
CHAPTER 7. OVERVIEW OF TESTING SCHEMES AND DESIGNS USED TO ESTIMATE HOST RANGES

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INTRODUCTION

Host range estimation for parasitoids and predacious insects draws on two bodies of past work: work with herbivorous insects used as weed biological control agents and basic studies of how entomophagous insects find, assess, and use hosts. Much of the following discussion on the relative merits of different types of tests comes from the weed biological control literature, in which there has been a lively debate about test methods for several decades – in contrast to the relative paucity of such debate for tests with entomophagous insects.

Some authors make a distinction between “host range” and “host specificity,” in which they use the former to mean the full list of host species attacked by an agent and host specificity to mean the relative degree of use likely for each of these hosts. Here, we focus on predicting only whether or not a test species is a possible field host (i.e., in the host range). Predicting the relative degree of use that is likely in the field is a more complex task, which weed biological control practitioners have approached by use of preference and, to a lesser degree, continuation tests. An herbivore may, for example, feed on six plants species, but show a strong preference for one species if given the choice. A critical question, however, is “will choice always be available?” We assert that it will not, and therefore even low rank hosts are potentially at risk. However, if continuation tests show that a low ranked host is not suitable for permanent maintenance of the biological control agent’s population across many generations, then it is legitimate to argue that such low ranked hosts are indeed not threatened and may be considered non-hosts.

Use of tests to assess plants as potential hosts for herbivorous insects began over 70 years ago and has long been routine. In contrast, interest in estimating parasitoid and predator host ranges lagged considerably behind. For herbivorous weed control agents, a variety of tests

have been developed, and those in favor have changed over time due to changes in the perspective of biological control practitioners and developments in the study of insect behavior. For herbivorous insects, tests used have focused on (1) oviposition, (2) adult feeding, (3) larval feeding and survival, (4) oogenesis and multi-generation population persistence, and (5) host preference (see Chapter 6 for a discussion of these parameters). These processes have been examined using tests with several different designs, principally (1) no-choice tests, (2) choice tests, and (3) open field tests. Individual projects often have used several types of tests in various combinations, including various kinds of controls. Tests used less often have included continuation tests, sequential choice tests (sometimes called sequential no-choice tests, see Chapter 13 [Combs]), preference-ranking tests (including a variation called “choice minus target”), and tests that investigate effects of specific aspects of insect behavior (time-dependent effects and behavior-dependent effects). In the following discussion, each of the major types of tests is discussed separately.

NO-CHOICE TESTS

DESIGN

These tests combine one or more specimens of the biological control agent with a single test species, for a fixed period of time, in cages (including petri dishes, plastic containers, or cages of various sizes) under standard laboratory conditions. Thus, if five non-target species are to be tested at one time with ten replicates of each, 50 cages would be required, plus appropriate controls.

As used in the past, test insects in no-choice design experiments may or may not have had contact with the target pest before the test. However, prior experience is a confounding effect because it may reduce response to lower ranked hosts, even if the preferred host is not present during the actual test. Thus, it may be better to avoid this complication and work with naive insects that have not touched or fed on the target pest (or for parasitoids have not had any host contacts or oviposition experiences) (see Chapter 5 for a discussion of such confounding factors).

Interestingly, scientists studying weed biological control agents and those studying parasitoids have treated this issue of prior experience differently. Most weed biological control practitioners assume that prior experience with the target weed will almost always automatically occur in the course of the insect’s life cycle, and thus they treat it as a given rather than examine its effects as a treatment variable in tests. Biologists studying parasitoids, in contrast, have shown extensive concern about the effects of prior experience and have routinely treated it as an experimental factor to be controlled and contrasted in experiments on parasitoids’ host preferences. This difference in assumptions affects how choice vs. no-choice tests are viewed by these two groups.

For oviposition and feeding trials, positive controls are essential to validate negative responses by showing that the group from which the test biological control agents were taken had the capacity for oviposition or feeding. Individuals used in controls should either be (1) different insects from the same rearing batch that are exposed to the target pest simultaneously with the main experiment or (2) the same insects used in the main experiment that, after expo-

sure to a nontarget species, are re-used by exposing them to the target host to demonstrate their physiological readiness to respond positively to a highly ranked host. Negative controls, in which test species are not exposed to parasitoids, are needed to detect mortality of test herbivores that is unrelated to the parasitoids (see Chapter 8 [Froud and Stevens] for an example). For feeding tests with predators, controls in which only water is provided show whether the test prey species provides any nutritional benefit to the predator by assessing survival time with prey versus water alone (Causton *et al.*, 2004; and Causton, this volume). For tests measuring survival and development of immature parasitoids or predators, performance on a host (or prey) of known suitability can be used as a standard against which reduced survival in a poorer host can be gauged.

HISTORY OF USE

Weed biocontrol agents The no-choice test design was the first approach used in early weed biological control projects and was the dominant method employed until the 1960s (e.g., see Briese, 1989; Thompson and Habeck, 1989; Turner *et al.*, 1990; Adair and Scott, 1993, 1997; Woodburn, 1993; Turner, 1994; Balciunas *et al.*, 1996; Peschken *et al.*, 1997; Scott and Yeoh, 1998). No-choice tests can be used with adults to assess feeding and oviposition and with larvae to assess feeding and development. In all tests, care must be taken to offer the appropriate plant stage to the test insects. For species with larvae that do not move between plants, use of no-choice tests is really the only appropriate model of the field biology since no host choice is exercised by the larvae. In early days, most weed biological control projects included larval feeding tests with no-choice designs.

It was observed, however, that in this type of test, immature herbivorous insects sometimes fed successfully on plant species that adult insects did not find or accept for oviposition. This observation caused emphasis in the testing of weed control agents to shift to the use of tests that focused on the oviposition choices of adults, as this step was believed to be the one that most often limited the host range. This shift toward oviposition tests was intended to reduce the chances that beneficial and safe species would be rejected for introduction because they were seen as unsafe based solely on data from no-choice larval feeding tests.

It was also observed that the range of plants insects laid eggs on was often smaller if a preferred plant (usually the target weed) was present in the test, as opposed to no-choice tests in which plants were each presented alone (e.g., Fornasari *et al.*, 1991; Willson and Garcia, 1992). This led to a strong preference on the part of biological weed control researchers to use choice designs in oviposition tests, starting around the 1970s. In some projects, preference for the target species in such choice oviposition tests was used to argue that attacks on less preferred nontarget species would be minimal in the field.

More recently, it has been recognized that such choice oviposition tests may not detect less favored hosts. For example, a seed bruchid that was recently introduced into New Zealand and Australia for control of broom (*Cytisus scoparius* [L.]) has been found attacking the non-target plant tagasaste (*Chamaecytisus palmensis* [L. Fil.] Link). While this plant was in the list of species tested to estimate the host range of this beetle, its status as a host was not detected (Fowler *et al.*, 2000). This occurred because only choice tests were used; when paired with the target weed, tagasaste was not attacked. A no-choice test would, presumably, have detected this

species' status as a possible host. To prevent such errors, no-choice tests are being re-emphasized in weed biological control projects (Heard, 2000), especially by conservation groups and regulatory agencies (Hill, 1999). Such use of no-choice tests also avoids the mixing of plant volatiles, which may mask the identity of co-presented plant species. This is likely also to be relevant to tests with entomophagous insects since their prey or hosts are often presented to them on host plants.

Entomophagous species Here we consider tests for (a) larval development, (b) oviposition, and (c) feeding by adult predators.

(A) Larval development tests For parasitoids, assessment of larval development (the analog to larval starvation tests with herbivores) is typically done by observing whether parasitoids develop and emerge from a test species that had been accepted by adult parasitoids in an oviposition test (e.g., Field and Darby, 1991; López-Vaamonde and Moore, 1998). Occasionally, immature stages of the parasitoid are artificially placed in or on the test host, as Fuester *et al.* [2001] did with eggs of the tachinid *Aphantorhaphopsis samarensis* [Villeneuve]) and Morehead and Feener (2000) did with eggs of the phorid ant parasitoid *Apocephalus paraponerae* Borgmeier, but this is the exception.

For predators (and a few parasitoids, such as those tachinids that scatter microtype eggs on foliage), larval development tests do not depend on successful oviposition on the test species because predator larvae are generally mobile and can readily be placed together with a candidate prey species to see if feeding occurs (Causton *et al.*, 2004).

Unlike the results seen with tests of herbivorous insects in which the host range found in larval feeding is often broader than the range of species accepted by adults for oviposition, the opposite is sometimes the case for parasitoids. For example, Bailey (1989) found the sciomyzid fly *Pelidnoptera nigripennis* (F.) laid eggs on the integument of millipede species in five families, but larval penetration only occurred in one family (Julidiidae), in part because eggs on some species failed to firmly adhere. Duan and Messing (2000) found that the restrictive step determining the host range of the braconid wasp *Dichasmimorpha kraussii* (Fullaway) was failure of immature stages to survive in plant-galling (as opposed to fruit-infesting) tephritids rather than rejection of the galling tephritids by ovipositing adults. Keller (1999) found that the aphid parasitoid *Aphidius rosae* Haliday, under choice conditions, attacked all four species of aphids tested but failed to develop in three of them. Mohamed *et al.* (2003) found that the braconid *Psytalia cosyrae* (Wilkinson) parasitized all six fruit flies presented, but due to encapsulation, the parasitoid developed in only two species. Similarly, the predatory derodontid beetle *Laricobius nigrinus* Fender oviposited on five species on which larvae failed to develop (Zilahi-Balogh *et al.*, 2002).

In conclusion, while plant tissues may be broadly eatable for herbivores (assuming no strong deterrent compounds or morphological barriers), the internal environment of host insects for parasitoids may require special adaptation for successful exploitation. Thus, no-choice larval development tests may be of greater value for predicting parasitoids' host ranges than larval starvation tests are for herbivorous insects. For idiobiont parasitoids (egg parasitoids and larval/pupal ectoparasitoids) and predators, this would not be the case, as there would be no requirement to defeat internal physiological host defenses.

(B) Oviposition tests Oviposition tests can be applied to both parasitoids and predators, and while these have many common features, there are important differences: chiefly, that predators oviposit near prey or in the prey habitat rather than in or on the host, as parasitoids usually do. There are two key elements in oviposition tests: host finding and host acceptance. Oviposition tests have frequently dealt only with host acceptance because they have been run in small cages that make expression of the full range of host finding behaviors unnecessary. Test cages can be as small as petri dishes (as used to assess the host range of *Trichogramma brassicae* Bezdenko by Babendreier *et al.* [2003]) or, more typically, are small plastic boxes or cages (Bailey, 1989; Field and Darby, 1991; López-Vaamonde and Moore, 1998; Porter and Alonso, 1999; Porter, 2000; Zilahi-Balogh *et al.*, 2002; Van Driesche *et al.*, 2003). Choice of the arena used will depend on the biology of the insect studied and the conditions needed to ensure natural behavior by the agent (see Chapter 5).

Aspects of host finding can be introduced into the testing process by including more space in the test arena, food for adult parasitoids (or predators), appropriate host plants of the test species, and circulating air. Duan and Messing (2000), for example, conducted their tests with tephritid parasitoids in larger cages (1 m on a side) in which hosts and host plants were placed. Keller (1999) conducted his assessment of the host range of a rose aphid parasitoid in a wind tunnel, which provided moving air and sufficient physical space so that flight toward the test aphid/plant complex was a required part of any positive responses in the assay. For tests run in quarantine in the receiving country, this is probably the largest practical sized arena that could be used. For tests run in the country of the parasitoid's origin, open field tests can be used (Porter *et al.*, 1995).

(C) Adult feeding tests No-choice tests can also be used with predators to assess which prey are used as food. Causton *et al.* (2004) used this approach to determine the host range of the Vedalia beetle, *Rodalia cardinalis* (Mulsant), relative to the native Homoptera of the Galápagos National Park. Alternatively, the goal may be to compare the level of consumption of a prey versus widely distributed foods such as honey dew or pollen. In such cases, it may be useful to note the number of prey of each test species eaten in the presence and absence of the non-prey food.

STRENGTHS

The strength of no-choice tests is that negative results are very robust and provide convincing evidence that a test species is not likely to be used as a field host, provided that the experimental design includes an environment that permits normal behavior of the biological control agent, as evidenced by a positive response to the normal host used as a control. Use of no-choice tests early in the testing sequence provides a strong rationale for classifying unattacked test species as non-hosts. Briese *et al.* (2002a), for example, followed this approach with European insects being screened as biological control agents for *Onopordum* thistles in Australia. Lack of oviposition and adult feeding, especially if coupled with lack of larval feeding, clearly indicate that the test species is not a host. This design guards against the risk of mislabeling a low-ranked host as a non-host, as can happen in choice tests when a preferred host deflects attention away

from a less preferred host that might have been attacked if presented alone (see the following section on Choice Tests).

For parasitoids, failure of the adult to oviposit in the appropriate life stage of a test species under no-choice conditions can be taken as fairly solid evidence that the species is not a host if the host is presented on its natural host plant, together with the usual associated kairomones, provided oviposition is observed in suitable controls. The same conclusion can be drawn if oviposition occurs but immature stages fail to develop successfully. Because negative data in no-choice tests are fairly unambiguous, regulators like this type of test. Porter and Alonso (1999) specifically chose a no-choice type test instead of a choice design for testing phorid parasitoids of ants because they wanted to find out whether a native species of ant would be attacked when the target ant was not present, as may happen if the biological control agent's range becomes larger than that of the pest or if the pest's density becomes very low.

WEAKNESSES

Weed biological control practitioners have long felt that weak positive responses to test species sometimes seen in no-choice tests are artificially induced by confinement and the lack of choice. In part, this is accurate – at least in regards the effects of confinement, which brings the agent into close contact with a test species such that important host finding steps may be skipped (since the insect is literally put on the host or very near it), allowing oviposition or adult feeding to occur on species that might not have been detectable in the field. This issue is likely to be particularly important in assessing the host ranges of parasitoids as some acceptable hosts are unfindable by particular parasitoids in nature and hence not really in their host range (e.g., *Pieris virginiensis* Edwards, for *Cotesia glomerata* [L.] [Benson *et al.*, 2003a]). Also, confinement with a non-host or a low ranked host may eventually lead to egg dumping as egg load increases. Without confinement, the agent would be free to disperse and would perhaps find a suitable oviposition site before egg load reached levels leading to egg dumping.

The second complaint, that lack of choice is unnatural, is misleading. Inclusion of choice in the test design is only appropriate if choice is universally present in the field. An insect may in fact not have a choice of hosts (1) if it expands geographically beyond the range of the target pest, (2) if it invades habitats not occupied by the target pest, (3) if the insect is partially out of synchrony with the target pest, or (4) if at the local scale, the target pest is absent for any reason (including biological control itself, chemical control, or even just chance). Thus, there is no reason to say that choice tests are “more natural” or more accurate than no-choice tests. Rather, the system and biology of the organisms in the particular case should dictate which test design is the better model of nature. The researcher, however, must anticipate the full range of these potential settings.

Conversely, even negative responses can be misleading under some circumstances. In particular, a false negative might arise if insects used in the treatment are not in good health or are infertile. This may happen, even with positive responses in the controls, because controls and treatments by design use different individual insects. This problem, however, decreases with suitable replication of both treatments and control or with the use of sequential choice tests.

CURRENT THINKING ON VALUE

Larval development tests No-choice tests are used to measure larval feeding, survival, and development on a potential plant or host. While larval starvation tests were out of favor for weed biocontrol agents in the 1980s and 1990s as too laborious and not focused on the most discriminating stage, their ability to detect the maximum physiological host range has led recently to the test's increased use in weed biological control. For parasitoids and predators, no-choice larval development tests are likely to be even more valuable as this step may be more restrictive than oviposition, particularly for internal hymenopterous parasitoids.

Oviposition tests No-choice oviposition tests have special value because they are able to identify low ranked hosts that can be missed in choice tests. Indeed, the notion that choice tests can "clarify" ambiguous results of no-choice tests in which low ranked species receive ovipositions (see Thompson and Habeck [1989]) is now seen as mistaken. Rather, choice tests can be used to rank hosts if this is desired (Withers, 1999).

For parasitoids, a key feature shaping host ranges will be long-distance attraction to the host via volatiles from the host/plant complex. While choice tests can be run using y-tube olfactometers, there is no advantage to using that design in place of a no-choice design employing olfactometers or wind tunnels. Indeed, misdirected behaviors caused by mixing of volatiles from two or more test species may easily complicate or invalidate test results.

CHOICE TESTS

DESIGN

In choice tests, two or more plant or host species are presented to the biological control agent simultaneously, and thus, the response is a measure of preference between the two options (e.g., McFadyen, 1983; Dunn *et al.*, 1989; Buckingham *et al.*, 1991; Forno *et al.*, 1992; Edwards, 1999). The target species is often, but not always, one of the choices offered. This approach is most commonly used to measure oviposition preferences but can also be used for feeding preferences of adults or even larvae, if these are mobile enough to move between hosts.

A variation on this design is called a sequential choice test (sometimes called a sequential no-choice test; see Chapter 13 [Combs]), in which the natural enemy is exposed to a series of test species, one at a time. Typically, exposure of the agent to a nontarget species begins the test, followed by exposure of the same test insects to the target weed (or host), then after that bout, to a second nontarget weed (or host), and so on. This process is believed to provide a positive control (periodic re-exposure to the target weed verifies continued ability of the tested insects to oviposit or feed) and eliminates the problem of cross contamination of the nontarget species with volatile chemicals from the pest species. A potential flaw of the sequential choice test design is that conditioning induced by experience with the target pest may persist long enough to reduce feeding or oviposition on nontarget species encountered later in the sequence.

While most use of sequential choice tests has been with herbivorous insects, the design has been applied in a few cases to parasitoids. Gilbert and Morrison (1997) used it to assess the host range of the phorid fly *Pseudacteon litoralis* Borgmeier relative to various ant hosts, and Sands

and Combs (1999) applied the method to the tachinid *Trichopoda giacomellii* (Blanchard) in tests with Australian pentatomids.

HISTORY OF USE

Weed biocontrol agents Choice tests are still commonly used to estimate the oviposition host ranges of herbivorous insects used for weed biological control (and to a lesser extent, the adult and larval feeding ranges). This design was adopted because practitioners felt that the other alternative – no-choice tests, especially larval starvation tests – was resulting in too many cases in which a species was a host in cage tests but did not seem to be attacked in the field. Oviposition choice tests, it was argued, corresponded better to circumstances in the field because the adult's host seeking was the most discriminating step in the chain of behaviors leading to host use. As a practical matter, these tests allowed some biological control agents to be introduced that might have been rejected based on no-choice tests alone, especially if just no-choice larval feeding data had been considered. Occasionally, workers combined the two, running choice oviposition tests followed by no-choice larval starvation tests (e.g., Dunn *et al.*, 1989; Forno *et al.*, 1992). Use of choice tests with only those species giving positive results in no-choice tests seems to have been done in the mistaken belief that subsequent lack of attack in choice tests would identify which of the positive responses in the no-choice data set were “erroneous” (e.g., Fornasari *et al.*, 1991; Willson and Garcia, 1992).

Currently, this line of reasoning is being re-evaluated by weed biological control practitioners because it has been recognized by some that choice tests are not a good model if, for any reason, the agent is found when and where the target pest is not found (hence, no choice can be made). In such cases (e.g., *Rhinocyllus conicus* [Frölich] on Platte thistle [*Cirsium canescens* Nuttall] in western Nebraska [Louda, 1998]), no-choice tests are a better model for the ecological circumstances the agent is presented with.

Entomophagous species No published examples were found of the use of choice tests for adult or larval feeding by entomophagous predators. Larval feeding and survival by most parasitoids cannot be assessed with this design because larvae have too little mobility, are internal, or both.

Oviposition responses have been measured with choice designs for both parasitoids (Bailey, 1989; Field and Darby, 1991; Keller, 1999; Porter, 2000; Fuester *et al.*, 2001; Babendreier *et al.*, 2003) and predators (Zilahi-Balogh *et al.*, 2002). In some cases, virtually all nontarget species offered were attacked at rates similar to a known host (e.g., *T. brassicae* and various nontarget Lepidoptera [Babendreier *et al.*, 2003]). In this case, it is fairly easy to draw the conclusion that the species is polyphagous. However, it may be useful to present some species to the biological control agent in a no-choice design to determine if volatiles from the target host might have, under choice conditions, contaminated non-target hosts, leading to their attack.

Sometimes in choice tests, the parasitoid shows a strong preference for the target pest (Porter, 2000 for phorid parasitoids of fire ants) or even fails to attack nontarget hosts at all (Fuester *et al.*, 2001, with a tachinid parasitoid of lymantriid moth larvae). These results were interpreted as meaning the parasitoid was strongly focused on the target pest.

With herbivores, the range of hosts that receive ovipositions by a candidate insect has generally been found to get smaller under choice conditions in which the choice includes the target pest. The same pattern was observed with the predatory beetle *L. nigrinus* when offered nontarget species either separately or together with the target pest, hemlock woolly adelgid (Zilahi-Balogh *et al.*, 2002). However, for several studies of parasitoids, the opposite pattern occurred, and the number of species accepted for oviposition, or the level of attack on less preferred hosts, increased rather than decreased under choice conditions (Bailey, 1989; Field and Darby, 1991; Barratt *et al.*, 1997; Keller, 1999). This suggests that for parasitoids the effect of choice conditions may primarily be to stimulate the parasitoid to attack (by providing kairomones from the target pest), causing attacks on hosts not themselves able to stimulate oviposition. For parasitoids, no published examples were found of attack on a nontarget species in no-choice tests where attack disappeared in choice tests containing the preferred host. If further examples demonstrate this pattern to be generally true, then the risk that choice oviposition tests with parasitoids would lead to false negatives would be smaller than it appears to be for herbivore responses to plants. Given that to be the case, negative data in choice oviposition tests would be a more robust indication of safety to nontarget species. In fact, there may be cause to worry that use of a choice-design will lead to false positive ovipositions. Thus, for parasitoids, it might be reasonable to use choice designs to screen a large number of test host species, following up with no-choice tests for all species receiving ovipositions in choice tests (to detect false positives).

STRENGTHS

The choice design is well suited to reveal if the agent shows a preference among potential host species (typically, the choice presented is between the target pest and one or several nontarget species). This design also allows a more rapid examination of many species of potential hosts than is possible if each must be studied separately. Finally, the rank order of preference among hosts can be established with removal of the most preferred host, followed with repetition of the test until all hosts have been ranked. This approach is called “choice minus target” (or sometimes “choice minus control”) and is discussed below under the heading Preference Ranking Test.

WEAKNESSES

The weakness of this design is that preference for host A over B, when the two are presented together, is often erroneously interpreted to mean that B is not a host. (To illustrate: a child presented with broccoli and a pizza will almost certainly eat the pizza only, but this should not be taken to mean that humans do not eat broccoli. A very hungry child presented only with broccoli will eat it, eventually.) For the results of a choice oviposition test to be predictive of field events, (1) the agent must experience the choice in the field – that is, the nontarget (low rank) host must not be the only possible host encountered or (2) the nontarget (low rank) host must be so non-preferred that even agents deprived of their preferred hosts for considerable periods will keep searching rather than attack the low ranked species. Since these conditions may not always be met, inferring that a species not attacked in a choice oviposition test is not a host will lead to some unpredicted impacts. To understand if choice tests are appropriate, one must look at the options likely to be available to a foraging individual of the released species.

For parasitoids, it may be argued that choice would rarely occur in the experience of individual insects because they are most likely to encounter potential hosts one species at a time and it may not be the most preferred species. Whether or not parasitoids live in a “multiple-choice world” would be a valuable research area.

Several examples have been noted in which preference in laboratory tests for the target pest proved not to be predictive of safety in the field for the non-preferred native or crop species. *Rhinocyllus conicus* has been found feeding extensively on Platte thistle (Louda, 1998) despite a preference in laboratory tests for the target pest, musk thistle (*Carduus nutans* L.) (Arnett and Louda, 2002). Similarly, the invasive weevil *Larinus planus* (F.) (since used as a biological control agent) is now attacking Tracy’s thistle (*Cirsium undulatum* [Nutt.] Spreng. var. *tracyi* [Rydb.]) in Colorado (Louda and O’Brien, 2002) despite predictions that it would not do so (McClay, 1990).

CURRENT THINKING ON VALUE

Some biological control practitioners still think that choice tests are useful as a means to determine if positive results found in no-choice tests are “real” by seeing if they still occur in a choice design that includes the target pest (presumably a highly preferred species) (Briese *et al.*, 2002a). This seems, however, to be a basic misunderstanding of the biology being studied (see the *Weaknesses* section, above). Rather, the value of choice tests is as a means to construct a rank order of preference within the list of possible hosts (using choice minus target pest tests, as described below). All members of the ranked host list should, however, be considered hosts unless larval starvation tests show that they do not complete development or continuation tests (see below) show that the species is so poor a host that population growth rate is below replacement and the agent dies out after a few generations.

The value of this design may be greater for parasitoids than for herbivores if it proves to be generally true that choice design tends to expand rather than shrink the set of test species attacked (see above). In such a case, negative data for nontarget species for tests with parasitoids may be a more robust indication of safety than is the case for herbivores. For plants, the predominant risk of choice tests is false negatives. For parasitoids, this risk seems lower, and there may even be a significant risk of false positives. The importance of these errors needs to be assessed by comparison of such results to data from no-choice tests for a series of parasitoid species.

OPEN FIELD TESTS

DESIGN

Open field tests have largely been limited to tests of herbivore oviposition on plants. These assays are uncaged tests run outdoors, either in a garden or in a natural stand of the target weed, where potted plants of the nontarget test species are interspersed among the target plants (Clement and Sobhian, 1991; Briese *et al.*, 1995; Clement and Cristofaro, 1995; Briese, 1999). The agent is either present as a natural population or additional individuals are released to augment the natural background density. The outcome of the test is usually measured as the number of eggs laid on each test plant.

HISTORY OF USE

Weed biocontrol agents The open field test was developed at the end of the 1980s (e.g., Clement and Sobhian, 1991) on the belief that removing test plants and insects from cages and letting their interactions occur in an open space eliminated erroneous results that occurred when test insects were denied the option to leave the test arena. (In cages, with emigration denied, oviposition sometimes occurs on plants believed to not really be hosts, or even on the cage itself.) Open field tests are typically described as being “more natural” than cage tests. An issue in tests with this design is effects caused by the pattern and sizes of the patches of the various test plants in particular tests. A variation of this test, called a “two-phase open-field test,” has been developed to determine what the test insects would do if the target weed’s population were suddenly not available (Briese *et al.*, 2002b).

Entomophagous species Use of open field tests as done for herbivores depends critically on being able to move test plants native to the region of proposed agent introduction to the agent’s country of origin. This is often possible if the test plants have already been moved, for economic or ornamental use, into the country where the tests are to be run or if plants are released from quarantine only in pots and care is taken not to allow seeds or plant fragments to escape.

For parasitoids and predators, however, it is typically impossible to move the nontarget test insects into the country of origin of the agent because these are usually herbivorous insects and might become pests. Consequently, the only way to employ open field tests in the areas of origin of parasitoids and predators proposed for introduction is to assess attack on local species that are phylogenetically close to the species of concern in the proposed area of introduction. This was done, for example, by Porter *et al.* (1995), who exposed a series of local species of ants in Brazil to phorid parasitoids. By this means, data were obtained suggesting that these flies were host specific at least to the genus level. Similarly, Fuester *et al.* (2001) collected 54 species of European caterpillars in 11 families to assess the frequency of parasitism by the tachinid *A. samarensis*, a proposed biological control agent for the gypsy moth, *Lymantria dispar* (L.). Both of these projects suggested a high level of specificity for the parasitoids under study. However, the inability to test the actual native species potentially at risk in the area where the agent is to be introduced remains an important limitation.

STRENGTHS

In open field tests, test insects do not experience any unnatural influences that might alter their behavioral responses to potential hosts, such as altered light quality within cages, increased egg loads, or stimulation or repression of their sensitivity to plant chemicals that might come from forced confinement on or near either the test plant (stimulation) or various non-target plants (sources of potential deterrents).

WEAKNESSES

Open field tests can only be done in the native range of the biological control agent that is being studied, as quarantine considerations prevent the test from being done in the area into which

the insect is proposed for introduction. Technical considerations that have been mentioned as potential defects are the relative balance of numbers of test plants and the target weed (likely to be strongly tilted toward the pest species) and the density of the test insects (likely to be low) (Briese, 1999). But the most important problem for these tests is the same as for choice tests run in cages (since open field tests, as used, have all been choice tests): namely, that what is measured is preference. Thus, like caged choice tests, open field tests are set up to miss low ranking hosts precisely because the preferred species, the target weed, is always present.

In theory, this might be corrected in the context of weed biological control if trials were run as no-choice tests. Groups of potted test plants of just a single species (either the target pest or one of a series of nontarget species) could be placed outdoors and test insects released onto or near by such test plants. Such tests would have to be made at sites isolated from stands of the target weed to avoid contamination by individuals of the test insect arising from wild plants and having a different set of past host experiences. However, there are no published examples of open field tests set up as no-choice tests. A partial approach to creating such conditions is found in a test termed a “two-phase open-field test” (Briese *et al.*, 2002b). Steps in the such a test are (1) creating a common garden plot containing the target weed and various nontarget test plants, (2) allowing the candidate biological control agents to colonize the plot, (3) taking data on the agents’ feeding and oviposition, and then (4) killing the target weed plants. This forces the agents to switch and accept lower ranked hosts, emigrate, or die. When this approach was used by Briese *et al.* (2002b) for four candidate species attacking the weed *Heliotropium amplexicaule* Vahl, a pest in Australia, it was found that three agents either left or died, but one (an undescribed flea beetle, *Longitarsus* sp.), switched to feeding on the nontarget species *Heliotropium arborescens* L.

CURRENT THINKING ON VALUE

Open field tests have not become widely used (Briese, 1999), in part because they must be done in the country of origin of the agent, in part because of the potential quarantine problems of moving test organisms to that region, and in part because they are seen as a final step, not a first step (and thus, are sometimes not needed to make a regulatory decision at that stage of the review). Practical difficulties that frequently result in poor quality test results are common. There may be too few test insects, for example, at the sites. Such considerations have discouraged the use of these tests in weed biological control.

Applying these tests to insect targets (as opposed to plants) is even less feasible because of quarantine concerns with the desired test species. Conceivably, simulated open field tests could be constructed using walk-in cages within naturally lighted, quarantine greenhouses, which would provide large spaces for natural insect behavior to occur and ample opportunity for test insects to leave the system if they so desired. For entomophagous species, use of open field tests would be further complicated in some cases by the mobility of the nontarget species used in the test (such as for active bugs or caterpillars). This would not be an issue for some groups such as scales, aphids, whiteflies, and psyllids, or if the stage attacked was relatively immobile (eggs, pupae, or very small larvae).

CONTINUATION AND OOGENESIS TESTS

DESIGN

These tests focus on the suitability of the host to support the test insect's population over the long term. Oogenesis tests determine whether the host is nutritionally adequate to promote the agent's egg development. Continuation tests measure whether the host can support a population of the agent indefinitely, with a growth rate greater than replacement. These things must happen if the agent's population is to survive with no other resources. These tests are no-choice in design, and combine the oviposition, feeding, and survival responses all together.

HISTORY OF USE

Neither of these tests is widely used, and all examples found were for herbivorous, not entomophagous, insects. Kok *et al.* (1979) included oogenesis tests in their study of the weevil *Ceutorhynchus trimaculatus* F. They showed that this species was able to develop eggs when it fed on various thistles or artichoke but not on safflower.

Continuation tests were run by Buckingham *et al.* (1989), who found that a population of the fly *Hydrellia pakistanae* Deonier died out within eight generations if reared exclusively on the nontarget pondweed *Potamogeton crispus* L., suggesting that this species is not a satisfactory host even though the insect can survive on it and produce offspring for several generations in decreasing numbers. The value of continuation tests to host specificity assessment has been discussed by Day (1999).

In some cases, experience with *de facto* continuation tests in one country may provide information valuable in assessing risk in another. In South Africa, the mirid bug *Eccritotarus catarinensis* (Carvalho) was released for control of waterhyacinth (*Eichhornia crassipes* [Mart.] Solms-Laub). This bug was found in laboratory assays to feed on pickerelweed (*Pontederia cordata* L.), a nonnative invader in South Africa. The mirid bug failed to establish persistent breeding populations on pickerelweed, both when released directly onto pickerelweed plants in cages and at sites where stands of waterhyacinth (with established populations of the bug) were close to stands of pickerelweed (Coetzee *et al.*, 2003). These results constitute a field continuation test and show that, if the bug were to be introduced to the United States (where waterhyacinth is an invasive pest but pickerelweed is a native plant), it would be unlikely to establish itself on pickerelweed.

STRENGTHS

These tests are a strong complement to larval starvation tests because they indicate the degree of risk that isolated populations of nontarget species might face if their habitat were to be invaded by the biological control agent, in the absence of the target pest. Failure of the population of an agent to survive for multiple generations on the target pest is a robust indication that in the field the agent could not threaten native species at sites isolated from the target pest.

WEAKNESSES

These approaches, especially the continuation test, are expensive to run as they extend for a longer time than other commonly used tests. Also, these tests may fail to predict impacts on

nontarget species that may occur in cases where the agent's population is sustained by the target pest and spills over onto the nontarget species. In this circumstance, even though the nontarget species did not support the agent's population, it might decline in its presence.

CURRENT THINKING ON VALUE

These tests are considered to be helpful in assessing risks to low ranked hosts. Another potential value of such multigenerational tests is to see if adaptation occurs between a parasitoid and a new host, such that progeny reared from a novel host accept that host more readily than did the parental generation, or have better growth or survival in it.

PREFERENCE RANKING TEST (CHOICE MINUS TARGET)

DESIGN

This test reveals the relative ranking of a particular host (within a test list) for a candidate biological control agent, from most to least attractive (usually for oviposition). In ecological studies, for example, it is of interest to understand the relative degree of attractiveness of various plants. It should be noted, however, that a lower ranking but more abundant plant might be a more important host to an agent – in terms of population level consequences – than a more preferred but scarcer host.

With herbivorous insects, rankings are inferred by presenting the insect with a group of plants all together in a cage, observing the species on which most eggs are laid, removing that species, and repeating the test. This process continues until all plants have been ranked. In practice, if the test list is very long, each test may contain only a random subset of the test plants. This also has the advantage of being more likely to average out any distorting effects introduced by one strongly stimulating or strongly inhibiting test species, whose presence in some subsets may change the insect's response to other plants in that grouping. The rankings within these subsets must then be fused into one master ranking.

A factor affecting the outcomes of such tests (indeed of all host preference tests, regardless of design) is the past experiences of the test insects with other hosts, especially the target pest. This important issue is discussed in Chapter 5.

HISTORY OF USE

The idea that an insect's hosts can be arranged in a hierarchy from most to least preferred was developed in basic studies of insect-plant interactions (e.g., Wiklund, 1975, 1981; Thompson, 1988; Jallow and Zalucki, 1996) and was later incorporated into biological weed control host range estimation as a variation on choice tests. The concept applies as well to ranking the hosts of a parasitoid or the prey of a predator, but published examples all concerned herbivorous insect assessment of plants.

An early step in this direction was simply to repeat a choice test with the target pest species omitted and compare the two data sets. Hill *et al.* (1995), for example, made this comparison for oviposition by the moth *Agonopterix ulicetella* (Stainton) (Oecophoridae), a gorse (*Ulex europaeus* L.) insect. All test species were present in the first series, while in the second, gorse

was removed. They found that removing gorse from the test resulted in increased oviposition on five of nine nontarget species tested (and reduced oviposition on four others, but these were mainly switches of trivial degree, such as from 1 to 0). Interestingly, these researchers also included a test in which test insects had no previous exposure to gorse. These insects also oviposited on a wider range of plant species (compared to tests including gorse), some of which were not attacked by individuals with prior exposure to gorse. Only five studies (Peschken and Harris, 1975; DeLoach *et al.*, 1976; Wapshere and Kirk, 1977; Cordo, 1985; and Withers *et al.* 1999) are mentioned in Edwards' (1999) review as having included both choice tests with the target weed present and with the target weed removed. Actual use of this approach is likely to be more common (e.g., Hill and Gourlay, 2000).

An extension of this process is then to specifically identify the first and second most preferred hosts in two rounds of testing, in which the most preferred species in round one is removed and the test continued with fresh plants (but the same insects) to identify the second-most preferred host. This was the approach taken by Solarz and Newman (1996) in establishing the host preferences of the native watermilfoil specialist weevil *Euhrychiopsis lecontei* (Dietz). This study also found that rearing the test insects on the target weed (Eurasian watermilfoil) induced a preference for this species in subsequent tests (see Chapter 5 on factors affecting tests). However, it should be noted that it is often nearly impossible to avoid doing so, given constraints of what the agent can be effectively reared on.

Marohasy (1998) later recommended that this process be further elaborated so that all hosts could be ranked by repeating the test multiple times, removing the most preferred species in each run, until only one host (the least preferred species) remains. Edwards (1999) has reviewed past choice tests and lists those studies that have run choice-minus-target experiments. So far few biological control studies have followed Marohasy's (1998) strategy for ranking hosts.

STRENGTHS

Preference ranking tests show in what order plants (or for parasitoids, hosts) would be accepted if an insect were aware of two or more potential hosts at the same time and place. All acceptable hosts, however, are in the host range, even the least preferred. Consequently, in practical terms this test design has limited value in countries that have a highly risk averse stance on biological control.

WEAKNESSES

Preference ranking tests, like choice tests in general, have been misinterpreted to imply that lower ranked hosts are non-hosts. This mistake has now been pointed out (Edwards, 1999). Preference ranking tests are also time consuming and only justified if there is a clear need to learn where particular species rank as hosts. Perhaps for these reasons, this procedure has not been widely adopted.

CURRENT THINKING ON VALUE

Preference ranking tests seem of greater value in the understanding the host plant choices of native insects than in estimating the safety of species proposed for introduction as biological

control agents because, by definition, all the hosts being ranked are inside the host range. The added knowledge of their precise place in the preference hierarchy is not information that can be used to change the release/reject decision for which host specificity data are being collected. The introduction of an insect for which a native plant is a very low ranked host might be acceptable if the pest weed caused great ecological or economic damage as the lesser of two evils – but this is a political decision. A low ranked host remains a host.

TESTS FOR TIME- AND BEHAVIOR-DEPENDENT EFFECTS

One of the directions in which host range testing has been moving in recent years for weed biological control projects is to pay more attention to physiological factors that can alter the test insect's response to plant species. (For a full discussion of these influences, see Chapter 5.) Among these factors are the age of the insect, its egg load, its experiential history, such as the insect's past contacts with stimulatory or inhibitory chemicals on plants, and the time that has passed since its last feeding or oviposition bout (leading to time-dependent changes in behavior). Study of these factors leads to new ways of testing insects. For example, to determine if there is an effect of age, both "young" and "old" insects must be considered in tests as distinct treatments. Similarly, if the effect of experience is to be understood, both naïve insects and those with experience with the target species must be tested separately. These issues have long been recognized as affecting oviposition choices of parasitoids and are explored in Chapter 5.

Particular attention has been paid to time-dependent influences (amount of time since the last feeding or oviposition bout) and how these would affect the outcomes seen in both choice and no-choice tests (Browne and Withers, 2002). Higher ranked hosts are likely to be eaten or used for oviposition earlier than lower ranked hosts. On higher ranked hosts, meals are likely to be larger and egg batches deposited more frequently. Since the refractory phase (the period after a bout on a preferred host during which a less preferred host elicits no response) can vary from minutes to days, the details of each agent's biology will influence the nature of responses observed in tests. If a species remains refractory to a less preferred host for a long time (relative to the length of the test or the period between contacts with the preferred host), then one predicts that the less preferred hosts will not elicit any response and be incorrectly scored as a non-host. Also, in no-choice tests, low ranked hosts will eventually be used if the test lasts long enough. Such responses may be viewed as false positives if one assumes that the target, preferred host would be contacted in the field before the less preferred host is used. However, if one projects that the biological control agent might enter a geographic or ecological zone where the more preferred hosts are not present, or that a partial asynchrony of the agent with its target hosts can cause a temporary lack of the suitable life stage of the preferred host, then this outcome in laboratory tests would not be a false positive, but would accurately foretell exploitation of a low ranked host.

POST-RELEASE VALIDATION OF PREDICTIONS

A final necessary step in the process of developing effective testing methods is to score the accuracy of predictions by post-release evaluations of realized field host ranges. In general, there have been only selective, partial attempts in this direction. One set of studies is that in

which an agent is discovered or suspected of attacking non-target species in the field and then studied more intensively (e.g., Louda, 1998; Louda and O'Brien, 2002; Benson *et al.*, 2003b). These studies, however, begin with prior knowledge of likely impact and therefore do not collectively estimate the average outcome.

More comprehensive reviews of whole sets of cases are needed. Sources of data, however, for such a wide range of cases are hard to come by. Pemberton (2000) reviewed the literature on weed biological control agents in North America, Hawaii, and the Caribbean and found that most nontarget species reported in the literature as fed on by biological control agents were congeners of the target weed. Willis *et al.* (2003) described a preliminary assessment of the impacts of weed biological control agents on nontarget plants in Australia; however, these authors' ability to assess the robustness of original predictions was reduced by lack of access to unpublished records of quarantine laboratories that did much of the testing. Only published records could be evaluated. Several studies currently underway in New Zealand and Australia are expected to provide additional much-needed post-release evaluations of realized versus predicted host ranges.

CONCLUSION

From the previous discussion, we can draw several conclusions. First, it is clear that tests should model the ecological contexts in which agents will interact with potential hosts. In many cases, this will be a no-choice context, making choice tests a less useful means of predicting outcomes. Second, unlike for herbivorous insects interacting with potential host plants, in which the discriminatory step is most often oviposition rather than larval feeding, the reverse may be the case for parasitoids. For parasitoids, larval survival in the host may be more discriminatory than adult oviposition. Third, oviposition tests should strive to include host finding as well as host acceptance in assays by using larger arenas, with natural host plants and air circulation. Fourth, use of naïve rather than experienced adults will better reveal the breadth of the potential host range. Fifth, open field tests and host preference tests seem of limited use for work with entomophagous insects: the former because of quarantine issues and the latter because a low host preference increasingly seems an inadequate reason for considering a native species as not a host or as not at risk. We expect there will be a need to reassess these tentative conclusions in the future as more studies seek to estimate host ranges of entomophagous insects, providing more data sets on responses in alternative experimental designs.

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