Host Specificity Testing: Why Do We Do It and How We Can Do It Better

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Abstract

Host specificity testing is universally used in weed biological control to predict non-target effects of potential agents. Despite this, there is some confusion regarding the role of host specificity testing in making such predictions. One possible role is as an assay of field host range. In this case, the ideal host specificity test will simulate conditions encountered in the field, and the result (the estimated field host range) will be judged according to how accurately it matches the realized field host range. An alternative approach is to separate the description of innate host specificity (which includes fundamental host range, the relative acceptability and suitability of hosts, the ability to learn, and time dependent effects) from the prediction of how it will be expressed in the post-release environment (in terms of field host range and relative attack). In this case, host specificity testing is used to describe properties of the insect, which are then used in combination with ecological information to predict where, when, and to what extent non-target attack would occur. I argue that the latter approach is more powerful because non-target effects under any particular environmental conditions are predicted, rather than being estimated by attempting to experimentally simulate the release environment.

Here I discuss this more basic approach to host specificity testing in some detail in relation to the meaning of the terms host specificity and host range, and I point out the implications of this approach for the way that we conduct host specificity testing. My approach to host testing can be divided into three steps: (1) identification of aspects of life history that need to be host-specific if the insect is to be safe for release; (2) description of the fundamental host range of the organism; and (3) if non-target species are included within the fundamental host-range, prediction of whether they will be attacked under field conditions and the frequency and severity of such attacks.

Keywords: biological control, host specificity testing methodology, innate host specificity, fundamental host range, realized host range, time-dependent effects, learning.
Introduction

All potential weed biological control agents need to undergo extensive host-specificity testing to ensure that their release would not result in unacceptable non-target impact. The biology of each potential agent is different, which means that the experimental methods used have to be modified for each species to ensure that our predictions of non-target attack are as accurate as possible. Decisions about testing include which aspects of the insect’s life history to focus on, what experimental designs to use, what combinations of tests to apply, whether to apply tests to the entire plant test list or just to a subset of it, the order in which tests are conducted, and the balance between laboratory and field trials. Each of these decisions can potentially affect the accuracy of our predictions. However, it is the purpose of this paper to consider the more fundamental issues of what role host specificity testing can and should play in the prediction of non-target attack, and what that means in practice. These are important issues, particularly as the scientific credibility of biological control and the accuracy of its predictions, come under increasing scrutiny (Thomas and Willis, 1998).

There are essentially two philosophical approaches to host specificity testing. The first seeks to predict non-target impact through experiments that attempt to simulate the field conditions likely to be encountered post-release (Fig. 1a). In this approach, trials conducted under field conditions are considered ideal because they are most realistic or “natural” (Wapshere, 1989; Cullen, 1990; Briese, 1999), although in practice laboratory trials are often necessary. Surveys and experiments seek to estimate the likely field host range in the proposed release environment. These methods are judged as successful if their predictions are accurate. For example, test designs are judged according to their likelihood of overestimating field host range (and thus generating “false positives”) or underestimating field host range (and generating “false negatives”) (Marohasy, 1998; Edwards, 1999; Hill, 1999; Heard, 2000).

This assay-based approach has a number of limitations. One limitation is that it is very difficult to simulate the field conditions that an agent would encounter in its introduced range, particularly in laboratory trials. A second limitation is that, even if accurate simulation were possible, the introduced range is likely to be heterogeneous with respect to the relative availability of target and non-target hosts, and this in turn can significantly affect relative attack (Courtney and Kibota, 1989). Estimates of relative effects on various non-target plants that are obtained through simulation assays apply to specific sets of field or experimental conditions and therefore it may be difficult or impossible to generalize...
such predictions to fit other conditions. A good illustration of this dilemma is the controversy surrounding the appropriate use of no-choice and choice trials (Harley, 1969; Cullen, 1990; Blossey, 1995; Harris, 1998; Edwards, 1999; Hill, 1999; Sheppard, 1999). Proponents of choice trials argue that they more realistically represent field conditions and that there is a danger of no-choice trials generating “false positives”. Proponents of no-choice trials argue that choice trials can generate “false negatives”, because the agent won’t necessarily be faced with a choice in the field. In reality, both arguments could be correct, sometimes. It will depend on the relative availability of target and non-target hosts, which could vary from all weed to all non-target species, with all possible ratios in between.

An alternative philosophical approach to host-specificity testing is to conduct experiments in order to describe the innate host-specificity of the insect (Fig. 1b). To achieve this goal, we need to describe what plant species an agent is capable of finding, accepting and using and how well it can do so, taking into account the plasticity of behavioral responses to deprivation and prior experience. Information thus gained can be used to predict non-target attack under the full spectrum of environmental conditions the insect would be likely to encounter once released (Fig. 1b). Such an approach also allows the host specificity of insects to be compared more objectively (van Klinken, in press) and provides a means for assessing the possibility of host-specificity evolving after the release of an agent in a new environment (van Klinken, 1999a).

In this paper I discuss the second approach. I first look at the terms “host range” and “host specificity” and how they relate to the innate host finding and accepting abilities of the insect, and to their expression under field conditions. I finish by examining methodological implications of this approach for the way we go about predicting non-target attack.

**What is Host Specificity and Host Range?**

The terms host specificity and host range are basic to the biological control lexicon, and it is important to understand what each means in relation to both the innate capabilities of the insect and what actually happens in the field.

Host specificity is used to rank insect species within a continuum, from specialists to so-called generalists (Fig. 2). It is commonly used synonymously with host-range breadth. However, the host-specificity of an insect can be further differentiated according to how acceptable or suitable hosts are relative to each other. For example, an insect that performs equally well on all host species would be less host specific than an insect for which only one of the same range of species is an ideal host, even though host-ranges are identical (Fig. 2). There are therefore two dimensions to quantifying how
host-specific an insect is - host range breadth, and the relative acceptability or suitability of hosts.

This two-dimensional concept of host-specificity is implicit in host-specificity testing, which aims to both define the host-range and obtain comparative data among hosts. However, this usage of the term differs from that in the behavioral literature, in which specificity refers specifically to differences in the discriminatory phase, as defined by Singer et al. (1992). As will be seen, the discriminatory phase (the time over which an individual accepts one plant while the lower-ranked plant is rejected) is only one of many possible ways in which the host-specificity of insects can be described. Limiting the description of host-specificity to comparisons of discriminatory phases is therefore unnecessarily restrictive.

**Host Range**

In the simplest terms, the host-range of an insect is the sum of plant species (or more precisely plant phenotypes) that are hosts. Host-range breadth will depend on the relatedness of those hosts. For example, herbivores are commonly categorised as being monophagous, oligophagous, and polyphagous, according to the degree of taxonomic relatedness of their hosts (Symons and Beccaloni, 1999). However, describing the host-range of an insect can be complicated by the fact that host-range is sometimes dependent on context. For example, the host-range observed in experiments is frequently broader than what occurs in the field (Shepherd, 1990; Olckers, 1999). Host range can even differ across an insect’s geographic range (Hodkinson, 1997).

One way to deal with this problem is to differentiate between fundamental and realized host ranges (Nechols et al., 1992). The fundamental host range is the most inclusive host range because it includes all the plant species that an insect is capable of accepting and/or utilising. It therefore represents the genetically determined limits to the host range of a particular insect species or, more precisely, insect genotype. The realized host range is how the fundamental host range is actually expressed under particular conditions (Nechols et al., 1992). In biological control we are concerned with predicting how the fundamental host range will be realized if the agent were to be released (the field host range).

**Fundamental host range.** The absolute limits to an insect’s host range, which circumscribe fundamental host range, are constrained by such factors as its metabolic and sensory capabilities, physical limitations and behavioral programming. For example, the location and acceptance of a host for oviposition is determined by an often complex catenary sequence of behaviours (Miller and Strickler, 1984; Wapshere, 1989). For some insects, this is highly constrained, with only a single plant species being accepted even when the insect is highly deprived and is offered no alternative (Adair and

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**Life History Stage**

<table>
<thead>
<tr>
<th>Life History Stage</th>
<th>Fundamental Host Range</th>
</tr>
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<tbody>
<tr>
<td>Egg Hatch</td>
<td>All test plants (2 families)</td>
</tr>
<tr>
<td>Nymphal Development</td>
<td>Tribe Mimoseae (Prosopis and Neptunia)</td>
</tr>
<tr>
<td></td>
<td>Late (instars 4-5)</td>
</tr>
<tr>
<td>Adult Feeding</td>
<td>Prosopis</td>
</tr>
<tr>
<td></td>
<td>(for oogenesis and longevity)</td>
</tr>
<tr>
<td>Oviposition</td>
<td>Possibly coupled with adult feeding (i.e., Prosopis)</td>
</tr>
<tr>
<td></td>
<td>Host finding cues</td>
</tr>
<tr>
<td></td>
<td>Final acceptance</td>
</tr>
<tr>
<td></td>
<td>All physically suitable test plants</td>
</tr>
</tbody>
</table>

Fig. 3. Fundamental host range estimated for different aspects of the life history of the psyllid, *Prosopidopsylla flava* Burchhardt (van Klinken, *in press*). *P. flava* inserts eggs into plant tissue and oogenesis occurs as adults. Host range breadth is represented schematically.
Scott, 1997). Constraints are likely to be primarily behavioral, although physical factors such as ovipositor length may also be constraining (Zwölfer and Harris, 1971). Similarly, nutritional requirements and metabolic limitations are likely to be important constraints to larval and adult development, although behavioral mechanisms can also play a critical role in determining whether an insect will commence feeding on an otherwise suitable host (Scriber, 1984; Slansky and Rodríguez, 1987).

In theory, fundamental host range can be described for any aspect of the insect's life history where interactions between insect and host occur. For example, the fundamental host range could be described separately for oviposition, egg development, larval development and adult feeding (Fig. 3). At a more refined level it could be described for each behavioral step within the catenary sequence of behaviors resulting in host acceptance (Wapshere, 1989; Keller, 1999). For most, if not all, insects the fundamental host range will differ dramatically for different aspects of its life history. For example, the pre-alighting cues of some insects such as certain aphids can be very general, and they discriminate primarily on the basis of post-alighting cues (Kennedy et al., 1959). Other insects appear to depend more on pre-alighting cues for host selection (Barton Browne et al., 1969; Johnson and Siemens, 1991). The fundamental host range could also be described for the life cycle of the insect (i.e. the plant species that fulfill all requirements for life and reproduction), which would represent the intersection of fundamental host ranges for each particular aspect of life history. For the psyllid, *P. flava*, it would be the genus *Prosopis* (Fig. 3).

In biological control, an insect's maximal host range is frequently described as its “physiological host range” (Cullen, 1990; McEvoy 1996; Ockers, 1998). This is misleading as it implies that the innate capacity of an insect to accept and use a host is constrained only by physiology. This is certainly not the case, even with larval development, for which the term physiological host range is most often applied. Larval development depends on the insect having innate behavioral responses to initiate and continue feeding, having nutritional requirements that can be met by the plant, having the physical ability to consume sufficient plant material to obtain necessary nutrients, and having the metabolic, behavioral and other capabilities to overcome any toxic properties (Scriber, 1984; Schoonhoven, 1987; Slansky and Rodríguez, 1987). The fundamental host range for larval development is therefore the consequence of all of these constraints combined. Even if desired, it is questionable whether we could decouple physiological constraints from all other constraints.

**Field host range.** Field host range is what actually happens in the field. Like fundamental host range, it can be described for different aspects of the herbivore’s life history. For example, field host range can differ between oviposition, breeding and adult feeding. An extreme example of differences between oviposition and breeding host range is a hepalid moth that oviposits indiscriminately over pastures (Barton Browne et al., 1969). Similarly, some psyllid species feed as adults on more plant species in the field than they breed on (Hodkinson, 1974).

Under field conditions, the realized host-range is frequently a subset of the fundamental host-range. That is, insects often accept or use only a proportion of those that they are capable of (Harris and McEvoy, 1995; Wapshere, 1989; Cullen, 1990). There are several possible reasons for the fundamental host range not being fully expressed (Fig. 4).

Clearly, for an insect to locate and use a host it must be sufficiently close to detect the necessary cues (Cullen, 1990). Spatial coincidence will depend on the geographic distributions of the insect and host, which in turn are determined by factors such as their respective abiotic requirements and the absence of geographic barriers. The distribution of the insect may also depend on the availability of plant species that can support a population. More locally, strong habitat preferences may prevent insect and host from co-occurring. For example, many insect species appear to restrict their search for hosts to particular vegetation types (Kibota and Courtney, 1991; van Klinken, 1996). A potential host must also be available at the correct time. For example, the weevil “*P. cordister*” requires rootstock in summer, and would therefore be unable to use winter annual species, even though some can support larval development (Cullen, 1990).

Even if a potential host is available, it may never be used because it is not included in the fundamental host range of a prior step in the insect’s life history. For example, a plant species on which larvae feed in the laboratory may never be used in the field because females do not recognize it as a host for oviposition...
(Wan and Harris, 1996). Similarly, host-specific pre-alighting cues might determine that only a small subset of available plant species is actually assessed through contact cues (Frick and Andres, 1967; Wapshere, 1989). Alternatively, a potential host may never be used because a more acceptable host is always available. Behavioral reasons for this include effects of prior experience and time dependent effects, and these are discussed further in the following section.

**Relative Acceptability or Suitability, and the Effect of Internal Status**

The relative acceptability or suitability of all hosts within the host range can be compared to give a more complete picture of an insect's host-specificity (Fig. 2). Like fundamental host range, hosts can be, and have been, compared for many aspects of the insect's life history. For example, hosts can be compared according to how acceptable they are for oviposition and initiation of feeding, or for support of pre-reproductive and reproductive development (Marohasy, 1998). The relative acceptability of hosts can also be compared for particular behaviors within the host location and acceptance sequence (Kennedy, 1965; Courtney and Kibota, 1989; Keller, 1999).

However, unlike fundamental host range, the relative acceptability and suitability of hosts to the insect is a dynamic property. It can be profoundly influenced by the internal status of the insect, particularly through time-dependent processes and effects of prior experience (Solarz and Newman, 1996; Newman et al., 1999; Heard, 2000; Withers et al., 2000). For example, deprivation can result in the acceptance of previously unacceptable hosts (Withers et al., 2000), and prior experience can reverse preference rankings (Hanson, 1976; Szentesi and Jermy, 1990). Behavioral plasticity is an innate property of the insect (Fig. 1b) and can be described experimentally.

Behavioral plasticity means that the expression of behaviors under field conditions can be complex (Rausher, 1980; Prokopy et al., 1987; Courtney and Forsberg, 1988). For some insects, prior experience and time dependent effects such as deprivation are likely to be greatly influenced by encounter rates with potential hosts, which in turn will depend on the relative availability of hosts (Prokopy and Lewis, 1993). For example, where the most acceptable host is relatively abundant, insects are less likely to become deprived enough to feed on a less acceptable host. Conversely, if the most acceptable host was relatively rare, then insects might become sufficiently deprived to begin accepting the lower ranked hosts. If prior experience results in a reversal in preference rank, then insects may in fact find the most abundant host more acceptable, regardless of their previous rank. However, regardless of the mechanism, relative attack rates can be profoundly influenced by relative host availability (Thompson, 1988; Blossey et al., 1994; Aeschlimann, 1997; Withers, 1998).

**Host Specificity Testing Methodology**

Non-target attack in the field is the consequence of the interaction between an insect's innate host-specificity and the environment (Fig. 1b). Host specificity testing can describe innate host specificity in terms of fundamental host ranges, the relative acceptability or suitability of each host, and how that is affected by changes in internal status. Exactly which aspects of the insect's innate host specificity need to be described will depend on what we need to know in order to be confident that there will be no “undue” non-target effects. When combined with knowledge of the release environment, these results can be used to predict field host range, and if relevant, when, where and to what extent non-target attack will occur.

This general approach to host specificity testing can be summarized as a three-step process. Firstly, the aspects of the life history that needs to be host-specific are identified in order to determine exactly what aspects of the innate host-specificity need to be described. Secondly, the fundamental host range is estimated for each such aspect. Finally, a prediction of the non-target consequences if the insect were to be released is made. The latter step may include further description of the insect's host-specificity such as the relative acceptability and suitability of hosts, and the effects of experience and deprivation.

In practice some steps might overlap. For example, comparative data (step 3) are often obtained as a by-product of determining the fundamental host range, and host-specificity testing often provides further insights into the insect's life history (step 1). Native range studies might also be conducted before determining the fundamental host range. This serves the dual purpose of ranking potential agents for subsequent study (according to their likely specificity,
likely impact and amenability to laboratory work [Schroeder and Goeden, 1986; Briese 1999] and obtaining a better understanding of their life history.

**Step 1. Identifying What Needs to be Host Specific**

The first step to host-specificity testing is identifying which aspects of the insect's life history need to be host specific. The requirement for host specificity will often depend on the life history of the insect and where it is to be released. The completion of larval development is an essential step in the life cycle of all insects, and for most insects larval feeding is also the most damaging aspect. Complete larval development, and possibly merely larval feeding, on non-target species in the field is therefore of primary concern. However, other aspects may also have to be considered. Late instar larvae can sometimes feed on more plant species than neonate larvae, and this may be important if there is a risk of them dispersing onto new plants (Cullen, 1990).

Where ovipositing females damage their host (such as twig-girdlers), oviposition on non-target species could be a potential problem, even if larval development cannot occur. Similarly, adult feeding on non-target species may be a concern, even if it does not result in oogenesis. Even exploratory feeding on non-target species could be a problem where adults are known virus vectors (Briese, 1988). Thus, for some insects we have to ensure that more than one aspect of their life history is sufficiently host-specific.

The potentially damaging aspects of an insect's life history need not, however, be studied directly. Sometimes the potentially damaging aspects of an insect's life history are preceded by prerequisite behavioral or developmental steps (Wapshere, 1989), and these steps could be studied instead. For example, if larvae depend on their mother to select the right host, it might be sufficient just to study oviposition (Heard et al., 1997). In some cases it may even be necessary to study the pre-requisite step, such as when the damaging step is not easily studied (for example when culturing is difficult) or is not sufficiently host-specific (Wapshere, 1989; Harris and McEvoy, 1995).

**Step 2. Estimating Fundamental Host Range**

Given that we have identified the parts of the life history for which we want to determine host-specificity, the fundamental host range can then be described for each. Since the fundamental host range represents the limits of an insect's ability, estimating it will identify all the plant species an insect is capable of accepting or using, regardless of the field conditions it may encounter. The better our estimate, the less chance we have of inadvertently excluding possible non-target field hosts. Carefully chosen plant test lists and experimental design will ensure our estimates are as accurate, and therefore as inclusive, as possible.

Theoretically, the fundamental host-range includes all the plant species (or more specifically, phenotypes) that are hosts. Given that it is not possible (or desirable) to test the total flora, it is necessary to subsample. The centrifugal-phylogenetic method is generally applied (Wapshere, 1989), although it assumes that host-range will correlate with phylogenetic relatedness, which may not always hold (Weidemann, 1991). Sometimes plant traits, such as plant architecture, that are not necessarily correlated to plant phylogeny, may therefore also need to be considered. Where possible, the quality of test plants should reflect what would be encountered in the field by using intact plants of the right age and reproductive stage. Sometimes particular fertilizer regimes (Cuda et al., 1995; van Klinken, 1999b) or prior exposure to sun (Cullen, 1990) may be necessary.

Both time dependent effects (Papaj and Rausher, 1983) and effects of prior experience (Szentesi and Jermy, 1990) can limit the full expression of the fundamental host-range (Marohasy, 1998; Heard, 2000; Withers et al., 2000). “Maximum likelihood” tests must therefore be designed to exclude this possibility. Time dependent effects can be excluded by conducting no-choice trials for the duration of the insect’s life. A no-choice design ensures that there is no alternative, more acceptable host, to confound the insect’s response, and conducting trials for the duration of the insect’s life ensures that the insect will be sufficiently deprived to accept a poorer host. Possible effects of prior experience can be

**Table 1. Possible methods for obtaining rapid, yet accurate, estimates of host ranges**

<table>
<thead>
<tr>
<th>Method Description</th>
<th>Benefits</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single test conducted on complete plant list</strong></td>
<td></td>
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</tr>
<tr>
<td>• Examine limited part of life history (e.g., first feeding instar)</td>
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<td></td>
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<tr>
<td>• Choice minus target trials</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Compromised test on complete plant list, test assumptions on a subset</strong></td>
<td></td>
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</tr>
<tr>
<td>• Use experienced individuals</td>
<td></td>
<td></td>
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<tr>
<td>• Limit the duration of tests</td>
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*See text for benefits and drawbacks of each test.
excluded to some extent by using neonate larvae and newly emerged adults. Further precautions against experience effects can be taken by washing eggs or dissecting out pupae. However, examples where prior experience has resulted in an irreversible change in fundamental host-range are rare (Ma, 1972; Renwick and Lopez, 1999).

Conducting experiments to determine necessary fundamental host ranges is relatively simple and rapid for some insect species, particularly for those which express their most discriminating host finding and acceptance behaviours under laboratory conditions (e.g., Gassmann and Tosevski, 1994; Adair and Scott, 1997; van Klinken, in press). However, for other insects, the required experimental design may not be possible in practice, or at the very least it might be unnecessarily strict and time-consuming. There are several ways of streamlining the estimation of fundamental host range (Table 1).

One approach is to describe the fundamental host range for a limited part of the insect's life history. For example, the fundamental host range for larval development could be estimated for just the first feeding instar, rather than for complete development. If larvae do develop through to the next instar a separate trial could be run to determine if complete development would occur on those species (e.g., van Klinken, 1999b). Another potential approach when studying host-acceptance is to conduct choice minus target trials (Heard and van Klinken, 1998; Edwards, 1999). If no plants are accepted, then the trial effectively becomes a no-choice trial. However, if at least one plant species is attacked then the trial would have to be repeated without that species to ensure lower ranked hosts have not been missed (Peschken and Derby, 1988; Marohasy, 1998). The compromise in this trial design is that deterrents from one plant species could completely mask an otherwise acceptable host (Marohasy, 1998), although I am not aware of any cases where this has been documented. The possibility could be minimised by randomising the combination of test plants presented in any one test.

Another approach to obtaining more flexibility in experimental design is to apply a less strict design on the entire plant test list, but then to test any resulting assumptions on a subset of plant species (Table 1). For example, if newly emerged adults are difficult to obtain it will sometimes be easier to use experienced adults when conducting feeding trials for life. The additional assumption that prior experience will not limit the insects' ability to feed on a test plant can be tested separately using naïve insects on a subset of plant species. Similarly, for insect species that are particularly long-lived, or are in short supply, conducting trials until all individuals are dead may not be cost-effective. In this case compromises can be made. For example, no-choice oviposition or feeding trials can be conducted sequentially (Heard and van Klinken, 1998), so that each plant is exposed for much less than the duration of the insect's life. It is however possible that these tests will result in an underestimation of the fundamental host range because insects never become sufficiently deprived to accept the non-target, or because of effects of prior experience. Once again, both assumptions could be tested on a subset of plant species.

Fundamental host ranges are already being estimated in most host-specificity studies under the guise of "laboratory host-range", "physiological host-range" or "experimental host-range" (Zwölfer and Harris, 1971; Gassmann and Tosevski, 1994; Olckers, 1998; Purcell et al., 1998; Hill et al., 1999). However, the aspect of the insect's life history for which the fundamental host range is being described is often not stated, and factors that could potentially limit the full expression of host range are rarely, if ever, explicitly excluded. Improved estimates of fundamental host ranges can generally be made by stating what it is that the fundamental host range is being described for, and by explicitly removing factors that could result in it not being fully expressed. Although it will not always be possible to entirely exclude such factors, what would result is the best possible estimate of what plant species the insect is capable of accepting and/or using.

**Step 3. Extrapolating to the Field**

Some insects have fundamental host-ranges which are essentially restricted to the target, and no further work is therefore required (e.g., Heard et al., 1997; van Klinken, in press; van Klinken and Heard, in press). However, if the insect is capable of attacking non-target species in a way that is considered potentially detrimental, then several avenues exist in order to predict what will happen after release. Predictions can be made as to whether those non-target species will actually be attacked in the field, the relative and absolute level of such attack, and its consequences. Only the prediction of field host range and relative non-target attack are considered below. The population dynamics of the insect would need to be predicted in
order to translate predictions of relative attack to one of absolute attack. Predicting the consequence of any non-target attack in terms of ecological, economic, social and/ or political impact are considered elsewhere (McFadyen, 1998; Waage and Kirk, 1999).

**Predicting field host range.** The fundamental host range will not necessarily be fully expressed in the field (Fig. 4). In practice, the main arguments for potential non-target hosts not being attacked after release are lack of coincidence with the potential distribution of the agent, and host-specificity of prerequisite steps. Lack of coincidence between insect and potential host can be demonstrated by predicting the potential distribution of the insect and comparing it with existing non-target distributions (Heard and Forno, 1996). However, potential changes in the distribution of both the target (if agent survival depends on it) and the non-target hosts (e.g., through changing land use) may also need to be considered. At a more local scale, vegetation associations of the agent and non-target species, and the phenology of the agent in relation to the non-target species, could be used to argue that non-target species will not be attacked (Frick and Andres, 1967; Harris and McEvoy, 1995).

Host-specificity of prior steps in an insect’s life history or host selection behaviour might limit or prevent an otherwise suitable non-target host from being attacked in the field (Harris and McEvoy, 1995; Wán and Harris, 1996). For example, larval attack on non-target larval hosts would not be expected if oviposition were restricted to the target and there were no possibility that larvae would disperse onto other larval hosts. Similarly, it is sometimes argued that species in which there is oviposition on non-target species in cages will still be host-specific in the field because distance cues that are effectively bypassed in cages are specific to the target. However, in each case the host-specific step must indeed be prerequisite, which may be difficult to prove conclusively in some cases.

A potentially confounding factor in using estimated fundamental host range to predict field host range is any difference between test plants and field plants that is biologically important. For example, Monochoria species supported complete development of Xubida infusellus (Walker) in the laboratory, but it was predicted that complete development would not occur in the field because plants are shorter-lived than those grown in the laboratory (Stanley and Julien, 1998). This represents a limitation imposed on the fundamental host-range estimate (because not all plant phenotypes were tested), rather than a discrepancy between fundamental and field host ranges.

**Predicting relative non-target attack.** If non-target species are likely to be attacked, then we can predict where, when and to what extent this may occur by considering the relative acceptability and suitability of hosts, and how that might be expressed in the release environment (Fig. 1b). If non-target attack is sufficiently “minor,” the agent could still be considered for use in biological control (Day, 1999; Hill, 1999). The theory and methodology necessary for describing relative acceptability and suitability of hosts and how it can be influenced by the internal status of the insect (through time dependent effects and prior experience) has received much attention in the general literature (Bernays and Chapman, 1994; Eigenbrode and Bernays, 1997), and in the biological control literature (Zwölfer and Harris, 1971; Marohasy, 1998; Heard, 2000; Withers et al., 2000). Acceptability and suitability can be compared in numerous ways, including acceptability for oviposition (during the discriminatory phase) (Singer et al., 1992;
Eigebrode and Bernays, 1997; Marohasy, 1998; Withers, 1998), total fecundity (where oogenesis results from larval feeding) (van Klinken and Heard, in press) and preference rank (Wiklund, 1975, 1981; Marohasy 1998; Stanley and Julien, 1999). Similarly, the suitability of plant species for complete larval development can be compared by determining the proportion of neonates which develop to adults, comparing growth parameters such as relative growth rate and developmental times, or comparing measures of “fitness” such as total fecundity, size and weight of emerging adults (e.g., Tabashnik, 1983; Wan et al., 1996). More detailed studies can be conducted to compare relative growth rates within instars, efficiency of conversion of ingested and digested food, and approximate digestibility (Berenbaum and Zangerl, in press).

The way hosts are compared will depend to a large degree on experimental design. No-choice trials can be used to compare various traits such as larval survival and development rates, the amount of adult feeding and resulting fecundity, and rates of natural increase (e.g., Blossy et al., 1994; Wan and Harris, 1997). Often these comparative data can be obtained when estimating the fundamental host range. Direct (continuous) observation or temporal sampling can often provide additional information such as duration of the discriminatory phases and feeding bouts (Solarz and Newman, 1996; Eigenbrode and Bernays, 1997; Withers, 1998; Singer et al., 1993). Choice trials can be used to rank hosts at particular relative densities (Wiklund, 1981; Marohasy, 1998; Briese, 1999; Stanley and Julien, 1999). In many experiments the internal status of the test insect can be manipulated in order to see how it influences relative acceptability and suitability. For example naive and experienced insects can be compared (van Klinken, in press), as can insects at different levels of deprivation (Withers et al., 2000).

Two potential qualifiers to the description of relative acceptability and suitability are effects of plant quality and intra-specific variation. Plant quality can differ between and among test plants and field plants in ways that affect their relative acceptability and suitability as hosts (Lowman and Box, 1983; Leather, 1989; Waring and Cobb, 1989; Cullen, 1990; Price et al., 1990; van Dam and Hare, 1998; Baars and Nesper, 1999; van Klinken, 1999b). This can make the interpretation of experimental results difficult. Similarly intra-specific genetic variation among herbivores can result in dramatic differences among individuals in their acceptance and use of different hosts (Wiklund, 1981; Papaj and Rausher, 1983; Singer et al., 1993).

Although rarely documented, such variation can have implications both in terms of immediate post-release attack and the rapid evolution of insect preferences and performances (Thompson, 1998).

When predicting relative attack in the field, relative acceptability and suitability must be considered in terms of the relative availability of target and non-target hosts. The simplest case is if non-target and target populations are far enough apart such that the non-target has to be a “sufficiently good host” to sustain a viable population (Heard and Forno, 1996). Prediction is not so straightforward if target and non-target species overlap. Plant (and insect) populations are typically heterogeneous and dynamic, and this needs to be understood in order to predict what environments the insect is likely to encounter post-release. Heterogeneity is particularly obvious for annuals, or in cases in which the weed is eventually brought under effective control in parts of its range. Under these circumstances, the challenge is to predict how availability of hosts will affect their relative acceptability and suitability, and thus relative attack.

In practice, concluding that a potential host is safe to release, despite the inclusion of non-target species in the field host range, will be easiest where differences between relative acceptability and suitability are great. Where non-target and target are likely to be sympatric, cases in which behavioral plasticity is limited would be easiest to interpret.

**Concluding Remarks**

Although host specificity testing is central to the prediction of non-target attack, confusion remains regarding its precise role. One approach is to view it as a direct estimate of field host range and relative attack. The primary limitation of this approach is that potentially profound effects of environmental variation (such as changes in relative host availability) on relative attack can only be determined by estimation, not prediction. That is, experiments need to realistically simulate each of the possible environments that an insect is likely to encounter post-release. The alternative approach is to use host specificity testing to describe the insect’s innate host-specificity, which might include its fundamental host range, and how the relative acceptability and suitability of hosts are influenced by changing internal status. The strength of this approach...
is that it concentrates on describing properties of the insect, which can in turn be used to accurately predict relative attack under any likely post-release conditions.

This second approach can be translated into a methodology for host specificity testing which produces generalizable results with which to make accurate predictions of non-target attack in the field. Fundamental host range, which represents the absolute limits of the insect’s innate host specificity, is described first for aspects of the insect’s life history that need to be host specific. If non-target species are included, predictions can be made as to whether non-target species within the fundamental host range will indeed be attacked in the field. If they will be, further host specificity testing can be conducted in order to describe relative acceptability and suitability of the different hosts and how possible learning or time dependent mechanisms modify them. These results can be used, together with a detailed knowledge of the release environment, to predict when, where and to what extent non-target attack is likely to occur.

This approach differs from existing experimental approaches in one or more of the following ways. It distinguishes between the innate capacity of an insect to interact with plants, and how that innate capacity is expressed under particular field conditions in terms of field host range and relative attack (Figs. 1b, 4). It describes host specificity as having two dimensions, host range and the relative acceptability and/or suitability of hosts (Fig. 2). It acknowledges that fundamental host range can be described for any aspect of an insect’s life history where the insect interacts with plants (Fig. 3). It accounts for possible behavioral plasticity resulting from prior experience or time dependent changes in internal status. Finally, it views the role of host specificity testing as describing innate host specificity (including fundamental host range, relative acceptability and suitability of hosts, and behavioral plasticity), rather than predicting field host range (Fig. 1).

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References


McEvoy, P.B. 1996. Host specificity and biological pest control: how well is research on host specificity addressing the potential risk of biological control? Bioscience 46:401-405.


van Klinken, R.D. Host-specificity constrains evolutionary host change in the psyllid *Prosopidopsylla flava*. *Ecological Entomology* (In press)

van Klinken, R.D. and T.A. Heard. Estimating the fundamental host range of *Evippe* sp. #1 (Gelechiidae), a potential biocontrol agent for *Prosopis* species (Leguminosae). *Biocontrol Science and Technology* (In press)


