HOST RANGE TESTING TECHNIQUES FOR PARASITOIDS AND PREDATORS

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INTRODUCTION

Information on the host range of parasitoids and predators intended for use as biological control agents is needed so that harm to nontarget native species is unlikely to occur after an agent is introduced to countries outside its native range. Information useful in forming estimates of host ranges may be obtained from the taxonomic placement of the agent and its known hosts, field observations made in an agent's native range, or data from laboratory host range tests. For many years, classical biological weed control programs have routinely conducted laboratory host range tests on exotic agents before they are released. At first, these efforts were intended only to avoid damage to economically important plants, such as crops or species used as ornamentals. Later, testing programs evolved to include the goal of also protecting non-economic native flora, especially species closely related to the target weed (Wapshere, 1974; Harley, 1979; Withers, 1999, Pemberton, 2000). The efficacy of the methods used to forecast host ranges of weed biological control agents varies and these are still undergoing further development (Hill, 1999; Palmer, 1999).

Until the 1980s, parasitoids or predators were not subjected to laboratory host range tests unless they were thought able to attack beneficial organisms (Ertle, 1993). In the 1990s, use of host range tests to assess safety of parasitoids and predators became recognized as essential for sound future work (Van Driesche and Hoddle, 1997), in view of claims that harm had occurred to native insects (Howarth, 1991; Simberloff and Stiling, 1996). In some instances, highly polyphagous agents have attacked diverse native species. The tachinid *Compsilura cocinnata* (Meigen), which was introduced into the United States in1905 for control of the gypsy moth, *Lymantria dispar* (L.), parasitizes more than 200 lepidopteran hosts, and currently appears to be an important source of mortality for larvae of swallowtail butterflies and giant silk moths (Boettner *et al.*, 2000).

Some natural enemies, emerging in large numbers from a target pest, may periodically "spill over" and cause periodic but important mortality in nontarget species. This is principally of concern in cases in which the biological control program is not very successful and the target species remains abundant and supports a large population of the natural enemy (Follett *et al.*, 2000; Henneman and Memmoth, 2001). This effect is thought to possibly depress populations of rare species or even drive them to extinction (Howarth, 1991; Holt and Hochberg, 2001), especially at the edge of their range where the opportunities for recolonization are reduced (Cullen, 1997). In the northeastern United States, the geographic range of a native butterfly, *Pieris napi oleraceae* Harris, is believed to have been reduced by the braconid *Cotesia glomerata* (L.), introduced against the pest species *Pieris rapae* (L.) (Benson *et al.*, in press).

We believe that host range testing of parasitoids and predators is likely to quickly become standard practice in all countries, required by regulatory governmental authorities. However, the methods used to estimate host ranges of parasitoids and predators are not as well developed as those used for weed biological control agents (Van Driesche and Hoddle, 1997). We see an urgent need to develop better methods for improving the accuracy of such laboratory tests. We should attempt to benefit from insights gained in assessing host ranges of herbivores, but we should not presume that all

the underlying principles will be the same or that current techniques are the most appropriate or even sufficient for this new group of agents. Finally, we must recognize that predators may require different testing approaches than parasitoids (Sands, 1997, 1998; Sands and Van Driesche, 2000; Kuhlmann *et al.*, 2000).

Here we discuss methods used to estimate the host range of parasitoids and predacious arthropods, including inferences that can be drawn from taxonomic information alone, inferences from host associations observed in the agent's native range, and techniques for use in laboratory tests.

INFERENCES FROM TAXONOMIC LITERATURE

The taxonomic placement of the target pest or other known hosts of a parasitoid, together with knowledge of the fauna in the area proposed for the parasitoid's introduction, can suggest which native species might be attacked by an introduced natural enemy. Similarly, the taxonomic placement of a parasitoid itself may indicate something of the limits of a species' likely host range, based on that of the host ranges of other closely related parasitoids.

The taxonomic placement of the known hosts of a proposed agent can be used to select native species from the area proposed for the introduction that are in the same genus, tribe, or subfamily, and which have not previously been exposed to the parasitoid. Such native taxa are obvious candidates to include in host range tests. For example, before the introduction of the braconid *C. glomerata* to the Americas, native species in the genus *Pieris* (in North America) or other related genera in the Pierini (in South America) would have been the logical species to assess as non-target native species at potential risk. This approach was used by Barratt *et al.* (1997) to assess risks to native New Zealand weevils from introduced euphorine braconids in the genus *Microctonus.* The same process can also be used to identify important non-native species, such as previously introduced biological control agents, that might be at risk. For example, if authorities in Australia wanted to mount a biological control project against *Hypsipyla robusta* (Moore), a major pest of red cedar (*Toona ciliata* M. Roem), the pest's taxonomic placement in the tribe Phyticini (Lepidoptera) (Shaffer, 1996) would dictate that the biological control agent *Cactoblastis cactorum* (Berg), found in the same tribe, should be included in lists of potential hosts for laboratory testing because protection of *C. cactorum* is critical for continued biological control of exotic pest cacti (*Opuntia* spp.) in Australia (Sands and Murphy, 2001)*.*

The taxonomic placement of a parasitoid also provides information on its likely host range. Many groups of parasitoids characteristically attack hosts belonging to a particular taxonomic group. For example, encyrtids in the genus *Metaphycus* are parasitoids of soft scales (Coccidae) and coccinellids in the genus *Rodolia* are predators of fluted scales (Margarodidae). However, exact host ranges are species-specific, and in many cases even closely related species of parasitoids (e.g., the same genus) can attack and develop on quite unrelated hosts (different genera or tribes) (Sands, 2000).

Several practical problems arise with use of taxonomic information. First, the literature contains errors due to misidentifications of parasitoids or their hosts and inaccurate host information may accompany pinned parasitoids in collections. Second, if the receiving region is large (a continent rather than an island), there may be so many species related to the hosts of a prospective agent at the higher taxonomic levels (tribe or subfamily) that testing them all is not possible. Third, some species of parasitoids may be mislabeled by their taxonomic names when several cryptic species or biotypes that vary in their host ranges are lumped under one name. To guard against such problems, agents collected from geographically isolated populations should be treated as potentially distinct entities that may have the same or different host ranges.

DNA analyses can be used to obtain markers for identification of each entity (e.g., Goolsby *et al.*, 2000). *Ageniaspis citricola* Logvinovskaya (Encyrtidae), for example, collected in Taiwan and

Thailand for introduction to Florida against citrus leafminer, *Phyllocnistis citrella* Stainton (Lep.: Gracillariidae) proved on close analysis to be separate species (Hoy *et al.,* 2000; Alvarez and Hoy, 2002). To determine if these entities have the same or different host ranges will require that host range tests be conducted for each stock separately. For example, Madeira *et al.* (2001) elected not to release a weed biological control weevil, *Oxyops vitiosa* Pascoe, from a second source (other than the one subjected to host range testing). Even though no tangible differences could be recognized, the populations differed slightly in color and size, suggesting a need for a second evaluation of host specificity before release of individuals from this second population. Such complexity needs to be recognized, so that the populations introduced are actually the same as the ones whose host ranges have been estimated in laboratory tests, to ensure that desired levels of host specificity are obtained.

INFERENCES FROM HOST/HABITAT SURVEYS IN THE NATIVE RANGE

Field studies in a biological control agent's native range can provide further useful information on its host and habitat preferences. Kuhlmann *et al.* (2000), for example, used field surveys in Europe of mirid plant bugs and their parasitoids to define the host and habitat ranges of various mirid parasitoids being considered for introduction to North America. Similarly, information on adaptation of the tachinid *Trichopoda giacomellii* (Blanchard) to grasslands and open crop habitats in Argentina proved to be valuable for predicting the non-target species of pentatomid bugs likely to be attacked when it was introduced into Australia for biological control of green vegetable bug, *Nezara viridula* (L.) (Sands and Coombs, 1999).

Such surveys greatly amplify the body of knowledge about a candidate agent's hosts. This new information can then be used in the same ways as described previously for information taken from the literature. If there are uncertainties about the significance of infrequently observed host-parasitoid combinations, their validity can be assessed by tests run in outdoor cages or in field plots where both target and non-target organisms can be exposed together under natural conditions. Such test conditions have the advantage over laboratory tests in that the full range of behaviors leading to host acceptance, feeding, and oviposition can occur under the actual environment found in the parasitoid's habitat. However, these field studies have the disadvantage that native insects from the proposed area of introduction cannot be included unless they are also native to the area in which the parasitoid is being collected (as such test species might escape from the tests and become invasive themselves). A further problem that may occur is that the hosts of many parasitoids or predators have low natural densities, or may be difficult to locate because we have too little information about their habitat preferences or seasonality.

TECHNIQUES FOR LABORATORY ESTIMATION OF HOST RANGES

Once a parasitoid that is a prospective biological control agent has been imported into quarantine and its identity confirmed, the hypotheses about its host range generated from the literature and any field surveys done in the native range can be tested in formal laboratory host range tests. The first step is to be sure that all unwanted parasitoids or pathogens have been removed from cultures of natural enemies and prospective hosts before any host range tests are carried out. If it is suspected that a parasitoid may act as a facultative hyperparasitoid or kleptoparasitoid, then the range of hosts to be considered in testing has to include other primary parasitoids of the target pest, as well as those of the native relatives of the pest.

Host range tests aim to demonstrate if adult agents feed on or oviposit in a test species, or if the agent's immature stages can feed and develop on a given host or prey. Such tests are complicated by the fact that some natural enemies use environmental cues as part of their host recognition process and by the fact that the expression of host preferences is influenced by both the choices presented, as

well as the agent's experience and current physiological state. As a consequence, it is unfortunately possible to construct host range tests that are not accurately predictive of field host ranges if these factors are not taken into account. In the following sections, we discuss the influence of several key factors that affect parasitoid host acceptance and preference in such tests. These are (i) the physical features of the test arena (size, lighting, etc.), (ii) the design of the test (sequence in which agent encounters choices, if any), (iii) the agent's past contacts with hosts (experience) and its current physiological status (egg load, hunger level), and finally, (iv) the host plant on which the parasitoid-host encounter is staged.

First we make some broad points to bear in mind for all host range testing.

1. **Use controls.** All host range tests should include the exposure of some parasitoids or predators to the target host as controls to demonstrate that the group of parasitoids or predators used in a given test had adequate health and fertility to successfully parasitize suitable hosts. In the absence of such controls, failure to parasitize or prey on a given non-target species because it was not an acceptable host could not be distinguished from failure to attack it merely because the parasitoids used were of too low a quality to attack any host species.

2. **Use high quality agents.** Agents used in tests must be of suitable age and reproductive maturity so as to be ready behaviorally to recognize hosts and oviposit in them. Agents should have had adequate food and water before the test, and the test must use appropriate temperatures, humidity, and light regimes. Depending on the agent, water on moistened pads and honey droplets may be required during the test.

3. **Use high quality test host species.** It is preferable to culture the non-target species used in host range testing, but if field-collected specimens must be used, it is important that they be healthy and not already parasitized. Hosts used in tests must be free of pathogens, which sometimes infect laboratory cultures or field populations.

4. **Be alert for host-induced diapause in parasitoids.** On occasion, immature parasitoids enter diapause in their hosts and their failure to develop could be mis-interpreted as lack of host suitability. For example, when immature adults of the coccid *Ceroplastes destructor* Newstead are parasitized in late summer by the encyrtid *Anicetus communis* Annecke, no larval development commences until the host matures in the warmer spring months, often more than six months after parasitization (Sands *et al*., 1986).

5. **Note parasitoid behavior toward potential hosts.** Differences in the behavior of adult female agents when exposed to target versus non-target species may provide useful indicators of host recognition or acceptance. For example, some scelionid egg parasitoids in the genus *Telenomus* visibly mark the corion of their hosts to prevent superparasitism. Also some braconids, such as *Cotesia erionotae* (Wilkinson), exhibit specific pre-ovipositional displays with their larval hosts, but not with non-target species (Sands *et al*., 1993). This parasitoid only displays when preparing to oviposit in its true hesperiid host, *Erionota thrax* (L.). It does not display with non-target hosts, although some palpating, probing, and even oviposition does occur with *Cephrenes augiades websteri* Evans, a related non-target hesperiid in the same subfamily.

6. **Choose appropriate instars.** Parasitoids of larval or nymphal hosts frequently oviposit in specific instars and complete development in later instars or pupae. When a non-target species is morphologically similar or related to a target species, it is most logical to select for testing the same life stage and instars as for the target species. For non-target species that are not closely related to the

target species, the appropriate life stage or instar to use in tests may be more difficult to select. An obvious approach is to match by instar number, but if this results in larvae that differ strongly between target and nontarget species in size, it may be better to match by size.

7. **Select conditions that limit host mortality.** For parasitoids attacking young larvae, if the exposure time is too long or the parasitoid-host ratio too high, many hosts are likely to die from the trauma of superparasitism. This problem can be corrected by exposing hosts to only one gravid parasitoid at a time and then removing the test larva from the test cage immediately after parasitization to prevent subsequent attacks. This approach was used by Sands *et al*. (1993) with the braconid *C. erionotae*, a parasitoid of banana skipper (*E. thrax*). They placed a single larva at the tip of a wood sliver, introduced it into a cage with gravid parasitoids, and then withdrew the host after one parasitoid had landed on the host and oviposited, but before other parasitoids did so. Such parasitized larvae can then be reared on appropriate plant material until they pupate or develop signs of parasitization. The method is also valuable for assessing the behavior of parasitoids to larvae because small portions of plant hosts, plants with feeding damage, or host fecal pellets can be added one at a time and the parasitoid's response observed.

Now we return to our discussion of factors that may affect laboratory estimates of host ranges.

Arena Effects

Choice of a testing arena with appropriate size, lighting and constituent materials is an important feature of the design of host range tests. Confinement of agents with target or non-target organisms in small or poorly lighted cages sometimes results in the disruption of the agent's behavior, leading to host choices (both acceptance and rejection) that are not consistent with actual field host preferences (Field and Darby, 1991; Sands, 1993; Sands and Papacek, 1993). Various aspects of the arena used for conducting host range tests can have such effects, including cage size, the lighting provided, and chemical contamination of cage materials.

Size. The size of the arena can affect the level of parasitism in particular species of test insects. For example, parasitism of both *Listronotus bonariensis* (Kuschel) and *Irenimus aequalis* (Broun) by *Microctonus hyperodae* Loan was higher in 9 cm petri dishes than in 1.75 liter containers (McNeill, 2000). Similarly, parasitism of the weevil *Nicaeana cervina* Broun by *Microctonus aethiopoides* (Loan) was higher in small cages (16 x 15 x 75 cm) than in field cages (45 x 90 x 50 cm) (Evans *et al.*, 1997). Host specificity of the chrysomelid beetles (weed biological control agents) *Leptinotarsa texana* (Schaeffer) and *Leptinotarsa defecta* (Stål) that was evident in choice tests run in walk-in cages (250 x 180 x 140 cm) largely disappeared in smaller test cages (40 x 40 x 60 cm) (Olckers *et al.*, 1995). Presumably, small cages promoted more frequent contact with test species. What has not been investigated is whether these arena size effects act equally on all test species, or if preferences between species vary with arena size.

Light. Light quality can be affected by cage materials, which may then affect oviposition behavior of some parasitoids. For example, many white fabrics produce glare, which may cause aggregation or disorientation of male parasitoids within cages, reducing mating. This problem can be solved by use of black, semi-transparent or transparent materials that cause less light scatter. Such black fabric was used successfully to obtain mating of the eulophid parasitoid *Aprostocetus ceroplastae* (Girault), after repeated attempts in cages made with white fabric had failed (D. Sands, unpublished). When white cage materials were used, male *A. ceroplastae* aggregated at the top or in corners of the cages, males failed to mate, and all offspring from the caged females were males.

When fine black nylon was used, males visited plants infested by the scale insect hosts, searching for females. Mating was observed and both sexes of parasitoids were reared from the scale hosts.

Sunlight (directed through windows) rather than artificial light may also stimulate mating in some species. For example, the sessiid moth *Carmenta mimosa* Eichlin and Passoa, introduced into Australia for biological control of the woody weed *Mimosa pigra* L., only mated when exposed in direct sunlight (R. Kassulke pers. comm.). For some species, such as the braconid *Cotesia rubecula* (Marshall), fluorescent lighting such as is typical in growth chambers reduces the level of oviposition compared with that under incandescent lighting (Van Driesche, pers. obs.). Again, what is not known is if these effects act in ways able to alter preference ranking of test host species.

Chemical contamination. Residues of host kairomones can also affect results of cage tests. Some kinds of cage materials may retain absorbed materials from previously held organisms, which may cause subsequently tested non-host species to be accepted for oviposition due to the simultaneous contact with the novel host and the expected kairomone of the true host. If such species are not accepted for oviposition in new cages, kairomone contamination may be suspected. In some cases, rigorous cleaning may remove the contamination from cages between trials.

Still other unrecognized forms of cage-induced effects on parasitoids are likely to exist. Several cases are recognized in which results of cage tests conflict with field results, but where no causative factor was identified. For example, a biotype of the pteromalid *Scutellista caerulea* (Fonscolombe) parasitized and successfully developed in both *Ceroplastes sinensis* Del Guerco (Coccidae) and *C. destructor* in cleaned cages in the laboratory. But after the parasitoid's field release, it was only recovered from the target host, *C. destructor*, despite the presence of both species in the field (Sands, 1993). Similarly, *C. rubecula* readily parasitizes both *P. napi oleraceae* and *P. rapae* in small cages (under no-choice design), but has not been recovered from North American *P. napi* in the field (Benson *et al.*, in press; Van Driesche *et al.*, in press).

Effect of Overall Experimental Design

Both choice and no-choice tests have been widely used to evaluate host ranges of herbivorous insects (Edwards, 1999; Sheppard, 1999), and both methods have been applied to arthropods (Barratt *et al.*, 1999; Sands and Coombs, 1999; Van Driesche *et al.* in press). Choice tests (target and non-target organisms exposed to the agent at the same time in the same container) and no-choice tests (target and non-target organisms exposed to the agent separately) can give different levels of host acceptance among target and various non-target species exposed to the same agent. The presence of a host in the same cage as a non-target species may either artificially induce attacks on non-target species (Field and Darby, 1991) or deflect attacks away from a true, but less preferred host.

A third test design is to alternately expose a parasitoid to the target host, followed by exposure to a test non-target species, and then repeat with the target host and another nontarget species. This procedure, called the sequential method, allows one to assess the strength of the parasitoid's preference to each nontarget species, given recent but not concurrent contact with the target host. This approach was used, for example, for testing non-target adult pentatomids, coreids and scutellerids with the tachinid *T. giacomellii*, a parasitoid released against *N. viridula* (Hemiptera: Pentatomidae) (Sands and Coombs, 1999). In sequential tests with this parasitoid, five of the 15 non-target species tested received ovipositions, but of these only three pentatomids supported development of the parasitoid. In this design, parasitoids previously exposed to *N. viridula* for two hours were then exposed for two hours to one non-target species, followed by repeated exposure for two hours to *N. viridula*, and so on for all test species. This test was followed by a no-choice test in which each non-target

species that attracted oviposition was exposed separately to a naive parasitoid (one with no previous host contacts). Naive females used in tests with non-target species were then exposed to the target species for two hours to confirm that they were physiologically and behaviorally ready to oviposit. In these tests, there were no differences in ovipositional preferences, between naive parasitoids and those with prior exposure to the target species.

Comparisons between two or more test designs can improve interpretation of test results. For example, if results of both choice and no choice design experiments on a parasitoid are available for the same set of test host species, we can ask if they agree. If under both choice and no-choice test designs a non-target species is not parasitized, it can reasonably be assumed to be a non-host. Similarly, if a non-target species is parasitized both in the presence and absence of the target species, the non-target species is likely to be a host and should be viewed as at risk. However, cases of this sort are known (*C. rubecula/P. napi*), in which positive laboratory data are contradicted by negative field results (Van Driesche *et al.,* in press).

However, there will be cases in which choice and no-choice tests give conflicting results (see Marohasy, 1998 for examples). How are these conflicts to be resolved? For example, if a test species is not attacked when it is presented to a parasitoid alone, but is attacked in the presence of the target species (e.g., two non-target millipedes, *Akamptogonus novarae* [Humbert and Saussure] and *Pogonosternum nigrovirgatum* [Carl] in Bailey, 1989), we suggest that chemical cues from the target species may be facilitating acceptance of the second species. This can be confirmed by further experiments using kairomones extracted from the target species or its products instead of actual hosts. If for example, parasitization of a non-target host can be manipulated at will by adding or deleting contact with some host-derived material like feces or silk, then the non-target species is unlikely to be a field host, except accidentally when populations of the two species are closely intermixed in the field.

The other case of disagreement between choice and no-choice results would be when it is the no-choice test in which parasitization occurs. If a non-target species is immediately parasitized when presented alone, but *not* parasitized when presented together with the target host, we suggest that the non-target species is inside the host range, but that it is a less preferred host that is ignored in the presence of a more preferred species. Such differences during testing have previously been misconstrued to suggest that less preferred hosts are outside the host range. Parasitism of such less preferred hosts in the field is likely to occur because parasitoids commonly encounter host species one at a time.

If, however, attacks on hosts in no-choice tests only occur after a prolonged period, we may suspect yet another scenario. In such cases, the non-target species is either a non-host or a very low ranked host that only becomes acceptable when parasitoids are very host-deprived. Field attacks on such hosts are likely to be uncommon in areas where the preferred host exists, but such non-target hosts might become hosts if parasitoids were to invade new biogeographical regions where no preferred host species existed.

Effects of Learning and Physiological State

Learning. Many parasitoid species have preferred and less favored hosts and have flexible behaviors responsive to recent experiences that allow them to focus on those species that have proven to be appropriate and locally available hosts (Vet, 1985). Influences of prior exposures to hosts may affect outcomes of host preference tests. Consequently, previous host contact must be a variable that is explicitly identified and controlled in such tests. For example, when adults of *M. aethiopoides* (reared from the target weevil *Sitona discoideus* Gyllenhal) were exposed to either the target pest or the non-target native weevil *N. cervina* and then later were exposed to mixtures of the target and non-target species, the parasitoids preferred to attack the species to which they were first

exposed (Barrett *et al*., 1999). When the aphelinid parasitoid *Aphytis melinus* DeBach was reared on *Aspiditus nerii* Bouché on squash, preference of adults with this history was reduced towards the target species *Aonidiella aurantii* (Maskell), in favor of *A. nerii* (Hare, 1996). The mechanism for this induced change in preference is contact by the emerging adult parasitoid with chemicals on the surface of the scale species from which it emerges.

Such examples illustrate that if emerging parasitoids have contacts with cadavers or products of the host from which they emerge, their subsequent host preferences may be altered. Consequently, the preference ranking of a given species will depend to some degree on what host the parasitoid emerges from. In laboratory tests, one can control for this issue either by trying to deny emerging parasitoids any contacts with hosts or host-derived materials (going as far, in some cases, as dissecting parasitoid pupae out of structures tainted with host residues), or by making such experience a variable and rearing the test parasitoids in all possible combinations of hosts used in the test. Duan and Messing (1999), for example, in assessing the host preferences of the braconid *Diachasmimorpha tryoni* (Cameron) between the target host (*Ceratitis capitata* [Wiedemann]) and the non-target species *Eutreta xanthochaeta* Aldrich, found that prior exposure of wasps to *E. xanthochaeta* increased their probing response 7 to 11 fold. This level of increase was possible because response to this host was innately weak, but could be stimulated by experience. In contrast, response to the principal host, *C. capitata*, was high under all combinations of rearing hosts and was not significantly increased by a prior exposure to *C. capitata*, suggesting that this was a strong, ingrained response that did not need induction.

Physiological state. Poorly nourished parasitoids may not search for or parasitize hosts, or may respond only to the most preferred host species. To avoid such false negatives, parasitoids in tests should be pre-fed with honey. Similarly, egg load can affect host choices. At very high egg loads, low ranked hosts or even nonhosts may receive ovipositions (see Minkenberg *et al.,* 1992 for a review). To eliminate this as an effect on tests, parasitoids should not be held without hosts for more than a few days before use in tests.

Effects of Plants on Host Preference by Parasitoids

The influence of plant species on a parasitoid's performance may require specific evaluation because some plants that are hosts for the target pest may have a strong effect on the parasitoid's host acceptance behavior. For example, *Eretmocerus* spp. from Spain and India performed well as parasitoids of *Bemisia tabaci* (Gennadius) (biotype B) in all crops. In contrast, *Encarsia* sp. nr. *pergandiella* Howard performed well on melons, but not on cotton or kale (Goolsby *et al*., 1998). Specific plant features such as volatile compounds may strongly attract parasitoids toward hosts on some plants, while the same host on a non-attractive plant would be missed (Read *et al.,* 1970; Roland *et al*., 1989; Romeis *et al.,* 1997). The choice of plants on which to assess hosts must be taken into account when designing host range tests. In some cases, oviposition by the test species can be induced to occur on inert substrates (e.g., cage materials). This then allows the potential influence of plants on parasitoids to be assessed by exposing the test species with and without various plant substrates (Sands and Van Driesche, 2000). In no-choice or sequential tests, plants or other important host-derived materials providing cues for parasitoid oviposition should be included so as to appropriately match a test species' biology. It is not appropriate to use the host plant of the target species as a substrate on which to expose the eggs of a non-target species, unless the non-target species is also associated with that plant species.

When an insect's life stage of interest cannot be separated from the plant (such as the cases of embedded eggs or nymphs of sedentary Homoptera), the plant will necessarily confound comparison between insect test species, unless the hosts to be examined share common host plants.

Estimating Prey Ranges of Predacious Arthropods

Predators are likely to have broader prey ranges than the host ranges of parasitoids. Data are needed both on the numbers of a given species of prey that are eaten and the value of each prey species in supporting predator growth and oviposition. Predators may show preferences both as adults and larvae, and these may differ. Consequently each must be determined separately. The adults of some predators may be generalists but have immature stages that are more specific in their prey requirements. For example, larvae of *Rodolia cardinalis* (Mulsant) are specialized to feed on only a few species of margarodid scales, but adults of *R. cardinalis* can subsist on a wider range of other insects and nectar for up to three months (V. Brancatini, unpublished; Sands and Van Driesche, 2000). The host range of this coccinellid has been recently estimated, relative to native scales on the Galápagos Islands (Causton *et al.,* in press).

As with parasitoids, predators may be affected by associated plants. Predators, for example, may be evolved to search for prey preferentially on certain kinds of plants. Some predators that appear to have broad prey ranges may have more restricted prey ranges due to a strong association between the predator and a specific plant or group of plants (e.g., species of phytoseiids in the genus *Neoseiulus* in Australia) (Beard and Walter, 2001). If plants of the target and non-target prey differ, each should be tested on its natural host. Predators are also sometimes adapted to forage under specific environmental conditions. Some coccinellids that prey on woolly hemlock adelgids (*Adelges tsugae* Annanad), for example, prefer to forage on spruce (*Picea* spp.) foliage over that of pine (*Pinus* spp.) or broadleaf trees.

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