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

Ulrich KUHLMANN[1], Urs SCHAFFNER[1], and Peter G. MASON[2]

[1]CABI Bioscience Centre
Rue des Grillons 1
2800 Delémont, Switzerland
u.kuhlmann@cabi.org

[2]Agriculture and Agri-Food Canada, Research Centre
Central Experimental Farm
Ottawa, Ontario, Canada K1A 0C6

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.

Agent and Target	Selection Criteria Used	# Non-target Selected	Reference
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 Cecidomyidae 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* (Coquillett) [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. [Lepidoptera : Pieridae] *Plutella xylostella* (L.) [Lepidoptera : Plutellidae]	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 Plutellidae 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).

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

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

Agent and Target	Selection Criteria Used	# Non-target Selected	Reference
Agent: *Aphantorhaphopsis samarensis* (Villeneuve) [Diptera: Tachinidae] Target: *Lymantria dispar* (L.) [Lepidoptera: Lymantriidae]	Ecological: European spp. collected in wild in areas of target occurrence; NA species collected from field and reared	56 Lepidoptera spp.: 45 European spp.: 5 Arctiidae 1 Drepanidae 8 Geometridae 2 Lasiocampidae 1 Lycaenidae 5 Lymantriidae 1 Nemeobiidae 10 Noctuidae 2 Notodontidae 6 Nymphalidae 2 Saturniidae 1 Sphingidae 1 Thaumetopoeidae 11 North American spp.: 4 Arctiidae 1 Danaidae 1 Lymantriidae 2 Noctuidae 3 Saturniidae	Fuester *et al.* (2001)
Agent: *Trichogramma platneri* Nagarkatti [Hymenoptera: Trichogrammatidae] Target: *Cydia pomonella* (L.) [Lepidoptera: Tortricidae]	Phylogenetic: Lepidoptera (known hosts) and non-Lepidoptera Biological: host egg characteristics Availability: 9 spp. from commercial cultures; 7 spp. from field-collected specimens reared in laboratory	2 Coleoptera spp.: 1 Cerambycidae 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 1 Neuroptera sp.	Mansfield and Mills (2002)
Agent: *Trigonospila brevifacies* (Hardy) [Diptera: Tachinidae] Target: *Epiphyas postvittana* Walker [Lepidoptera: Tortricidae]	Ecological: community interactions Availability: field collections	14 Lepidoptera spp.: 12 Tortricidae 2 Oecophoridae	Munro and Henderson (2002)

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

Agent and Target	Selection Criteria Used	# Non-target Selected	Reference
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 [Lepidoptera: Crambidae]	Phylogenetic: representative Lepidopteran spp. 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 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. [Lepidoptera: Pieridae]	Socioeconomic: endangered status	2 Lepidoptera spp.: 2 Pieridae [in same genus (Pieris) as target]	Benson *et al.* (2003)
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 1. Summary of selection criteria used in recent studies assessing host-specificity of entomophagous biological control agents (continued).

Agent and Target	Selection Criteria Used	# Non-target Selected	Reference
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)

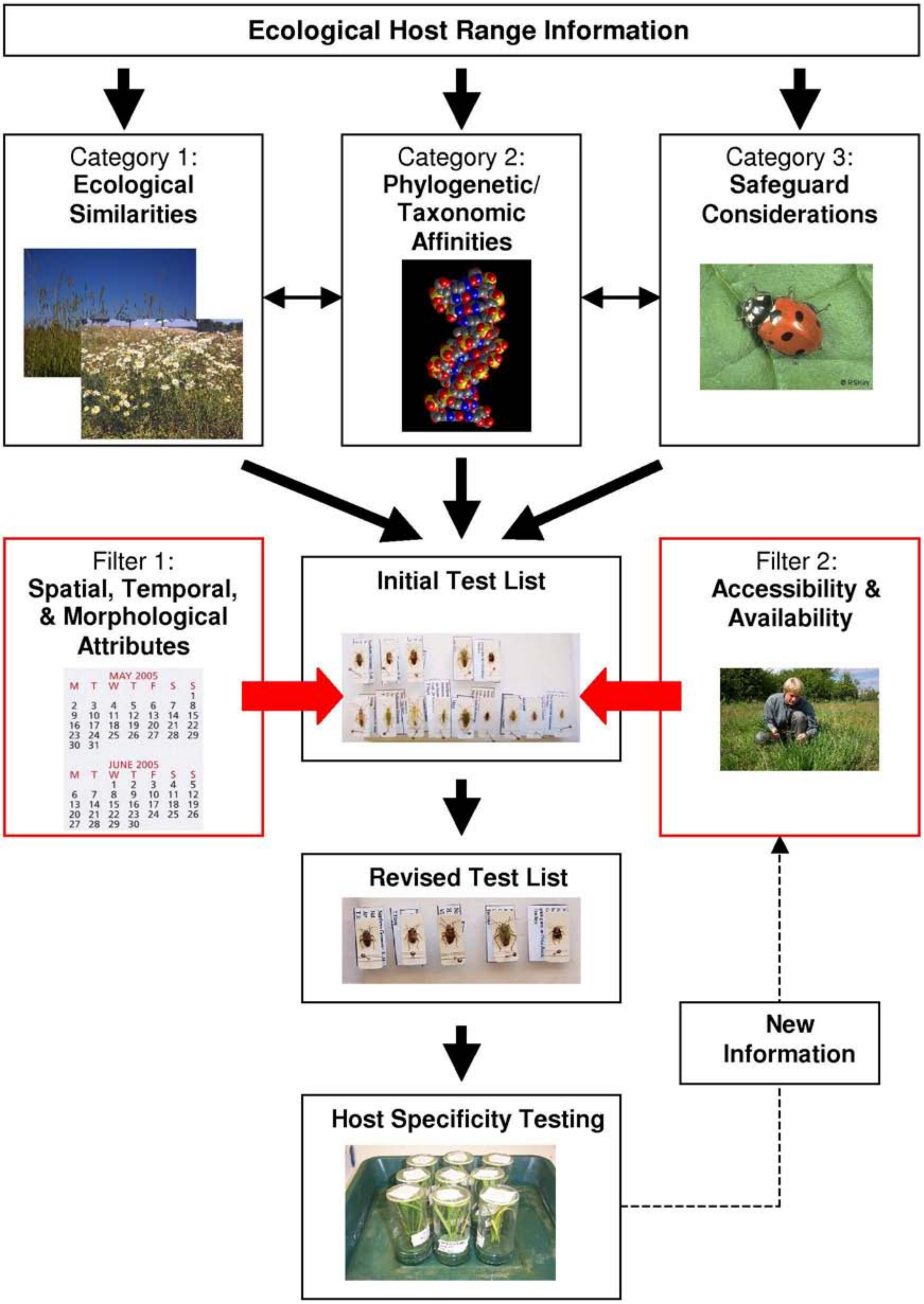

Figure 1. Recommendations for selecting non-target species for host specificity testing of invertebrates for biological control of arthropods.

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.

Available 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 information 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.) (Hemiptera: Aphidae). 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. rosae*.

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 parasitoid 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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HOST RANGES OF NATURAL ENEMIES AS AN INDICATOR OF NON-TARGET RISK

Joop C. VAN LENTEREN[1], Matthew J. W. COCK[2],
Thomas S. HOFFMEISTER[3], and Don P. A. SANDS[4]

[1]Laboratory of Entomology, Wageningen University
Binnenhaven 7
6709 PD Wageningen, The Netherlands
Joop.vanLenteren@wur.nl

[2]CABI Bioscience Centre Switzerland
Rue des Grillons 1
CH-2800 Delémont, Switzerland
m.cock@cabi.org

[3]Institute of Ecology and Evolutionary Biology, University of Bremen
Leobener Strasse NW2
D-28359 Bremen, Germany
hoffmeister@uni-bremen.de

[4]CSIRO Entomology
120 Meiers Road
Indooroopilly, Queensland 4068, Australia
don.sands@csiro.au

ABSTRACT

Potentially, the introduction of exotic natural enemies or mass release of biological control agents may lead to unwanted non-target effects. Whether or not such effects occur will mainly depend upon the host range of the biological control agent and the presence of non-target species in the area of release. Host-specificity testing is an important aspect of host-range assessment – perhaps the most important, and the easiest conceptually for regulators. Usually, laboratory based manipulative experiments will form the core of host-range assessments, but there is little information on how to determine host ranges. Here, we present a framework for step-wise host-range testing with levels of increasing complexity that should allow to avoid over- and underestimation of the host range of a biological control agent. Next, the interpretation of data obtained with host-range testing is discussed and conclusions are drawn about the importance of host-range testing in future biological control projects.

INTRODUCTION

Contrary to the thorough host-range evaluations applied in the search for natural enemies of weeds (Wapshere 1974), host ranges of biological control agents for insect or mite control were usually not extensively studied until recently. The earlier lack of concern for non-target effects combined with the fact that very few non-target effects were ever found in insect biological control resulted in hardly any host-range assessment or screening studies before the 1990s with the exception of Australia, which started in the 1980s.

Several publications have appeared in which ideas or methods for host-range testing are presented; they are reviewed in van Lenteren *et al.* (2006a). Aspects of risk assessments have been developed and applied during the past two decades, though often in a preliminary way and not always satisfactorily (van Lenteren *et al.* 2006b). Decisions about release of exotic natural enemies are still often based on short term decisions strongly influenced by financial and social benefits reflecting national priorities, and tend to ignore environmental ethics especially where risks are difficult to quantify. However, there are several positive developments taking place currently, which commenced with the design of a Code of Conduct for the import and release of exotic biological control agents (IPPC 1996). A recent review in which the implementation and use of this Code of Conduct is evaluated (Kairo *et al.* 2003) led to the following conclusions: (1) the CoC is widely used currently, (2) with the CoC several requests for importation could be rejected based on good reasons, (3) the CoC made evaluation procedures generally more rigorous and lengthy, but did not lead to fewer introductions, (4) most users were positive about the implementation of the CoC, but also that (5) the CoC lacks procedures for, among others, host-range assessment schemes and host-range testing methods that need to be developed with high priority.

Although there is still much debate on how to test host specificity, several protocols for host-range determination have been designed and used during the past decade (Barratt *et al.* 1997; Sands 1998; van Lenteren *et al.* 2003). An important conclusion from recent papers on risks of releasing exotic biological control agents is that host-range assessment should form the focus of every natural enemy risk assessment, because the width of the host range will, together with the numbers of natural enemies that are released and the dispersal capacity of the natural enemy, determine the probability that non-target effects will occur. Several sources of information may be incorporated into a host-range assessment, including literature records, field observations in the area of origin, and physiological, behavioral and ecological observations and experiments; all these aspects are reviewed in van Lenteren *et al.* (2006a). Usually though, laboratory based manipulative experiments to test host ranges will be performed.

Developing a list of appropriate nontarget species is a difficult task and is discussed in detail by Kuhlmann *et al.* (2006). In addition to what one would logically select as potential indigenous non-target species, species should be included that are of *conservation concern* or important biological control agents, i.e. any non-target species considered to be at risk from introduced biological control agents, causing declines in distribution or density, or local and regional extinctions. Species of conservation concern may not necessarily be taxonomically closely related to the target species but their ecological, cultural or conservation significance are considered sufficient to justify an expansion of the host-range testing schedule.

In this paper we attempt to answer the question of how to test the host specificity of arthropod biological control agents, and we present a framework for host-range testing. Next we will discuss the interpretation of data obtained with host-range testing, and finally some conclusions are drawn.

DEVELOPING HOST-RANGE TESTS

Hypotheses about host ranges of natural enemies generated from the literature and field surveys can be tested in formal laboratory host-range tests (Sands 1998). Host-range tests aim to demonstrate if a natural enemy can feed, develop or reproduce on a nontarget species. Laboratory testing can become quite complicated as a result of multitrophic chemical communication, learning and wide host ranges, involving many host plant species. Host preferences are determined not only by the choice of species offered, but also by the physiological condition and experience of the natural enemy under investigation. Host-range testing is relevant only if proper controls are included. Hence, before a specific testing scheme is designed, knowledge needs to be obtained about the multitrophic system in which the natural enemy forages in order to make the tests meaningful. Particularly, behavioral variation including learning, intraspecific variation and genetic changes occurring during laboratory rearing of natural enemies, may complicate host-range testing and these are reviewed in van Lenteren *et al.* (2006a).

FRAMEWORK FOR HOST-RANGE TESTING

The above-mentioned considerations may lead to the conclusion that host-range testing is too complicated and produces unreliable results. But based on the very limited number of negative non-target effects known in biological control, we may conclude that biological control workers have generally done an excellent job in making predictions about such effects in the past. However, with an increasing number of non-specialists involved in biological control work, there is great need for a basic methodology to perform host-range testing.

Below we present a design for a testing scheme to determine host ranges of insect natural enemies. Because of the large variation in natural enemy – host relationships, this testing sequence should be considered as a basic approach, which will need to be adapted for specific situations. The test sequence we present may be simplified if this can be based on the biology of the natural enemy. Depending on the multitrophic system under consideration, one does not necessarily have to start with step 1, but can start with approaches in e.g. large arenas that allow a much more precise estimate of the host range. So, the tests described below are examples. There are a great many potential designs, and these will be determined by the nature of the interaction between the natural enemy (parasitism, predation) and the habitat occupied by the organism.

Step 1: Small arena no-choice black-box test. The aim of this test is to answer the question: does the biological control agent attack the non-target organism in the appropriate stage on the relevant part (e.g., the leaf or a root) of its natural host plant? A positive control is performed with the target species; a negative control is done with the target and non-target species without the natural enemy to check survival of target species under test conditions. Consider that extensive stinging and superparasitism can lead to host mortality and prevent parasitoid development, and thus potentially underestimate the host range. For predators, consider the effect of cannibalism on prey range.

If none of the non-targets is attacked and the target species (=positive control = pest species) is attacked at a rate approaching that in the field, one can stop testing, because no direct effects on the tested non-target species in field are expected. If non-target hosts are attacked, even at very low rates, further testing is mandatory.

Step 2: Small arena no-choice behavioral test. The aim of this test is to answer the question: does the biological control agent consistently attack the non-target organism on the appropriate substrate of its natural host plant? A positive control is performed with the target species; a negative control is done with the target and non-target species without the natural enemy. Superparasitism in the confines of a small arena may lead to unnatural mortality of the host. Therefore, special precautions may be necessary to deprive individual hosts from repeated oviposition after first oviposition to avoid host mortality. With predators, the occurrence of cannibalism in small arenas need to be taken into account. This no-choice test can overestimate the risk of including the non-target species in the host range of the natural enemy.

If the target host (= positive control = pest species) is attacked at a rate approaching that in the field, and the non-target host is not attacked at all, one can stop testing, because no direct effects on non-target species in the field are expected. If attack rates are above zero for target and non-target host, but the attack on non-target hosts is significantly lower than on target hosts, the hazard to non-target hosts under field conditions might be low to acceptable, and further testing should be considered. If non-targets are only attacked at the end of the observation period (long latency time), then the risk of direct effects on these species is small. If non-target species are consistently attacked, with a latency time similar to the target, and attack rates on target and non-target hosts do not differ significantly, non-target effects might be considerable and further testing is mandatory.

Step 3: Large arena choice test. The aim of this test is to answer the question: does the biological control agent attack non-targets when target and non-target species are present in a semi-natural situation on their natural host plants? Present multiple host plants each with their own non-target species and the target species in a large arena. Offer target and non-target hosts in as natural a situation as possible and on their natural host plants. Positive controls are done in the same type of cage with the natural enemy and the target host only and the natural enemy and the non-target host only; a negative control is done with the target species and non-target species, but without the natural enemy. Care should be taken that the same number of total hosts is present at the start of each treatment. The experiments should be terminated before the target host is eliminated, or in case of parasitoids before most target hosts are parasitized. Consider that extensive stinging and superparasitism can lead to host mortality and prevent parasitoid development, and thus potentially underestimate the host range. With predators, consider the occurrence of cannibalism.

Non-target species that are easily attacked on their natural host plants, i.e. with similar latency times as target hosts and with similar attack rates, pose a high risk for non-target effects. If latency times of attack on a non-target species are much higher and attack rates are much lower than in the target control, the natural enemy displays a strong preference for the target species, but may be prone to attack the non-target species under situations where the target species is not present. If latency times in the choice test and the non-target control are much higher than in the target control and the attack rates are much lower in the choice test and non-target control than in the target control, the risk of direct effects on the non-target species under field conditions is small.

Step 4: Field test. The aim of this test is to answer the question: does the biological control agent attack the non-target when the non-target and the target species are present in their respective habitats? This test can only safely be done in the area of release if the biological control agent cannot establish in this area (e.g., agents from tropical areas to be used in greenhouses in temperate climates). The test can be done in the native area of the natural enemy if the non-target species also occur in this area. Release the natural enemy in the non-target habitat, and determine if there is attack of non-target species. Control: put target species on target host plant in the non-target habitat. Replicate the approach in a number of plots.

If the target species is easily attacked, and no or low attack of non-target species occurs, a low risk for direct effects on non-target species is expected. If the biological control agent easily attacks non-target species on their host plants in their natural habitat, it poses a very high risk for non-target effects.

INTERPRETATION OF HOST-RANGE DATA

Interpretation of host-range data is difficult, among others because of the confusing effect of test conditions. Regularly observed confusing effects of test design are: (1) overestimated host ranges, in which non-hosts are used by agents when deprived for long periods from their normal hosts, (2) overestimated host ranges in which non-hosts are used when in close proximity to the normal host due to transference of stimuli, and (3) underestimated host ranges in which valid, but less preferred, hosts are ignored in the presence of a more preferred host. The disruption of insect behavior when they are held in confinement, or outdoors in cages, is well known for biological control agents generally (Sands and Papacek 1993). Sometimes a particular host will be accepted in laboratory trials but when released into the field, the agent will ignore it. This anomaly commonly leads to overestimated host-range predictions for an agent and may lead to discontinuation of evaluation studies that, if continued, may have shown high degrees of host specificity. Not all potential agents are affected by confinement during tests for host preference or specificity but it is important to be wary of this problem arising and, depending on the suspected nature of the problem, adjust the design of experiments to minimize or prevent overestimated host ranges in agents. If laboratory host-range tests remain inconclusive decisions whether or not to release an agent may depend on information from its native range or countries where it has already been introduced (van Lenteren *et al.* 2006).

For mono- or slightly oligophagous and for clearly polyphagous biological control agents, the above host-range testing framework will usually lead to clear answers about risks for non-target species. Indeed, in a number of cases, host-specificity data from mono- or slightly oligophagous species found in the literature were confirmed when exposed to new non-target host species (e.g., Cameron and Walker 1997). But exceptions do occur. For example, natural enemy species that were considered to be monophagous or that had a rather restricted host range, were found to attack a number of other host species in the area of release (e.g., Barratt *et al.* 1997; Brower 1991). Conclusions about host specificity can, therefore, seldom be made alone on data collected in the area of origin of the biological control agent, although this is an important first step.

The most difficult group for interpretation of host-range data will be the more pronounced oligophagous and slightly polyphagous biological control agents. These agents might first of all not be the most efficient natural enemies and result in intermediate or partial control, and may also show more severe non-target effects when compared to strongly monophagous species. This group of natural enemies needs to be studied with high priority.

Host-range data have earlier been used to reject introductions. For example, Sands and Van Driesche (2000) reported that certain egg parasitoids were not released in the United States for control of pest Hemiptera because they were shown to attack at least 20 species of unrelated native Hemiptera. The decision not to release them was based on their wide host ranges and lack of evidence that they were effective in suppressing the target pest in their native ranges (Jones 1988).

Frustratingly little information is available about potential changes in host preference over time. While to our knowledge no recent example is available for insect parasitoids, some herbivorous insects like tephritid fruit flies that attack fruits of their host plants provide a well known example for an evolutionary host race formation in ecological time dimensions (Berlocher and Feder 2002). Apple maggot flies seem to have switched to cherries within the last century (Jones *et al*. 1989). Nevertheless, such host-range expansions, host shifts, or host race formations seem not to occur so often that they represent a major concern for the release of otherwise host specific insect natural enemies.

CONCLUSIONS

Determination of host specificity, particularly of generalist natural enemies, will always be a complicated and time-consuming affair. First there is the problem of the selection of appropriate non-target species to be tested and which set of tests to use. We propose to use the sequential test that is summarized above for the determination of host ranges of new exotic natural enemies. We already indicated that, depending on the type of natural enemy and the ecosystem where it will be released, the testing sequence might need to be adapted. We also realize that this sequential design will undergo changes with growing experience.

After host-range testing, there is the issue of interpretation of data obtained with the various tests. For all these phases, arthropod biological control workers have just started to develop a theoretical and methodological background. Finally, the risk posed by and the benefits resulting from the release of the exotic biological control agent should be weighted against the risks and benefits of any other control method under consideration (van Lenteren *et al*. 2003; van Lenteren *et al*. 2006b; van Lenteren and Loomans 2006).

An exhaustive data search of Lynch *et al.* (2001) in which more than 5000 recorded biological control cases were analyzed and 30 international biological control experts were contacted for additional information, and information provided in van Lenteren *et al.* (2006b), has underlined our ignorance of the degree to which non-target effects occur. Host-range testing combined with pre- and post release studies need to become standard procedures in each biological control project (Coombs 2003). That this does not necessarily result in fewer introductions of exotic biological control agents has been shown by the recent evaluation of the IPPC Code of Conduct (Kairo *et al*. 2003). But it does lead to higher costs and delay of introduction. However, if higher costs and later introduction do result in fewer serious mistakes, the investments are certainly justified.

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EFFECTS OF TEMPERATURE ON THE ESTABLISHMENT OF NON-NATIVE BIOCONTROL AGENTS: THE PREDICTIVE POWER OF LABORATORY DATA

Jeffrey BALE

School of Biosciences
University of Birmingham
Birmingham B15 2TT, UK

j.s.bale@bham.ac.uk

ABSTRACT

The European Union (EU) and its constituent governments are committed to increasing the success of biocontrol in Europe, and are currently seeking a pan-European balanced regulatory system to aid this objective. The concept of 'balance' recognizes that (a) the complexity of any licensing system must be proportionate to risk, (b) industrial producers of biocontrol agents have limited RandD budgets, but (c) there can be no compromise on environmental safety. Whilst there is common agreement between agencies responsible for environmental protection, regulators and industrial producers, about the ecological information required to assess the establishment potential of non-native species, the research methods by which such data can be generated (if not available in the literature), have not been fully developed or tested. The inappropriate use of 'climate matching' between native and introduced ranges as a 'proxy' for cold tolerance and overwintering ability is one example of this problem.

Most predatory insects and mites used in glasshouse biocontrol in the UK originate from tropical and semi-tropical climates. For this reason, the licensing system for the introduction of non-native species has operated under the assumption that winter would act as a natural barrier to the establishment of such species outside of glasshouse environments. This view has been challenged by the establishment in the wild of the predatory mite *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) and the discovery of the predatory mirid *Macrolophus caliginosus* Wagner (Hemiptera: Miridae) outside of glasshouses in winter. Whilst the impact of these species on native ecosystems is unknown, their establishment is considered undesirable. This paper describes a series of experiments used to determine a range of thermal characteristics (developmental threshold, day-degree requirement per generation, supercooling point, lethal times and temperatures, field survival) of five non-native biocontrol agents. A strong correlative relationship was found between the time at which 50% of populations die in the laboratory at 5°C (LTime$_{50}$) and duration of winter survival in the field. The comparative data provide a retrospective ecophysiological explanation for the establishment of *N. californicus* and occurrence of *M. caliginosus* outside of glasshouses, and also indicate that *Delphastus catalinae* (Gordon) (Coleoptera: Coccinellidae), *Eretmocerus eremicus* (Rose and Zolnerowich) (Hymenoptera: Aphelinidae) and *Typhlodromips montdorensis* (Schicha) (Acari: Phytoseiidae) would not survive outdoors in the UK under current climatic condi-

tions, and would therefore be 'environmentally safe' introductions. The experimental protocol applied to these species could be used as part of a routine, stepwise testing procedure for 'establishment potential' in the licensing system of non-native biocontrol agents in the UK and other parts of the world.

INTRODUCTION

Biological control has a long history of use in pest management, both as a method of control in its own right, and in combination with other techniques as part of IPM programs. In some respects, the importance and success of biological control has been overshadowed, historically by pesticides, and more recently, by the prospect of insect-resistant GM crops, both of which have been viewed as a more generic approach to pest management, capable of being targeted against a range of pests in different climatic zones. However, there is now widespread international agreement on the need to reduce over-reliance on chemical pesticides, at the same time as the future of GM crops looks uncertain, particularly in Europe, not least because of public concern over risks to human health and the environment. By contrast, biological control is regarded as safe and environmentally friendly.

The definition of 'success' in biological control depends in part, on the environment into which an organism is released. In classical biological control, where relatively low numbers of a non-native predator or parasitoid are released into a new country or region of the world, often against an exotic pest, success can usually be defined by the ability of the introduced species to suppress numbers of the target pest below economic levels, and to become permanently established in the new area, thus reducing the need for re-releases. In inundative biological control, where large numbers of non-native natural enemies are released into glasshouses, pest suppression is again a criterion for success, but there is also the expectation that any organisms that escape from the protected environment will die out rapidly, and not cause any disruption to the native ecosystem. This is the 'paradox of establishment': in classical biological control, establishment is a key feature of success, whereas in inundative biological control in glasshouses, establishment outside of the protected environment is considered potentially deleterious.

INTERNATIONAL CONTEXT

Over the last 10-20 years there has been a developing trend toward international regulation for the import and release of non-native biological control agents, including the International Plant Protection Convention and the Convention on Biological Diversity. At the same time, various countries have introduced their own legislation to regulate importation of exotic species (United States, Canada, Australia, New Zealand, United Kingdom). Recently, a number of organizations have developed guidelines for the import and release of non-native biological control agents, in which an environmental risk assessment forms a central component. At the present time, the guidelines previously issued by FAO, EPPO and OECD are being harmonized to provide comprehensive guidance for EU member states and European countries under the auspices of IOBC-WPRS (Bigler *et al.* 2005) whilst the International Standard for

Phytosanitary Measures (ISPM3) will soon provide revised advisory guidelines for all introductions of non-native biological control agents worldwide.

It is evident that biological control practitioners, programs and producers will become subject to greater regulation in the future than hitherto. It is however acknowledged that any new regulatory framework should be 'balanced', whereby the complexity of the licensing system is proportionate to the risk, without compromising environmental safety. However, there is already an identified problem that may hinder the implementation of new regulations: whilst the guidelines provide clear statements about the range of information that should be included in an environmental risk assessment, they do not indicate the methods by which such information should be obtained, especially when it is not available from the published literature.

ENVIRONMENTAL RISK ANALYSIS

It is self evident, but not always recognized, that there can be no long term negative effects on native species and ecosystems unless exotic species become permanently established in new environments; transient 'summer only' survival is unlikely to have any major impact. For this reason, an environmental risk analysis should first focus on the likelihood of successful establishment of non-native species.

The two most important factors affecting the establishment of non-native biological control agents are climate (especially temperature) and availability of prey. This knowledge can be utilized in the design of risk assessment protocols. In a step-wise testing procedure to assess the outdoor establishment potential of non-native species released into glasshouses in cool temperate climates, a case can be made for firstly investigating the effects of temperature on development and winter survival, followed by experiments on host range and non-target effects, in those species that appear to be capable of developing in summer and surviving through winter.

The difficulties of assessing the establishment potential of non-native biological control agents intended for inundative release in glasshouses are well illustrated by recent experience in the UK. Successful biological control has been implemented in glasshouses with the management of the whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) by the parasitoid *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae), and the spider mite *Tetranychus urticae* (Koch) (Acari: Tetranychidae) by the predatory mite *Phytoseiulus persimilis* (Athias-Henriot) (Acarina: Phytoseiidae). These two schemes have operated successfully over decades without any recorded establishment outside of the glasshouse in the cool climates of western Europe, or any deleterious effects on native fauna. Over the last 10-15 years, a number of 'new' species have been licensed for release in UK glasshouses. Although the UK licensing system requires companies to compile an environmental risk assessment dossier containing physiological and ecological information on the subject species, including overwintering ability and host range, this 'critical information' is often unavailable. As a classic example, in the absence of any direct assessment of cold tolerance, it has been assumed on the basis of 'climate matching', that winter would be an effective barrier to establishment in the UK of species originating from warmer climates. This assumption is incorrect, as evidenced by the outdoor establishment of the predatory mite *Neoseiulus californicus* after a first release in 1991, and occurrence outside of glasshouses in winter of the predatory mirid *Macrolophus caliginosus* following release in 1995.

In the light of the definite establishment of *N. californicus* and the possible establishment of *M. caliginosus*, a series of studies were undertaken to investigate the thermal biology of these two species, and two other species that had been licensed in the UK for the same periods of time, and for which there had been no reports of establishment or outdoor occurrence in winter (*Eretmocerus eremicus* and *Delphastus catalinae*). The same series of experiments were then conducted on a further species (*Typhlodromips montdorensis*) that was currently under study as a candidate for release in the U.K. (see Hart *et al.* 2002a,b; Hatherly *et al.* 2004; 2005; Tullett *et al.* 2004; for full details). It was envisaged that a comparative analysis of the thermal biology of established and non-established species might identify indices with 'predictive power' that could be applied to future candidate species in a step-wise risk analysis protocol.

MATERIALS AND METHODS

DEVELOPMENTAL THRESHOLD AND THERMAL BUDGET

Individuals of *N. californicus, M. caliginosus, E. eremicus, D. catalinae and T. montdorensis* were reared from egg to adult at a range of temperatures (5° to 35°C depending on the species) and the time taken to complete development recorded. The data were analyzed by weighted linear regression and the developmental threshold estimated by extrapolation of the linear relationship between development and temperature to the x (temperature) axis, and the thermal budget (day degree requirement per generation) by taking the reciprocal of the slope (Campbell *et al*. 1974).

Annual voltinism. The developmental threshold temperature and thermal budget values for each species were compared with daily temperature records over a 10 year period to calculate the annual number of available day degrees and hence the number of generations that could be completed each year. The temperature data were further divided into nominal summer (April to September) and winter (October to March) periods to indicate if development could continue throughout the year or was restricted to summer.

COLD TOLERANCE

All experiments were carried out on both immature stages (larvae, nymphs) and adult organisms of the five species, with and without a period of prior acclimation (usually 7 days at 10°C). This regime was known to increase the cold tolerance of other species and was intended to identify any acclimation ability, rather than to produce 'fully acclimated, winter hardy' populations.

Supercooling points. The freezing temperature (supercooling point or SCP) was measured by cooling the organisms (n = 20 to 50 depending on species) at 1°C min^{-1} in a Peltier cooling device, alcohol bath or differential scanning calorimeter, depending on the size of the specimens. The SCP was detected by the release of heat (exotherm) when the organisms froze.

Lethal temperatures. Replicate samples (3-5 x 10-50 specimens, depending on species) for each exposure temperature were cooled at 0.5 or 1°C min^{-1} in a programmable alcohol bath to range of sub-zero temperatures (-5° to -20°C, depending on the species), exposed at the minimum temperature for 1 min, and then warmed back to the rearing temperature at the same rate. Survival was assessed 24h after exposure.

Lethal times. Replicate samples (3-10 x 10-50 specimens, depending on species) were maintained with and without target prey for increasing periods of time (days, weeks or months as appropriate) at -5°, 0° or 5°C, and mortality assessed 24h after return to the culture temperature.

FIELD EXPOSURES

Replicate samples (5 x 40-50 specimens) were placed in the field within sealed 'quarantine boxes', with and without prey, for increasing periods of time (days, weeks or months depending on the species), and returned to the laboratory after different exposure periods. Survival was assessed within 24h.

DIAPAUSE

The occurrence of diapause was investigated by maintaining different life cycle stages of *N. californicus* and *T. montdorensis* in various 'diapause-inducing' regimes (different LD cycles and temperatures), and monitoring reproduction in the emerging adults after return to normal rearing conditions.

RESULTS

The results for the two predatory mites, *N. californicus* and *T. montdorensis* are presented in Table 1 as examples of the types of data obtained in the range of experiments conducted on the five species. Full details on all species are given in Hart *et al.*, 2002a, b; Tullett *et al.*, 2004; Hatherly *et al.*, 2004, 2005.

The developmental threshold is lower in *N. californicus* than *T. montdorensis*, though both species can complete an average of 6 generations under UK summer conditions; a key difference between the species is the ability of a non-diapausing strain of *N. californicus* to both develop and reproduce in winter. The freezing temperatures of adult females of the two species were similar and did not change after a period of acclimation. In both species there was evidence of substantial pre-freeze mortality with $LTemp_{50}$ values considerably above the mean SCP. However, the most striking differences between the species were in the $LTemp_{50}$, $LTime_{50}$ (at 5°C), and maximum survival times in the field in winter. In all these indices, *N. californicus* was clearly the more cold hardy species.

The data in Table 1, together with that for *M. caliginosus*, *E. eremicus* and *D. catalinae* were then analyzed (Pearson product moment correlation with Bonferroni correction for multiple comparisons), to identify any relationship between laboratory indices of development and cold tolerance (developmental threshold, thermal budget, SCP, $LTemp_{50}$ and $LTime_{50}$)

and survival in the field in winter. The only significant correlation was between the LTime$_{50}$ (at 5°C) and maximum survival time in the field ($r = 0.97$, $P < 0.005$, Fig. 1; Hatherly *et al.* in press).

In the laboratory, *N. californicus* had the longest LTime$_{50}$ and survived for the longest in the field (over 3 months); the mites also reproduced before dying. By contrast, *T. montdorensis* has a short LTime$_{50}$ and died out quickly in the field. Also, provision of prey extended the survival time of *N. californicus*, with 10% still alive after about 4 months, when observations ended.

Table 1. Ecophysiological data for *Neoseiulus californicus* and *Typhlodromips montdorensis* as part of a risk assessment protocol.

Index	***N. californicus***	***T. montdorensis***
Development		
Developmental threshold (C)	8.6	10.3
Thermal budget (DD)	142.9	108.7
Mean annual voltinism	7	6
Development in winter	Yes	No
Cold tolerance		
Mean SCP ± SE (C)		
Acclimated female	-22.2 ± 0.4	-22.4 ± 0.5
Non-acclimated female	-21.6 ± 0.3	-24.1± 0.6
LTemp50 ± 95% fiducial limits (C)		
Acclimated female	-17.7 ± 0.3	-11.5 ± 1.0
Non-acclimated female	-13.9 ± 0.3	-6.7 ± 1.1
LTime50 ± 95% fiducial limits (days at 5 C)		
Acclimated female	65.4 ± 2.5	11.6 ± 1.1
Non-acclimated female	38.6 ± 1.9	9.5 ± 1.1
Field survival		
Maximum survival time (days)		
Without prey	100	35
With prey	112*	35
Reproduction in winter	Yes	No
Ability to diapause+	No	No

*10% still alive after 112 days, +Refers to tested strain

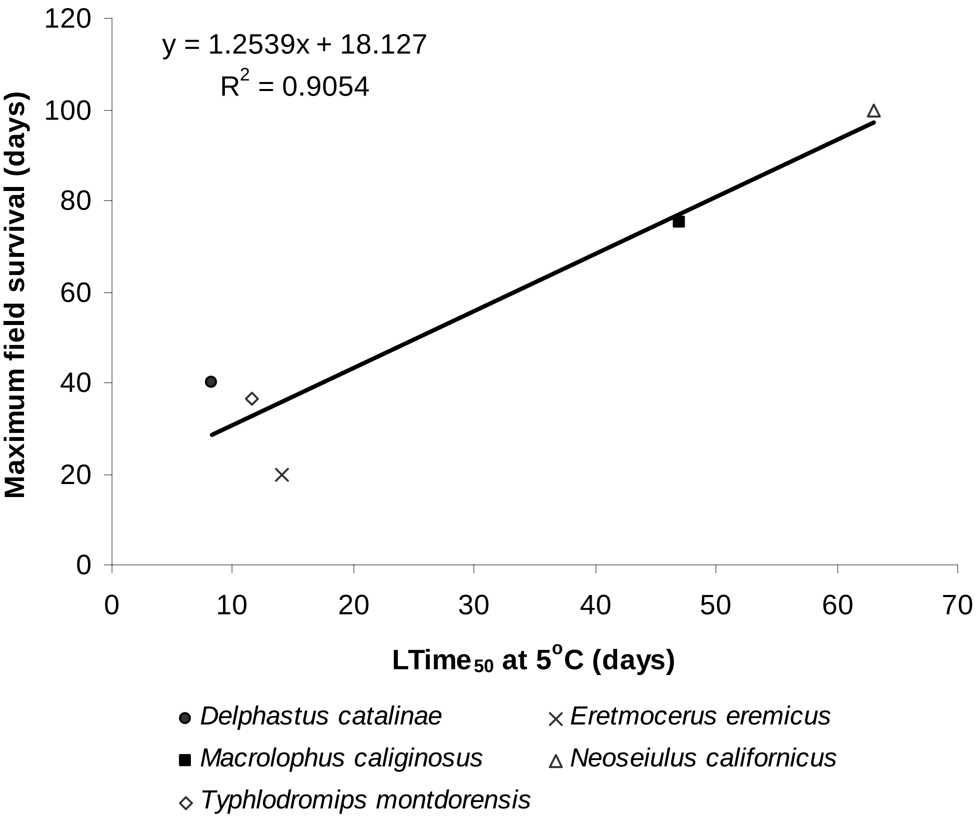

Figure 1. Relationship between maximum field survival (days) and LTime_{50} at 5°C (days) for five non-native biological control agents (data refer to unfed adults of all species except *E. eremicus* that were exposed as unfed larvae).

DISCUSSION

Environmental risk assessment (ERA) of non-native biological control agents, regulated by worldwide, European or country-specific legislation, is an inevitable reality over the next 5-10 years. Irrespective of the proven historical safety of biological control with non-native species, the 'precautionary principle' is now pervasive across all methods of pest management. The common task of scientists, the biological control industry, regulatory bodies, and environmental agencies, is to design and implement a system where the complexity and level of testing in the ERA is proportionate to the risk, without compromising environmental safety.

The recent IOBC-WPRS guidelines (Bigler *et al.* 2005), based on similar documentation from OECD (OECD 2004) and EPPO, together with a comprehensive review of risk assessment of non-native biological control agents (van Lenteren *et al.* in press) have all highlighted an essential requirement for any ERA: the testing should be conducted in a 'step wise' manner, such that species that are either demonstrably safe, or likely to establish and impact on native species or ecosystems, are identified early in the process. The likelihood of establishment is clearly a crucial component in an ERA, especially for inundative releases into glasshouses.

In cool temperate climates, two dominant factors will determine the establishment potential of species escaping from glasshouses: overwintering ability and sources of prey. These two factors must therefore be the central focus for any risk assessment. However, the 'step wise' concept suggests that the first stage of the assessment should focus on overwintering, because if a species is unable to survive through winter, establishment is impossible, and hence, any consideration of effects on non-target prey becomes irrelevant.

The analysis presented in Fig. 1 indicates that the LTime_{50} at 5°C is a reliable predictor of the winter field survival of five non-native biological control agents, representing different taxonomic groups and trophic guilds. It is important to stress that this predictive relationship should not be viewed in isolation; it is one component of an ERA. Also, there is clearly a limit to the sensitivity of the system in terms of estimating maximum survival times in the field. The real value of this approach is that it enables candidate agents be classified into different 'risk categories'. For example, *D. catalinae, E. eremicus* and *T. montdorensis* are representative of a 'low risk' group, where 100% field mortality occurs within four weeks and any establishment is highly unlikely. An 'intermediate risk' group would contain *M. caliginosus*, where survival may persist for extended periods outdoors in winter with limited establishment. *Neoseiulus californicus* would fall into a 'high risk' group where some strains are able to overwinter in diapause and non-diapause strains survive long enough to develop and reproduce.

An indication that a non-native species is able to survive through winter in a new environment is not in itself a reason to reject a licence application. Other forms of risk assessment should then be carried out, on host range and dispersal (van Lenteren *et al.* 2003; in press), and in the final analysis, it may be decided that the overall benefits of release outweigh the risks.

In critically reviewing the contribution that studies on thermal biology, cold tolerance and overwintering can make to an ERA, there are a number issues to consider, including: the possibility that the observed relationship may have occurred by chance, that other indices have similar predictive power, and the extent to which the system is applicable to insects and mites with different levels of cold hardiness.

There are sound ecophysiological reasons to believe that the observed relationship is based on a representative index of cold tolerance that links the laboratory to the field and is not a 'chance occurrence'. It is known that the vast majority of insects show some pre-freeze mortality, in some cases, with 100% death above the SCP. For this reason, the SCP temperature in isolation is not a reliable indicator of cold hardiness, and hence, no correlation with field survival would be expected (and was not found). For insects and mites that originate from warm climates, where pre-freeze mortality is extensive, it is intuitive to predict that the duration of survival at low temperatures (0° to 5°C) in the laboratory would be reflected in field survival, and this was shown to be case. A similar relationship has been reported for a range of native and non-native crop pest species in the UK (Bale and Walters 2001).

It is interesting that no other laboratory index of thermal biology was correlated with field survival. In some respects, the most misleading information relates to the estimation of the developmental threshold and annual number of available day degrees. Both *N. californicus* and *T. montdorensis* can complete an average of 6 generations in UK summers, but their winter survival is markedly different. Estimates of annual voltinism are clearly important, but are not a reliable indicator of winter survival or establishment potential.

The final consideration concerns the applicability of this system to other insects and mites with different levels of cold tolerance. The current analysis includes species in which pre-freeze mortality occurs after exposures of days or a few weeks (*D. catalinae*, *E. eremicus* and *T. montdorensis*) up to several months (*M. caliginosus* and *N. californicus*). These two groups would be classified as 'chill susceptible' and 'chill tolerant' respectively according to Bale (1996). In terms of the world-wide distribution of insects and mites, there are very few 'true' freeze susceptible species (where there is no mortality above the SCP), and only a small number of freeze tolerant species. These species tend to inhabit the coldest regions of the world, and none have ever been used as biological control agents. In summary, it seems reasonable to conclude that the current protocol is applicable to virtually all insects and mites that are likely to be considered as non-native biological control agents, and can make a valuable contribution to a step-wise environmental risk assessment.

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HOW TO ASSESS NON-TARGET EFFECTS OF POLYPHAGOUS BIOLOGICAL CONTROL AGENTS: *TRICHOGRAMMA BRASSICAE* AS A CASE STUDY

Dirk BABENDREIER and Franz BIGLER

Agroscope FAL Reckenholz
Swiss Federal Research Station for Agroecology and Agriculture
Reckenholzstr. 191
8046 Zürich, Switzerland

dirk.babendreier@fal.admin.ch
franz.bigler@fal.admin.ch

ABSTRACT

We show key elements of the risk assessment conducted for *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae), an egg parasitoid which is successfully used for control of the European corn borer in European countries. The main factors that we addressed in this study were: the potential of establishment; acceptance and parasitism of non-target butterflies under laboratory, field-cage and field conditions; the searching efficiency in non-target habitats; the dispersal capacities; and the potential for effects on other natural enemies in maize.

Although high parasitism of non-target butterflies and other natural enemies were observed under laboratory conditions, very few eggs of the non-target species were attacked in the field. These findings may be explained by a low host searching efficiency and the observation that female *T. brassicae* do disperse only a few meters per day. We conclude that the possibility of using invertebrate agents with a broad host range in inundative biological control should not *a priori* be excluded, however, a thorough environmental risk assessment should be performed prior to release.

INTRODUCTION

Egg parasitoids of the genus *Trichogramma* are used for inundative biological control against a range of agricultural pests. In fact, *Trichogramma* spp. are the most widely used natural enemies in inundative biological control worldwide and both native and exotic species have been mass reared and released The vast majority of *Trichogramma* species are known to be polyphagous attacking a wide range of lepidopterans as well as insects belonging to other orders (e.g., Thomson and Stinner 1989). Due to this wide host range concerns have been expressed already several years ago that mass released *Trichogramma* may threaten non-target species in natural habitats (Andow *et al.* 1995; Orr *et al.* 2000).

Concerns about detrimental effects of introduced species on the native fauna have been increasingly expressed over the last two decades. There is now general agreement that the potential for non-target effects has to be evaluated before releasing biological control agents. During the last 10 years, several guidelines addressing non-target effects have been developed. For instance, the Organisation for Economic Co-operation and Development (OECD) developed guidelines to provide 'light regulation' for invertebrates used in classical and inundative biological control (OECD 2004). Despite these initiatives which basically aim to provide guidance on what data should be considered for environmental risk assessment, there is still a debate on how these data can be obtained. Van Driesche and Reardon (2004) provided a 'guide to best practice' on how to conduct host specificity testing which generally forms an important part of the risk assessment. Babendreier *et al.* (2005) recently published a comprehensive review on the methods used to assess non-target effects in biological control and a book in which questions on environmental risk assessment of arthropod biological control are addressed will be published in the near future (Bigler *et al.* 2006).

In this paper we summarize key elements and results of an environmental risk assessment project conducted for *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) in Switzerland from 1998 to 2002 which was part of the EU funded project 'Evaluating Environmental Risks of Biological Control Introductions into Europe' (ERBIC).

PRIME FACTORS FOR NON-TARGET EFFECTS

Host specificity is one of the bottom lines in the assessment of non-target effects (Van Driesche and Reardon 2004; Van Lenteren *et al.* 2006) and hence, only agents with a narrow host range are considered for release in classical biological control. However, less specific agents are sometimes used in inundative biological control. One example is *T. brassicae* which is used since many years in European countries for control of the European corn borer, and it is known that this species attacks eggs of other lepidopterans and other non-target insects. We tested host acceptance and parasitism of *T. brassicae* on non-target butterflies and predators in maize fields under laboratory, semi-field and field conditions. Further we hypothesized that host searching efficiency in non-target habitats could be another important factor responsible for adverse effects on non-target butterflies.

In contrast to classical biological control, overwintering and establishment are negative properties of non-native agents if used for inundative biological control. If establishment does not occur, the risk for non-target species is limited to the period of release and possibly the following weeks if females can reproduce on target or non-target hosts in the crop or in other habitats. Therefore the risk of non-target impacts is spatially limited and of transient nature (Lynch *et al*. 2002). If *T.brassicae* would be able to survive the winters, reproduce on non-target host eggs in the area of introduction and disperse, there is potential for permanent effects on a large geographical scale.

OVERWINTERING

In order to test for the ability of *T. brassicae* to establish in Switzerland, two experiments were conducted. The first one was designed to study whether *T. brassicae* would survive outdoor winter conditions in Switzerland. Eggs of six non-target host species parasitized in the laboratory by *T. brassicae* were exposed under outdoor conditions (in Zurich) every two weeks between 26 September and 7 November. Control eggs were kept in an environmental chamber at 25 °C, 70% RH (for details see Babendreier *et al*. 2003a).

We found that *T. brassicae* is able to overwinter successfully on eggs of six lepidopteran species in the families Tortricidae, Noctuidae, Plutellidae, Pyralidae and Crambidae. Between 75% and 100% emergence was observed in the following spring for all of the six host species exposed on 26 September. On later exposure dates, spring emergence decreased significantly and no development of *T. brassicae* occurred from host eggs parasitized on 7 November.

In a second experiment, we evaluated at what time of the year diapause induction under field conditions occurs. Eggs of the flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were offered to *T. brassicae* females at five consecutive dates at weekly intervals from 27 August to 24 September. After parasitization in the laboratory, the eggs were exposed under outdoor conditions until emergence occurred. We found that the period of diapause induction is equal to the dates which allowed successful development and overwintering of *T. brassicae*. In order to evaluate the effect of overwintering on the fitness of females that had spent the winter in diapause inside the eggs of *E. kuehniella* under outdoor conditions from 17 September 1999 to beginning of May 2000, we measured the fecundity of 30 females. Fecundity of females that overwintered outdoors was not significantly different from the fecundity of females that were reared in the laboratory without diapause at 25 °C.

Our results demonstrate that the egg parasitoid *T. brassicae* is able to overwinter successfully in northern Switzerland and that is has the potential to establish if host eggs were available.

PARASITISM OF NON-TARGET BUTTERFLIES

Since *T. brassicae* was known to be polyphagous, we concentrated on butterflies because of the strong environmental concerns for this group of insects. We exposed eggs of 23 non-target lepidopteran species, including nine endangered species of Switzerland, to single *T. brassicae* females under no-choice conditions in the laboratory (Babendreier *et al*. 2003b). Most of the species were well accepted and parasitized at the same level as the target, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae). In addition to oviposition, we also measured the number of times a female rejected a host egg before acceptance as well as the time from first host egg contact to acceptance.

In a next step, we investigated parasitism of six non-target butterfly species by *T. brassicae* in field cages of 2 x 2 x 2 m (Babendreier *et al*. 2003c). Eggs of the non-targets were glued on host plants together with *E. kuehniella* eggs (multiple choice) and exposed for 24 hours to the females. Parasitism of non-target species in field cages ranged between 2.5% and 18.7%. We found that parasitism was density dependent.

Field trials were then carried out in maize fields and adjacent meadows (Babendreier *et al*. 2003c). We released 30,000 female *T. brassicae* in a plot of 50 x 50 m. This corresponds to the number of females released in commercially treated maize fields. All release plots were situated inside the maize fields but bordering the meadows. We exposed eggs of two non-target hosts together with eggs of *E. kuehniella* as a control. Eggs were exposed for 3 days at 2 m distance inside the maize field and at 2 m and 20 m distance outside the maize field in the meadow. At each distance, we attached 30 single eggs of the non-targets and 30 egg masses of *E. kuehniella* (50 –100 eggs each). As a control for natural occurrence of *Trichogramma* spp., we placed 30 egg masses of *E. kuehniella* on leaves of maize plants in two fields that were 1-2 km away from the treated fields.

Parasitism rates of *E. kuehniella* egg masses inside maize fields averaged 40% compared to significantly lower parasitism rates of 26.2% and 12.6% for eggs of the two non-targets. In the meadow, at 2 m distance from the maize field, parasitism rates decreased to 2.3% and 6.1% for the non-targets and 9.8% for *E. kuehniella* while no single egg was found parasitized in the meadow at 20 m distance from the maize field.

HABITAT SPECIFICITY

In order to evaluate whether the low parasitism in meadows can be generalized and to understand the underlying mechanisms, we studied the searching efficiency of *T. brassicae* in several non-target habitats such as meadows, flower strips and hedgerows. At the same time, *T. brassicae* was released at rates of 120,000 females/ha in plots of maize and one of the selected non-target habitats (plot size 24x24 m). Sentinel egg clusters of *E. kuehniella* were applied to the plants and recollected after 3days. Parasitism of sentinel egg clusters was 1.6 - 3.6% in meadows and 2.0 - 4.0% in flower strips while the respective figures were 57.6% – 66.7% and 19.2% - 46.9% in maize (Babendreier *et al*. 2003d). Subsequent field cage experiments confirmed the higher parasitism rates in maize compared to meadows, flower strips and hedgerows.

To investigate the factors responsible for the low parasitism in non-target habitats, the behavior of individual *T. brassicae* females was observed on common meadow plants. Single females were directly observed on different plants and parameters such as mean walking speed, turning angles and number of wasps leaving the plants were measured (Babendreier *et al*. 2003d). Significant differences in these variables were found between maize and four meadow plants. The most pronounced effects were found between maize and red clover, a very common plant in meadows in Switzerland with very hairy leaf surface. In a laboratory choice experiment, carried out with all five host plant species together in cages, we obtained highest parasitism on maize and lowest on red clover, confirming the behavioral observations.

DISPERSAL

While dispersal is a prerequisite of a successful classical biological control agent, it may be a negative feature in the context of non-target effects of inundatively released agents. The ultimate question is how many released biological control agents will enter a given non-target habitat or, more precisely, what densities of the agent can be found in certain distances from the release fields. To answer this question, experiments were carried out to investigate the dispersal behavior of *T. brassicae* (Babendreier *et al.* 2002; Kuske *et al.* 2003; 2004; Mills *et al.* 2006). The first experiment aimed to establish the degree to which *T. brassicae* will leave maize fields where they were released. Traps consisting of a plastic transparent sheet (30 x 21 cm), sprayed with glue on both sides, were placed at the edge of a maize field. These traps were mounted on wooden sticks at a height of 40-70 cm and positioned inside the field (0.8 m from the edge), at the edge of the field and outside the field (0.8 m from the edge). After one week the numbers of male and female *T. brassicae* on each side of the trap were counted. The results indicated a strong decrease in numbers from inside to outside of the maize field.

Kuske *et al.* (2003) increased the scale of this experiment and placed traps of the same type at distances up to 40 m away from the edge of maize fields. Traps were placed directly above the vegetation and exposed for one week before and during the first and the second commercial release of *T. brassicae* as well as for three weeks following the second release. A strong decrease in numbers with distance was observed and, altogether, it can be concluded from these experiments that a large fraction of *T. brassicae* will not leave the field. Moreover, the experiments have shown that *T. brassicae* will be present in non-target habitats close to the release field only for one or two weeks after releases.

In order to investigate the distance that individual *T. brassicae* travelled in a given time period, about 100,000 wasps were released from parasitized eggs from a central release point in a meadow (Babendreier *et al.* 2002). Sticky traps that had been placed at distances of 2, 4, 8, 16, 32 and 64 m in four directions from this release site were changed daily and all *T. brassicae* that had been collected were counted. This experiment revealed that *T. brassicae* only flies a few meter per day (Mills *et al.* 2006). Finally, sticky traps were used to study whether hedgerows may act as a barrier for dispersing *T. brassicae* (Babendreier *et al.* 2002).

INTRAGUILD PREDATION AND INDIRECT EFFECTS

After demonstrating that non-target effects will most likely be restricted in space and time, we decided to conduct a final experiment on potential effects on populations of other natural enemies in maize. In a tiered approach, experiments were conducted on the host acceptance of *T. brassicae* towards eggs of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), *Episyrphus balteatus* (De Geer) (Diptera: Syrphidae), *Coccinella septempunctata* L. and *Adalia bipunctata* L. (both Coleoptera: Coccinellidae) under laboratory, greenhouse cages and field conditions (Babendreier *et al.* 2003e). While no offspring emerged from eggs of *A. bipunctata* and *C. septempunctata,* high parasitism rates were obtained for *C. carnea* and *E. balteatus* eggs in laboratory experiments. However, we observed significantly increased mortality on *A. bipunctata* eggs, compared to the control and also found young instars of *T. brassicae* inside *A. bipunctata* eggs. In a second experiment where the host acceptance behavior of the parasitoid female was directly observed for 10 min, 10% of *T. brassicae* females were found to oviposit in eggs of *A. bipunctata* but development of parasitoid offspring failed.

In greenhouse cages, parasitism rates of *C. carnea* eggs (7%) and *E. balteatus* eggs (0.4%) were significantly lower than parasitism of *E. kuehniella* eggs (21 and 27%, respectively) that were used as a control in the two experiments. In the field, only 3.1% of *C. carnea* eggs were parasitised by *T. brassicae*. This was significantly less than the observed parasitism rate of *E. kuehniella* egg clusters (64%). From direct observations of the parasitoids host acceptance behavior and the low parasitism rates observed in cages and under field conditions we conclude that ecologically relevant adverse effects of mass released *T. brassicae* on natural enemies in maize are unlikely to occur.

Finally, we aimed to assess the potential for negative effects on the native larval parasitoid *Lydella thompsoni* Hert. (Diptera: Tachinidae) (Kuske *et al.* 2004). In Switzerland, this tachinid was found to develop the first generation on the two non-target lepidopteran species *Archanara geminipuncta* Haworth (Lepidoptera: Noctuidae) and *Chilo phragmitellus* Hb. (Lepidoptera: Crambidae) living on common reed plants, *Phragmites australis* (Cav.), while subsequent generations attack the European corn borer in maize. Severe parasitism of the two non-target lepidopterans by *T. brassicae,* immigrating from maize fields into reed habitats could lead to negative effects on the tachinid due to competition. Under laboratory conditions, both non-targets were found to be suitable hosts for *T. brassicae*. However, parasitism rates were low, either because eggs are hidden between leaf sheaths and the stalk of the host plant or because of low attractiveness of the eggs. Field experiments and surveys of the two non-target lepidopteran species were conducted in common reed habitats located amongst maize fields with *T. brassicae* releases. No single egg of the two non-target species was found parasitized, indicating that negative effects on the native tachinid due to mass releases of *T. brassicae* are unlikely.

CONCLUSIONS

We have provided an example on how to conduct a full environmental risk assessment for a polyphagous biological control agent. The study on non-target effects of *T. brassicae* mass releases demonstrates that the final conclusion on environmental risks could be drawn only after investigating host range, establishment, dispersal and competition in laboratory and field experiments. We have evidenced that low dispersal capacities and low host searching efficiency in non-target habitats were the main determinants to explain the relatively low level of risk associated with this egg parasitoid. Our results indicate that the structural complexity of the plants and of the habitat play a role for the low searching efficiency. We conclude that the possibility of using agents with a broad host range in inundative biological control should not *a priori* be excluded, however, a thorough environmental risk assessment should be performed prior to release.

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TOOLS FOR ENVIRONMENTAL RISK ASSESSMENT OF INVERTEBRATE BIOLOGICAL CONTROL AGENTS: A FULL AND QUICK SCAN METHOD

Antoon J. M. LOOMANS[1] **and Joop C. VAN LENTEREN**[2]

[1]Plant Protection Service
P.O. Box 9102
6700 HC Wageningen, The Netherlands
a.j.m.loomans@minlnv.nl

[2]Laboratory of Entomology, Wageningen University
P.O. Box 8031
Wageningen, The Netherlands
joop.vanlenteren@wur.nl

ABSTRACT

The deliberate or accidental introduction of species from their native ranges to new environments is a major threat to biological diversity. Biological control is both an important management tool for controlling threats to agriculture an the environment as well as - in rare cases - a potential threat to the environment itself. The newly adopted International Standard for Phytosanitary Measures No. 3 (ISPM3) offers a framework for risk assessment and focuses specifically on the shipment, import, export and release of biological control agents. Guidelines for information requirements of exotic natural enemies and methods for risk assessments are currently in development. The major challenge in developing risk assessment methodologies is to develop protocols and guidelines that will prevent serious mistakes through import and release of potentially harmful exotics, while at the same time still allowing safe forms of biological control to proceed. We expect that a risk assessment methodology for biological control agents will integrate information on the potential of an agent to establish, its abilities to disperse, its host range, and its direct and indirect effects on non-targets. In this presentation, we first propose a comprehensive risk evaluation method (full scan) for new natural enemies and, second, a quick scan method for natural enemies already in use. The outcome of our evaluation of150 biological control agents, commercially available in northwest Europe, will be discussed.

INTRODUCTION

Measures to protect the environment, and people in it, have involved a wide variety of approaches and underlying principles (Calow 1998). Risks posed to human and animal health and to ecosystems from chemicals, genetically modified organisms and from biological introductions are widely assessed, based on scientific methods and procedures (Simberloff and Alexander 1998). Risk assessment is a tool that can be used to support exclusion of invasive species as well as to assess the potential impact of those that have become established. Risk assessment can be used in decision-making to help determine if action should be taken, and, if so, what kind (Wittenberg and Cock 2001). There is, however, still a great need for research on risk assessment procedures and methods to evaluate biological introductions. Although regulations for biological control agents of weeds have been more strict than those of pests, risk assessments have not always been accurate enough to prevent ecological side effects on nontarget hosts (Louda *et al.* 2003). Invertebrate biological control agents (IBCAs) are applied across the world to control pest species in agricultural, urban and natural ecosystems. In the past 100 years many exotic natural enemies have been imported, mass-reared and released as biological control agents for pest control in areas outside their origin. In few cases, negative effects of these releases have been reported, mostly of generalist predators, often vertebrates (Lynch and Thomas 2000; van Lenteren *et al.* 2005). The current popularity of biological control may, however, result in problems: an increasing number of projects will be executed by persons not trained in identification, evaluation and release of biological control agents, an increasing number of agents and products will become available for the control of pest organisms, and the internet increasingly lowers access, sales and demands for public use.

The International Plant Protection Convention (Rome 1951; IPPC 1997) and the Convention of Biological Diversity (CBD 1992) are the two conventions which are most relevant for biological introductions of economical and environmental concern. Obligations on contracting parties include development of scientifically based risk assessment procedures and methods. Whereas for plant pests there is a long history of such procedures and measures, for introductions of organisms of environmental concern these are relatively new (IPPC 2004). Since 1992 more and more countries have put legislation in place concerning biological introductions that threaten species habitats and biological diversity. This has increased the international interest in risk assessment as a legislative tool. The FAO Code of Conduct (FAO 1996) has brought about important changes in the regulation of IBCAs in developed (EPPO 1999; 2000; NAPPO 2001) and developing countries (Kairo *et al.* 2003), but these were still largely non-legislative instruments. The recently revised ISPM3 (IPPC 2005) includes assessment of environmental risks and offers contracting parties a minimal standard when putting regulation in place. In addition, its recognition by the WTO-SPS agreement, provides that ISPM3 will be an international binding instrument that offers a format for trade in and release of biological control agents (WTO 1994). Except that there is a need for generic risk assessment schemes for all types of biological introductions, there is a specific need for schemes tailored for biological control and other beneficial organisms. Here we summarize new tools for assessing environmental risks of biological control agents that have been developed recently (van Lenteren *et al.* 2005; van Lenteren and Loomans 2005), consisting of a full and a quick scan analysis.

ECOLOGICAL DETERMINANTS

Various qualitative methods are used to generate a cumulative risk index for potential quarantine pests by adding qualitative or quantitative scores, such as low, medium, high (APHIS 2000; NRC 2002), assign numerical scores in a questionnaire (EPPO 1997; MacLeod and Baker 2003) or using successive matrices (Biosecurity Australia 2001; Murray 2003). Similarly quantitative risk assessment models have been developed for weed introductions (Pheloung *et al.* 1999; Williams and Newfield 2002) and their biological control agents (Wapshere 1974). Risk assessment procedures for inoculative and inundative biological pest control need to be more tailored to its specific requirements and needs, and support a well-balanced decision making process, properly weighting its principal beneficial and potential detrimental impact (Sheppard *et al.* 2005). Environmental risk assessment should preferably be placed in a general framework for regulation of import and release of biological control agents (OECD 2004), including

- characterization of the agent (taxonomic, biological characteristics),
- risks posed to human and animal health,
- efficacy, quality control and benefits of use, and
- environmental risks.

The latter category, assessment and analysis of environmental risks, demands integration of many aspects of their biology, as well as information on ecological interactions identified above. The risk posed by introduced species, whether invasive and of ecological or of economic concern, including biological control agents (Simberloff and Alexander 1998; van Lenteren *et al.* 2003), is determined by the following ecological factors:

- the potential of an agent to establish in its novel environment,
- its abilities to disperse,
- its host range, and
- its direct and indirect effects on nontarget species.

Any risk-assessment of IBCAs should include information on these factors. The first three factors mainly determine to what extent the intrinsic attributes of a species determine its environmental impact (direct and indirect effects). The intrinsic factors of successful invaders and of successful biological control agents partly have common denominators. It is a critical issue to develop risk assessment schemes that recognize these potential conflicts of interest and distinguish keystone values subsequently.

ENVIRONMENTAL RISK ASSESSMENT TOOLS

The following account is largely summarized from van Lenteren and Loomans (2005) and van Lenteren *et al.* (2005), with additional references. In contrast to most PRAs for pests of phytosanitary importance, pathway analysis for biological control agents is of secondary importance as they are mostly deliberately introduced. Performing an ecological risk assessment prior to first introduction is then essential as addressed in ISPM3, thus avoiding undesired establishment of an IBCA. Nevertheless, potential IBCAs also are entering a country by range expansion or by accident as stowaways on infested plants and hosts and are discovered when they already passed ports of entry. When there is no legal justification for eradication measures, as for most IBCAs which are not of phytosanitary importance, regulation of an exotic IBCA present but still contained in a country, can be covered indirectly by performing a risk assessment prior to its commercial release (IPPC 2005).

Depending on the stage of the regulatory process, either a comprehensive full scan can be used as a tool for risk assessment, or a quick scan, based on the same environmental determinants as indicated above. A quick scan is an initial screening of available information for known nontarget impact to exist or to expect, revealing any invalid, missing or incorrect information. It is supposedly fast, less costly than a full scan, but mostly indicative and qualitative in its results. A full scan, on the other hand, includes all these elements as well, but is more thorough, comprehensive, evaluating and extrapolating potential hazards, including the use of generated data and performing a complete risk-analysis.

FULL SCAN

Any comprehensive environmental risk assessment will first identify the hazards (intended as potential to cause harm), subsequently estimate the risk (intended as the likelihood of that potential being realized) of environmental importance (intended to refer to the routes of exposure for both humans and animals) (Calow 1998). Risk assessment includes a risk identification and evaluation procedure and should be closely tied to risk management, risk-cost-benefit analysis and risk communication. Van Lenteren *et al.* (2003) proposed a first general framework of the first step, a risk assessment methodology for import and release of inundative biological control agents. Their method integrates and indexes the five ecological determinants mentioned above. A numerical value (1-5) is assigned to the likelihood (L) and magnitude (M) of each of the five elements to quantify risks. The overall ecological risk index (ERI) was based on multiplying values for L and M for each element and adding the values of all five elements. The minimum score was thus 5 (5 * 1x1) and the maximum score 125 (5 * 5x5). Thirty-one cases of natural enemy introductions where thus analyzed in retrospect. Although a clear categorization was obtained with an ERI ranging from 7-105, we encountered some practical and intrinsic drawbacks: calculation and evaluation of such a cumulative ERI would require a substantial amount of information and experimentation before any evaluation can be made, and when these are not available (mis)interpretation could lead to manipulation in decision making. In addition, the ecological elements are not independent and not equal in importance, they should not be rated equally and cannot be indexed in a cumulative way as we previously did. To optimize the process and avoid unnecessary research efforts and costs, we suggest a more advanced, stepwise risk assessment procedure (van Lenteren and Loomans 2005).

In contrast to the procedure of the cumulative risk assessment method described above, the decision to release is based on a tiered approach of each of the ecological determinants, using successive individual matrices of L*M matrix as indicated before. Prevention of entry and establishment is the first and most cost-effective line of defense against biological introductions, such as plant pests or other invasive species (Baker *et al*. 2005; Wittenberg and Cock 2001). Establishment is therefore considered as the first factor in line. When establishment (survival, reproduction, over-wintering) in the novel environment is aimed at, like in classical biological control (CBC) programs, host specificity (and host range testing) is considered the most relevant element, etc. The step-wise procedure of environmental risk assessment is shown in Table 1. For some steps (3, 4 and 6) successive ERI levels (L*M) are calculated according to

Table 1. Generic key to procedures of environmental risk assessment of invertebrate biological control agents (after van Lenteren *et al.* 2005).

Step #	Topic/Condition	Go To
Step 1	**Origin**	
	native to area of release	Step 6
	exotic to area of release	Step 2
Step 2	**Biological Control Program**	
	import and release for permanent introduction (CBC)	Step 4
	establishment not intended (ABC)	Step 3
Step 3	**Establishment**	
	certain	Stop
	possible	Step 4
	not possible	Step 6
Step 4	**Host Range Includes**	
	attack of related and non-valued nontargets	Release
	attack of related + unrelated and/or valued species	Stop
Step 5	**Dispersal**	
	local, moderate	Step 6
	extensive	Stop
Step 6	**Ecological Impact (direct and indirect effects)**	
	likely - permanent	Stop
	unlikely - limited - transient	Release

the approach of van Lenteren *et al.* (2003) and depending on its outcome, the procedure stops or continues.

When we applied the proposed stepwise risk assessment procedure (Table 1) to biological control agents commercially available in Europe (EPPO 2002), obviously risky species were eliminated early in the process. Other species that scored - erroneously - a high cumulative index in the first quantitative risk assessment procedure (van Lenteren *et al.* 2003), such as *Trichogramma brassicae*, were not eliminated early in the new procedure. In concordance with recent experimental data these are recommended for further release. See van Lenteren and Loomans (2005) for a full report.

QUICK SCAN

Under certain conditions a more qualitative ‘quick scan’ method could be used to assess potential adverse environmental effects based on currently available information only:

1. For *newly introduced organisms* a quick scan can act as an initial screening step for governments to initiate the evaluation process and to assess the status and of the species or population. level of containment prior to first import of a new organism into their country for research or production. For the applicant it helps before first introduction of a natural enemy to quickly evaluate the biological and ecological characteristics and to determine the potential research effort he will have to make to get an approval after efficacy testing is resolved. When after a thorough evaluation on efficacy a release is still considered, a comprehensive risk analysis would apply.
2. In countries developing new regulations a quick scan would allow governments to assess the environmental risk for *natural enemies already in use* to distinguish IBCAs with minor effects from those with large effects, based on evidence of ecological impact. Species considered safe for continuation of release can thus be exempted from further regulatory measures.
3. A quick scan can be used to assess the environmental risk of mass-releasing natural enemies originating from areas within the same ecoregion, but not present in the area of release itself (initial step 1 and 6 in Table 1). Thus, the results of a quick scan could help to establish lists of species that can be used in certain, specified regions or (parts of) ecoregions of the world (*ecoregional "white lists"*). This would result in strongly reduced costs for regulation of the major part of biological control agents currently used and continuation of current biological control programs.

We applied the quick scan method, based on the information requirements and ecological determinants as outlined above, to 150 species of natural enemies currently commercially available in The Netherlands (EPPO 2002; Loomans and Sütterlin 2005). About 5 % of the species were considered too risky for (continuation of) release and 80 % of the species were considered safe. For the remaining 15% information initially was either still partly inadequate, inappropriate or lacking to complete the quick scan. However, when no evidence was available on any significant nontarget effects, or not foreseen, it was advised for most species to continue release. In 2005, 134 species were placed on a "white list", which will be exempted from further regulatory measures in The Netherlands. All other species, IBCAs and other beneficial organisms, will need authorization by derogation.

CONCLUSIONS

The intrinsic factors of successful invaders and of successful biological control agents partly have common denominators. An environmental risk assessment (ERA) can help to reveal, and where possible distinguish, potential conflicts of interest in the application for certain taxa, guilds, species or populations of biological control agents and to distinguish keystone values subsequently. Thus, we can increase efficacy and avoid direct and indirect nontarget effects. In order to be of practical use, the risk evaluation method in a full scan should preferably be 1. quantifiable, so that the environmental effects of different biological control agents can be compared and choices can be made, and 2. consist of a tiered or stepwise procedure so that the clearly safest agents or the unequivocally hazardous natural enemies will be identified quickly and with lowest possible costs involved. The applicant needs to provide sufficient and reliable information to issue a permit or derogation for import and release. For natural enemies already in use (~200 species worldwide), the quick scan risk evaluation method consists of steps and questions which are the same as in the advanced method, but will be based on available data only. The results of a quick scan could help to establish lists of species that can be used in certain, specified areas or (parts of) ecoregions of the world.

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