HOST SPECIFICITY TESTING OF BIOCONTROL AGENTS OF WEEDS

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Abstract. A range of test designs is available to biocontrol practitioners. Their suitability depends on the biology of the potential agent being tested. Tests are conducted on many aspects of the biology of agents including oviposition, adult feeding, larval feeding, larval development, adult longevity and fecundity, and field utilization under natural conditions. Commonly used designs are choice, no-choice and choice-minus-target, in parallel or in sequence. Many behavioral factors affect the results of host specificity testing. These factors can be divided into 1. the sequential behavioral responses to host plant cues, 2. learning and 3. the effects of time-dependent factors. These behavioral effects can be complex, can be caused by a variety of mechanisms and can produce opposing effects. Biocontrol workers should be familiar with the behaviors that affect the results of these tests and apply their knowledge of them appropriately. That is, biocontrol workers responsible for host specificity testing should recognize that they are applied behavioural biologists as well as applied ecologists.

Keywords: host range testing, risk assessment, applied behavioral theory.

INTRODUCTION

Host specificity testing is a critically important step in the process of introducing natural enemies for classical biological control. Most direct non-target effects can be predicted if a sound understanding of the host specificity of potential biocontrol agents is gained. Host specificity testing provides the basic information upon which the safety of a proposed biocontrol agent can be assessed. Sound host specificity testing also prevents another problem—the rejection of safe and potentially effective agents because of an inability to prove their high level of specificity. Hence in host-specificity testing, we are trying to avoid false results, both false positive results and false negative ones. False positives refer to the attack of a plant in the test when there is no potential for attack on that plant in nature. That is, the host range is over-estimated. False negative results indicate no attack in the test where there is potential for attack in the field; the host range is thereby under-estimated (Marohasy 1998).

In this paper, I review experimental designs used in host-specificity testing of potential biological control agents. I then list some of the more important behavioral and biological factors that affect the validity of tests and discuss the strengths and limitations of the various tests used in light of these behavioral and biological factors. I do not cover the topic of the selection of plants for the host test list as this is well covered in the literature. This talk focuses on arthropod agents of weeds but the results and analysis are applicable to host specificity testing of predators and parasitoids of arthropod pests. Host specificity testing of pathogens will continue to rely on inoculation of test plants because spore dispersal of most potential weed control agents is passive. However, where insects are the vectors of pathogens, appropriate testing must account for insect behavior.

REVIEW OF METHODS USED IN HOST-SPECIFICITY TESTING

Aspects of biology examined

Many experimental approaches to determination of the host range are used. Various experiments examine aspects of host selection and use by agents as indicated by oviposition, adult feeding, larval feeding, larval development, adult longevity and fecundity. Some tests are conducted in the field and determine which hosts are used under natural conditions (Heard & van Klinken 1998).

Test designs

Tests are traditionally divided into two designs-- choice and no-choice--depending on whether the organism can select from a range of species or is restricted to a single species. In a no-choice test, groups of insects are placed on each plant species in separate arenas such as cages, jars, petri dishes, etc (Figure 1). In choice tests, insects are located in arenas in the presence of a choice of plant species including the target weed (the control). A separate category of design is the choice-minus-target (or choice-minus-control) test that includes a choice of test plant species excluding the target. The results of no-choice tests allow us to predict the realized field host range in the absence of the target weed. Choice tests do the same in the presence of the weed. Both of these scenarios are possible in nature so both types of tests have a role.

No-choice and choice-minus-target tests are subdivided into sequential or simultaneous depending on whether the target species and the test species are offered in sequence to the same insects or at the same time to different insects. If done simultaneously, separate groups are placed on test plants at the same time. Sequential tests differ in that the same group of adults is moved from plant to plant. In the sequential tests, the insects are not naïve but have experience of other plants. The significance of this will be discussed in the section on learning below.

Choice-minus-target tests are useful and perhaps under-used designs. A powerful, efficient test with few behavioral shortcomings is one in which the target weed is offered as no-choice and done simultaneously with the choice of test plants.

We can gain a clearer picture of the suitability of different host specificity tests by viewing tests as a combination of biological responses measured under different experimental designs (Table 1). The test design used depends on the biological

Table 1. Frequency of occurrence of test designs versus biological response measured as determined from a literature review of 38 papers published over the past 20 years (Heard & van Klinken 1998).

	No-choice	Choice	Choice-minus-target	Total
Oviposition	15	26	6	47
Adult feeding	13	15	3	31
Larval feeding	8	6		14
Larval development	30			30
Adult longevity	9			9
Adult fecundity	7			7
Field utilisation (from surveys)		5		5
Field utilisation (from open-field tests)		4		4
Total	82	56	9	

response under investigation. Tests on larval development, adult longevity and fecundity can be only of the no-choice design, while field-survey and open field tests are necessarily choice tests under natural or semi-natural conditions. Other responses (oviposition, adult feeding and larval feeding) may be addressed in any of the test designs. From the final column, we see that tests commonly examine oviposition and larval development. In many studies these two tests are done together for a particular agent to assess the behavioral preferences of adults (acceptability) and the suitability of the plant for larval development and production of viable adults. If both responses are measured together, results may be confounded. If, for example, the eggs are laid internally in the plant and not easily scored, larval presence or damage or adult emergence are the only indications of oviposition. In this case, one cannot discern whether a negative score is the result of unacceptability of the host for oviposition, or unsuitability for larval development, or both.

When these two responses are measured separately, some practitioners subject all test plants to both tests. Others only subject species accepted in oviposition tests to larval development tests. A third option is to assess larval development on all plant species and to assess suitability for oviposition only on those species that support larval development. Because most immature insects have limited dispersal ability, larval development trials are most important for plant species on which oviposition occurs. Withers (1999) recommends that all test plants be subjected to both tests to fully assess risk.

Larval development tests are prone to false positive results, because the developmental host range is often wider than the range of plants used in nature. Hence by themselves, larval development tests can result in the rejection of safe agents. Sometimes, when tests assessing oviposition behavior are difficult (e.g. in many Lepidoptera and Hemiptera), larval development tests cannot be done in conjunction with oviposition tests. Larval development tests are still routinely used in the USA where they often are called starvation tests. Where standardized testing procedures are required, it is the only test that can be applied to nearly all agents.

Adult feeding tests are commonly investigated. These trials are restricted to species that feed destructively and so are not used with those species that do not feed (e.g., Cecidomyiidae) or that feed non-destructively (e.g., most Lepidoptera, Bruchidae, Tephritidae). Adult feeding may not be important *per se*, in that only minimal damage can be done to plants if larvae cannot develop on them. However, even cosmetic damage can affect public perception of biocontrol results. In India, a small amount of feeding on sunflower by *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) a biocontrol agent of *Parthenium hysterophorus* L. (Asterales, Asteraceae) has almost shut down the use of biocontrol agents to control weeds in that country.

Adult fecundity tests (also known as oogenesis tests) examine the ability of a plant to support egg production. Their use is restricted to insects that depend on adult feeding for continued egg production (Schwarzlaender *et al.* 1996). They differ from adult feeding or oviposition tests that test the acceptability of a host. Adult fecundity tests examine both the acceptability of the food (behavior) and its suitability for egg maturation (physiology). These tests are relevant only for plant species that adults accept for feeding. Adult longevity tests often are performed also with adult fecundity tests.

Field surveys and open-field tests are being used only occasionally but increasingly. In open-field tests, the test plants are placed in the field in the native

range of the agent. Sometimes the local abundance of agents is increased. The absence of a cage in these tests allows the assessment of all aspects of the host selection process, so the results are more likely to predict the realized host range when the agent is introduced into a new geographic range. Field surveys are fundamentally the same as open-field tests, but in the latter plant spatial arrangements and agent densities are not manipulated.

A STRATEGY FOR HOST-SPECIFICITY TESTING

This array of test combinations must be integrated into an overall experimental strategy. More than one test usually is needed to determine host specificity. The sequence in which tests are employed may vary; field tests can be done early, for preliminary screening, or late, for clarification of equivocal lab results. Several tests may be applied to all plant species on a list or one type of test can be used to eliminate plants from further testing before a second test is applied to the reduced set of plants. Alternatively a subset of plants may be tested initially to decide whether the agent is sufficiently promising to justify further testing.

Some attempts have been made to combine the results of several tests to calculate an index of plant suitability – a bottom line (Wan & Harris 1997). This is a step towards a risk assessment approach, in which the risk to non-target plant species is quantified. For example, the probability that a plant is accepted for oviposition multiplied by probability of larval development estimates the probability of an adult producing a pupa on the host plant. All plants on the test list should be tested for both these components to make comparable calculations. Some practitioners resist this approach because it would not seem necessary to test the suitability of a plant for larval development if it is never accepted for oviposition. (Withers 1999). It may not be desirable to develop a formulaic approach to host specificity testing. The current lack of standardization in the type of test used reflects the unique aspects of the biology of each agent as well as associated practical considerations.

BIOLOGICAL FACTORS AFFECTING TESTING STRATEGIES

Insect behavior involved in host selection is expressed during the conduct of tests and can influence the results. Understanding the host selection behavior of potential agents is the key to more effective host specificity testing.

Sequential behavioral responses to host plant cues

There is a long held and widely accepted recognition that insects use a sequence of behavioral responses to cues in host selection. The sequence of steps in host selection includes habitat location, host location, host acceptance, and host utilisation (Keller 1999).

Consideration of the sequential behavioral steps to host selection raises a number of issues that have consequences for host specificity testing. Possibly the most important point is inability to express the early steps of the host selection sequence in many experimental arenas If each step eliminates a certain number of potential hosts, then the removal of that step from a host test may generate false positive results. This process has been demonstrated with *Drosophila magnaquinaria* (Diptera, Drosophilidae) (Kibota & Courtney 1991). In nature, this insect first selects its habitat (low lying areas); within that habitat it then selects its only natural host (skunk

cabbage). If the insect is confined to a cage, it selects many more plant species for oviposition than it does in nature, producing false positive results. Testing for host range in insects such as this should incorporate this early step in the host selection behavior.

Use of larger, more natural arenas, field surveys and open-field testing may alleviate this problem. Less well-known methods to minimize the false positives in lab tests include the use of wind tunnels or olfactometers, or simply provision of good airflow through cages. Long-distance cues used in host location often rely heavily on olfaction and the still air in cages does not allow for the upwind response of insects to olfactory cues. If a potential agent gives a negative response to a plant species in an olfactometer, that plant species may be eliminated as a potential host. That plant species may have been accepted when the insect was confined to it in a cage. Increased airflow through cages may provide a simple solution to allow some insects to include olfactory cues in the host selection process (Keller 1999).

It is thus important to understand the critical steps in the host selection process for the insect being tested; and include these steps in the test design. Some insects are very specific in their habitat choice, e.g. the *Drosophila* on skunk cabbage. Others, such as many Lepidoptera, use distant, pre-alighting host plant cues. Still other insects, such as *Aphis fabae* Scop. (Homoptera, Aphididae), passively locate plants, but show high specificity for chemo-tactile cues such as surface chemicals. Insects that depend on pre-alighting host plant cues probably will not be tested accurately in small arenas, but insects that passively locate hosts can be.

Learning

Learning can be defined as the modification of behavior due to the effects of prior experience. The effects of experience (learning, memory and forgetting) are important behavioral components of the host-selection process. Hymenopterous parasitoids are well known for their abilities to learn. A smaller proportion of phytophages are known to learn; they include Lepidoptera (adults and larvae), tephritid flies, Orthoptera and Coleoptera (Papaj & Lewis 1993). A number of mechanisms are involved in learning; I will examine two-- habituation and induction of preference.

Habituation is the decrease in response to a stimulus with repeated exposure to that stimulus. Habituation to feeding deterrents is a common phenomenon in which an insect initially is deterred but, after more exposure, begins to accept a plant. Habituation can have consequences for host specificity testing. For example, insects may habituate to deterrents of non-hosts through repeated contact while confined with them in cages resulting in eventual acceptance of those plants. False positive results are produced in such cage tests. In nature, the insects are likely to leave the plant before habituation occurs.

Induced preference is the effect of experience on changes in food or oviposition preferences. For example, in many species, newly hatched larvae will only accept plants they first experience for further feeding. Induced oviposition and adult feeding preferences are influenced by early experience in many insects. For example, an adult may respond to cues she learned when she emerged from her pupa to show oviposition or feeding preference for the same species of plant on which she emerged. Induced larval feeding preferences can lead to false negative results, if insects begin their feeding on the target weed and are then transferred to test species.

Other mechanisms of experience and learning can affect the results of host specificity tests (Marohasy 1998, Heard, 2000). In general several tactics help avoid

unwanted consequences. First, whenever possible use naïve insects that have no experience of any plants. Second, be aware of the false results certain tests can generate and incorporate this information into their interpretation. Third, use different test designs and compare results to assess which mechanisms may be operating.

Time-dependent effects

Time-dependent effects are reversible changes in the responsiveness of an organism to a potential host resulting from food or oviposition site deprivation. As an insect becomes more deprived it may become more likely to accept lower ranked hosts. A hungry insect may feed on a wider range of hosts than a satiated one. An insect that has recently laid an egg may reject a lower ranked host for a considerable period (Withers *et al.* 2000).

The main consequence of this effect is a false negative result in choice tests. For example, *Bruchidius villosus* (F.) (Coleptera, Bruchidae), a seed feeder introduced into New Zealand and Australia against broom, is attacking tagasaste (*Chamaecytisus palmensis*), a non-target plant in New Zealand. This attack on tagasaste does not represent a host range expansion, but a failure of host specificity testing to predict field host range (Fowler *et al.* 2000). Testing relied on choice tests alone and under the conditions of this test, tagasaste was not attacked. Choice tests can generate false negative results due to time-dependent effects. Insects may never reach a sufficiently deprived state in the presence of the host plant to accept lower ranked hosts. However, when in a no-choice situation, as happens in the field because tagasaste fruits are available before broom, the insect may oviposit and feed on non-target species

A recent review of open-field testing has shown that this effect can take place under natural conditions (Briese 1999). The results of open-field tests vary depending on the experimental design. For example when the density of test plants is too low, false negatives results are obtained because the insects never reach a sufficiently deprived state.

To minimize these consequences, temporal patterns of feeding and oviposition should be understood for each insect. This information should be incorporated into the design of the host specificity tests. Second, no-choice trials should be continued for the whole of the insect life to ensure that the insects become sufficiently deprived to accept lower ranked hosts. Third, in open-field tests and field surveys, the target plant should be removed to achieve a deprived state in insects.

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Figure 1. Some test designs used in host specificity testing of biocontrol agents. Circles represent plants, squares represent cage, and arrows represent the addition of a group of insects.