmann et al. (2005) |
Figure 1. Recommendations for selecting non-target species for host specificity testing of invertebrates for biological control of arthropods.
The general consensus is that experiments must be performed in order to thoroughly
determine the ecological (realized) host range of a potential biological control agent (Hopper 2001). This can be achieved through carefully planned field studies to determine parasitoid-host complexes in the area of origin of the candidate biological control agent. Knowledge of the host species attacked by the candidate agent and its close relatives in its native range will facilitate the selection of appropriate test species for host range testing in the proposed area of introduction (Kuhlmann and Mason 2003; Kuhlmann et al. 2000). It is also recommended that comparable field studies be conducted in the area of introduction to provide insight into which herbivore species would be exposed to the candidate biological control agent, both ‘in space and time’. If little is known about the target pest (see Barratt 2004), these initial studies are especially necessary in order to generate the information required for selection of appropriate non-target test species.

An initial test species list can then be established based on this knowledge of ecological host range of the candidate biological control agent in its native habitat. We propose three different categories from which test species should be selected (the category order holds no relevance):

Category 1: Ecological Similarities: Species, which live in the same/ adjacent habitat (e.g., on arable land and adjacent field margins) or feed in the same micro-habitat (e.g., on same plant species, or in galls) as the target species;

Category 2: Phylogenetic/ Taxonomic Affinities: Species which are taxonomically/ phylogenetically related to the candidate biological control agent (according to modern weed biological control programmes);

Category 3: Safeguard Considerations: ‘Safeguard’ species, which are either beneficial insects (e.g., pollinators, other biological control agents) or rare and endangered species that belong to the same family or order. Additionally, host species of congeneric species of the candidate biological control agent could be selected when appropriate.

A vailable information may be limited such that it becomes necessary to focus on selecting species that fit into one category more than another category. However, the selection of species that are associated with more than one category should be a priority.

It is likely that the initial non-target test list will consist of at least 50 species, as is often the case for the final plant test list in weed biological control programmes. The rearing of such a number of insect species is unrealistic, however, being far more laborious and time-consuming than growing the equivalent number of plant species. Field collection of suitable stages for testing would provide an alternative to laboratory rearing, although confirmation that the collected species are not already parasitized or diseased would be required.

It has been suggested by Sands (1997) that testing more than 10 species of non-target arthropods may be impractical, and in those cases where the non-target species test list is long, often the number of species could be reduced to a more manageable size. In addition, carefully designed tests on a few species related to the target will provide adequate informa-
tion relating to the host specificity of candidate agents (Sands 1998). We therefore propose that the test species list can be reduced by filtering out those species with certain attributes (listed below) that do not overlap with those of the target species and are thus not suitable hosts. Attributes that can lead to the elimination of certain species from the list include; non-overlapping geographical distribution, different climate requirements, phenological asynchronization and host size which is outside of the range that is accepted by the candidate biological control agent (Filter 1 in Fig. 1). The latter attribute can be tested by offering target species or other host species of different size classes to the candidate biological control agent. Phenological asynchronization of the potential non-target test species can be determined by studying the herbivore complex that inhabits the potential area of introduction of the biological control agent. Species that are neither available nor accessible in large enough numbers for adequate experimental replicates to be conducted should also not be considered for host specificity testing (Filter 2 in Fig. 1). For rare and endangered species, it is acceptable to test congeners as surrogates.

Following this filtering process, the host-specificity test list might focus on approximately 10 to 20 non-target species. However this should not necessarily be considered as a final test list. Results from on-going host specificity testing and parallel studies to assess the chemical, visual and tactile cues emitted by the host or its host-plant(s) and involved in the agent’s host-selection behaviour may shed new light on which non-target species may be at risk of being attacked by the candidate biological control agent. As is the case for weed biological programmes we propose that the revised test species list should be periodically revisited during the pre-release studies of arthropod biological control programmes (indicated by the feedback loop in Fig. 1). In North American weed biological control programmes, test plant lists that have been submitted to and approved by the Technical Advisory Group at the beginning of a programme may be subject to revision during later stages of the pre-release studies. New information gathered during the pre-release studies may lead to scientifically based justification for removal or addition of test species.

It is our belief that this reiterative process is of greater relevance in arthropod biological control programs because of the requirement to keep the test list as short as possible while still providing a reliable host range profile for the candidate biological control agent.

RESULTS AND INTERPRETATION OF HOST-SPECIFICITY TESTS WITH PARASITOID BIOLOGICAL CONTROL CANDIDATES

The usefulness of selection criteria for setting up test species lists depends on the type of results that are generated by host-specificity tests, and the ease of their interpretation. The goal of host-range testing should be to carefully select test species and choose host-selection bioassays so that the biological control agents will reject at least some of the tested species. The interpretation of results from host-specificity tests is notoriously difficult when a large number of test species are accepted. This is also true for those cases where significant differences in attack rates among the test species were found, because spatial and temporal distribution of preferred and less-preferred hosts in the area of introduction is usually highly variable (e.g., Schaffner 2001).
Based on the experience from pre-release studies in weed biological control projects, one might expect that a discriminating host-selection behavior under confined conditions can be plausible in host-specificity studies with more or less specialised parasitoid species that are considered as classical biological control agents. However, general concern has been expressed about the interpretability of results from laboratory host-specificity tests with parasitoid species, since parasitoids may display a more indiscriminant host-selection behaviour in containment than herbivorous insects (e.g., Sands 1997).

The limited number of published host-specificity studies available to date suggests, though, that tests on the basis of a carefully selected test species list can indeed provide reliable data on the fundamental host-range of parasitoid biological control candidates with a supposedly narrow host-range. The selection criteria used in these studies for setting up the test species list are reviewed in an additional paper (Kuhlmann et al. submitted); here we focus on the interpretability of the results obtained in the host-specificity tests.

Using multiple-choice cage experiments, Neale et al. (1997) exposed 17 non-target leafmining species on their respective host-plants to three parasitoids of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracilariidae). The test species were selected on the basis of taxonomic and ecological criteria. No adult parasitoids were recovered from any of the non-target species exposed to the three biological control candidates.

Barratt et al. (1997) tested the laboratory host specificity of two classical biological control agents, *Microctonus aethiopoides* Loan and *Microctonus hyperodae* Loan (Hymenoptera: Braconidae), which had already been released into New Zealand. Two of the twelve weevil species exposed to *M. aethiopoides* and 7 of the 11 weevil species exposed to *M. hyperodae* were not accepted or not suitable for larval development. The narrower host-range of *M. hyperodae* displayed in the no-choice cage experiments was corroborated by data from a field study assessing the realized host-range of the two species in the area of introduction. A single record for each of two non-target species were reported for *M. hyperodae*, while *M. aethiopoides* was recovered from 13 different non-target species.

The host-specificity of the supposedly specialist parasitoid *Cotesia rubecula* (Marshall) and of *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae), which had previously been recorded from several Lepidoptera species, were experimentally assessed by Cameron and Walker (1997). In the laboratory no-choice host-specificity tests, *C. rubecula* readily accepted the target host, *Pieris rapae* L. (Lepidoptera: Pieridae), for oviposition, but none of the other nine Lepidoptera species offered. In contrast, *C. plutellae* oviposited in all species tested, and completed its development in 8 out of the 13 test species. The authors concluded that laboratory tests based on suitability of hosts for parasitoid development are appropriate for demonstrating high degrees of specificity such as found in *C. rubecula*.

Kitt and Keller (1998) studied the host-specificity of *Aphidius rosae* Haliday (Hymenoptera: Braconidae), a parasitoid of the rose aphid *Macrosiphum rosae* (L.) (Homoptera: Aphididae). In no-choice and choice experiments, only *M. rosae* and *Macrosiphum euphorbiae* (Thomas) were frequently attacked; single attacks were observed on each of two additional aphid species, while three aphid species were not attacked at all. Host suitability tests revealed that *M. euphorbiae* is not a suitable host for *A. rosae*. In wind-tunnel experiments females
were strongly attracted to roses, but not to the odours of various other plant species. The results of these laboratory studies provide strong evidence for a very narrow host-range of *A. rosalae*.

A series of no-choice and choice tests with 21 different ant species were carried out by Porter (2000) to study the host-specificity of the decapitating fly *Pseudacteon curvatus* Borgmeier (Diptera: Phoridae), a biological control agent against the invasive fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). In these tests, which were conducted in small plastic trays, no *P. curvatus* larvae or pupae resulted from any of the 19 ant species from 12 non-host genera. Two congeneric, native fire ants were successfully parasitized by *P. curvatus*, indicating that the host-range of this parasitoid is likely to be restricted to fire ants of the genus *Solenopsis*.

Fuester et al. (2001) carried out field and laboratory studies to assess the host specificity of the tachinid fly *Aphantorhaphopsis samarensis* (Villeneuve), a biological control agent against gypsy moth. In choice oviposition tests, one out of eleven North American non-target species was attacked and supported larval development. The susceptible non-target species belongs to the same family as the target species, the Lymantriidae. In tests where nine European non-target Lepidoptera were artificially inoculated with maggots of *A. samariensis*, no puparia were obtained. These findings were in agreement with extensive field studies in Europe, during which no verifiable recoveries of *A. samariensis* from non-target species resulted. One questionable recovery each was made from two lymantriid species.

Kuhlmann et al. (2005) applied the recommendations outlined above for host specificity testing of *Celatoria compressa* Wulp (Diptera: Tachinidae), a candidate biological control agent of the western corn rootworm, *Diabrotica virgifera virgifera*. The final test list comprised nine Coleoptera species. Naïve and experienced *C. compressa* females did not parasitize eight non-target species but they did accept the red pumpkin beetle, *Aulacophora foveicollis* Lucas was attacked regardless of the presence or absence of *D. v. virgifera*. These studies showed that *C. compressa* has a high degree of host specificity and is restricted to a few genera in the tribe Luperini of the subfamily Galerucinae within the family Chrysomelidae.

In contrast, Haye (2004) selected seven non-target species to define the fundamental host range of *Peristenus digoneutis* Loan (Hymenoptera: Braconidae), a parasitoid of *Lygus* plant bug species in Europe. Laboratory choice and no-choice tests demonstrated that all selected non-target species were attacked and were largely suitable for parasitoid development. Haye (2004) also studied the ecological host range in the European area of origin to compare laboratory and field results. It was shown that *P. digoneutis* was reared from ten hosts in the field, including three *Lygus* species and seven non-target hosts from the subfamily Mirinae. However, the proportions of *P. digoneutis* in the larval parasitoid guild of non-target hosts were less than 5%.

In general, the published studies that report laboratory assessment of the host-specificity of supposedly specific entomophagous agents provide evidence that a careful selection of non-target test species and host-specificity tests based on host-selection behavior and host suitability allow a thorough assessment of the fundamental host-range of parasitoid biological control candidates. However, it is too early to draw any general conclusions from such a limited set of published host-specificity studies as shown by Haye (2004). Several of the para-
sitoid species which have been thoroughly tested up to date may have been selected because they were likely to display a very discriminating host-selection behavior in containment. As in weed biological control projects, it appears to be much more challenging to predict the ecological host-range when parasitoid biological control candidates do not display a discriminating host-selection behavior in containment, or when they have a relatively broad fundamental host-range. A relatively broad host-range may be particularly common in parasitoids aimed for use in inundative biological control projects. In these cases, laboratory host-range studies may be of limited value, and a thorough risk assessment will need to consider additional aspects, such as dispersal as well as long-distance and short-distance host-searching behaviour of the biological control candidate (Babendreier et al. 2005; Orr et al. 2000).

CONCLUSIONS

Selection of non-target species for inclusion in host range testing for exotic entomophagous biological control agents must be done carefully to ensure that appropriate species are chosen. While phylogenetic relationship (taxonomic relatedness) is a useful starting point, other attributes such as ecological similarities, biological habits, socio-economic considerations, and test species availability are of primary importance and have been used in the limited number of studies conducted to date. Because the number of plant species screened in weed biological control (typically 40-100) would be prohibitive for testing entomophagous biological control agents one of the key aspects in host specificity testing in arthropod biological control programmes lies in setting up a test species list that is both scientifically sound and manageable. This is a challenging task, particularly since host-selection by parasitoids is often triggered by an additional trophic level (host and host-plant) than that by herbivores.

The recommendations proposed will help improve the host specificity testing of entomophagous biological control agents. Compilation of a test species list is in itself a valuable step in the pre-release assessment because it provides a mechanism for assembling and synthesising relevant information and knowledge. Hopefully, new evidence from thorough host specificity tests will accumulate relatively quickly so that the proposed recommendations for the non-target selection procedure, which are based on a relatively small data set of experimental parasitoid host range assessments, can be thoroughly tested and refined as necessary.

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