

Evaluating the Host Range of Agents for Biological Control of Arthropods: Rationale, Methodology and Interpretation

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Abstract

Before releasing exotic natural enemies for biological control of weeds, host range tests are almost universally required by authorities, to ensure that agents are unlikely to have detrimental impacts on non-target plants. However, for biological control of arthropod pests, tests to determine the potential host range of exotic agents have not been so widely practiced, leading to concerns that agents once established may have undesired impacts on beneficial organisms and native fauna. The rationale for host range tests is similar for weeds and arthropod projects and the centrifugal method for selecting non-target taxa related to a target is applicable to both, but the taxonomic relationships for arthropods are often not as well known as for plants. The number and range of non-target arthropods to be tested with an exotic agent must be selected carefully, since it is impractical to maintain in culture an extensive range of taxa. Non-target beneficial or threatened arthropod taxa may be priorities for testing as potential hosts but their life histories are sometimes unknown or appropriate stages may be difficult to obtain or culture. Tritrophic agent/ host/plant interactions are not uncommon and difficult to evaluate, and predators need special evaluation when compared with parasitoids. Although adults of predacious arthropods are sometimes generalists, their immature stages may be sufficiently specific to be acceptable. Current methods for evaluating the host range of agents for biological control of arthropod pests are discussed, taking into account issues of insect taxonomy and behavior that influence testing procedures, as well as some environmental and faunistic considerations that need to be considered in making decisions relating to safety or risk assessments of potential agents.

Introduction

The selection of non-target plants for testing the host range of agents for biological control of weeds has been implemented for many years (Wapshere, 1974; Harley, 1979). Before weed agents are translocated from another country and released, they are tested to demonstrate that they will not damage: (a) plants of economic importance or ornamentals, or (b) have a significant impact on native flora, particularly rare or threatened species. For parasitoids and predators used for biological control of arthropods, the host range has been considered differently and tests were usually

conducted before the release of such species only if they were thought likely to attack beneficial organisms (Ertle, 1993). However, the justification for host range tests on arthropod agents has gained further attention (Van Driesche and Hoddle, 1997), following claims that some undesirable impacts have occurred to non-target organisms (Howarth, 1991). As a result of these concerns, pre-release studies on introduced arthropod agents have been adopted in New Zealand (Barratt et al., 1999) and Australia (Keller, 1999).

For weed projects, guidelines for testing non-target plants have been available and summarized (e.g. Waterhouse, 1991; Harley and Forno, 1992), but due to lack of detailed studies, specific procedures for arthropod agents have not been well developed (Sands and Papacek, 1993). The criteria needed for assessing the host range of arthropod agents may also differ from those applied to weed agents (Sands, 1997), and some of the procedures for predicting host ranges of arthropod agents have recently been discussed (Barratt et al., 1999).

The rationale, methodology, and interpretation of tests for determining the host range of arthropod agents are discussed.

Rationale for Testing the Host Specificity of Arthropod Agents

Biological control agents with narrow host ranges are generally considered to have few, if any, detrimental effects on beneficial or indigenous organisms, even when non-target species are used as hosts (Waterhouse, 1991). However, this may be attributed to the lack of documentation, especially when monitoring for effects in non-target organisms would require sampling in habitats different from those occupied by the target pest (Simberloff and Stiling, 1996). For weeds projects, there is evidence that some narrowly-specific agents have attacked rare or endangered native plants (Louda et al., 1997). Although the impact of these herbivores on the native plant populations has not yet been shown to be definitely detrimental (Herr, 1999), their attacks have been cause for concern (Louda et al., 1998).

In the case of arthropod agents with broad host ranges, their development on non-target taxa has sometimes been considered to be beneficial; such agents are seen as “lying in wait” ready for an opportunity to parasitise or prey on a pest when outbreaks occur (Murdoch et al., 1985). A further extension of this strategy has been to establish agents in readiness for exotic pest incursions. For example, attempts were made in Australia to establish the polyphagous parasitoid *Aphelinus varipes* (Foerster) on the aphid *Rhopalosiphum padi* (L.) in preparation for the possible arrival of the Russian wheat aphid, *Diuraphis noxia* (L.) (Hughes et al., 1994).

High densities of natural enemies maintained by exotic prey species are claimed to have the potential to drive rare non-target species to extinction (Simberloff and Stiling, 1996). Other aspects of the safety of arthropod

agents are being debated, and methods to predict their host ranges have recently been reviewed (Barratt et al., 1999). In particular, tests are recommended when the biology of a candidate indicates that it has a wide host range, poses risks to economically-important, endangered, or ecologically significant non-target species (Van Driesche and Hoddle, 1997).

Potential Impacts on Other Biological Control Agents

There is a risk that parasitoids introduced to control a pest might also attack biological control agents of weeds and reduce their efficacy (Table 1). This is particularly possible if a target pest is closely related to a beneficial agent. For example, the parasitoids *Tamarixia leucaenae* Boucek and *Psyllaephagus yaseeni* Noyes, used for biological control of the psyllid pest *Heteropsylla cubana* Crawford, were deliberately not introduced into Australia to avoid risk to the effectiveness of another psyllid (*Heteropsylla spinulosa* Muddiman, Hodkinson and Hollis), an agent established for control of the weed *Mimosa invisa* Martius ex Colla (Waterhouse and Norris, 1987). Similarly, pyralid shoot-borers in the genus *Hypsipyla* are serious pests of trees in the family Meliaceae, and biological control agents have been introduced from India into Central America in attempts to control these borers (Rao and Bennett, 1969). In Australia, *Hypsipyla robusta* (Moore) has been considered as a potential target for biological control, since a much wider range of natural enemies are known from elsewhere than currently exist in Australia. However, the phylogeny of the subfamily Phycitinae, to which *Hypsipyla* spp. belongs has not been satisfactorily resolved (M. Horak, *personal communication*). Shaffer et al. (1996) note that *Hypsipyla* Ragonot is related to *Cactoblastis* Ragonot, and belongs to the same tribe, Phycitini. Since *Cactoblastis cactorum* (Bergroth) is an important agent for control of prickly pear (*Opuntia* spp.) in several countries including Australia, it might be an alternative host for

Table 1. Rationale for host range tests

Avoid detrimental effects to:
A. Exotic biological control agents and other beneficial organisms (e.g., parasitoids, predators, pollinators).
B. Native species, especially those which are threatened, rare, and of conservation concern.
C. Organisms of commercial, cultural, or aesthetic significance.

parasitoids introduced for control of *Hypsipyla* spp., unless such parasitoids were narrowly specific to only this genus. Any reduction in efficacy of the beneficial non-target species *C. cactorum* would be unacceptable. This example illustrates the importance of understanding taxonomic relationships in biological control projects.

Native natural enemies play an important role in the control of many pests. Room (1979) for example, listed many natural enemies of *Helicoverpa armigera* (Hübner) in cotton crops in Australia. Predators included the pentatomid bugs *Cermatulus nasalis* (Westwood) and *Oechalia schellebergii* (Guerin-Meneville), both also known to be important in a wide range of other crops. When introducing agents for control of pest pentatomids, effects on these important predators are considered, to avoid a possible decrease in their effectiveness. For example, tests were conducted that showed that neither of these predators would be attacked by the tachinid *Trichopoda giacomellii* (Blanchard) before it was approved for release as a biological control agent for *Nezara viridula* (L.) (Sands and Coombs, 1999).

Potential Impacts on Non-target Indigenous Species

Few detrimental effects have been recorded from deliberately introduced arthropod natural enemies with a broad host range. An egg parasitoid, *Trissolcus basalis* (Wollaston), is said to have had an impact on native Pentatomidae in Hawaii as well as on its exotic target pest, the green vegetable bug, *Nezara viridula* (L.) (Howarth 1991). In Hawaii and other closed geographical populations, non-target organisms are believed to be more susceptible to effects of exotic generalists than those on larger land masses (Howarth and Ramsay, 1991). In Australia, impacts by *T. basalis* leading to decline in abundance of non-target taxa have not been reported, even though the eggs of many native species are parasitized by the parasitoid, including important predatory species (Waterhouse and Norris, 1987).

Although non-specific agents may sometimes develop on native non-target taxa, it is very difficult to predict the levels of attack or possible detrimental impacts before their introduction. For example, the tachinid *Bessa remota* (Aldrich), a generalist parasitoid of moth larvae, was reared from the zygaenid *Amuria catoxantha* (Hampson) and introduced from Malaysia to Fiji to

control the zygaenid coconut pest *Levuana iridescens* Bethune-Baker (Tothill et al., 1930), but it also had impacts on non-target species, including the related moth *Heteropan dolens* Druce. These two moths, *L. iridescens* and *H. dolens*, are said to have become extinct in Fiji (Robinson, 1975), but there is some debate as to whether both have disappeared in Fiji, or merely continue to occur at very low densities (Paine, 1994; Sands, 1997). In Hawaii, of the 679 agents deliberately introduced for control of pests between 1890 and 1985, 243 agents became established and 20 have been recorded attacking non-target species (Funasaki et al., 1988). However, these authors considered that only the generalist tachinid *Lespesia archippivora* (Riley), introduced for control of armyworms, may have contributed to the extinction of a non-target species, the noctuid *Agrotis crinigera* (Butler).

In Guam, generalist parasitoids introduced to control lepidopterous pests parasitize the eggs and pupae of indigenous butterflies, including the nymphalids *Hypolychnas anomola* (Wallace) and *Hypolychnas bolina* (L.), but neither species is threatened as a consequence of attacks by these introduced parasitoids (Nafus, 1993). Up to 40 percent of eggs and 25 percent of pupae of *H. bolina* were attacked by the exotic parasitoids, but only the pupal parasitoid was considered to have had an adverse effect on the butterfly by reducing its abundance.

Even when levels of parasitism of a non-target host are higher than parasitism rates on the target species, impacts on the non-target species' population may not occur. For example, the native New Zealand weevil *Irenimus aemulator* (Broun) is parasitized by the exotic parasitoid *Microctonus aethiopooides* Loan at a level equal to or greater than that of the target, *Sitona discoideus* Gyllenhal (Barratt et al., 1996; 1997), but detrimental effects on populations of *I. aemulator* have not been demonstrated.

Potential Impacts on Organisms of Conservation, Commercial and Aesthetic Significance

Non-target taxa known to be rare or threatened may require special consideration, especially if they are taxonomically related to the target (Van Driesche and Hoddle, 1997). However, the biologies of such species are often poorly known or unknown and the logistics of testing them with a potential agent may prove to be impractical. If target species are abundant, their

populations may support large populations of an agent, increasing the chances of “spill over” onto non-target species. This effect may locally depress populations of such rare species or even drive them to extinction (Howarth, 1991), especially at the edge of their range (Cullen, 1997). The reduction of distribution of *Pieris napi oleraceae* Harris in Massachusetts by the braconid *Cotesia glomerata* (L.), (introduced against the still common *Pieris rapae* L.), may be such a case (Benson and Van Driesche, unpublished). Evidence for effects on rare taxa is lacking and it has been suggested that they may not be so prone to impacts of exotic agents in their native habitat, where they avoid attack because of their low numbers (Cullen, 1997).

The need to test potential biological control agents against species of commercial or aesthetic significance is not based only on their relationships to a target pest but rather their perceived value. Testing is justified by the claims that: (a) any impacts on commercially important taxa are unacceptable, and (b) aesthetically important organisms are well known and valued by the public and may be “flagship” species that serve as symbols for invertebrate conservation activities. For example, representatives of the commercially valuable birdwing butterflies (*Ornithoptera* spp., Papilionidae) were tested to ensure that their larvae would not be parasitized by the braconid *Cotesia erionontae* (Wilkinson) before release of this species in Papua New Guinea for biological control of the Asian banana skipper, *Erionota thrax* (L.), was approved (Sands et al., 1993).

Methods and Interpretation

Selecting Non-Target Taxa for Testing with Agents

When conducting specificity tests with weed agents, potted non-target plants can usually be maintained for exposing to agents, but it is impractical to maintain cultures of many non-target arthropods for such testing. The number of non-target taxa that can be tested in an arthropod biological control project cannot, therefore, be as great as the numbers of potted plant species tested against weed control agents. To successfully run such tests, sufficient numbers of the appropriate stages of each non-target species of interest must be available from cultures, or obtained from the field and exposed to the agent in a way that will provide evidence of host suitability, which can then be compared with the suitability of the target host. Collection of suitable stages of non-target species from the field requires careful

establishment of identity of the species and evidence that stages collected are not already parasitised or diseased. Risks will remain that unrecognized effects influence host acceptance or development of an exotic natural enemy in a particular non-target species.

Information on the degree of taxonomic relatedness of non-target taxa is important when selecting species for use in centrifugal testing but a major impediment for selecting non-target species is lack of systematic knowledge of insects when compared with plants (Kuhlmann et al., 1998). Species of the same genus as the target, followed by ones in related genera, tribes or subfamilies can be used for appropriate testing (Table 2) (Sands, 1998). Difficulties arise when testing the host range of an agent if little is known of the taxonomic relationships of a target with indigenous fauna. Also, without knowing the phylogenetic relationships between a target species and its relatives, it may be very difficult to select for testing, related non-target taxa in the proposed country of introduction. Use of molecular methods for identifying phylogenetic relationships may be an option when conventional morphological features do not adequately clarify relationships, or when complementary information is required (Maley and Marshall 1998). Misidentifications of agents and their hosts, which sometimes occur in the literature and data attached to specimens, can affect conclusions about an agent’s host range, especially if taxonomic studies on the agents and their hosts are lacking.

Difficulties and costs of maintaining cultures of rare or threatened taxa for testing can be serious constraints to evaluating the host range of agents (Kuhlmann et al., 1998). For example, two major pests in Australia,

Table 2. Criteria for selecting non-target taxa for host range tests

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| <p>A. Phylogenetic centrifugal methods (morphological or molecular) can be used to determine the relationships</p> <p>B. The inclusion of threatened or other taxa of conservation significance may be desirable even when such species are not closely related to the target organism.</p> <p>C. Appropriate numbers of specific stages must be available by collecting or culturing.</p> <p>D. Tests may not be necessary when the host range is known from elsewhere and if taxa closely related to the target (e.g., in the same tribe, genus) are unknown in the receiving country.</p> |
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Helicoverpa armigera (Hübner) and *Helicoverpa punctigera* (Wallengren), have been targets for classical biological control but the introduction of at least one potential agent, the braconid *Microplitis croceipes* (Cresson), has been deferred in Australia due to difficulties in testing it with related, non-target taxa (D. Murray, *personal communication*). Although *M. croceipes* is specific to *Heliothis* and *Helicoverpa* species, the very rare *Helicoverpa prepodes* (Common) has not been cultured, its life history is unknown, and it cannot therefore be evaluated as a potential host for exotic biological control agents. If *M. croceipes* became an abundant parasitoid as a result of attack on the other two target pest species, the rare *H. prepodes* might be at risk.

Also, the results from laboratory tests in which test species are chosen based on their phylogenetic relationship to the target species may fail to detect distantly related or unrelated potential hosts that are suitable for development by a natural enemy (Van Driesche and Hoddle, 1997). In such cases, the life history, plant hosts, or habitat of the target may be more important in influencing the foraging and selection behavior of a parasitoid than the taxonomic relatedness among potential hosts.

Barratt et al. (1999) suggest that even organisms unrelated to the target should be tested with a potential agent if they occupy a similar ecological niche, for example, species that feed on related plant species, or all develop as leaf miners, or all are seed feeders, grassland dwellers, or canopy feeders. However, the logistics of testing a wide range of organisms on the basis of their similar ecological niche is likely to be impractical. A number of taxa, carefully selected on the basis of their relatedness to a target, their life history and choice of habitat, should, however, provide an indication of the likely degree of safety of an agent. When the hosts of natural enemies closely related to an agent are known, predictions for the agent's host range can sometimes be made. However, if the group of agents include in their host range unrelated taxa, such predictions are of little value (Sands, *in press*). If a potential agent can be shown to be unlikely to develop on any non-target taxa, or only on exotic pest species (given literature records of known host groups and occurrence of such groups in the fauna of the area targeted for introduction), there may be little need for any formal host specificity testing.

Table 3. Evaluating the host ranges of exotic arthropod agents

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| A. Information on hosts may be available from an agent's country of origin or where it has already been introduced |
| B. Laboratory experiments contribute to predicting the likely host range of an agent after its release in a receiving country. |
| C. Monophagous agents are preferred candidates, but many with "narrow" host ranges are often the most effective agents. |
| D. Generalist natural enemies with "broad" host ranges are not acceptable unless the benefits outweigh possible risks to non-target species. |

Assessing the Degree of Host Specificity of Potential Agents

Information on the host range of potential biological control agents may initially be compiled, based on records from the agent's country of origin (when known), from countries where it has been introduced, or from pre-release studies conducted in a quarantine facility (Table 3). Monophagous agents that complete development and reproduce only on one target species are preferred, but in practice most potential arthropod agents attack more than one species in their native range. While strictly monophagous parasitoids are rare or unknown for some groups (Zwölfer, 1971), some stenophagous species become functionally monophagous if introduced into countries where taxa closely related to the target do not occur.

Host specific biotypes (or races) of agents may be overlooked if specimens reared from samples from different hosts are not distinguished in surveys and subsequent colonization in quarantine. For example, a biotype of the pteromalid egg-predator *Scutellista caerulea* (Fonscolombe), originally from South Africa, developed only on the scale *Ceroplastes destructor* Newstead in the field in Australia, but in the laboratory it also developed on other exotic *Ceroplastes* species (Sands, *in press*). Another biotype of *S. caerulea* (morphologically distinguishable, Sands et al. 1986), developed in the field on several other Coccidae but not on *C. destructor*. Despite the importance of selecting biotypes specific to the target pests, there are no simple ways to detect such biotypes other than by extensive field and laboratory evaluation. The frequency of

parasitoid biotypes in nature indicates that they are likely to be overlooked when agents are collected and pooled only on the basis of their morphology. However, molecular methods may be useful for identifying host specific biotypes and separating them from a polyphagous species.

Multiple Choice and No-Choice Tests to Determine the Host Range

When conducting host range assessments in the laboratory, the pattern and sequence of contact between the agent and test species can affect the response observed. Tests that present each test species separately to an agent (usually naive individuals with no previous host contacts) is a “no choice test”. In contrast “choice tests” present several potential hosts to the agent simultaneously (Table 4). Choice tests typically, but not always, include the target pest in the mix of species presented to the agent.

As with the response of herbivores to plants, those of parasitoids or predators to arthropod hosts are affected by the test design. Among possible effects (see Edwards, 1993; Marohasy, 1998 for review) of test design are: (1) false positives, in which non-hosts are used by agents when deprived for long periods from their normal hosts, (2) false positives in which non-hosts are used when in close proximity to the normal host due to transference of stimuli, and (3) false negatives in which valid, but less preferred, hosts are ignored in the presence of a more

preferred host. For discussion we refer to these as: (1) “desperation” effects, (2) “spillover” effects, and (3) “diversion” effects.

Neither choice or no-choice testing is universally superior to the other and often there are advantages to running tests of both designs on the same agent. Some thought needs to be given to interpretation of the outcomes of sets of tests of varied designs. We can recognize four cases (Table 4):

Case I. Choice and no-choice tests both suggest that a given species is not a host for an agent. If no attack by an agent occurs in either design on a non target species, it may be assumed to be outside of the host range. Control tests are needed subsequently with the agent and target to confirm the ability of the agent to oviposit or feed, unless the target was included in the choice test.

Case II. Choice and no choice tests both suggest that a given species is a host for an agent. If a potential host is utilized under both choice and no choice designs, the test species may be assumed to be in the host range.

Case III. Choice test is positive, but no choice test is negative. If a species is utilized *only* in a choice design (but not in a no choice design), the positive result in the choice test is likely to be a spillover effect caused by stimuli from presence of the target host. In such cases, the non-target tests species is likely to be outside the fundamental host range.

Table 4. Interpreting test programs in which both choice and no choice designs are employed for the determination of insect host ranges

	Choice Test Results (-/+) and Interpretation Relative to Result (-/+) in No-Choice Test	
	- Result	+ Result
No-Choice Test, - Result	Case I Test species <i>outside</i> host range	Case II Test species is outside host range and positive result in Choice Test is likely due to “spillover effect”
No-Choice Test, + result, immediately	Case IV-A Test species is inside of host range and negative result in Choice Test is likely due to “diversion effect”	Case III Test species <i>inside</i> host range
No-Choice Test, - result, + result, after several days deprivation	Case IV-B Test species is outside of host range and positive result in No-Choice Test is likely due to “desperation effect”	

Case IV. Choice test is negative, but no-choice test is positive.

- **Subcase A.** A positive response to a test species is present immediately in the no-choice test. In this case the test species is likely to be a valid host and failure to detect it in the choice test is due to the “diversion effect” caused by presence of a more strongly preferred host.
- **Subcase B.** A positive response to a test species in the no-choice test is not initially present, but only develops after extended periods (e.g. several days) of deprivation. In this case, the positive response in the no choice test is likely to be erroneous, due to the “desperation” effect. The negative result is then a reliable indication that the test species is outside the host range.

Effects of Confinement on Natural Enemy/Host Interactions

Confinement in cages or the laboratory may disrupt the normal behavior of parasitoids or predators and is equally of concern in weed biological control projects (Cullen, 1989). False positive results are commonly experienced when agents encounter non-target species in the laboratory under environmental circumstances that would not occur in the field (Table 5). For example, Field and Darby (1991) found that in choice tests with the target species *Vespula germanica* (Fabricius), the parasitoid *Sphexophaga vesparum* (Curtis) parasitized two non-target species of *Ropalidia*, but in no-choice tests, the non-target species were not attacked. Apparently the parasitoids were stimulated into attacking the non-target species, by the presence of the host or saliva from the larvae of the natural host (Field and Darby, 1991). In another example, a biotype of the pteromalid egg predator *S. caerulea* that was adapted to the soft scale *C. destructor* was easily reared on the related *Ceroplastes sinensis* Del Guercio in the laboratory (when its host *C. destructor* was not available for culture). However, after *S. caerulea* became established in the field, only *C. destructor* was attacked (Sands 1993). In this example, close proximity of agent and non-target apparently disrupted the host recognition, leading to false positive results.

False negatives, in which an agent failed to attack or develop on a species in the laboratory but subsequently did so on the species after release, have not been well documented for arthropod agents. However, Barratt et

Table 5. Effects of confinement on host range tests with arthropod agents

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| A. Choice tests are more susceptible to false positive results than no-choice tests when carried out in cages. |
| B. Cages may inhibit mating and induce false positives for false negative host recognition and acceptance. |
| C. Tri-trophic interactions and behavior are often disrupted when agents, plants and potential hosts are confined in cages. |
| D. Habitat specialists may be very difficult to evaluate as habitats often cannot be reproduced in laboratory tests. |
| E. Cage design or materials may influence agent/target interactions. Controls must be included by presenting agents with suitable targets, to avoid false negative results with non-targets taxa (when parasitoids fail to oviposit for physiological reasons). |

al. (1997) suggested that cage tests may have underestimated the host range of the braconid *M. aethiopoidea*, which failed to attack the weed biological control agent *Rhinocyllus conicus* (Froehlich) in the laboratory but did so after its establishment in the field.

For weed biological control agents, false positive results seen in choice tests are believed to have been induced by experience-dependent changes in the agents' responsiveness, adsorption of volatile kairomones onto test plants, or indiscriminate behavior of agents when confined in cages (Marohasy, 1998). In the case of arthropod agents, major problems may arise if agents are held in confinement with other organisms that would rarely if ever, be found naturally in close proximity with the host. One or more behavioral phenomena may then lead to acceptance of an organism as a host by a natural enemy under such conditions (e.g., example in Field and Darby, 1991). Moreover, the presence of kairomones from a host in close proximity with another test species may induce a natural enemy to mistakenly recognize the non-target species as a host. Cages used for routine rearing of an agent may not be appropriate for host range testing because the amount of space required for mating and oviposition in a favored host may not be comparable with the requirements for expression of an agent's behavior towards a non-target species. For example, confinement can disrupt diapause in some parasitoids, especially if diapause is regulated by the host physiology. Such circumstances would require a

more detailed study of the natural enemy and its host to avoid misinterpretation of host specificity tests.

The physiological state of a non-target host may change in a laboratory environment and affect parasitoid development. Such effects need to be considered when assessing host range tests. For example, the first instars of the parasitoid *Anicetus communis* (Annecke) only break diapause in the host scale *C. destructor* when the adult scale is in a pre-ovipositional state; otherwise, no development of the parasitoid occurs in hosts for up to 8 months on plants in the field (Sands *et al.*, 1986). In the laboratory if the plant host is stressed, diapause in *A. communis* is broken and the parasitoid develops in the pre-ovipositional scale. In such cases, failure of a parasitoid to develop when in diapause in a non-target host on an unstressed plant, could easily be misinterpreted as host unsuitability, rather than being attributed to the predisposing condition of the plant.

Cage design, size, materials, and access to light may all influence the responses of natural enemies to their hosts, and each species of agent and host may require specialized treatments. For example, cage size influenced the levels of parasitism in weevils by the braconid parasitoids *M. aethiopoidea* and *Microctonus hyperodae* Loan (Barratt *et al.*, 1999). Food offered to both agents and the target species in the cages is also important. For example, the longevity and fecundity of an agent may be reduced by poor nutrition, affecting responses to non-target species being tested. Overcrowding of agents may inhibit their mating or host recognition. The number of agents and stages of hosts, or their ratios, may require adjustment to avoid anomalous results in both choice and no-choice tests (Barratt *et al.*, 1996). Care must be taken to ensure that the non-target species is not presented on a plant that would not be its natural host under field conditions.

Cage materials, especially synthetic substances, may adsorb kairomones from contact with a target species. In choice and no-choice tests these adsorbed compounds on cages may promote attack by agents on non-target species exposed in affected cages. These errors are most likely to occur in choice tests but can be avoided if necessary by replacing the cage materials after each test. Confinement in cages may also disrupt mating behavior. Sometimes this problem can be corrected by using black materials that transmit light instead of white cage materials, which scatter light. For example, pairs of *Aprostocetus ceroplastae* (Girault), a parasitoid of soft scales, failed to mate in white cages but mated

immediately when exposed to sunlight in cages made from black organdy (Sands unpublished). Fine black materials transmit light in a different way than do white or pale colored cage materials, which scatter light, sometimes affecting both mating and ovipositional behavior of parasitoids. Plexiglass may also be useful for replacing white materials.

Superparasitism leading to host mortality frequently results from confinement of several gravid agents with hosts. To avoid crowding effects, the exposure period must sometimes be adjusted so that an individual attracts oviposition by only one agent before it is removed and transferred to its own host plant. This effect was also observed when the braconid *M. aethiopoidea* attacked the alfalfa weevil, *Hypera postica* (Gyllenhal), in the laboratory (Neal, 1970). Tests may require withdrawal of a host immediately after exposure and parasitoid oviposition, to ensure that optimal chances are provided for the development of a parasitoid.

Choice tests may exacerbate effects of confinement on selection of hosts by an agent. These problems can sometimes be avoided by no-choice tests using sequential, separate exposures of target host and test species. For example, Sands and Coombs (1999) conducted no-choice tests by exposing gravid females of *T. giacomelli* (Tachinidae) alternatively to the target host, *N. viridula*, for two hours and then to each non-target species, each for two hours, to record oviposition. The number of eggs deposited on the target host were then compared with the number of eggs (if any) deposited on the non-target species, for each two-hour period. In this way, false positive responses due to the “spillover” effect (Table 4) were avoided. Possible effects of conditioning by prior exposure to the target were separately evaluated by exposing gravid naive parasitoids only to the non-target species tested.

Tri-trophic Effects May Influence Host Acceptance

Host range tests can be designed to take into consideration the kinds of tri-trophic interactions that often affect host recognition of agents. Such effects are important especially when a plant host of a target organism is a cue for host location (Table 6). Without a particular plant substrate, the searching activity of some parasitoids may be severely reduced. For example, *Eretmocerus* spp. from Spain and India performed well as a parasitoid for *Bemisia tabaci* (Gennadius) (biotype B) in all crops; however, *Encarsia* sp.

nr *pergandiella* Howard performed well on melons but not on cotton or kale (Goolsby et al., 1998).

Some plants stimulate or inhibit ovipositional responses in parasitoids. For example, *Trichogramma* spp. rarely parasitize eggs of *H. armigera* on pigeon pea (*Cajanus cajan* [L.] Millsp.), but levels of parasitism are much higher if the eggs are deposited on other plants (Romeis et al., 1997). Similarly, the eggs of *Leptocorisa oratorius* (F.) (Hemiptera: Alydidae) on rice were more heavily parasitised by the scelionid *Gryon nixonii* Masner than were eggs deposited on six other plant hosts of the bug (Morrill and Almazon, 1990). Characteristics of pigeon pea that inhibited parasitism included volatile compounds (emitted by leaves and pods) that repelled or deterred the parasitoids, leaf trichomes that inhibited the parasitoid searching behavior, and exudates that trapped the adult parasitoids. Feeding damage on plants may also affect the behavior of parasitoids. For example, Steinberg et al. (1993) demonstrated the attraction of the braconid *Cotesia glomerata* (L.) to cabbage damaged by larvae of its host, *Pieris brassicae* (L.). In host range tests, non-target species should be presented to parasitoids both with and without their associated host plants.

It may be necessary to evaluate an agent with a target on several of the target's plant hosts, and false positives may occur if non-target species are presented on host plants of the target species because the agent may be stimulated to oviposit in the test species by the plant substrate. For example, the egg parasitoid *Ooencyrtus erionotae* Ferriere was introduced from southeast Asia into Guam, Saipan, Mauritius, and Hawaii for control of the banana skipper, *E. thrax* (Waterhouse and Norris, 1987; Sands et al., 1993). When laboratory tests were conducted with *O. erionotae* in Papua New Guinea, the presence of banana leaves affected the species of hosts attacked. Parasitoids, in the presence of leaves, oviposited in eggs of *Cephrenes augiades* (Felder), a species belonging to the same subfamily (Hesperiinae) as *E. thrax* (Sands, 1991) and also attacked other Lepidoptera that were not taxonomically related to the target or attacked in the field (Sands, unpublished).

Table 6. Responses of arthropod agents to plants

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|---|
| A. Physical or chemical characteristics of plants may predispose host recognition by an agent. |
| B. Plants may stimulate, reduce, or prevent host recognition, leading to false positive or negative interpretations of the specificity of agents. |
| C. Plant damage from feeding by herbivores and their feces may stimulate responses in agents. |
| D. Plants form part of the habitat specialization of agents. |

Using Developmental Parameters to Assess Host Suitability

Differences in the life history parameters of natural enemies have been used as indicators of host suitability and, thus, the likely levels of impact on non-target taxa compared to that in the target host. For example, Wright and Kerr (1988) compared the development of the parasitoid *Encyrtus saliens* Prinsloo and Annecke in two scales. *Pulvinaria delottoi* Gill was shown to be less suitable than *Pulvinaria mesembryanthemi* (Vallot) because: (a) development of *E. saliens* in *P. delottoi* required more thermal units, (b) from the same initial host size, immature parasitoids in *P. delottoi* developed more slowly than *P. mesembryanthemi*, (c) adult parasitoids emerging from *P. delottoi* were smaller and less fecund, and (d) small *P. delottoi* received fewer parasitoid eggs, and these were deposited at a lower rate when compared to oviposition rates on *P. mesembryanthemi*. The authors concluded these results demonstrated that *P. delottoi* was a less suitable host for development and maintenance of *E. saliens* than was *P. mesembryanthemi*. The authors also predicted that *E. saliens* would persist better on *P. mesembryanthemi* and attack *P. delottoi* only when it occurred with the primary host in mixed infestations. Field evaluation of these predictions is lacking, but the approach provides a framework for using laboratory data to predict field outcomes among host and parasitoid populations.

The same approach has been used to infer that a natural enemy might have a greater impact on a target than on non-target species after the natural enemy is established. Sands and Coombs (1999), when evaluating the tachinid *T. giacomellii* for control of *N. viridula*, found that although the parasitic fly laid eggs on six non-target pentatomids, only three supported its immature development. On the three suitable non-target hosts, parasitoid fecundity and longevity were reduced when compared with that on the target species, *N. viridula*.

These findings plus the reduced size of the puparia from these non-target species, indicated that those species were sub-optimal hosts for the parasitoid. If an agent's population size depends on its utilization of sub-optimal hosts, it is likely to have little effect on density. However, if the geographical ranges of abundant, high quality and sub-optimal hosts overlap, population densities of sub-optimal hosts may be reduced via "spillover" of parasitoids deriving from the better host.

The quality and health of the agent, the target species, and non-target organisms need to be monitored when host specificity tests are undertaken, since they affect the developmental parameters (Barratt et al., 1999). Infection with micro-organisms may affect natural enemy/host interactions, and these pathogens must be removed from cultures if meaningful tests are to be carried out. Microsporidia are commonly found in insect cultures and are well known in parasitoids. For example, Sheetz et al. (1997) identified a species of *Nosema* infecting the ovaries of the parasitoid *E. nr pergandiella*, that lead to a steady decline in parasitoid fecundity. An antibiotic was used to effectively treat the microsporidium infection in the parasitoid culture, indicating that there may be a place for more routine use of antibiotics in insect cultures, to ensure that host range tests are not biased by the presence of similar infections. Such infections if overlooked, might easily influence the assessment for host suitability of a non-target species.

Effects of Conditioning and Prior Experience

The process of locating a host and confirming its suitability by a parasitoid is a progressive response to environmental and host cues that lead finally to acceptance when oviposition occurs (Vinson, 1976). Although many parasitoid species have both preferred and less favored hosts, adaptive behavior allows parasitoids to focus on those species that have already proven to be appropriate and locally available hosts (Vet, 1985). The individual, prior experience of an agent is known to sometimes affect its behavior towards another host and may affect the outcome of both choice and no-choice tests. Tests with agents can be designed to determine if prior exposure of parasitoids to a target host influences the subsequent acceptance of a non-target species (Sands and Coombs, 1999).

Arthropod Predators and Their Evaluation

Arthropod predators are second only in importance to parasitoids as agents for classical biological control of arthropods. Although many species of exotic predacious arthropods have been established in various countries without host range testing, few examples of detrimental non-target effects are recorded. Two important groups, coccinellids and mites, are often generalists, adapted to groups of hosts or a particular type of habitat. Adaptation to certain habitats or plant hosts may be important when considering the acceptability of species otherwise considered generalist predators.

In some species of predators, adults may be generalists, but immature stages may be more specific in their choice of prey. For these cases, separate tests with the appropriate stages of prey may be needed for the different predator life stage (Table 7). A number of adult coccinellids are generalists, but have immature stages that are much more specific. For example, *Rodolia cardinalis* (Mulsant) is specialized to feed and breed on only a few species of margarodid scales, but adults of *R. cardinalis* can subsist on a wide range of other insects and nectar for up to three months. However, margarodid scales are strictly required for development of the immature stages (V. Brancatini, unpublished). Despite the close adaptation of this coccinellid to its prey, the undoubted value of *R. cardinalis* for controlling *Icerya purchasi* Makell might be questioned if only the host range of adults were tested. There is little evidence available from field or laboratory studies on *R. cardinalis*, to demonstrate any preference for *I. purchasi* over *Icerya aegyptiaca* (Douglas), even though on some tropical atolls *R. cardinalis* is unable to maintain control of the latter species (Waterhouse 1993).

Field association of a predator with a target prey is a common means of choosing an agent for introduction against a particular pest. The coccinellid, *Curinus coeruleus* (Mulsant), for example, was imported to Hawaii from Mexico in 1922 for control of the coconut mealybug, *Nipaecoccus nipae* (Maskell) (Waterhouse and Norris, 1987). Such field associations may, however, give misleading impressions about an agent's actual preference among a broader range of potential prey. In the case of *C. coeruleus*, the subsequent invasion of Hawaii by the leucaena psyllid, *Heteropsylla cubana* Crawford, presented *C. coeruleus* an additional prey. A preference by *C. coeruleus* for the psyllid over the mealybug, rapidly became evident as *C. coeruleus* populations, formerly present only at low densities, increased significantly on the psyllid. Such cases argue strongly that predator preferences need to be

Table 7. Evaluating predators

A. Habitat specialization may be a primary cue for prey location.
B. Host ranges of adults may differ from those of immature stages.
C. Phoretic mites may require specific evaluation of intermediate carrier agents.

determined by testing and not merely surmised from field associations.

When separate host range tests are carried out on adult and immature stages, differences in host/prey interactions can be detected. Predators, in common with other natural enemies, might be adapted to prefer to forage under certain environmental conditions or on certain plant substrates. These affinities can be revealed by tests in the laboratory and results used to evaluate the association of a natural enemy with a non-target prey. For example, woody stems of the host plants for the ortheziid scale *Orthezia insignis* Browne were shown to affect the performance of the coccinellid *Hyperaspis pantherina* Fursch when cultured in the laboratory (Booth *et al.*, 1995). The coccinellid was easily cultured when its prey was held on plants with woody stems, but on whole infested plants held in large cages, very few larvae of the predator matured due to scarcity of woody stems infested with the host, and their development was impossible to monitor. Such adjustments to selecting a plant substrate to rear prey with its predator in the laboratory need to be developed before meaningful host specificity tests can be applied to non-target species on their own host plants.

Determining the host range of predatory mites, a group of predators cultured for suppression of pest tetranychid mites, poses difficulties. Micro-habitat and tri-trophic cues are likely to be important to consider when testing their responses to prey, and species adapted to forage in host plants of a target may provide an appropriate choice. Phoretic mites, such as those used for biological control of dung-breeding flies, may require that we also evaluate the suitability of the micro-habitats and of symbiotic carrier agents.

Risk Assessment: Acceptance or Rejection of a Potential Agent

What is the breadth of host range that should be considered unacceptable? Should agents that develop on non-target host or prey of a particular taxonomic level

of relatedness to target be excluded? For example, should an agent be considered unacceptable if it feeds on other members of the same subfamily, tribe or genus as the target? Most difficulties arise when making decisions about agents that complete development on a limited number of non-target taxa in the laboratory. Such tests demonstrate the potential to develop on non-target taxa even though the agent may fail to do so in the field. Agents may be considered to be acceptable if they complete development and reproduce only on non-target species that are closely related (same genus or tribe) to the target pest. However, if more distantly related (different genus or tribe), unrelated (e.g., different family or order), or beneficial organisms are shown to be suitable hosts, the potential for detrimental effects on unrecognized organisms should be considered before release of an agent.

When an agent develops on one or more non-target taxa, the benefits need to be carefully weighed against any risks of undesirable effects. Such risk assessment aims to reduce risks, but not to completely eliminate them (Bourchier and McCarty 1995). The likely benefits, i.e., effective control of the target pest and associated benefits, need to be compared against possible declines or extinctions of the non-target species that might be attacked. Estimates of host ranges of potential agents that are based on results of tests carried out in the laboratory influence governmental decisions about whether or not to release the agents. For example, four egg parasitoids in the same genus *Ooencyrtus* were not released in the United States for control of *N. viridula* 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).

Information on the range of habitats used by an agent in the country of origin may provide evidence to suggest that a non-target taxon that only occurs in different habitats would not be likely to be at risk. If an agent and target are known to be adapted to an environment different from that in a receiving country where a non-target species is present, it can be argued that the risks of the agent adapting to that environment are minimal.

Environmental criteria were used to evaluate non-target risks after the release of *Cotesia flavipes* Cameron, a braconid wasp released in Kenya against the stem borer *Chilo partellus* (Swinhoe). Host searching of *C. flavipes* was limited to plant

communities of long-stemmed grasses in natural and agricultural habitats, where the only acceptable hosts present were lepidopteran stem borers. Natural grasslands were occupied by several native parasitoids, including *Cotesia sesamiae* (Cameron), which was possibly at risk of displacement by *C. flavipes* in the agricultural habitats. Displacement of *C. sesamiae* was less likely in the grasslands since its response to native grasses was stronger than to sorghum (Overholt et al., 1994). It was suggested that some displacement of *C. sesamiae* by *C. flavipes* might occur where its habitat overlaps with that of *C. partellus*, but that *C. sesamiae* would persist in native habitats where the dominant host species were unsuitable for *C. flavipes*.

Assuming tests can demonstrate the host range of potential agents and that only agents with “narrow” host ranges are candidates, the process of risk assessment begins with making a decision whether or not some complete development of an agent on a non-target species is acceptable, as few parasitoids are strictly monophagous. Secondly, the likelihood and nature of any detrimental effects (e.g., decline in density, extinction) need to be balanced with the benefits of controlling a pest. For agents proposed for release that are not narrowly specific, more comprehensive assessments of potential impacts and benefits are needed before reaching decisions. Polyphagous agents, while often undesirable, may be necessary and beneficial in specific contexts.

Discussion

Assessment of the host range of potential arthropod parasitoids and predators before they are introduced from another country is usually necessary to reduce risk of harm to related non-target organisms. For some agents, laboratory host range tests may not be required if appropriate information is available from overseas and when there are no species related to the target in the receiving country. In other cases, tests with non-target organisms may be required to estimate the likely host range before introduction of the agent. Some host range tests are easily implemented, but others are difficult to conduct or evaluate. In some cases, it may be impossible to approve the release of potentially valuable agents because non-target taxa or their appropriate stages needed for testing are not available, or because there are anomalies in the behavior of an agent when confined in cages or when tri-trophic responses and other difficulties affect interpretation of results.

The interactions of parasitoids with plants need to be considered when designing host range tests. For example, the effects of different food plants used by target or non-target species may influence conclusions about performance of an agent, when it, a potential host and the host's food plant are brought together. It may be necessary to evaluate performance of an agent with the target species on its various plant hosts before comparative studies are initiated with non-target species.

Some instances of development of introduced agents on non-target taxa must be considered acceptable if classical biological control of arthropods is to continue, since mono-specific agents are few and often are not available. If the use of host range tests is to be realized, they must be limited to a few representative non-target taxa or those of special conservation significance. While each assessment will be made on a case by case basis (Barratt et al., 1999), a framework for testing procedures exists that could be adopted as the basis for such testing protocols. Caution will be needed when agents are shown in the laboratory to complete development on beneficial and other non-target taxa (Van Driesche and Hoddle, 1997). There is a need to review case histories where non-target taxa support development of exotic natural enemies to determine the nature and dynamics of impact on their populations. Excellent examples are available for case studies to quantify impacts on non-target taxa. For example, *T. basalis* has been introduced to control *N. viridula* in many countries, in some of which it also develops on eggs of many unrelated pod-sucking bugs. Its actual impact on these non-target species has not been fully investigated.

While biological control is the most cost effective and safe alternative to pesticides and genetically modified plants for the management of pest arthropods, resources for prolonged detailed studies on the interactions of agents and non-target species are not readily available. In cases where non-target species are shown by laboratory evaluation to be attacked by an agent, the likely benefits of pest control must be weighed against the possibility of some detrimental effects. Without neglecting the importance of protecting non-target taxa, more evidence of detrimental impacts is required before the release of only mono-specific species becomes a priority and a major limiting factor for arthropod biological control.

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