
CHAPTER 5. BEHAVIORAL AND PHYSIOLOGICAL PROCESSES AFFECTING OUTCOMES OF HOST RANGE TESTING

T. M. Withers¹ and L. B. Browne²

¹Forest Research, Rotorua, New Zealand
toni.withers@forestresearch.co.nz

²CSIRO Entomology, Canberra, Australia

INTRODUCTION

Estimation of the host range of entomophagous biological control agents (parasitoids and predators) is more complex than for phytophagous weed biological control agents. This is primarily because there is an additional trophic layer involved and often an intimate and specific relationship between the target and test organisms and their substrate (usually their food plant). An important consequence of this intimate and specific relationship between the host or prey of entomophagous agents and their substrate is that prior experience of the substrate can affect the organism's responsiveness to cues from this and other substrates.

A second complicating factor for endoparasitoids is that it is not possible, in most cases (although exceptions do exist: Morehead and Feener, 2000; Fuester *et al.*, 2001), to inoculate all test organisms with eggs or neonates to determine "suitability." Thus, a program to determine the host range of parasitoids is denied one of the most powerful tools (the so-called physiological host range test) used in determination of host range of phytophagous agents (Hill, 1999; van Klinken, 2000). This means that, in the host range testing of parasitoids, it is important to employ test procedures that will maximize the probability that the test species will be accepted for oviposition. This is vital for an accurate risk assessment.

In this chapter, we discuss the effects of experience and physiological state on the host range expressed by parasitoids and predators. We suggest ways the potential agent might be treated before testing and the form the test should take to maximize the expression of host acceptance. It must be understood that effects of experience, deprivation, and age often are not clearly separable in any given circumstance. Nevertheless, we have taken the approach of discussing the known effects of each separately to illustrate the processes that might be involved.

INFLUENCE OF INFORMATIONAL AND PHYSIOLOGICAL STATES OF PARASITOIDS

EFFECTS OF EXPERIENCE

Comprehensive reviews exist that thoroughly cover the phenomena of learning in hymenopterous parasitoids (Turlings *et al.*, 1993; Vet *et al.*, 1995). Thanks to the high quality of the literature now available, we have a good appreciation of the complexity of experience effects on host-related behavior in parasitoids.

The nature of experience-induced changes in responsiveness There are two key effects of experience that can influence the outcomes of various designs of host specificity tests. First, there is change in the responsiveness of females to a previously experienced (i.e., familiar) host and/or the substrate host complex (SHC). Second, there is any change in the responsiveness to an unfamiliar host or SHC.

Generally, responsiveness to a familiar host or SHC is enhanced by experience. This phenomenon has been observed in relation to the rearing host and to the rearing host's substrate. This phenomenon has also been demonstrated in relation to experience later in life of the complete SHC or some of its components, with or without oviposition.

Experience-induced changes in responsiveness have been demonstrated most unequivocally in no-choice assays comparing the behavior of naive and experienced females. A number of studies have shown that the probability of upwind flight is higher in experienced females than in naive females, and that the relevant experience may come from (1) the odors of a familiar host or SHC (e.g., Monge and Cortesoro, 1996; Hérard *et al.*, 1988), (2) effects of the rearing substrate (Monge and Cortesoro, 1996), (3) effects of host remains (Parra *et al.*, 1996; Du *et al.*, 1997), (4) contact with the SHC but without oviposition (Du *et al.*, 1997; Potting *et al.*, 1999; Daza-Bustamante *et al.*, 2002), or (5) the effect of oviposition experience on the SHC. Other studies have demonstrated an increased probability of host acceptance (e.g., Ambriz *et al.*, 1996; Bjorksten and Hoffman, 1998) or of probing (e.g., Kerguelen and Cardé, 1996) in response to a familiar host or to components of a familiar SHC in experienced females. Others, again, have shown that searching times on a familiar host or SHC is greater in experienced females than in naive ones (e.g., Iizuka and Takasu, 1998).

There is much less directly comparable information available in relation to experience-induced changes in responsiveness to an unfamiliar host or SHC from no-choice assays. In theory, responsiveness to unfamiliar hosts or SHCs may be enhanced, unchanged, or reduced. Enhanced responsiveness could come about as a result of priming or sensitization (see Turlings *et al.* [1993] for discussion of this phenomenon). At least two instances of enhanced responsiveness to an unfamiliar SHC have been shown in no-choice assays (Turlings *et al.*, 1989; Eller *et al.*, 1992). We are aware of only one study that provides evidence for reduced responsiveness to unfamiliar hosts. Kitt and Keller (1998) showed that naive females of *Aphidius rosae* accepted several non-target species of aphid in no-choice tests, whereas females that had oviposition experience on the target species did not. This indicates that the possibility of reduction in responsiveness to unfamiliar hosts or SHCs by experienced parasitoids must be borne in mind when designing host testing protocols.

There is strong but indirect evidence that any enhancement in responsiveness to a familiar host or SHC is generally greater than any enhancement in responsiveness to an unfamiliar host or SHC. This comes from the result of choice assays in which the choices made by naive females have been compared with choices made by experienced females. Typically, the experienced females are more biased toward the familiar host than naive females. For example, naive females of *Cotesia kariyai* Watanabe strongly preferred the odor of host-infested corn plants over the odor of infested kidney bean plants (about 80% flight to infested corn). In contrast, females that had oviposited on hosts on kidney bean plants showed a preference for the odor of infested kidney bean (less than 40% of flight to infested corn) (Fujiwara *et al.*, 2000). Comparable results have been obtained by Pettit *et al.* (1992) for the braconid *Opius dissitus* Muesebeck. Similar results with respect to host acceptance following experience during eclosion have also been obtained with *Aphytis melinus* DeBach (Hare, 1996).

A substantial proportion of studies of the effects of experience on the foraging behavior of parasitoids (and phytophagous insects) has employed the classical induction-of-preference paradigm. In this, the choices made between host (or SHC) “A” vs. “B” are determined in insects that have had prior experience of either “A” or “B”. Frequently, it has been found that the choices made are relatively biased towards the familiar host or SHC (e.g., Pettit *et al.*, 1992; Geervliet *et al.*, 1998; Fujiwara *et al.*, 2000; Daza-Bustamante *et al.*, 2002). As pointed out by Bernays and Weiss (1996), results of this kind do not distinguish between enhanced responsiveness to the familiar host (or SHC) and reduced responsiveness to the unfamiliar. Nevertheless, results from this type of experiment have often shown profound effects of experience. In one striking example of relevance to the use of choice tests, it was shown that females of the aphidiid parasitoid *Aphidius ervi* (Haliday) that have oviposited in the aphid *Acyrtosiphon pisum* (Harris) on alfalfa exclusively chose the odor of the familiar SHC over the odor of *Sitobion avenae* (F.) on wheat, but females with experience of *S. avenae* on wheat exclusively chose the odor of this SHC over the odor of *A. pisum* on alfalfa (Daza-Bustamante *et al.*, 2002).

Implications of experience-induced changes for host range testing It follows from the aforementioned articles that the general expectation is for a parasitoid to be biased toward a familiar host or SHC in host range testing programs. What this means in practical terms depends (i) upon the history of the parasitoids used in the tests, (ii) how the test organisms (target and non-target) are presented in the tests, (iii) the form of the test(s) (choice versus no-choice; see Chapter 7), and (iv) the magnitude and nature of the effects of previous experience. For example, one likely scenario is for a parasitoid to be reared on the target organism feeding on a particular host plant. The experience gained of the rearing host and its host plant during eclosion and perhaps during larval development is likely to result in enhanced responsiveness to cues from this SHC. It is likely that this would be reinforced by continued contact with, and possibly oviposition experience on, the rearing SHC, if the parasitoids were not removed from the rearing colony before or shortly after eclosion.

Therefore, if the parasitoid had a greater innate preference for a SHC consisting of the target species on its host plant compared with a SHC consisting of a non-target species on a different host plant, then experience of the target SHC would, in effect, exaggerate the apparent difference between the rankings of the two SHCs. This would have somewhat different implications for the interpretation of results from choice and no-choice tests, particularly when the target species and non-target species are presented on different hosts. In choice tests, increased

contrast in ranking between the SHCs would, in itself, increase the probability that there would be no attack on the non-target species. In the case of no-choice tests, the results of tests could be influenced by any reduction or enhancement in responsiveness to unfamiliar, non-target SHC caused by experience of the target SHC. If responsiveness to the unfamiliar SHC were reduced, this would also decrease the probability of attack on the non-target species. On the other hand, if responsiveness to the unfamiliar, non-target SHC were enhanced (e.g., by priming), this would increase the probability of attack on the non-target species. Both outcomes would be influenced by the duration of the no-choice assay compared to the duration for which the effect of experience remains.

Strategies that might minimize the undesired effects of experience There are ways that experience-induced bias towards the target species can be reduced or even eliminated. The most difficult effect to avoid is any enhanced responsiveness to the rearing host, unless high quality parasitoids can be reared on an alternate host. This is particularly useful if there is an influence of experience acquired during pre-imaginal development, a phenomenon that has very rarely been demonstrated either for a host species or its substrate (Gandolfi *et al.*, 2003). More commonly, it has been shown that the apparent influence of the larval host or its substrate has been the result of early adult experience acquired at eclosion or shortly afterwards (e.g., Hérard *et al.*, 1988; Monge and Cortesoro, 1996). For crucial tests in these circumstances, the effects of the rearing environment may be avoided or reduced by such methods as dissecting the parasitoid pupae out of the host and washing the pupae prior to eclosion.

Bias in favor of the target species' host plant can be avoided if it is possible to rear the target species on two or more hosts or on a synthetic diet. This opens the possibility of presenting the target species in tests on a different substrate from that which the parasitoids experienced during pre-imaginal stages and/or during eclosion. A more practical approach to avoiding a possible bias towards the host's substrate used in the rearing of the parasitoids may be to present target and non-target species to the parasitoid on an "inert" substrate such as glass. However, this is impossible wherever test species are inseparable from their hosts, such as with internally feeding larvae, scale insects, or mealybugs.

We recommend that any bias as a result of experience of the SHC, with or without oviposition, later in the life of the parasitoid could readily be avoided by collecting the parasitoids immediately at or soon after eclosion and storing them in the absence of hosts and plant material. As indicated above, whether avoidance of oviposition experience on the target species would be desired would depend on the type of test and the effect of such oviposition on responsiveness to unfamiliar non-target hosts or SHCs. We suggest that, if no-choice tests are employed, ideally they should be done both with female parasitoids that have had oviposition experience on the target species and also comparably with females that have been denied the opportunity to oviposit.

EFFECTS OF HOST DEPRIVATION

The responsiveness of female insects to cues associated with oviposition sites is known to be affected by host deprivation (Papaj and Rausher, 1983; Barton Browne and Withers, 2002). The general expectation is that there will be a positive correlation between readiness to oviposit and elapsed time since the female last oviposited or since she emerged. The most important

practical result of this is that lower ranked hosts are more likely to be accepted as the period of deprivation increases.

Most of the evidence for this in parasitoids comes from the finding that host-deprived females (e.g., Klomp *et al.*, 1980; Hubbard *et al.*, 1999) and/or females that have had low encounter frequency with unparasitized hosts (e.g., Babendreier and Hoffmeister, 2002) show increased acceptance of hosts already parasitized by conspecific females. In the species studied, females were able to rank parasitized hosts lower than pristine hosts because of the presence of host-marking pheromones and/or because of internal changes induced within the host (see review in Nufio and Papaj, 2001). However, there is evidence that the probability of accepting lower ranked host species also increases with the period of host deprivation. Host-deprived females of the chalcidid *Brachymeria intermedia* (Nees) showed a 30% probability of acceptance of a higher ranked host, the pupae of the gypsy moth, *Lymantria dispar* (L.), about three days after eclosion. The same rate of acceptance of a lower ranked host, pupae of *Holomelina lamae* Freeman, was displayed only after about 10 days, by which time the acceptance rate of *L. dispar* pupae had increased to more than 70% (Drost and Cardé, 1992).

Life history theory predicts that readiness to oviposit would be influenced by egg load and/or host encounter rate during some period immediately preceding the current encounter (Mangel, 1989). Empirical data for parasitoids and other insects support this prediction (e.g., Minkenbergh *et al.*, 1992; Hughes *et al.*, 1994; Babendreier and Hoffmeister, 2002). Host deprivation always has the potential to give the insect a low expectation of encountering hosts and, hence, an increased probability of accepting lower ranked hosts. However, the effect of host-deprivation on the egg load, and therefore the potential contribution of this factor to any increased readiness to oviposit, is dependent on ovarian physiology. For example, a female of a pro-ovigenic species, by definition, will not increase its egg load over a period of host deprivation, and any increase in readiness to oviposit in pro-ovigenic species cannot be attributed to this factor. On the other hand, there is a potential for females of synovigenic species to increase their egg load, at least up to point, during a period of host deprivation (e.g., Eliopoulos *et al.*, 2003). The extent to which this happens, if at all, would be dependent on the nutritional reserve stored within the body and/or the availability of suitable foods during the period of host deprivation. This is particularly relevant in host-feeding species when host deprivation deprives the females of nutrients for oogenesis as well as depriving them of the opportunity to oviposit. For example, when the host-feeding species, *A. melinus*, is maintained on honey but deprived of hosts, there is a reduction in egg load due to oosorption (Collier, 1995). Reduction in egg load has also been suggested as a probable explanation for the decline in readiness to oviposit seen in *B. intermedia* (Drost and Cardé, 1992) after a prolonged period of host deprivation.

Host deprivation could be generally expected to increase the probability of acceptance of lower ranked hosts except, perhaps, when host deprivation results in a reduction in egg load, as is likely in host-feeding species. It is relevant to note, however, that the egg load in *A. melinus* did not decrease in females maintained on yeast in addition to sucrose (Heimpel and Rosenheim, 1995). Thus, any reduction in egg load during host deprivation in host-feeding species might be avoided or minimized by the provision of a suitable nitrogen-containing food in addition to a source of carbohydrate.

AGE AND LIFE EXPECTANCY

We discuss the effects of age and life expectancy in terms of “ovigeny” characteristics of a species and a female parasitoid’s “perception” of its likely life expectancy.

Ovigeny index Jervis *et al.* (2001) have refined the concepts pro-ovigenic and synovigenic proposed by Flanders (1950) by devising an “ovigeny index.” This is defined as the proportion of the maximum potential egg complement that is mature when an adult female emerges into the environment. They designated species that have an index of 1 as “strictly pro-ovigenic” and those species with an index less than 1 as exhibiting varying degrees of “synovigenicity.” The ovigeny index has a profound influence on the age-specific fecundity exhibited, especially early in adult life and assuming females have continuous or at least daily access to an abundance of high ranked hosts. Under such conditions, strictly pro-ovigenic species have been found to lay most of their lifetime egg complement within one or two days of emergence (e.g., Fleury and Boulétreau, 1993; Garcia *et al.*, 2001). In contrast, species that have an index of zero (no mature eggs at emergence) have been shown to lay few, if any, eggs one or more days after emergence. After daily egg laying begins, it continues at approximately the same rate over a considerable proportion of the female’s life span (e.g., Cohen and Mackauer, 1987; Donaldson and Walter, 1988). Therefore, the proportion of eggs available to be laid early in adult life would be positively correlated with the ovigeny index.

What implications do these differences in reproductive strategies have for the design and interpretation of host range tests? The main one relates to species that emerge with no or very few mature eggs. There is a risk that young females may not oviposit in any host because of a lack of mature eggs. Furthermore, even young females carrying some mature eggs might not oviposit in lower ranked hosts because of a low egg load. This highlights the need in no-choice tests for the use of rigorous positive controls, in which females from the same rearing group are exposed to the target pest to confirm egg laying ability of the parasitoid cohort used.

It is clearly desirable to know approximately the ovigeny index of the parasitoid in question or, at least, the egg load at emergence. In the absence of such knowledge, a possible alternative strategy is to keep female parasitoids for a few days without hosts prior to using them in host range tests. With this strategy, there is a slight risk that strictly pro-ovigenic species might reduce their egg load as a result of resorption (see above). It is relevant to note, in this context, that pro-ovigenic species have a significantly shorter adult life span than synovigenic species (Jervis *et al.*, 2001). Therefore, a standard period of deprivation would constitute a greater proportion of the life span in pro-ovigenic species, increasing their risk of resorption.

Life expectancy At least three studies have provided evidence that female parasitoids with a “perception” of reduced life expectancy display a higher incidence of superparasitism. This implies that such females are more likely to accept parasitized hosts than females of the same age with longer life expectancy. In two studies with *Leptopilina heterotoma* (Thomson), a higher incidence of superparasitism was seen in females that received cues indicative of a shorter life expectancy. The cues provided were a photoperiod typical of autumn, as opposed to a photoperiod typical of mid-summer (Roitberg *et al.*, 1992) and a drop in barometric pressure, an indicator of an imminent storm (Roitberg *et al.*, 1993). In the third study, food-deprived females of *Venturia canescens* (Gravenhorst) were found to have a higher incidence of superpara-

sitism than females that had been fed honey when both groups were tested 24 hours after eclosion (Fletcher *et al.*, 1994). The food-deprived females lived for about 2 days whereas the fed females lived for about 4 days.

The above results suggest that female parasitoids might express a wider host range when their life expectancy is reduced. Thus, it might be beneficial to test females given treatments that reduce their life expectancy (presumably by a period of food deprivation).

EFFECTS OF MATING STATUS

The mating status of a female parasitoid in those species where males commonly exist may also influence responsiveness to host stimuli. For several arrhenotokous aphidiid parasitoids, mated females remained longer in host patches, parasitized more aphids per unit time, and laid more eggs per parasitized host than did virgin females (Michaud and Mackauer, 1995). Parra *et al.* (1996) found that mated females showed enhanced upwind flight in response to the SHC. In those species where both sexes exist and can be easily distinguished, it is highly advisable, therefore, to either ensure mating occurs beforehand or have both sexes present during tests.

EFFECTS OF FEMALE SIZE

It is common in insects that egg load is positively correlated with size for females of similar physiological age and nutritional history. For example, this has been shown in the parasitoids *Aphytis lingnanensis* Compere (Rosenheim and Rosen, 1991) and *Anaphes nitens* Girault (Carbone and Rivera, 2003), and we suspect this would be so in all parasitoid species. As discussed above in the section on effects of host deprivation, it is widely accepted that readiness to oviposit is correlated with egg load (Minkenbergh *et al.*, 1992). It is logical to believe, therefore, that there would be a positive relationship between size and readiness to oviposit, even if the variation in readiness to oviposit is mediated by egg load rather than by size *per se* (Rosenheim and Rosen, 1991). This suggests that females used in tests should be as large as possible.

INFLUENCE OF EXPERIENCE AND PHYSIOLOGICAL STATE OF PREDATORS

EFFECTS OF EXPERIENCE

Relatively few studies have investigated the effects of experience on the foraging behavior of predators. Enhanced responsiveness has been demonstrated in the anthocorid *Anthocoris nemoralis* (F.) (Drukker *et al.*, 2000) and the predatory mite *Phytoseiulus persimilis* Athias-Heriot (de Boer and Dicke, 2003) to a previously experienced volatile semiochemical. In addition, it has been shown that host selection in a predaceous wasp is influenced by larval/early adult experience (Rayor and Munson, 2002). As these results are strikingly similar to those seen in some parasitoids, we believe that experienced-induced changes in responsiveness could be expected to occur more or less generally in predators. Any bias in favor of the previously consumed species and/or its substrate can be avoided or reduced by analogous strategies that we have suggested for parasitoids (see above).

EFFECTS OF PREY DEPRIVATION

As with phytophagous insects, the likelihood of acceptance of hosts for feeding is expected to increase with increasing periods of food deprivation (Barton Browne and Withers, 2002). The consequence of a deprivation-induced increase in acceptance that is most relevant to host specificity testing is that deprived predators might accept a wider range of hosts than non-deprived individuals would. This will have a direct influence on experimentally deduced host range, and has been demonstrated in stonefly larva preying on mayfly larvae (Molles and Pietruszka, 1983, 1987).

Our present understanding of the influence of food deprivation on host acceptance behavior (Withers *et al.*, 2000; Barton Browne and Withers, 2002) suggests that satiated predators introduced into choice tests would be in danger of showing reduced attack and feeding on less preferred but otherwise acceptable test species. Satiated predators might also potentially fail to accept test species in no-choice tests if the period of access to the non-target species was short. Consequently, such tests would fail to reveal the fundamental host range (*sensu* Nechols and Kikuchi, 1985; van Klinken, 2000), and there is a risk, therefore, that they would produce a false negative result. Hence a period of food deprivation prior to initiating testing would be strongly advisable.

INFLUENCE OF THE TEST ENVIRONMENT

EFFECTS OF TYPE OF TEST

The attributes of the various kinds of tests are discussed in Chapter 7. Here, we will briefly discuss the instances in which a non-target species is accepted in choice tests including the highly ranked target species and is rejected in no-choice tests (i.e., a wider host range is expressed in choice tests than in no-choice tests) (Marohasy, 1998). As pointed out by Barton Browne and Withers (2002) in their analysis of the effects of time-dependent changes in responsiveness, by Papaj and Rausher (1983), and also in Chapter 7 of this volume, there is a general expectation that a wider host range will be expressed in no-choice tests than in choice tests.

There are, however, at least four known examples in parasitoids in which one or more non-target species have been attacked in choice tests yet not attacked in no-choice tests (Bailey, 1989; Field and Darby, 1991; Barratt *et al.*, 1997; Kitt and Keller, 1998). Two explanations can be suggested to account for this type of occurrence. The first is that volatile kairomones from the highly ranked target species have condensed on or in the immediate vicinity of the non-target species. The second is that stimulation elicited by kairomones of the target species have generated an excitatory state in the female parasitoid's central nervous system, leading her to accept non-target species providing a lower level of stimulation ('central excitation' *sensu* Dethier *et al.*, 1965).

In one of the examples, the parasitoid *Sphecophaga vesparum* Curtis (Ichneumonidae) oviposited in (and then successfully developed in) two adjacent cells of a non-target wasp when unguarded cells were held in a choice situation within 10 cm of cells of the target wasp, *Vespula*

spp. In contrast, no-choice tests found no parasitism occurred on the non-target wasp *Ropalidia plebeiana* Richards (Field and Darby, 1991). In the field, these species are unlikely to nest in close proximity, leading to the conclusion that the result of the no-choice test is the true one in this case.

Whether a wider host range is expressed in no-choice or choice tests depends on the relative strengths of any time-dependent effects, on the one hand, and the effects of kairomonal contamination and/or central excitation, on the other. Since there is no way of predicting the outcome of the above processes, it would seem advantageous to perform both no-choice and choice tests including the target species before making conclusions.

EFFECTS OF SIZE AND FORM OF THE TEST ARENA

It is generally accepted that, in the field, there is a sequence of behaviors leading to host location and acceptance. This is especially true of the natural enemies of phytophagous arthropods (Vet *et al.*, 1995). Cues from the host habitat perceived at a distance (e.g., olfactory, visual) are important at the early steps in the sequence, and contact cues (e.g., gustatory) are important later in the sequence, particularly in the final acceptance or rejection stage. In most laboratory testing situations, at least some steps of the early sequence are prevented by the small size and lack of natural complexity of the test arena. There is a possibility, therefore, that the range of hosts accepted in small arenas will be wider in the laboratory than in the field if failure to respond to one or more distance cues is the factor responsible for the non-host status of any species in the field (i.e., a failure to locate the host occurs under natural conditions, which does not occur in the laboratory assay).

The scientist has a choice between attempting to simulate the field situation in host range tests or attempting to provide conditions where the maximal host range is likely to be expressed. We have stated in the introduction that we have recommended taking the latter approach, at least initially, in order to fully assess non-target species at risk of attack (see van Klinken, 2000). We believe it is adequate for the test arena to be small and simple in structure. The main consequence of predicting host range using only the final stages of host location and acceptance is to predict a host range that may be broader than would actually occur in the field (Keller, 1999). Assays incorporating more natural conditions indicative of the field should only need to be employed when such false-positive results (*sensu* Marohasy, 1998) are strongly suspected.

EFFECT OF PARASITOID DENSITY

It is possible to test parasitoids singly or in groups. The results reported in a recent paper suggests that female parasitoids display a greater readiness to oviposit when in groups than when tested singly. Carbone and Rivera (2003) found that the egg parasitoid *A. nitens* laid 50% more eggs per female when tested in groups than when tested singly. There was also a higher incidence of superparasitism when females were in groups (33% vs. 15%).

This result was interpreted as an adaptive response to the “perception” of competition for hosts. If this is indeed so, the occurrence of this kind of response should be widespread among parasitoid species. In the interests of revealing the widest realistic host range for oviposition, we

recommend, therefore, that the parasitoids should be tested in groups rather than singly. Naturally separate experiments using individual females would be required in order to obtain accurate data on attack rates, etc.

EFFECTS OF PROPORTION OF THE TARGET SPECIES RELATIVE TO NON-TARGET SPECIES

The results obtained by Cornell and Pimentel (1978) demonstrate that the outcomes of choice tests may be affected by the relative proportions of test species presented. They found that when the parasitoid *Nasonia vitripennis* (Walker) was given a choice of puparia of two blowfly species, the apparent preference shown for a species was positively correlated with the proportion of that species within the test arena. This phenomenon of frequency-dependent attack rate has implications for the design of choice tests. For example, if the target species were to outnumber one or more test species, there may be a reduced probability of attack on a lower ranked but acceptable non-target species. Thus, the most challenging choice test would one presenting only a small proportion of the presumably high ranked target species compared to the non-target species. In the interests of revealing the widest realistic host range for oviposition, we recommend, therefore, presenting only a small proportion of the presumably high ranked target species compared to the non-target species. The down-side of this approach is that statistical analysis will be compromised compared to situations in which equal proportions of test species are presented.

CONCLUSIONS AND RECOMMENDATIONS

Given the overall objective to maximize the probability of attack on non-target species in a laboratory test, we recommend the following practices or conditions.

FOR TESTS WITH PARASITOIDS

1. To take account of the potentially opposing effects of various behavioral and physiological processes:
 - Perform no-choice tests with both naive and oviposition-experienced females because it has been shown that oviposition experience can reduce responsiveness (through a specific learning process) or enhance responsiveness (through priming).
 - Perform both choice and no-choice tests because parasitoids can display wider host ranges in choice tests (contrary to the general expectation) because of time-dependent processes.
2. To minimize any experience-induced bias in favor of the rearing host, particularly in the context of choice tests:
 - Rear parasitoids on a host other than the target species, whenever possible.
3. To minimize any experience-induced bias in the favor of the rearing host's substrate (food), particularly in the context of choice tests:
 - Rear parasitoids on hosts on a different substrate from those used in the test.
 - Present target and non-target species on an inert surface whenever possible.

4. To avoid any experience-induced bias in favor of rearing SHC or components thereof due to continuing contact with the rearing environment after eclosion, particularly in the context of choice tests:
 - Remove parasitoid from the rearing environment before or shortly after eclosion.
5. To take advantage of any increase in readiness to oviposit induced by host deprivation *per se* and/or any associated changes in egg load:
 - Keep parasitoids separate from hosts but with a source of suitable food for a few days after eclosion before the test, especially in synovigenic species with a low ovigeny index.
 - If oviposition is permitted, allow a period of host deprivation before the test.
 - In host feeding species, provide a source of nitrogen-containing food (in addition to carbohydrate) during a period of host deprivation.
6. To take advantage of any increase in readiness to oviposit induced by a perception of competition for hosts:
 - Test parasitoids in groups rather than singly.
7. To take advantage of any increase in readiness to oviposit induced by a perception of a reduction in life-expectancy:
 - Subject females to a period of food deprivation before using in tests.
8. To take advantage of any increased expressed host range in environments that do not require parasitoids to respond to distance cues to establish contact with potential hosts:
 - Use small arenas that are simple in structure.
9. To take advantage of the relationship between female size and egg load and of any correlation between egg load and readiness to oviposit:
 - Use females that are as large as possible.
10. To take advantage of any frequency-dependent attack rate:
 - Provide only a small proportion of the target species compared to non-target species in choice tests.

FOR TESTS WITH PREDATORS

1. To take advantage of any increase in the tendency for food-deprived insects to accept lower ranked food:
 - Deprive predators of prey for a period before the test.
2. To minimize any experience-induced bias in favor the target species, especially in the context of choice tests:
 - Rear and maintain predators on a species other than the target species, if possible.

ACKNOWLEDGMENTS

We are grateful to John Goolsby and Sarah Mansfield for comments on this manuscript. The research was funded by the NZ Foundation for Science, Research and Technology.

REFERENCES

- Ambriz, S. J., M. R. Strand, and W. E. Burkholder. 1996. Behavioral response of the parasitoid *Lariophagus distinguendus* (Forst) (Hymenoptera: Pteromalidae) to extracts from cocoons of *Lasioderma serricornis* Fab. (Coleoptera: Anobiidae) and their effects on subsequent oviposition responses. *Biological Control* 6: 51-56.
- Babendreier, D. and T. S. Hoffmeister. 2002. Superparasitism in the solitary ectoparasitoid *Aptesis nigrocineta*: the influence of egg load and encounter rate. *Entomologia Experimentalis et Applicata* 105: 63-69.
- Bailey, P. T. 1989. The millipede parasitoid *Pelidnoptera nigripennis* (F.) (Diptera: Sciomyzidae) for the biological control of the millipede *Ommatoiulus moreleti* (Lucas) (Diplopoda: Julida: Julidae) in Australia. *Bulletin of Entomological Research* 79: 381-391.
- Barratt, B. I. P., A. A. Evans, C. M. Ferguson, G. M. Barker, M. R. McNeill, and C. B. Phillips. 1997. Laboratory nontarget host range of the introduced parasitoids *Microctonus aethiopoidea* and *M. hyperodae* (Hymenoptera: Braconidae) compared with field parasitism in New Zealand. *Environmental Entomology* 26: 694-702.
- Barton Browne, L. and T. M. Withers. 2002. Time-dependent changes in the host-acceptance threshold of insects: implications for host specificity testing of candidate biological control agents. *Biocontrol Science and Technology* 12: 677-693.
- Bernays, E. A. and M. R. Weiss. 1996. Induced food preferences in caterpillars: the need to identify mechanisms. *Entomologia Experimentalis et Applicata* 78: 1-8.
- Bjorksten, T. A. and A. A. Hoffman. 1998. Plant cues influence searching behaviour and parasitism in the egg parasitoid *Trichogramma nr. brassicae*. *Ecological Entomology* 23: 355-362.
- Carbone, S. S. and A. C. Rivera. 2003. Egg load and adaptive superparasitism in *Anaphes nitens*, an egg parasitoid of the *Eucalyptus* snout-beetle *Gonipterus scutellatus*. *Entomologia Experimentalis et Applicata* 106: 127-134.
- Cohen, M. B. and M. Mackauer. 1987. Intrinsic rate of increase and temperature coefficients of the aphid parasite *Ephedrus californicus* Bakr (Hymenoptera: Aphididae). *The Canadian Entomologist* 119: 231-237.
- Collier, T. R. 1995. Host feeding, egg maturation, resorption, and longevity in the parasitoid *Aphytis melinus* (Hymenoptera: Apheliniae). *Annals of the Entomological Society of America* 88: 206-214.
- Cornell, H. and D. Pimentel. 1978. Switching in the parasitoid *Nasonia vitripennis* and its effects on host competition. *Ecology* 59: 297-308.
- Daza-Bustamante, P., E. Fuentes-Contreras, L. C. Rodríguez, C. C. Figueroa, and H. M. Neimeyer. 2002. Behavioural differences between *Aphidius ervi* populations from two tritrophic systems are due to phenotypic plasticity. *Entomologia Experimentalis et Applicata* 104: 321-328.
- de Boer, J. G. and M. Dicke. 2003. Experience with methyl salicylate affects behavioural responses of a predatory mite to blends of herbivore-induced plant volatiles. *Entomologia Experimentalis et Applicata* 110: 181-189.
- Dethier, V. G., R. L. Solomon, and L. H. Turner. 1965. Sensory input and central excitation and inhibition in the blowfly. *Journal of Comparative and Physiological Psychology* 60: 303-313.

- Donaldson, J. S. and G. H. Walter. 1988. Effects of egg availability and egg maturity on the ovipositional activity of the parasitic wasp, *Coccophagus atratus*. *Physiological Entomology* 13: 407-417.
- Drost, Y. C. and R. T. Cardé. 1992. Host switching in *Brachymeria intermedia* (Hymenoptera: Chalcididae), a pupal endoparasitoid of *Lymantria dispar* (Lepidoptera: Lymantriidae). *Environmental Entomology* 21: 760-766.
- Drukker, B., J. Bruin, and M. W. Sabelis. 2000. Anthocorid predators learn to associate herbivore-induced plant volatiles with presence or absence of prey. *Physiological Entomology* 25: 260-265.
- Du, Y., G. M. Poppy, W. Powell, and L. J. Wadham. 1997. Chemically mediated associative learning in the host foraging behavior of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). *Journal of Insect Behavior* 10: 509-522.
- Eliopoulos, P. A., J. A. Harvey, C. G. Athanassiou, and G. J. Stathas. 2003. Effect of biotic factors on reproductive parameters of the synovigenic endoparasitoid *Venturia canescens*. *Physiological Entomology* 28: 268-275.
- Eller, F. J., J. H. Tumlinson, and W. J. Lewis. 1992. Effect of host diet and preflight experience on the flight responses of *Microplitis croceipes* (Cresson). *Physiological Entomology* 17: 235-240.
- Field, R. P. and S. M. Darby. 1991. Host specificity of the parasitoid, *Sphécophaga vesparum* (Curtis) (Hymenoptera: Ichneumonidae), a potential biological control agent of the social wasps, *Vespula germanica* (Fabricius) and *V. vulgaris* (L.) (Hymenoptera: Vespidae) in Australia. *New Zealand Journal of Zoology* 18: 193-197.
- Flanders, S. E. 1950. Regulation of ovulation and egg disposal in the parasitic Hymenoptera. *The Canadian Entomologist* 82: 134-140.
- Fletcher, J. P., J. P. Hughes, and I. F. Harvey. 1994. Life expectancy and egg load affect oviposition decisions of a solitary parasitoid. *Proceedings of the Royal Society of London B* 258: 163-167.
- Fleury, F. and M. Boulétreau. 1993. Effects of temporary host deprivation on the reproductive potential of *Trichogramma brassicae*. *Entomologia Experimentalis et Applicata* 68: 203-210.
- Fuester, R. W., M. Kenis, K. S. Swan, P. C. Kingsley, C. López-Vaamonde, and F. Hérard. 2001. Host range of *Aphantorbaphopsis samarensis* (Diptera: Tachinidae), a larval parasite of the gypsy moth (Lepidoptera: Lymantriidae). *Environmental Entomology* 30: 605-611.
- Fujiwara, C., J. Takabayashi, and S. Yano. 2000. Oviposition experience on a host-infested plant affects flight and antennal searching behavior of *Cotesia kariyai* towards the host-plant complex. *Entomologia Experimentalis et Applicata* 97: 251-256.
- Gandolfi, M., L. Mattiacci, and S. Dorn. 2003. Preimaginal learning determines adult response to chemical stimuli in a parasitic wasp. *Proceedings of the Royal Society of London B* 270: 2623-2629.
- Garcia, P. V., E. Wajnberg, M. L. M. Oliveira, and J. Tavares. 2001. Is the parasitization capacity of *Trichogramma cordubensis* influenced by the age of the females? *Entomologia Experimentalis et Applicata* 98: 219-224.
- Geervliet, J. B. F., A. I. Vreugdenhil, M. Dicke, and L. E. M. Vet. 1998. Learning to discriminate between infochemicals from different plant-host complexes by the parasitoids *Cotesia glomerata* and *C. rubecula*. *Entomologia Experimentalis et Applicata* 86: 241-252.
- Hare, J. D. 1996. Priming *Aphytis*: behavioral modification of host selection by exposure to a synthetic contact kairomone. *Entomologia Experimentalis et Applicata* 78: 263-269.
- Heimpel, G. E. and J. A. Rosenheim. 1995. Dynamic host feeding by the parasitoid *Aphytis melinus*: the balance between current and future reproduction. *Journal of Animal Ecology* 64: 153-167.

- Hérard, F., M. A. Keller, W. J. Lewis, and J. H. Tumlinson. 1988. Beneficial arthropod behavior mediated by airborne semiochemicals. IV. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson. *Journal of Chemical Ecology* 14: 1597-1606.
- Hill, R. L. 1999. Minimising uncertainty - in support of no-choice tests, pp. 1-10. *In: Withers, T. M., L. Barton Browne, and J. Stanley (eds.). Host Specificity Testing in Australasia: Towards Improved Assays for Biological Control.* Scientific Publishing, Department of Natural Resources, Brisbane, Australia.
- Hubbard, S. F., I. F. Harvey, and J. P. Fletcher. 1999. Avoidance of superparasitism: a matter of learning? *Animal Behaviour* 57: 1193-1197.
- Hughes, J. P., I. F. Harvey, and S. F. Hubbard. 1994. Host-searching behavior of *Venturia canescens* (Grav.) (Hymenoptera: Ichneumonidae): superparasitism. *Journal of Insect Behavior* 7: 454-464.
- Iizuka, T. and K. Takasu. 1998. Olfactory associative learning of the pupal parasitoid *Pimpla luctuosa* Smith (Hymenoptera: Ichneumonidae). *Journal of Insect Behavior* 11: 743-760.
- Jervis, M. A., G. E. Heimpel, P. N. Fern, J. A. Harvey, and N. A. C. Kidd. 2001. Life-history strategies in parasitoid wasps: a comparative analysis of 'ovigeny'. *Journal of Animal Ecology* 70: 442-458.
- Keller, M. A. 1999. Understanding host selection behaviour: the key to more effective host specificity testing, pp. 84-92. *In: Withers, T. M., L. Barton Browne, and J. Stanley (eds.). Host Specificity Testing in Australasia: towards Improved Assays for Biological Control.* Scientific Publishing, Department of Natural Resources, Brisbane, Australia.
- Kerguelen, V. and R. T. Cardé. 1996. Reinforcement mechanisms of olfactory conditioning during parasitization by the parasitoid *Brachymeria intermedia* (Hymenoptera: Chalcidae). *Journal of Insect Behavior* 9: 947-960.
- Kitt, J. T. and M. A. Keller. 1998. Host selection by *Aphidius rosae* Haliday (Hym., Braconidae) with respect to assessment of host specificity in biological control. *Journal of Applied Entomology* 122: 57-63.
- Klomp, H., B. J. Teerink, and W. C. Ma. 1980. Discrimination between parasitized and unparasitized hosts in the egg parasite *Trichogramma embryophagum* (Hym.: Trichogrammatidae): a matter of learning and forgetting. *Netherlands Journal of Zoology* 30: 254-277.
- Mangel, M. 1989. An evolutionary interpretation of the "motivation to oviposit." *Journal of Evolutionary Biology* 2: 157-172.
- Marohasy, J. 1998. The design and interpretation of host specificity tests for weed biological control with particular reference to insect behaviour. *Biocontrol News and Information* 19: N13-N20.
- Michaud, J. P. and M. Mackauer. 1995. Oviposition behavior of *Monoctonus paulensis* (Hymenoptera: Aphidiidae): factors influencing reproductive allocation to hosts and host patches. *Annals of the Entomological Society of America* 88: 220-226.
- Minkenbergh, O. P. J. M., M. Tatar, and J. A. Rosenheim. 1992. Egg load as a major source of variability in insect foraging and oviposition behavior. *Oikos* 65: 134-142.
- Molles, M. C. J. and R. D. Pietruszka. 1983. Mechanisms of prey selection by predaceous stoneflies: roles of prey morphology, behavior and predator hunger. *Oecologia* 57: 25-31.
- Molles, M. C. J. and R. D. Pietruszka. 1987. Prey selection by a stonefly: influence of hunger and prey size. *Oecologia* 72: 473-478.
- Monge, J. P. and A. M. Cortesoro. 1996. Tritrophic interactions among larval parasitoids, bruchids and Leguminosae seeds: influence of pre- and post-emergence learning on parasitoids' response to host and host-plant cues. *Entomologia Experimentalis et Applicata* 80: 293-296.

- Morehead, S. W. and D. H. Feener, Jr. 2000. An experimental test of potential host range in the ant parasitoid *Apocephalus paraponerae*. *Ecological Entomology* 25: 332-340.
- Nechols, J. R. and R. S. Kikuchi. 1985. Host selection of the spherical mealybug (Homoptera Pseudococcidae) by *Anagyrus indicus* (Hymenoptera Encyrtidae): influence of host stage on parasitoid oviposition, development, sex ratio and survival. *Environmental Entomology* 14: 32-37.
- Nufio, C. R. and D. R. Papaj. 2001. Host marking behavior in phytophagous insects and parasitoids. *Entomologia Experimentalis et Applicata* 99: 273-293.
- Papaj, D. R. and M. D. Rausher. 1983. Individual variation in host location by phytophagous insects, pp. 77-124. *In*: Ahmad, S. (ed.). *Herbivorous Insects: Host Seeking Behaviour and Mechanisms*. Academic Press, New York.
- Parra, J. R. P., S. B. Vinson, S. M. Gomes, and F. L. Cônsoli. 1996. Flight response of *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) in a wind tunnel to volatiles associated with infestations of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). *Biological Control* 6: 143-150.
- Petitt, F. L., T. C. J. Turlings, and S. P. Wolf. 1992. Adult experience modifies attraction of the leafminer parasitoid *Opius dissitus* (Hymenoptera: Braconidae) to volatile semiochemicals. *Journal of Insect Behavior* 5: 623-634.
- Potting, R. P. J., G. M. Poppy, and T. H. Schuler. 1999. The role of volatiles from cruciferous plants and pre-flight experience in the foraging behaviour of the specialist parasitoid *Cotesia plutellae*. *Entomologia Experimentalis et Applicata* 93: 87-95.
- Rayor, L. S. and S. Munson. 2002. Larval feeding experience influences adult predator acceptance of chemically defended prey. *Entomologia Experimentalis et Applicata* 104: 193-201.
- Roitberg, B. D., M. Mangel, R. G. Lalonde, C. A. Roitberg, J. J. M. van Alphen, and L. E. M. Vet. 1992. Seasonal dynamic shift in patch exploitation by parasitic wasps. *Behavioral Ecology* 3: 156-165.
- Roitberg, B. D., J. Sircom, C. A. Roitberg, J. J. M. van Alphen, and M. Mangel. 1993. Life expectancy and reproduction. *Nature* 364: 108.
- Rosenheim, J. A. and D. Rosen. 1991. Foraging and oviposition decisions in the parasitoid *Aphytis lingnanensis*: distinguishing the influences of egg load and experience. *Journal of Animal Ecology* 60: 873-893.
- Turlings, T. C. J., J. H. Tumlinson, W. J. Lewis, and L. E. M. Vet. 1989. Beneficial arthropod behaviour mediated by airborne semiochemicals. VIII. Learning of host-related odors induced by a brief contact experience with host by-products in *Cotesia marginiventris* (Cresson), a generalist larval parasitoid. *Journal of Insect Behavior* 2: 217-225.
- Turlings, T. C. J., F. L. Wäckers, L. E. M. Vet, W. J. Lewis, and J. H. Tumlinson. 1993. Learning of host-finding cues by hymenopterous parasitoids, pp. 51-78. *In*: Papaj, D.R. and A.C. Lewis (eds.). *Insect Learning: Ecology and Evolutionary Perspectives*. Chapman and Hall, New York.
- van Klinken, R. D. 2000. Host-specificity testing: why do we do it and how we can do it better, pp. 54-68. *In*: Van Driesche, R. G., T. Heard, A. S. McClay and R. Reardon (eds.). *Host-Specificity Testing of Exotic Arthropod Biological Control Agents: the Biological Basis for Improvement in Safety*. USDA Forest Service, Forest Health Technology Enterprise Team, Report FHTET-99-1, August, 2000. Morgantown, West Virginia.
- Vet, L. E. M., W. J. Lewis, and R. T. Cardé. 1995. Parasitoid foraging and learning, pp. 65-101. *In*: Cardé, R.T. and W. J. Bell (eds.). *Chemical Ecology of Insects*. Chapman and Hall, New York.

Withers, T. M., L. Barton Browne, and J. Stanley, 2000. How time-dependent processes can affect the outcome of assay, pp. 27-41. *In*: Van Driesche, R. G., T. Heard, A. S. McClay and R. Reardon (eds.). *Host-Specificity Testing of Exotic Arthropod Biological Control Agents: the Biological Basis for Improvement in Safety*. USDA Forest Service, Forest Health Technology Enterprise Team, Report FHTET-99-1, August, 2000. Morgantown, West Virginia.