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X International Symposium on Biological Control of Weeds
Bozeman, Montana, USA
July 4-14, 1999

Proceedings of Session: Host Specificity Testing of Exotic Arthropod Biological Control Agents - The Biological Basis for Improvement in Safety

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Abstract

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Estimating accurately the field host ranges that are likely to occur after biological control agents are released is a key feature in promoting the safe use of biological control. Several aspects of the experimental designs used to estimate host specificity under laboratory conditions can affect the validity and meaning of test results. Papers presented here explore several such issues, including the effect of the physiological state of the test insects, the nature of the test design (choice, no choice, etc.) and genetic variability of the individuals used in tests. Estimation of the degree of host specificity of natural enemies is important both for herbivorous insects used in weed biological control and for parasitoids and predators used for arthropod biological control. While many similarities exist among testing methods for these groups, there are also significant differences, some of which are discussed here in the final two papers in this session.

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Photo Credit

On the cover: Bernd Blossey, Assistant Professor and Director, Biological Control of Non-Indigenous Plant Species Program, Department of Natural Resources, Cornell University, Ithaca, NY, took the cover photo. Photo shows garden beds used to grow host specificity test plants (*note shade cloth for forest species*).

Thursday, July 8th — Session: Host Selection and Specificity

Time (P.M.)	*Event*	*Presenters*
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2:00-2:20	The Role of Coincidence in Host-Range Expansion	***Douglas W. Tallamy***
2:20-2:40	Time-Dependent Changes in Responsiveness Can Influence the Outcome of Both No-Choice and Choice Assays	***Toni M. Withers***
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3:00-3:20	Afternoon Break	
3:20-3:40	Developing an Hypothesis Driven Approach to Host- Specificity Testing, with Examples from Mesquite Biocontrol	***Rieks Dekker van Klinken***
3:40-4:05	Evaluating Host Specificity of Agents for Biological Control of Arthropods: Rationale, Methodology, and Interpretation	***D.P.A. Sands***
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Concepts in Insect Host-Plant Selection Behavior and Their Application to Host Specificity Testing

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Abstract

Testing the host specificity of potential agents is an important part of biocontrol methodology. An understanding of the behavioral processes involved in selection of a host plant can be used to improve the accuracy of host specificity testing by biocontrol practitioners and others interested in predicting field host use. These behavioral processes include the sequential nature of host selection behavior, the effects of experience, and time-dependent changes of host acceptance or rejection. Each of these three aspects of behavioral science is reviewed and its potential effect on the outcome of host testing is examined. The means by which practitioners can incorporate these concepts into the design, implementation and interpretation of host specificity tests are discussed. Practical matters affected by these issues include: (a) choice of arena size and design (e.g., small cages versus wind tunnels versus open field tests), (b) duration of tests, (c) use of behavioral observations to examine the process instead of the end result, and (d)interpretation of the results of choice vs no-choice tests, sequential versus parallel tests, and open field versus cage tests. Because of the diversity of behavioral factors and the inconsistent ways in which they can produce false results in host specificity tests, guidelines cannot be generalized. Hence, all biocontrol practitioners are encouraged to become familiar with the relevant concepts and apply them appropriately.

Introduction

Serious concerns about the non-target effects of biocontrol agents are increasingly being expressed by ecologists, the wider scientific community and biocontrol practitioners themselves (Thomas and Willis, 1998; Anonymous, 1999). Many of these warnings call for biocontrol practitioners to better understand the effects of releases of biocontrol agents. The best single way of predicting both direct and indirect non-target effects is to understand the host specificity of agents (*see* Secord and Kareiva, 1996). Host specificity testing provides the primary information for making decisions on whether to release an agent.

Given the importance of host specificity testing, can we improve it? In this paper, I argue that the application of behavioral concepts gives us an opportunity to improve the design, conduct, and interpretation of host specificity testing. Insect behavior is a large and fast moving area of research. The practice of host specificity testing has benefited much from such basic studies but we can continue to fine tune testing methodology by applying the latest information and concepts (Marohasy, 1998; Withers et al., 1999; Withers et al., 2000).

Biocontrol practitioners have argued that their testing is sufficiently rigorous because they have made few mistakes. Several recent examples show, however, that the outcome of host specificity tests can be influenced by behavioral phenomena that express themselves differently in tests of different designs. The mirid

Eucerocoris suspectus Distant completed development on several plant species including guava (*Psidium guajava* L.) in open field tests when all feeding sites on the target weed, the paperbark tree, *Melaleuca quinquenervia* (Cavier) Blake were destroyed. Extensive cage choice tests and field surveys did not reveal any attack on guava (Purcell et al., 2000). This insect will not be released against paperbark trees in Florida but could have been if the former tests were not done. Later I will show how behavioral factors were responsible for this serious disparity in the results of different types of tests.

A second example is of greater concern because the insect has already been released. *Bruchidius villosus* Fabricius, a seed bruchid, was recently released in New Zealand and Australia against broom (*Cytisus scoparius* [L.]) but is attacking tagasaste (*Chamaecytisus palmensis* [L. Fil.] Link), a non-target plant. 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. Many examples are known of the expression of a broader host range in no-choice tests compared to choice tests (Hill et al., 1995; Marohasy, 1998). Behavioral factors generate these results. When the design and interpretation of trials fail to recognize and understand these factors, problems can and do arise. Other studies show differences between host ranges measured in the field compared to the laboratory (Balciunas et al., 1996) but this is not always the case (Cordo et al., 1995).

In this paper, I first review the proximate behavioral factors that influence host acceptance and choice. I divide the host selection behavior into: (1) sequential behavioral responses in host plant selection (and use), (2) effects of time dependent factors, and (3) effects of experience. I treat the effects of time dependent factors very briefly as they are covered by Withers et al., 2000. To illustrate the above points, I give an example of a hypothetical insect and follow its life and behavior. I then discuss how the behavioral factors can influence the results of host tests and what we can do to improve our testing using this information.

Host Plant Selection in Phytophagous Insects

Sequential Behavioral Responses in Host Plant Selection

There is a long held and widely accepted view that insects use a sequence of behavioral responses in host selection. This was first recognized in parasitoids and later in phytophagous insects (e.g., Kennedy, 1965). The sequence of steps in host selection includes habitat location, host location, host acceptance, and host use. Insects use a number of sensory cues in host selection including visual, olfactory, gustatory, and tactile stimuli as well as humidity and light intensity (Bernays and Chapman, 1994). These cues stimulate receptors, generating sensory input and finally behavioral responses. A large number of sensory receptors of different modalities receive stimulation at each step in the host selection process. This information must be processed and integrated by the central nervous system, interpreted as a positive or negative signal and a decision made as to whether to make a certain behavioral response. Courtney and Kibota (1990) critically review host plant selection for oviposition while Mayhew (1997) reviews adaptive patterns of host plant selection.

Different species express high specificity at different stages in the host selection process. High specificity early in the host selection process has been demonstrated in nature with *Drosophila magnaquinaria* Wheeler, which shows very high specificity to its habitat: wet, low-lying areas. Low levels of host specificity are expressed at later stages – pre-alighting attraction to cues from many plant species occurs and larval survivorship on many substrates is very high. However, high host specificity in the field occurs because skunk cabbage is the only suitable substrate in its preferred habitat (Kibota and Courtney, 1991). After habitat selection, distance cues are used in host location. For example, adult apple maggot flies, *Rhagoletis pomonella* (Walsh), show positive responses to host odor within a few meters of the source (Aluja and Prokopy, 1992). Post-alighting cues are the most important stage in host selection for some insects, including the bean aphid, *Aphis fabae* Scopoli. This insect alights with equal frequency on host and non-host plants proving the lack of a role for pre-alighting cues. After contact with non-hosts, the aphids leave non-hosts but remain on hosts. Antennation of the leaf surface allows contact cues to be assessed (Kennedy et al., 1959).

It will be shown later that the testing methods that are best to determine the host specificity of agents will depend on the behavioral stage in the agent's host selection sequence in which it expresses the greatest specificity.

Experience and Learning

The effects of experience – learning, memory and forgetting – are important behavioral components in the host selection process. Learning is the modification of behavior due to the effect of prior experience. Learning can happen very quickly. The effects of experience can be short-lived or prolonged: from seconds to days. Learning allows animals to infer correlations among stimuli in order to predict the future occurrence of resources (Smith, 1993). A constant resource favors the evolution of innate responses to cues. Hence hymenopterous parasitoids, many of which use hosts that occur on different plant species, are well known for their abilities to learn. A smaller proportion of phytophages are known to learn but they include Lepidoptera (adults and larvae), tephritid flies, Orthoptera, and Coleoptera (Prokopy and Lewis, 1993). Similarly host selection behavior by host specific insects may be less affected by learning than in insects with a broader host range. However, even relative specialists are affected by learning, e.g., associative learning has been demonstrated in the cabbage butterfly, *Pieris rapae* L., and will be described in a later section.

In the following paragraphs, I will describe the mechanisms involved in learning. Generally these mechanisms can be divided into two groups: associative and non-associative. Non-associative learning includes habituation and sensitization.

Habituation. Habituation is the decrease in response to a stimulus with repeated exposure to that stimulus. Habituation to deterrents may be very common in phytophagous insects as many plant secondary compounds are deterrent but not toxic. The acceptability to grasshoppers and caterpillars of foods treated with deterrents has been shown to increase over time as they habituated to the deterrents (Bernays, 1995).

Sensitization. In one sense, sensitization is the opposite of habituation. It is the gradual increase in response to a stimulus with repeated exposure to that stimulus. An example is a feeding deterrent that allows feeding to occur for a few minutes on the first encounter but prevents all feeding on the second encounter. Another example is the positive response to a previously neutral phagostimulant following contact with that phagostimulant. Priming is a related concept that occurs where experience with an innate stimulus makes the insect more responsive to other stimuli such as other foraging cues (Turlings et al., 1993).

Central excitation and central inhibition. Contact with a highly ranked host will increase the responsiveness and readiness of an insect to oviposit or feed. Central excitation is a similar effect to sensitization but is shorter lived and the underlying physiological mechanism is different (Barton Browne et al., 1975).

Associative learning. Associative learning, also known as classical conditioning, is the association of a neutral stimulus with an innately meaningful stimulus that produces a positive or negative effect. When the insect next encounters the previously neutral stimulus it responds to it. Conditioning is well known in parasitoids. An example from a phytophagous insect is demonstrated by the cabbage white butterfly. Adult females were given paper discs of two colors, only one of which was impregnated with an oviposition stimulant, sinigrin. When later given a choice of the two colors without the stimulant, they chose the color that had previously been associated with the chemical (Traynier, 1984; 1986). A mechanism related to associative learning is aversion learning, the learning by an animal to associate a negative internal effect with the taste of a food (Bernays, 1993).

Induction of preferences. It is often difficult to deduce the exact mechanism that explains why experience has changed behavior and several learning mechanisms could be involved (Bernays, 1995). The process of induced preference provides an example. Induced preference is the effect of experience on changes in food or oviposition preferences such that the relative acceptability of plants already fed or oviposited upon is increased. Induced feeding preferences in larvae are an outcome potentially caused by a number of behavioral and physiological mechanisms including habituation to deterrents, associative learning and sensitization (Bernays and Weiss, 1996). Induced oviposition and adult feeding preferences have been shown for many insects. For example, adult females of some species respond to cues on the host plant on which they have emerged from their pupae, and later these insects may show a preference to oviposit or feed on that host plant.

Time-Dependent Effects

Time-dependent effects are, with experience, important internal factors affecting host selection. These effects have been defined as the changes in responsiveness in relation to time elapsed since the insect last fed or oviposited (Papaj and Rausher, 1983). In general terms, theory predicts that as time from completion of feeding or oviposition passes, the responsiveness of an insect to lower ranked hosts will increase. For a further discussion of these factors, see Withers et al. (2000).

Biography of a Phytophagous Insect

To illustrate the influence of the above factors and to help us appreciate them, I will relate the life story of an insect: a hypothetical phytophagous weevil with a narrow host range. An adult female emerges from its pupal case in the early wet season in the monsoonal tropics. She is soon ready to begin searching for habitat, food and mates. She initially responds very strongly to a combination of humidity, temperature, and light that is characteristic of her habitat: low lying, semi-inundated, open fields. Once she has found an acceptable habitat, she responds more strongly to host kairomones, volatile chemicals emitted by her preferred host plant. She may have learned this olfactory cue when emerging from her pupal case from trace host chemicals present. She locates a host, a *Mimosa* species that is currently in flower. She learns the visual cue of pink flower color through association with her strong response to the plant kairomone and uses this cue in the future to help find hosts. She lands on the plant and further assesses it by contact chemoreception; that is, she tastes chemicals present on the surface of the plant with receptors on the feet and mouthparts. Further gustation will occur through exploratory feeding. She accepts the plant for feeding and later mates on the plant.

She reaches a full complement of eggs and her motivation to oviposit has risen to a peak but she finds no suitable oviposition sites (young seeds) on this plant. She leaves the plant and flies to many other plants of the same species but fails to find any with suitable seeds. She doesn't find all available *Mimosa* plants, because their chemical signatures are masked by deterrents from surrounding non-hosts. She arrives at another host plant, a *Neptunia* species, which shares the habitat and some olfactory and visual cues with *Mimosa* spp. The *Neptunia* species is not a preferred host and is rarely used, but it is accepted on this occasion. She lays eggs on the seeds.

She finds another *Mimosa* plant where she feeds on leaves and matures more eggs. She again searches for suitable oviposition sites. This time she finds a *Mimosa* plant with many seeds of the most attractive stage. She responds strongly to the seeds and lays several eggs. Because of the positive chemical, tactile, visual cues she has recently encountered, she is now primed, sensitized and in a state of central excitation such that it will respond more readily to cues that previously would not have stimulated her. As a result she makes an oviposition mistake, laying an egg on a seed of an intertwining legume vine.

Upon egg hatch, her larvae use contact chemoreception to assess the palatability of the plant on which they find themselves. If suitable phagostimulants are sensed by receptors on the head of the larvae, they begin to feed. This first experience with stimuli associated with this host will lead to induced preference to this host so that if the larvae needs to move to another feeding site, its preference for the same host species is increased, even to the point where it will starve to death rather than eat another host. Development is rapid on this *Mimosa* plant and many larvae reach the pupal stage. Their siblings on the *Neptunia* plant also developed successfully. The larva on the legume vine, however, failed to develop because the required phagostimulants were not present.

This life story illustrates many of the behavioral elements that play a role in the life of an insect in a natural setting. Many of these elements may influence the host specificity testing of the insect as will be seen in the next section.

Impact and Management of Behavioral Factors on Host Specificity Tests

Here I will discuss how the behavioral responses listed above can influence the results of host tests and what we can do to improve our testing using this information. The types of tests often done in host specificity testing examine oviposition, adult feeding, larval feeding and development, adult longevity, and fecundity. Tests may be done in laboratory cages of various sizes and designs, or in open fields. Field surveys to detect attacks on a range of plant species may be considered a type of test. Test designs are similarly varied. They may be choice, no-choice, or choice-minus-control (where control is normally the target weed). No-choice tests may be done

in parallel or in sequence (Heard and van Klinken, 1998).

Are the behavioral effects discussed in this paper relevant to insects with narrow host ranges? Our preliminary null hypothesis in host specificity testing must be that all plant species are equally good hosts for each agent. Our design must set out to test this hypothesis. The most rigorous design will account for these behavioral effects.

Tests can generate false positives and false negatives. False positives refer to the attack of a host in the test when there would be no attack on that plant in nature. False negatives results indicate no attack in the test when there is potential for attack in the field (Marohasy, 1998). Many of the behaviors listed above may produce false negatives or false positives in host specificity testing. A difficulty with these terms is that the "true" response is unknown or is variable in the field. "False" results can also occur in the field, e.g., a plant may be rejected in some natural circumstances when there is potential for it to be accepted under other circumstances (van Klinken, 1999a). Despite this limitation these terms are useful for alerting researchers to potential problems with the interpretation of these tests.

Sequential Behavioral Responses in Host Plant Selection

Consideration of the sequential behavioral steps in host selection raises a number of issues that have consequences for host specificity testing. Much of the progress in applying the concepts of insect behavior to host specificity testing has been made by examining this process (Wapshere, 1989; Cullen, 1990; Marohasy, 1998). Possibly the most important consequences are those that stem from the absence of early steps in the host selection sequence in experimental arenas. If a certain number of potential hosts are eliminated at each step in the testing sequence, then the omission of that step from a host test may generate falsely positive results leading to over-estimation of field host range (Table 1). If the early steps are important in host selection, e.g., habitat selection by *D. magnaquinaria* mentioned earlier, then false positives will occur in cages in the absence of these "behavioral filters".

Tests applied to insects such as this need to incorporate as many steps in the host selection behavior as possible. Options to achieve this include using large arenas (Wan et al., 1996), more natural arenas (Cullen, 1990), open field testing (Clement and Cristofaro, 1995), or testing sequences that take a smaller more selective group of plant species to less restricted arenas (Wapshere, 1989).

Other methods to minimize false results in laboratory tests include the use of wind tunnels, olfactometers, or simply good air flow through cages (Keller, 1999). Wind tunnels and olfactometers are well known tools for determination of host finding cues in parasitoids, phytophagous insects, and mosquitoes but have been largely ignored by biocontol workers. Pre-alighting cues used in host location often rely heavily on the sensory modality of olfaction. The still air in cages does not allow for the upwind response of insects to olfactory cues. Air flows through cages provide a simple solution that may allow some insects to include this important step in the host selection process. A negative response to a plant species in an olfactometer test may allow the elimination of plant species that were accepted by herbivores when confined with the plant. The use of these tools is reviewed by Eigenbrode and Bernays (1997).

These precautions do not need to be taken for all insects. Many insects do not show high levels of specificity until they alight on the plant and receive contact cues. For example, the aphid *A. fabae* passively locates plants but then shows high levels of specificity to the chemo-tactile cues such as surface chemicals (Kennedy et al., 1959). Insects such as this can often be accurately host tested in small cages. Similarly, the psyllid *Prosopidospylla flava* Burchkhardt accepts a wide range of plants for oviposition, but larval development will only occur on *Prosopis* spp. (van Klinken, 2000). Experiments on the host selection behavior of each insect will be needed to determine where highest specificity is expressed.

Other factors may be important in the host selection process and may only be revealed by careful study of the process in each species under study. For example, Wan and Harris (1996) found that attraction by adults to larval feces and to adults of the same species was an important cue that limited field host range to one plant species.

Volatile chemicals may cause false negatives and false positives in tests (Table 1). The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is attracted upwind by the odor of its host plant. In a wind tunnel, Thiery and Visser (1986) showed that the odor of non-host plants

blocks the upwind responses of non-experienced and experienced females to the odor of their host. Such effects can cause false negatives in choice tests and even in no-choice tests if the test plants are close to target plants. The effects can occur in both field and cage tests.

The opposite result, false positives, can occur when volatiles from hosts are absorbed onto surfaces of test plants (Table 1). Insects may respond positively to these cues, resulting in the acceptance of non-hosts for oviposition or feeding (Withers and Barton Browne, 1998).

Experience and Learning

The various mechanisms of experience can have many effects on the results of host specificity tests (Table 1). Associative learning can affect choice tests if test insects associate kairomones from hosts with non-hosts, thereby learning to accept those non-hosts (resulting in false positives). Habituation can also cause false positives in tests, particularly no-choice tests, if insects habituate to deterrents of non-hosts through repeated contact with them, resulting in acceptance of those plants.

Two other mechanisms can cause false positives in choice tests: sensitization (including priming) and central excitation. Sensitization to stimuli of normal hosts may lead to acceptance, in choice tests, of less-stimulating plants that would not be normally acceptable as hosts. The same results could occur in sequential no-choice tests in which the insects are transferred from target weed to a test plant. Sensitization is likely to be a bigger problem in cage tests rather than open field tests and field surveys as the necessary repeated contact with hosts is more likely to occur in cages. Priming could have a greater effect than sensitization as the insect is generally more responsive to all stimuli, not just the specific stimulus that elicited the response. Central excitation, in which the short term responsiveness to stimuli is increased by contact with a host stimulus, is another mechanism that can lead to false positives in choice tests.

Induced preferences of adults and larvae can cause false negatives in tests if the insects have experienced the target weed or any other plant which induces a strong preference for that plant. These adults may then reject plants that inexperienced adults would have accepted. Naive and experienced insects are both routinely used in host specificity testing. Naive insects are generally preferable for most standard tests, as there is less potential for the effects of experience to induce preferences. Naive insects may not always be readily available; e.g., for a long-lived weevil that is difficult to rear, there is a strong motivation to re-use adults which are already experienced. Whether this is appropriate needs to be carefully considered for each case. In some situations, it may be necessary to use experienced individuals, for example, to determine whether a late instar larva can complete its development of a test plant species after initially feeding on the target weed. Occasionally it is not feasible to use naive adults for

Table 1. Insect behavioral mechanisms and their consequences for the design and interpretation of host specificity tests

Behavior	Consequence
Host location stimuli	
Absence of early steps in host selection behaviour.	False positives in all cage tests.
Volatile chemicals from non-hosts mask those of hosts.	False negatives in most tests
Volatile chemicals from hosts are absorbed onto non-hosts.	False positives in cage choice tests
Experience and learning	
Associative learning	False positives in cage choice tests
Habituation to deterrents of non-hosts	False positives in cage choice and no-choice tests
Sensitization (including priming) to stimuli of hosts	False positives in choice tests and sequential no-choice tests
Central excitation	False positives in choice tests
Central inhibition	False negatives in cage and open field choice tests
Induced oviposition or adult feeding preferences	False negatives in all tests if adults experienced with test plant are used
Induced larval feeding preferences	False negatives in larval feeding and development trials
Time-dependent effects	
Insects increase their response to lower ranked hosts as they approach a deprived state	False negatives in cage and open field choice tests Choice trials run for short times may not be appropriate
Age: females become less discriminating as they age.	False negatives in all tests if old insects are not used
Other behaviors	
Inhibitory cage environment / escape responses	False positives in all cage tests

tests. For example, some adult insects need the target weed to mate and mature eggs. In this case, oogenesis tests to assess the ability of test plant species to support egg development in adult females may be more appropriate for determination of host specificity.

Induced preferences are shown in larvae as well as adults. Larvae that initiate their feeding on a particular plant species may not accept other species that inexperienced larvae would accept. The use in tests of naive individuals (that is unfed, first-instar larvae) minimizes the consequences of induced preferences. There is still a role for the use of older larvae in tests, if in nature larvae of the particular species are able to move between individual plants.

Time-Dependent Effects

Withers et al. (2000) explain how some temporal patterns of feeding and oviposition should be understood for each insect and how this information can be incorporated into setting the duration of host specificity tests. They recommend the use of no-choice trials that last for the whole of the insect's life. In open field tests and field surveys, it is recommend to destroy the target weed to create a situation in which the insects reach a state of deprivation so that they may accept lower ranked hosts.

Other Behaviors

The cage environment may inhibit normal behavior and/or stimulate escape responses. Inhibited insects or those trying to escape may not respond to oviposition or feeding cues until they reach a very high state of deprivation. They then respond to poor stimuli, resulting in false positive results (Withers and Barton Browne, 1998). Methods available to avoid obtaining such false results include the use of large arenas, more natural arenas, or open field testing.

Strengths and Limitations of Test Designs

Choice vs No-Choice Tests

The host specificity testing of many insects uses a combination of choice and no-choice tests. Often disparate results are obtained in these tests (Marohasy, 1998). Understanding of key behavioral concepts can assist in the interpretation of these differences.

Choice tests provide us with information as to how an insect may select hosts in a situation where the target weed is present or abundant. Because insects will use a variety of behaviors to ensure that they remain in the presence of host plants (e.g., long distance attraction, arresting of locomotion in presence of positive stimuli), choice tests should predict the host range of most individual insects most of the time. However, insects can find themselves in situations where the target weed is not present or is encountered so infrequently that insects become responsive to lower ranked hosts. Insects may accept a wider number of plants as hosts under these conditions. No-choice tests provide us with the best tool to predict the outcome of this situation. A number of causes will create this condition in the field. One cause is seasonal asynchrony, e.g., the broom seed beetle, *B. villosus*, emerges before suitable broom pods are available. Pods of tagasaste are available and they are accepted for oviposition in this no-choice field situation even though they are not accepted in choice tests (Fowler et al., 2000). Similarly, *Parthenium hysterophorus* L. plants die towards the end of the season, denying agents their preferred host (R. E. McFadyen, *personal communication*). Another cause is the destruction of the host plants by large number of biocontrol agents. This caused adult feeding by the lantana leaf beetle, *Teleonemia scrupulosa* Stål, on sesame (Greathead, 1968). In all these examples, insects cannot locate host plants causing them to reach a high level of host deprivation, hunger, egg load, and old age: all factors which could lead to oviposition on less preferred hosts. Again, no-choice tests will predict this propensity to accept lower ranked hosts under these circumstances.

Parallel vs Sequential No-Choice Tests

No-choice tests may be run in sequence, with all insects moving between target weed and test plant species, or in parallel, with groups of insects being placed simultaneously on the target and test plants. The control (target weed) may be less valid in the case of the sequential tests as it is not done at the same time as the tests and the insects may be in a different behavioral or physiological state (Withers et al., 2000).

Open Field Tests and Field Surveys

Open field tests and field surveys both suffer from some problems related to insect behavior, and they do not always necessarily provide the most accurate prediction of field host range in a new environment. Environmental factors can influence the results such that the genetically determined fundamental host range cannot be ascertained, and hence one can never be completely sure of full scope of the realized host range

as expressed in a new environment. In contrast, the fundamental host range can be measured accurately in laboratory tests (van Klinken and Heard, 1999; van Klinken 2000a).

Some recent studies have illustrated the behavioral limitations of open field tests (reviewed in Briese, 1999). These studies show that the results of open field tests vary depending on the experimental design. Designs in which the density of test plants is too low relative to the target plant have given false negatives probably because the insects never reached a sufficiently deprived state to accept lower ranked hosts. Briese (1999) proposes a two-phase open field test design. The first phase is a choice design. In the second stage, the target weed is removed to create a choice-minus-control design. This design will cause insects to become food- and oviposition-site-deprived and this design will remove the effects of central excitation.

Conclusion

Behavioral factors can effect the results of host specificity tests in many, complex ways. Behavioral mechanisms can theoretically produce opposing results. For example, time dependent effects may cause choice tests to underestimate the host range (generate false negative results), but the effects of experience may cause choice tests to overestimate the host range (generate false positive results). Biocontrol workers should be familiar with the behavioral processes that might affect the results of these tests and use this knowledge to design the most appropriate tests and interpret them with greater insight. Biocontrol workers responsible for host specificity testing need to recognize that they are applied animal behaviorists.

Often experiments will need to be done to understand how the behavior of each particular agent is being expressed in tests. For example, experimenters could follow the rate of acceptance of a number of hosts through time in a no-choice test to determine if time dependent factors are influencing host range. It may be instructive to compare the results of choice versus no-choice tests, or to compare the results of tests from different arenas. Most host tests rely on counting the output, i.e., the number of eggs or degree of feeding at the end of a test. It may be useful to make behavioral observations during the test, looking at the process rather than merely the end result.

The point in the host selection process that has the highest specificity should be determined for each agent being tested. The most discriminating phase must be given the heaviest weight when interpreting the results of host specificity tests. For example, if an insect relies heavily on habitat or distance cues to locate hosts, then the investigator must be aware that these cues are omitted from a cage test. But if the insect passively locates plants, then leaves non-hosts or stays on hosts, then cage tests will probably yield accurate results. Less discriminating phases of the host selection process may be the initial focus of testing if this is easiest. The more laborious, more discriminating phases of the testing process may then be done later with the resulting reduced subset of plants.

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Physiological Issues in Host Range Expansion

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Abstract

Host range in a natural system is determined over evolutionary time and constrained through ecological time by behavioral, neurophysiological and physiological exaptations, by biogeographic isolation, exposure to a restricted suite of plant communities, inter- and intraspecific competition, predation, parasitism, and by influential stochastic events. When a biological control agent is transported to a novel environment, some of the evolutionary constraints and many of the behavioral constraints on host use are relaxed, encouraging host range expansion that could have occurred in its native habitat. Host-specificity tests are designed to identify plants that might serve as acceptable hosts in the new environment, but behavioral plasticity, both in host discrimination and in the physiological ability to develop on a given host, makes the task difficult at best. One particularly critical challenge lies in the choice of potential hosts to be screened. Selections are justifiably based on knowledge of the relatedness of such plants to native hosts, on the similarity of their primary allelochemicals to those found in native hosts, or both. I will argue that these criteria risk underestimating host-range (i.e., risk false negatives) because host shifts to chemically or genealogically novel plants by newly introduced agents can occur through coincidence alone. Recent evidence from luperine chrysomelid beetles and other pharmacophagous insects suggests that evolutionary novel compounds can elicit feeding or oviposition responses when their polarity, molecular configuration and stereochemistry at binding sites meet the criteria for depolarization of stimulatory input at peripheral neuroceptors. Mechanisms for identifying plants with such compounds will be discussed.

Introduction

After more than a century of support from the lay, governmental and scientific communities, the concepts and practices of classical biological control are currently being reconsidered with respect to potential effects on non-target species (Howarth, 1983; 1991; Pimentel et al., 1984; Lockwood, 1993; Simberloff and Stiling, 1996; Louda, 1997; Strong, 1997; Thomas and Willis, 1998). A number of purported "host shifts" or "host expansions" (Marohasy, 1996), primarily by vertebrate biocontrol agents (Pimentel et al., 1984) have been cited as evidence that biological control may not be a "green" alternative to chemical control, as it has historically been touted. The timing of these criticisms is particularly ironic in view of a growing reliance on biological control as the most viable and perhaps only remaining weapon against invasive alien plant pests. Now recognized as one of the most significant threats to North America's native biota (Randall and Mannelli, 1996), introduced plants such as leafy spurge (*Euphorbia esula* L.; Fornasari, 1997; Jackson, 1997; Cristofaro et al., 1998), purple loosestrife (*Lythrum salicaria* L.; Blossey et al., 1994; Blossey and Hunt, 1999), and mile-a-minute weed (*Polygonum perfoliatum* L.; Okay, 1995) are just some of the invasive species that have been targeted for new biocontrol initiatives. The increasing demand for the biological control of weeds together with the current criticism of such efforts by reputable biologists has placed biocontrol practitioners and their standard protocols under the most intense scrutiny in their history.

At issue in the debate is the ability of biocontrol screening procedures to avoid false negatives (Simberloff and Stiling, 1996). That is, when prospective control agents are screened for host specificity (Blossey, 1995; Marohasy, 1998), the experimental designs involved must minimize the risk of incorrectly accepting the null hypothesis: that the agent poses no threat to non-target organisms. Advances in our knowledge of insect-plant interactions (Strong et al., 1984; Bernays and Chapman, 1994; Dobler et al., 1996), the evolution of host specificity (Mitter et al., 1991; Hopper et al., 1993; Futuyma et al., 1995; Mardulyn et al., 1997; Kopf et al., 1998), and the behavioral ecology of host switching (Futuyma, 1986; Karowe, 1990; Hawkins and Marino, 1997), however, are justifiably creating the impression that this task is extraordinarily difficult (Roitberg, 2000). Biocontrol practitioners must be concerned with a prospective agent's potential to expand its host range over both ecological and evolutionary time scales. In natural systems, host range is ecologically constrained by the behavioral, neurophysiological and physiological traits shared by members of a population, by the suite of plant species that have been and are currently within the geographic range of the population, and by the intensity of inter- and intraspecific competition, predation, and parasitism that the population must endure. Changes in host specificity are most likely to occur when one or more of these constraints is relaxed during periods of allopatric (Mayr, 1963) or allochronic (Wood and Keese, 1990) isolation. Unfortunately, a successful biological control introduction also relaxes these constraints. The agent is transported to a new environment, isolated from gene flow with the parent population, released from constraining interactions with predators, parasitoids, and interspecific competitors, and exposed to communities of novel plant species. If successful in reducing the population density of the target weed species, the agent faces diminishing host availability and, in turn, increasing intraspecific competition (Marohasy, 1996). Consequently, selection to oviposit or feed on plant species outside the normal range of acceptability becomes exceptionally intense.

Making matters worse for biocontrol practitioners seeking to identify prospective agents that might attack non-target organisms is latent behavioral plasticity triggered by stress similar to what might be encountered during an introduction. The degree to which parasitic Hymenoptera and insect herbivores discriminate among potential hosts is highly condition-dependent (Papaj and Rausher, 1983; Bernays and Chapman, 1994; Roitberg, 2000). Host specificity has been shown to vary with photoperiod (Roitberg et al., 1992), barometric pressure (Roitberg et al., 1993), hunger (Schoonhoven, 1987; Fletcher et al., 1994), the presence of conspecifics (Visser, 1995), egg load (Minkenberg et al., 1994), and host availability (Fitt, 1986; Speirs et al., 1991; Singer et al., 1992). One can easily imagine other variables that might alter host acceptability as well. But the problem goes beyond identifying all of the factors that affect host choice, for even if this is accomplished, complex factorial screening designs are then required to identify interactions between these factors. Unfortunately, variation introduced into the analysis by each additional factor examined causes a serious loss of analytical power (Roitberg, 2000). This can be somewhat ameliorated by increasing the number of replicates in the screening design, but such solutions are almost always constrained by resources and time. Thus, at first glance it would seem that the quest to improve the accuracy of pre-release host specificity assessments has created an impossibly complex challenge for biocontrol practitioners.

For the first time, these issues have stimulated the interest of evolutionary biologists (Holt and Hochberg, 1997; Roitberg, 2000). Roitberg (2000), for example, has made a convincing case for the use of state-dependent dynamic life history models (Mangel and Clark, 1988; Mangel and Ludwig, 1992) as a first step in biocontrol screening procedures. By calculating lifetime reproductive success that results from various ecological (Heimpel et al., 1998; Roitberg, 2000) and evolutionary (Travis, 1989; Roitberg, 1990; Carrière and Roitberg, 1996; Roitberg, 1998) host-acceptance "decisions," these models can be used to determine what combination of conditions might cause a prospective biocontrol agent to expand its host range. This approach conveniently permits the analysis of several interacting factors at the theoretical level when similar empirical analyses are all but impossible (Roitberg, 2000). Thus, dynamic life history modeling designed to identify the likelihood of adaptive host switches can simplify screening protocols by identifying life history periods during which a particular candidate will be most susceptible to such switching.

Biocontrol practitioners have also recognized the logistical limitations and risks of potential false negatives that are associated with current screening protocols

(Dunn, 1978; Cullen, 1990; Marohasy, 1998). In response to these problems, Marohasy (1998) has suggested that screening procedures for the biological control of weeds can be improved by paying closer attention to behavioral mechanisms underlying host-finding and acceptance. Factors targeted as being critical to screening designs include (1) the time elapsed between the most recent period of eating (or oviposition) and testing (Papaj and Rausher, 1983), (2) pre-alighting opportunities for host discrimination (Wapshere, 1989), (3) experience-related phenomena such as short-term central nervous excitation (Menzel et al., 1993), longer-term sensitization (Marohasy, 1998), habituation to inhibiting inputs from non-hosts (Jermy et al., 1982), associative learning (Bernays and Wrubel, 1985); (4) cross contamination of non-hosts by host volatiles (Jayanth et al., 1993), and (5) loss of host-discrimination behavior due to cage effects (Cullen, 1990). If these empirical advances can be married to life history modeling as suggested by Roitberg (2000), the accuracy and efficiency of pre-release screening procedures must surely improve.

As promising as they are, however, both theoretical and empirical advances in biocontrol screening procedures have little practical value if the selection of plants to be screened is flawed. Clearly if species that could support an expansion of host range by the prospective agent are not included in the evaluation of its host specificity, the results of such evaluations will be inaccurate and false negatives may occur. In this chapter, I discuss two physiologically-based phenomena that may frequently influence host range expansion by insect herbivores and consequently may have important ramifications for the selection of plant species to be included in screening procedures for potential agents in the biological control of weeds.

Plant Selection Criteria and Their Problems

The objective of host specificity assessment in weed biocontrol is to quantify the ability of prospective biological control agents to attack non-target plant species after introduction (Schroeder, 1983; Marohasy, 1998). It has been agreed through international consensus (Greathead, 1995) that plant species should be chosen for host range screening on the basis of their taxonomic relationship to the target species (Wapshere, 1989; Harley and Forno, 1992). By sequentially exposing prospective agents to progressively less related plant species (i.e., to different varieties of the target host, then to different species in the same genus, then to different genera in the same tribe, and so on) it is thought that all plant species that might support populations of the agent will be identified. A related approach (although one rarely done in practice) is to expose the agent to plants with similar signatures of secondary metabolic compounds, regardless of their taxonomic affiliation with the target species (Blossey, 1995). Both of these criteria for plant selection are based on the contention that "*Natura nonfacit saltum*" (nature does not make leaps), particularly when it comes to the evolution of host ranges in phytophagous insects (Futuyma, 1994). Ever since Guignard first noted in the 1890s that insect host range is mediated by the presence of common feeding stimulants (Feltwell, 1982), a great deal of evidence has been accumulated in support of the hypothesis that host shifts or expansions by phytophagous insects are often constrained to plant species sharing a common suite of phytochemicals (Ehrlich and Raven, 1964; Berenbaum, 1990; Futuyma, 1991; Farrell et al., 1992; Feeny, 1992; Becerra, 1997). Such plants typically (but not always: Berenbaum, 1981; Menken et al., 1992) are also close relatives (Mitter and Farrell, 1991).

The primary problem with this line of reasoning is that it disregards host shifts by phytophagous insects to unrelated plants with vastly different chemistries. The literature is replete with descriptions of closely related insect species that specialize on plants from different families or even different orders with widely differing secondary metabolic compounds (reviewed by Jermy, 1984). Some of these examples involve insects with haustellate mouthparts (e.g., aphids, Eastop, 1973, Müller, 1978; treehoppers, Tilmon et al., 1998), whose actual xylem and/or phloem foodstuffs may differ very little in chemical makeup among unrelated plants. However, many others involve mandibulate insects that cannot consume plant material without full exposure to the entire suite of allelochemicals present in the tissues. Table 1 provides 24 examples within the Coleoptera and Lepidoptera in which closely related species or populations within species expanded their host range not only to plants in different genera, families, or orders, but to plant species in a different subclass, or in 17 extreme cases to a different plant class. Clearly current screening procedures would have detected none of these host expansions.

Table 1. Evidence that closely related mandibulate insect species are physically capable of host shifts to genetically and chemically disparate plant taxa.

Taxon	Host Plant	Subclass (Class)	Family	Dominant Secondary Compounds[1]	Reference
Coleoptera					
Chrysomelidae					
Diabrotica virgifera complex	grasses	Commelinidae	Poaceae	ferulic acid; C-glycosylflavones	Branson & Krysan, 1981
Diabrotica fuscata complex	cucurbits	Dilleniidae	Cucurbitaceae	triterpenes; pyridine alkaloids; cucurbitacins	
Gonioctena subgenus *Goniomena interposita* Franz & Palmén	alder	Hamamelidae	Betulaceae	nontannic phenolics	Mardulyn et al., 1997
Goniomena pallida (L.)	willow	Dilleniidae	Salicaceae	phenol heterosides	
Goniomena intermediate Hell.	cherry	Rosidae	Rosaceae	cyanogenic compounds; triterpenoid saponins	
Phratora (=*Phyllodecta) polaris* (Schneider)(race1)	willow	Dilleniidae	Salicaceae	phenol heterosides	Kopf *et al.,* 1998
Phratora polaris (race 2*)*	birch	Hamamelidae	Betulaceae	highly tanniferous with galic acid	
Lochmaea capreae L. (race 1)	willow	Dilleniidae	Salicaceae	phenol heterosides	Mikheev & Kreslavsky, 1980
Lochmaea capreae (race 2)	birch	Hamamelidae	Betulaceae	highly tanniferous with galic acid	
Syneta betulae (Fabricius) (race 1)	birch	Hamamelidae	Betulaceae	highly tanniferous with galic acid	Jolivet, 1954
Syneta betulae (race 2)	pine	(Conopsida)	Pinaceae	diterpene acids; phenolics (pinosylvan)	
Lepidoptera					
Pyralidae					
Cactoblastis cactorum (Bergroth) (cactus abundant)	cactus	Caryophyllidae	Cactaceae	isoquinoline alkaloids; triterpenoid saponins	Dodd, 1940
Cactoblastis cactorum (cactus depleted)	tomato	Asteridae	Solanaceae	tomatine (glycoalkaloid)	
	melons	Dilleniidae	Cucurbitaceae	triterpene cucurbitacins; pyridine alkaloids	
Hedylepta Blackburni (Butler) (ancestral)	Pritchardia palm	Arecidae	Arecaceae	polyphenols; pyridine alkaloids	Zimmerman, 1960
Hedylepta (5 sister spp) (derived since the introduction of banana to Hawaii 1000 y.)	banana	Zingiberidae	Musaceae	tanniferous	
Tortricidae					
Laspeyresia (Cydia) *pomonella* (L.) (race 1)	apple	Rosidae	Rosaceae	cyanogenic compounds; triterpenoid saponins	Philips & Barnes, 1975
Laspeyresia (Cydia) *pomenella* (race 2)	walnut	Hamamelidae	Juglandaceae	napthaquinones	

[1]*Chemical signatures from Cronquist (1981)*

Table 1 (continued)

Taxon	Host Plant	Subclass (Class)	Family	Dominant Secondary Compounds[1]	Reference
Epinora caprana (Fabricius)					
normal host	*Myrica gale*	Hamamelidae	Myricacae	tanniferous; triterpenes; sesquiterpenes	Winter, 1974
new host	*Pinus contorta*	(Conopsida)	Pinaceae	diterpene acids; phenolics (pinosylvan)	
Clepsis senecionana (Hübner)					
normal host	*Myrica*	Hamamelidae	Myricaceae	tanniferous; triterpenes;	Winter, 1974
	Vaccinium	Dilleniidae	Ericaceae	sesquiterpenes; phenol heterosides (arbutin); triterpene urolic acid; diterpene andromedotoxin	
new hosts	*Picea, Pinus, Larix*	(Conopsida)	Pinaceae	diterpene acids; phenolics (pinosylvan)	
Ptycholoma lecheana (L.)					
normal hosts	*Quercus* spp.	Hamamelidae	Fagaceae	highly tanniferous with galic acid; triterpenes	Winter, 1974
new host	*Picea sitchensis*	(Conopsida)	Pinaceae	diterpene acids; phenolics (pinosylvan)	
Philedone gerningana (Denis & Schiffermüller)					
normal hosts	*Vaccinium*	Dilleniidae	Ericaceae	phenol heterosides (arbutin); triterpene urolic acid; diterpene andromedotoxin	Winter, 1974
	Potentilla	Rosidae	Rosaceae	cyanogenic compounds;	
new hosts	*Picea sitchensis*	(Conopsida)	Pinaceae	diterpene acids; phenolics (pinosylvan)	
Philedonides lunana (Thunberg)					
normal hosts	*Potentilla*	Rosidae	Rosaceae	cyanogenic compounds; terpenoid saponins	Winter, 1974
	Myrica	Hamamelidae	Myricacea	tanniferous with triterpenes; sesquiterpenes	
new hosts	*Picea, Pinus, Larix*	(Conopsida)	Pinaceae	diterpene acids; phenolics (pinosylvan)	
Acleris caledoniana (Stephens)					
normal hosts	*Myrica*	Hamamelidae	Myricaceae	tanniferous; triterpenes; sesquiterpenes	Winter 1974
new host	*Pinus contorta*	(Conopsida)	Pinaceae	diterpene acids; phenolics (pinosylvan)	
Acleris hyemana (Haworth)					
normal hosts	*Calluna, Erica*	Dilleniidae	Ericaceae	phenol heterosides (arbutin); triterpene ursolic acid; diterpene andromedotoxin	Winter, 1974
new host	*Picea sitchensis*	(Conopsida)	Pinaceae	diterpene acids; phenolics (pinosylvan)	

[1]*Chemical signatures from Cronquist (1981)*

Table 1 (continued)

Taxon	Host Plant	Subclass (Class)	Family	Dominant Secondary Compounds[1]	Reference
Cochylidae					
Eupoecilia angustana (Hübner)					
normal hosts	*Calluna,Erica*	Dilleniidae	Ericaceae	phenol heterosides (arbutin); triterpene ursolic acid; diterpene andromedotoxin	Winter, 1974
new hosts	*Picea sitchensis*	(Conopsida)	Pinaceae	diterpene acids; phenolics (pinosylvan)	
Lasiocampidae					
Lasiocampa quercus callunae (Palmer)					
normal host	*Calluna vulgaris*	Dilleniidae	Ericaceae	phenol heterosides (arbutin); triterpene ursolic acid; diterpene andromedotoxin	Winter, 1974
new hosts	*Pinus, Picea*	(Conopsida)	Pinaceae	diterpene acids; phenolics (pinosylvan)	
*Macrothylacia rubi (*L.)					
normal hosts	*Myrica*	Hamamelidae	Myricaceae	tanniferous, triterpenes sesquiterpenes	Winter, 1974
	Calluna, Erica, Vacinium	Dilleniidae	Ericaceae	phenol heterosides (arbutin), triterpene ursolic acid; diterpene andromedotoxin	
new host	*Picea sitchensis*	(Conopsida)	Pinaceae	diterpene acids, phenolics (pinosylvan)	
Geometridae					
Entephria caesiata (Denis & Schiffermüller)					
normal hosts	*Vaccinium, Calluna Erica*	Dilleniidae	Ericaceae	phenolic heterosides (arbutin); triterpene ursolic acid; diterpene andromedotoxin	Winter, 1974
new hosts	*Pinus contorta*	(Conopsida)	Pinaceae	diterpene acids; phenolics (pinosylvan)	
Hydriomena furcata (Thunberg)					
normal host	*Vaccinium*	Dilleniidae	Ericaceae	phenolic heterosides (arbutin); triterpene ursolic acid; diterpene andromedotoxin	Winter, 1974
new host	*Pinus contorta*	(Conopsida)	Pinaceae	diterpene acids; phenolics (pinosylvan)	

[1]*Chemical signatures from Cronquist (1981)*

Table 1 (concluded)

Taxon	Host Plant	Subclass (Class)	Family	Dominant Secondary Compounds[1]	Reference
Noctuidae					
Blepharita (=Eumichtis) adusta (Esper)					
normal hosts	*Myrica*	Hamamelidae	Myricaceae	tanniferous; triterpenes; sesquiterpenes	Winter, 1974
new hosts	*Salix*	Dilleniidae	Salicaceae	phenol heterosides	
	Picea, Pinus	(Conopsida)	Pinaceae	diterpene acids; phenolic (pinosylvan)	
Pieridae					
Pieris rapae (L.) (fed cabbage as neonates)	cabbage	Dilleniidae	Brassicaceae	glucosinolates	Renwick & Huang, 1995
Pieris rapae (fed nasturtium or wheat germ diet as neonates)	nasturtium	Rosidae	Tropaeolaceae	chlorogenic acid; glucosinolates	

[1]*Chemical signatures from Cronquist (1981)*

The argument can be made that host shifts such as those that occurred in *Diabrotica* and *Goniomena* chrysomelids (Table 1) over evolutionary time are so infrequent that the risks of such events are negligible in time frames of interest to people. Possibly, but in the case of race specialization within *Phratora polaris*, *Lochmaea capreae*, and *Syneta betulae* (Table 1), host expansion happened so recently that further population divergence has not yet occurred. Moreover, it is difficult to dismiss the host switches that have occurred before our eyes among the 19 species of Lepidoptera listed in Table 1 as being too rare to worry about. In the case of *Cactoblastis cactorum* (Bergroth), host expansion occurred as a consequence of host deprivation; when normal cactus hosts were overexploited (as one would hope for in the case of a biocontrol agent), *C. cactorum* readily switched to tomatoes (Dodd, 1940). Whether *C. cactorum* is capable of permanently adapting to tomatoes remains to be seen, but the tortricid *Laspeyresia pomonella* L. clearly had the capacity to permanently adopt walnut (*Juglans regia*) as a new host without selection from host deprivation (Philips and Barnes, 1975) as did fifteen species of Lepidoptera that expanded from a variety of unrelated hosts to various conifers shortly after they were exposed to them (Winter, 1974).

Many of these taxa expanded their host ranges sometime during the course of their evolutionary histories (measured in millions of years), but others, prompted by man-induced perturbations, adopted new hosts within a single generation in recent decades. Given that host switches without constraint from lineage or chemistry can and do occur, it is essential that we develop methods for predicting: (1) which prospective biocontrol agents have the innate capacity to adopt chemically unrelated host groups, and (2) what phytochemical signatures fall within the range of acceptability for such agents. Without this knowledge there will always be some probability that potentially acceptable plant species will be inadvertently omitted from screening designs.

Malleable Gustatory Receptors

One way to improve the chances of identifying plant species that might serve as suitable non-target hosts for biocontrol agents is to design screening procedures for maximum sensitivity. That is, screen potential hosts under conservative no-choice conditions that occur under field conditions whenever an egg is either mistakenly or purposely laid on a "non-host." This is not likely to be the rare event it was once thought to be. Gravid females may relax their efforts to discriminate among hosts if preferred species are in short supply (Wiklund, 1981; Fitt, 1986), if the period since the last oviposition has been unusually lengthened by, for example, bad weather (Papaj and Rausher, 1983, Schoonhoven, 1987; Singer et al., 1992), or during the last days of life when receptors, flight or other vital processes no longer function well. Once an egg is deposited on a novel host, the larva that emerges from that egg typically does not have enough energy reserves to leave the plant and search for another more

appropriate host. Its only real options are to attempt to eat the plant at hand or starve — a classic no-choice scenario.

Recent studies of feeding deterrents in Lepidoptera suggest that such "no choice" situations involving neonate larvae may have important implications for biocontrol screening procedures. Using the imported cabbageworm, *Pieris rapae* L., as a model, Renwick and Huang have developed good evidence that the gustatory receptors of neonate larvae are initially so malleable that the chemical signature of a novel host may not deter feeding and successful development if it is the first signature encountered (Huang and Renwick, 1995ab; Renwick and Huang, 1995, 1996; Huang and Renwick, 1997). For example, nasturtium (*Tropaeolum majus* L.) contains substantial quantities of a phenolic compound, chlorogenic acid, that deters feeding in cabbage-reared *P. rapae* larvae to the point of starvation (Huang and Renwick, 1995b). If, however, *P. rapae* hatch and feed as neonates on nasturtium without first tasting cabbage, or are fed a wheat germ diet upon hatching, larvae readily accept nasturtium as a viable host and complete development without loss of fitness (Renwick and Huang, 1995; Huang and Renwick, 1997). Induction of food preference (Szentesi and Jermy, 1989) has been ruled out as an explanation of this phenomenon since transfers from nasturtium to cabbage do not cause subsequent rejection of cabbage. Instead, Renwick and Huang believe that sensitivity to nasturtium's chlorogenic acid develops while neonates feed on cabbage. If they are never exposed to the chemical signature of cabbage, larvae never develop sensitivity to deterrents in nasturtium or wheat germ diet.

Furthermore, cross habituation occurs readily in young *P. rapae* larvae. Early exposure to strophanthidin, cymarin, erysimoside, digitoxigenin, digitoxin, cucurbitacins E and I, and rutin (all powerful deterrents) suppressed the development of sensitivity in larvae to chlorogenic acid and thus rendered nasturtium an acceptable host (Huang and Renwick, 1995).

The mechanisms by which sensitivity is induced or suppressed in young larvae are not yet known, but available evidence suggests that, for some period after hatching, the peripheral gustatory receptors of neonate larvae can be permanently molded in ways that affect the acceptability of leaf tissue as a food source. Apparently, it is the lack of chemical suppressors in plant tissue that permits the normal development of sensitivity in the peripheral receptors of neonates. Conversely, the presence of one or more deterrents in a novel host can permanently suppress the development of sensitivity to these and other compounds, enabling larvae to consume them without ill effects (Renwick and Huang, 1996). Obviously there are limits to the degree to which neonate peripheral receptors can be molded by the chemical signature of the first tissues consumed. The point to emphasize here, however, is that neonate larvae are far more plastic in their acceptance criteria than are older larvae, as long as they have not previously been exposed to food that lacks a particular deterrent. Thus, screening procedures will more accurately identify acceptable host species if tests are confined to hatching neonates, simulating the no choice conditions that occur every time eggs are laid on novel hosts.

One might protest that if some small percentage of eggs persistently ends up on "non-hosts" and if neonates hatching from these eggs have a greater chance of finding these plants to be suitable hosts than previously thought, why then are the host ranges of the vast majority of phytophagous insects narrowly constrained to only a few species (Bernays and Graham, 1988)? For the answer we must reconsider all of the ecological and evolutionary constraints on host range in natural systems discussed in the introduction. Oviposition mistakes and the malleability of neonate gustatory receptors might very well have played important roles in defining the current host ranges found in natural populations of phytophagous insects. But biocontrol introductions are not natural interactions. They are manipulated events that suddenly expose a phytophagous insect to an unprecedented array of novel hosts. If oviposition mistakes and neonate habituation ever influence host range expansion it should be during a biocontrol introduction.

"Loose" Gustatory Receptors

The neurophysiological basis of peripheral perception is extraordinarily complex in insect gustatory systems (Frazier, 1986; Simmonds et al., 1990; Schoonhoven et al., 1992; Städler, 1992; Mullin et al., 1994). In the simplest terms, feeding behavior is stimulated if the chemoreception of phagostimulants exceeds the chemoreception of feeding deterrents (Dethier, 1980). In caterpillars and possibly all insects, taste sensilla contain cells specialized for the production of either inhibitory or excitatory imputs to the central nervous system, upon detection of deterrent or stimulatory chemicals in foods (Frazier, 1986). Receptor sites on these cells can be highly specific (tight) or less specific (loose). Strychnine, for

example, is a compound novel to most phytophagous insects, but it readily depolarizes activation channels leading to inhibitory input in most insects; the binding requirements at these sites are sufficiently "loose" that a variety of molecular structures meet the polarity and configuration specifications for binding there. The loose characteristics of receptor sites with deterrent capabilities may be adaptive because they protect the central nervous system from exposure to damaging novel compounds (Frazier, 1992).

Of particular interest to students of host range expansion is that relatively loose binding properties of receptor sites can also enable novel and sometimes deleterious compounds to trigger feeding behavior (Tallamy et al., 1999). There are several mechanisms by which this can happen (Frazier, 1986, 1992). Some molecules bind at receptor sites leading to inhibitory inputs, but rather than depolarizing the activation channels, they simply block them. Without inhibitory inputs, even small amounts of phagostimulants, including amino acids present in the insects' saliva, are sufficient to activate the stimulatory inputs at the sensillum and elicit feeding. Activation leading to inhibitory inputs can also be prevented when particular molecules block the stimulus removal system. Finally, loose stimulatory receptor sites themselves can encourage phagostimulation by novel compounds with the appropriate configuration and polarity at binding sites. This is apparently the mechanism by which some *Atrichopogon* flies (Ceratopogonidae) respond to terpenes in which the heptane skeleton is associated with either a 2,3-dicarboxylic anhydride or a 2,3-γ-lactone (Frenzel et al., 1992) and by which the peptide aspartame mimics the carbohydrate sucrose at vertebrate receptors, a mimicry upon which much of the sweetener industry is based. We emphasize that considerable variability in response is the rule rather than the exception in insect chemosensory systems (Frazier, 1992). If this variability is even partly genetic, a typical insect population would theoretically be fertile ground for the advent of novel feeding preferences.

There are numerous examples in insects of inappropriate feeding responses that are presumably the result of imprecision at gustatory receptors. When presented with petunia (*Petunia integrifolia* [Hooker]) plants, *Manduca sexta* L. caterpillars voraciously eat the leaves, pausing only to regurgitate everything they have just eaten. This behavior may continue until the larvae starve to death (Dethier and Crnjar, 1982). Several haustellate arthropods are stimulated to eat in the presence of toxic cucurbitacins. *Tetranychus urticae* Koch, the two-spotted spider mite, prefers cucurbitacin-rich cucumber lines over cultivars without cucurbitacins, even though such behavior reduces mite fitness (Gould, 1978). Similarly, corn delphacids (*Peregrinus maidis* [Ashmead]), sycamore lace bugs (*Corythucha ciliata* [Say]), and pea aphids (*Acyrthosiphon pisum* [Harris]) are all stimulated to feed by exogenous coatings of cucurbitacin B, an evolutionarily novel compound to these species (Tallamy et al., 1997). Mafra-Neto and Jolivet (1994) report the eating by seven species of lace bugs (Tingidae) and plant bugs (Miridae), and one luperine chrysomelid beetle, *Diabrotica angulicollis* (Erichson), of the cantharidin-rich hemolymph oozing from the joints of disturbed *Epicauta aterrima* (Klug), a large meloid beetle from Brazil. Occasional predation is commonly exhibited by mirid plant bugs, but this is the first report of hematophagy among the phytophagous tingids and *Diabrotica* beetles. That this unusual response is triggered by cantharidin is supported by numerous studies in which traps baited with pure cantharidin attracted pyrochroid, endomychid, anthicid, and staphylinid beetles, ceratopogonid, sciarid, and anthomyiid flies, and braconid wasps (Young, 1984; Frenzel et al. 1992; Frenzel and Dettner 1994; Eisner et al. 1996).

The apparent ease with which loose gustatory receptors can lead to an association with novel compounds suggests that this mechanism may provide the missing explanation for host switches by phytophagous insects to plants with chemical signatures vastly different from those of parent hosts (Tallamy et al., 1999). If an insect with gustatory receptors that evolved in the context of meeting nutritional and pharmacological needs on one host species suddenly encounters a novel compound from a different plant, a feeding response could be elicited for one or more of the reasons discussed above. If such phagostimulation enhances the fitness of those that exhibit it, the response should rapidly move to fixation within the population. If the novel molecule (or any other compound present in the new plant) is toxic, early consumers will suffer reduced fitness. This will not, however, lead to a "tightening" of the responsible receptor's specificity unless selection to avoid the new compound exceeds selection to maintain the loose properties of the receptor in question. Host expansion should ensue when: (1) exposure to the novel compound is sufficiently frequent to select for physiological tolerance, and (2) gene flow diluting genetic change in tolerance is reduced.

A successful biocontrol introduction could create exactly this scenario. If an agent locally reduces the

target host population to the point where most dispersing individuals have nothing on which to oviposit or feed except evolutionarily novel plant species, there will be powerful selection favoring those agents with peripheral receptors that are sufficiently loose to enable acceptance of a new host. Gene flow in the succeeding generation between agents that have successfully adopted the new host and those that were able to locate target hosts could be restricted allochronically through differences in host phenology (Wood and Keese, 1990; Wood et al., 1990) or allopatrically if, for example, the collapse of the target host population had occurred in a relatively isolated valley (Mayr, 1963). But one needs to hypothesize the restrictions of gene flow for this mechanism of host range expansion to occur. It is probable that the loose properties of the appropriate receptors are shared by all members of the population because of their selective advantage. Thus, all members of the population are physiologically predisposed to finding any novel plant bearing the appropriate components to be stimulating by coincidence alone.

Strong et al. (1984) agree that host shifts can occur even without the collapse of the parent host's population. Close proximity of abundant parent host species and novel plants creates an ecological opportunity for insects physiologically capable of interpreting the compounds in novel plants as phagostimulants rather than deterrents. For example, proximity has been evoked to explain the seven species of British Lepidoptera that expanded their host range from native moorland plants in several genera (*Myrica* – Myricaceae; *Vaccinium*, *Erica* and *Calluna* – Ericaceae) to *Pinus contorta* Douglas that were planted extensively among them (Winter, 1974). In the same vein, laboratory experiments have repeatedly demonstrated that some phytophagous insects (presumably those with loose gustatory receptors) can rapidly adapt to novel hosts when under selection from repeated exposure (Schroder, 1903; Pictet, 1911; Harrison, 1927; Kozhanchikov, 1950; Brower et al., 1967; Gould, 1979). For example, Brower et al. (1967) created a line of monarch butterflies (*Danaus plexippus* L.) that developed entirely on cabbage rather than its normal milkweed hosts.

Do loose gustatory receptors have the potential to permit a shift to any nearby plant? Certainly not; host shifts are only possible when one or more key compounds in the chemical signature of a novel plant coincidentally share the molecular configuration, polarity and solubility of compounds in the parent host for which the insect's taste receptors originally evolved (Tallamy et al., 1999). But how can biocontrol practitioners predict which plants might produce such binding site matches? Advances in computerized molecular modeling programs have the potential to make this proposal more feasible than it sounds. The first step would be to characterize the chemical profile of the prospective agent's ancestral host species. Contributions from natural products chemists over the last three decades have been so substantial that the profile of secondary metabolic compounds in most angiosperms is readily accessible (Karrer, 1958; Hegnauer, 1962-1973; Tetenyi, 1970; Cronquist, 1981). Next, the chemical profiles of key plant species of agricultural, ornamental, environmental, and political value from the habitats of the target species should be determined. Quantitative structure-activity relationships (QSAR), a powerful technique for studying three-dimensional structure-function relationships between ligands and membrane receptors (Mullin et al., 1997; Kim and Mullin, 1998), can then be employed in conjunction with molecular modeling software to identify which compounds in these novel plants might match the binding site requirements of chemicals in the ancestral host. In essence, initial screening can be done relatively quickly and painlessly on the computer. Only plants that are found to contain compounds with similar configuration, stereochemistry, and hydrophobicity to the compounds of the ancestral host will be added to the list of plant relatives to be actually screened. Every time a new compound is modeled in this way its binding site characteristics can be stored in a cumulative data base. Eventually, the data base will be sufficiently complete that matches can be sought by quick searches rather than new modeling.

Summary

A growing awareness of environmental problems caused by the introduction of some biological control agents has created serious opposition to new biocontrol initiatives in both political and scientific circles. Despite the fact that most biocontrol mishaps have been caused by the irresponsible release of vertebrate predators, practitioners of the biological control of noxious weeds are under pressure to design infallible screening procedures to identify all non-target plants that might encourage host range expansion by prospective agents. Theoretical advances such as the use of dynamic life history modeling and empirical improvements in screening designs that incorporate behavioral mechanisms underlying host-

finding and acceptance have been proposed to address this goal, but these will increase screening accuracy very little if the proper plant species are not included in the population of non-targets to be screened. In practice, plants to be screened are selected almost exclusively on the basis of taxonomic relatedness to the target host. This approach ignores the fact that host range expansions to unrelated plants with chemical profiles that differ from the ancestral host occur over both evolutionary and ecological time frames and are well documented.

Recent studies suggest that some phytophagous insects may be physiologically capable of accepting and developing on evolutionarily novel plant species for two reasons. First, it appears that the discriminatory abilities of gustatory receptors in newly hatched larvae are shaped to an important extent by the array of chemicals those receptors encounter during the first feeding episodes of larval life. Early exposure to novel compounds that would normally deter older larvae can render such chemicals (and the plants that contain them) acceptable for life. Thus, ecological conditions that favor oviposition "mistakes" resulting in the deposition of eggs on novel plant species set the stage for the acceptance in nature of plants that would have been rejected in screening protocols using anything but unfed neonate larvae.

Second, there is growing evidence that phytophagous insects can adopt novel plants as acceptable hosts when one or more of the secondary metabolic compounds of such plants coincidentally possess the structure and polarity necessary to depolarize phagostimulatory binding sites on gustatory receptors. When this is the case, host plant acceptability is a function of the binding properties of particular compounds, not the taxonomic relatedness or class of chemical deterrents in a plant's tissues. Computer programs that model the 3-dimensional configuration of secondary metabolic compounds can be used to identify molecules in non-target plants with binding site properties similar to those of phagostimulatory chemicals in the ancestral hosts of prospective weed control agents. Only non-targets possessing such matches need to be included in actual screens. Thus, screening procedures can be simultaneously made more conservative and more efficient by designs based solely on no choice feeding responses by unconditioned neonate larvae that are exposed to a population of non-targets prescreened by computer searches of chemical libraries.

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How Time-Dependent Processes Can Affect the Outcome of Assays

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Abstract

In an insect, the level of responsiveness to sensory cues varies throughout its life and this variation affects the probability that a response to any given cue will occur at a particular time. Important sources of variation in responsiveness to sensory cues associated with food or oviposition sites are changes induced by food or oviposition-site deprivation. Such changes, which have been termed time-dependent, have the potential to affect the outcome of host specificity assays of various designs. Groups of a biological control agent, the parthenium leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae: Chrysomelinae), were tested in two different assays involving differently ranked plants. First, beetles differing in their time-dependent level of responsiveness were tested in two choice assays with plants in the subtribe Ambrosiinae of the Heliantheae. Second, groups of beetles were tested in no-choice sequential assays alternating exposure between the highest and lower ranked plants. These assays showed that time-dependent factors can influence the results of choice and no-choice feeding assays. In the choice test, *Z. bicolorata* that had fasted for only 3 hours, consistently rejected the lower ranked host, *Xanthium occidentale* Bertoloni (Noogoora burr), for feeding but accepted *Parthenium hysterophorus* L. This situation produced the false impression that Noogoora burr is not an acceptable host plant for feeding. However, if beetles entered similar choice tests in a food-deprived state (i.e., having fasted for 6 days), many beetles fed on *X. occidentale* when they encountered it first. The number of eggs laid on *X. occidentale*, however, was consistently less than on parthenium, irrespective of the food-deprivation state of the beetles. Summed over the whole experiment, the 6-day food-deprived beetles laid fewer eggs per day than did less food-deprived beetles. In sequential no-choice assays, beetles initially did not feed or oviposit on *X. occidentale* plants, but acceptance increased with time since the last exposure to parthenium. These data support predictions that choice tests using insects in a non-deprived state, and short duration sequential no-choice assays, will not adequately reveal the acceptability of lower ranked host plants.

Keywords: choice test, sequential test, behavior, biological control agent, host specificity testing, deprivation

Introduction

Ideally, host specificity testing and risk assessment methodologies should both prevent the release of any organism that is likely to have an unacceptable economic and/ or environmental impact and minimize the likelihood that safe and potentially useful agents will be rejected. Thus, the challenge for the practitioner is to identify and use host specificity testing methods that will provide a realistic estimate of the field host range of a proposed biological control agent (Withers et al., 1999).

In the last decade there has been increasing interest in the design and interpretation of the laboratory assays used to assess the host range of phytophagous insects (Cullen, 1990; Harris and McEvoy, 1992; McEvoy, 1996; Blossey, 1997; Marohasy, 1998). Debate over the virtues and shortcomings of the various assay methods has continued (Withers, 1997; Marohasy, 1998; Withers et al., 1999)

There is a range of assay designs that are commonly used for the host range estimation of biological control agents (Heard, 1997; Heard and van Klinken, 1998). In a recent review, Sheppard (1999) found that for the most commonly used groups of weed biological control agents, namely Coleoptera, Lepidoptera, and Diptera, feeding assays were dominated by no-choice tests, with choice tests that included the target weed in the array being used less often. In contrast, for oviposition tests, choice assays were used as commonly as no-choice assays (Sheppard, 1999). A common variation of the traditional no-choice assay (in which non-target plant species are presented separately to test insects using the same conditions as those for testing responses to the target weed) and choice assays, is the sequential no-choice design. Here non-target plants are presented to a group of insects one at a time, in a serial order alternating with the target plant. It is envisaged that host specificity testing programs will continue to be dominated by these types of assays - no-choice (sequential and parallel) and simple choice assays. But how much do we really know about the ability of these assay types to accurately predict the host range of a biological control agent in the field?

Marohasy (1998) discusses the useful concepts of false positives and false negatives in the context of host specificity testing. False positives occur when a test indicates that a plant species will be fed or oviposited on, when in reality it would not be attacked in the field. False negatives occur when a test indicates that a plant species is outside the host range of the insect species, when in reality it would be attacked in the field (Marohasy 1998). One source of both false negatives and false positives is that the responsiveness of insects to sensory cues from a potential host can change over their lives. The phenomena responsible for these changes in responsiveness fall into three general categories: reversible changes resulting from food or oviposition-site deprivation (termed time-dependent changes by Papaj and Rausher, 1983), changes induced by experience (Szentesi and Jermy, 1990; Bernays, 1995) and ontogenetic changes (Barton Browne, 1993,1995).

Of all the assay methods, the no-choice test is deemed to be the testing method least likely to produce false negative results (Cullen, 1990; Heard, 1997). It is widely believed, however, that no-choice tests of extended duration tend to over-estimate the field host range of insects (i.e., cause false positives). This is because increased acceptance often occurs as a result of effects of extreme deprivation and experience. Because of this perceived drawback, the choice test is frequently used for revealing the preference ranking for the target weed relative to other plants (Marohasy, 1998; Edwards, 1999), and/or for reducing the list of test plants required to be tested in further assays (especially when larvae are immobile and oviposition specificity decides the host range). Because of this, choice tests have been widely used and will continue to be used to measure the risk that test plants will be damaged in the presence of the target weed. The main concern with choice tests is their inability to reveal the acceptability of lower ranked host plants (Heard, 1997) because the insects can be expected to be in a low state of responsiveness due to their ready access to the highly ranked target species (Marohasy, 1998; Edwards, 1999). Also choice tests do not adequately predict the outcome of events in cases in which insects occur in localized areas where the target host is absent.

Predictions

There are two key features of time-dependent changes in responsiveness for insect feeding or oviposition. Firstly, the responsiveness of an insect to food and oviposition-related sensory cues increases with elapsed time since the last meal or oviposition. Secondly, responsiveness to sensory cues decreases following a meal or laying of eggs (Dethier 1982; Miller and Strickler 1984). These features form the basis of models of Singer (1982), Singer et al. (1992), Courtney et al.

(1989) and Courtney and Kibota (1990), which describe the increase in the number of hosts accepted for oviposition caused by such deprivations. In general terms, these conceptual frameworks predict (i) that an insect, upon completing a bout of feeding or oviposition on its most highly stimulating host (highest-ranked host sensu Courtney and Kibota, 1990), will, for a period, be unresponsive to sensory cues from this host (refractory phase) or lower ranked hosts (Simpson, 1982), (ii) that as time since feeding or ovipositing increases, the insect again becomes responsive to the higher ranked host but not to lower ranked hosts (discrimination phase), and (iii) that, if an insect is denied access to its highest ranked (or to any) host, it will progressively become more responsive so that it will, increasingly, come to accept food and oviposition sites providing lower and lower levels of stimulation (deprivation phase).

The consequences of time-dependent changes in responsiveness are that insects deprived of the opportunity to feed or oviposit for significant periods may feed or oviposit on hosts that are rejected by less deprived individuals. Evidence for this has been obtained in relation to feeding by the acridids *Locusta migratoria* (L.) (Bernays et al., 1976) and *Chortoicetes terminifera* Walker (Bernays and Chapman, 1973) and the psyllid *Cacopsylla pyricola* Foerster (Horton and Krysan, 1991). In the tephritid *Bactrocera tryoni* (Frogg.), it has been shown that host deprived individuals accept for oviposition, host species that are rejected by less deprived individuals (Fitt, 1986). When caged continuously in a no-choice situation with oviposition sites providing different levels of excitatory stimulation, female phytophagous insects may accept lower ranked hosts later than higher ranked hosts (Weston et al., 1992; Kostál, 1993). The relevance of such outcomes for host specificity testing is obvious.

In this paper we will examine the potential influence of time-dependent changes on the outcomes of two-choice assays and sequential tests. The example we use is one in which the higher ranked target weed is being compared with a non-target plant that is ranked lower than the target species for both oviposition and feeding. On the basis of the above conceptual framework, the following predictions can be made. If the insect is in a refractory or a discrimination phase when it enters a choice test that includes the two plant species, its first meal or oviposition will be on the higher ranked plant. Thereafter, it can be expected to fluctuate between the refractory and discrimination phases because of the continuous availability of the higher ranked plant. In this case, therefore, the insect would not be expected to feed or oviposit on the lower ranked plant over the course of the tests. In contrast, if the insect is in a highly deprived state when it enters the two-choice test, we predict that it will feed or oviposit initially on whichever plant species is encountered first. Thus, in this case, the expectation is that there will be some feeding or oviposition on the lower ranked plant early in a choice test, but that the incidence of this will decline to zero as the test proceeds.

For sequential no-choice tests, we predict that if the insect is in the refractory or discrimination phase when transferred from the higher ranked plant to the lower ranked plant, it will initially reject the lower ranked plant. It will, however, become progressively more responsive as the elapsed time since it last fed or oviposited on the higher ranked plant increases, until it reaches the state where it will accept the lower ranked plant. Thus, there will be little or no feeding or oviposition on the lower ranked plant for a period, but thereafter, it will increase with time. Our predictions require some simplifying assumptions, including that plant quality does not change during the assay, that no host-marking pheromone is deposited, that host selection is not influenced by long-range orientation behavior to the preferred host, and that the insect shows non-random movement in response to host plant cues, such that it will tend to remain and feed or oviposit on the higher ranked plant when it is located.

Our Model Insect-Plant System

The insect we used to test these predictions is a weed biocontrol agent that has caused controversy because adults have fed on non-target plants in areas where the target weed was rapidly defoliated (Jayanth and Visalakshy, 1994). This insect is the oligophagous leaf-feeding beetle *Zygogramma bicolorata* Pallister (Chrysomelidae), which has been released in Australia and India for the biological control of *Parthenium hysterophorus* L. (Heliantheae: Ambrosiinae) (Jayanth and Bali, 1994; Dhileepan and McFadyen, 1997), as well as for ragweed *Ambrosia artemisiifolia* L. (Heliantheae: Ambrosiinae) in Australia. This insect has been subjected to host range testing in quarantine in Australia (R.E. McFadyen, *unpublished data*), field studies in Australia and India (Jayanth et al., 1993; Jayanth and Bali, 1994), and detailed behavioral observations (Withers, 1998, 1999).

Previous studies have shown that time-dependent processes play a part in the host acceptance behavior of *Z. bicolorata* (Withers, 1999). Adult beetles readily accept parthenium and ragweed for feeding. Noogoora burr (*Xanthium occidentale* Bertoloni, Heliantheae: Ambrosiinae), which is also a weed in Australia, is acceptable but generally only after prolonged periods of food deprivation. Noogoora burr often receives eggs in the field (as do other Heliantheae at times), and supports adult survival. However larval mortality is extremely high on Noogoora burr. Probably because of this mortality, population densities on this host rarely become high in the field (T. Withers, unpublished data). Sunflower (*Helianthus annuus* L., Heliantheae: Helianthiinae) is accepted for feeding by only a small proportion of the adults in a *Z. bicolorata* population either under severe deprivation or when the plants' acceptability has been increased by covering the leaves with parthenium pollen (Jayanth et al., 1993; Jayanth and Visalakshy, 1994; Withers, 1998). Sunflower is not a host for larvae.

We predicted that, when introduced into choice tests containing both a higher ranked plant (parthenium), and a lower ranked plant (Noogoora burr), adult *Z. bicolorata* in a discrimination phase would exhibit little or no feeding on Noogoora burr over the course of the test. On the other hand, we predicted that insects contacting Noogoora burr first when introduced into the choice test in a deprived state would feed on Noogoora burr. We predicted that in sequential no-choice tests, adult *Z. bicolorata* would be in a discrimination phase when transferred from parthenium to Noogoora burr, and that Noogoora burr would not initially be accepted for feeding. With increasing duration of exposure to Noogoora burr, we predicted an increase in feeding during the test. We present the results of experiments designed primarily to examine the effects of food deprivation on the outcome of feeding assays. However, some data on number of eggs laid are also briefly presented.

Materials and Methods

Experiment 1 – Two Choice Assays

Insects. The *Z. bicolorata* population used in these experiments originated from adults collected from parthenium in Monterrey, Mexico in 1980 and reared in the laboratory on parthenium in Brisbane, Australia until release in 1983. In addition to its establishment on parthenium weed in central Queensland, the beetle also established on ragweed in Brisbane. For our tests, adult *Z. bicolorata* were collected in Brisbane in spring off ragweed, and their offspring reared for two to three generations on potted ragweed plants. As adults eclosed from pupation sites in the soil of these pots, they were collected and held in cages with ragweed plants in a greenhouse maintained at 26° C (±2°) and 85-95 % RH. They were two to three weeks old (mean of 10 days old) at the time of experimentation (January 1998). Ragweed ranks as highly as parthenium for feeding by *Z. bicolorata*. Using ragweed to rear the insects meant beetles were naïve to those plant species used in experiments.

Zygogramma bicolorata adults of three different levels of feeding responsiveness were obtained by depriving them of the opportunity to feed for one of three time periods:

- *Recently-fed beetles* were obtained from beetles on ragweed that were continuously observed until they were seen to have just completed a meal. These individuals were collected and used in tests within 30 minutes of the end of their meal.

- *Three hours post-meal beetles* were ones held on ragweed and continuously observed between 08:30 and 09:30 hrs. Immediately after each beetle completed a meal, it was placed into a 5 x 10 x 20 cm plastic container with a mesh insert in the lid containing a moistened sand/bark mixture. They were held for approximately 3 hours before testing. This interval was chosen because it is almost one inter-meal interval for both adult male and female beetles (T. Withers, unpublished data).

- *Deprived six days beetles* were ones removed from ragweed plants at 11:00 hours and placed into a container as above and tested six days later.

The state of responsiveness of beetles in each of these three groups at the start of the test were expected to be for:

- Recently-fed beetles, initially unresponsive to both higher and lower ranked host plants because the time since the last meal had not exceeded the intermeal interval for either male or female beetles (refractory phase)

- Three hours post-meal beetles, responsive to the highest ranked plant for feeding, but not the lower ranked plant (discrimination phase)

- Six days deprived beetles, highly responsive to

both the higher and lower ranked plants due to severe food deprivation (deprivation phase).

Responsiveness state of the beetles. No-choice behavioral observations were undertaken concurrently with the two-choice tests to indicate the level of responsiveness of each group of beetles to plant cues. On each day on which experiments were set up, two beetles from each treatment group were chosen and held in a cotton mesh covered cage (40 x 40 x 85 cm h, with an open front through which the observations were made) containing either a parthenium or a Noogoora burr plant. Behaviors were recorded directly onto a portable computer programmed with the behavioral recording software "The Observer, version 3.0" (Noldus, 1990). The observations took place alongside the two choice tests. Timing, to the nearest second, and the location of the beetle in the cage or on the plant were recorded as all behaviors were occurring. Behaviors recorded were sample biting, feeding, walking, or pausing. Observations were made on each beetle until it completed a meal or until 30 minutes elapsed, whichever occurred first (see Withers [1998] for details of the behavioral recording protocol). Due to some difficulty obtaining recently-fed beetles, the final sample sizes were 10 recently-fed, 12 three hours post-meal and 12 six days deprived beetles.

Procedure for choice tests. Inside the cotton mesh covered cages used for the two choice tests (55 x 90 x 85 cm H) a wooden frame with 4 large holes (20 cm apart) was placed over the pots containing parthenium and Noogoora burr plants. Brown paper was then placed on top of the wooden frame with slits cut in the appropriate place to allow the plants to protrude and to prevent the beetles from escaping down the sides of the cage and into the plants pots.

Recently-fed, three hours post-meal, or six days deprived beetles were introduced (generally 10 per cage) into one of three identical test cages containing two plants each of the higher ranked parthenium and the lower ranked Noogoora burr. The cages were situated in a naturally lit greenhouse (28 - 32 °C). Half the beetles were marked on the elytra with a whitening fluid (Tipp-Ex Germany, Malaysia). The marked beetles were introduced into each cage onto the young leaves of one Noogoora burr plant. The other half of the beetles (unmarked) were introduced onto leaves of one parthenium plant. The position of each beetle was recorded at 2-5 minute intervals for the first 20 mins, half hourly for the rest of the day, and hourly for the next two days, between the hours of 0830 and 1730. Each morning at 0830 hours the number of eggs laid, and the number of meals taken from each plant was recorded. After the third day (70 hours), the beetles were captured and after freezing, dissected to obtain the sex ratio within that test.

This procedure was repeated three times during three consecutive weeks in January 1998. The only difference between repetitions involved the sample size of beetles in the recently-fed treatment, i.e., sample size in this treatment was dependent upon the number of beetles observed taking a meal within the 30 minute period preceding the tests. This resulted in 6, 10 and 8 beetles per repetition, respectively, for the recently-fed treatments. The tests all began at 11:30 hrs on the first day and finished at 08:30 hrs on the third day. During this time, daily counts were made of the number of meals consumed from leaves (estimated by counting each scalloped area removed from a leaf edge), and the number of eggs laid, both without disturbing the beetles on the plants.

Data were expressed as the number of meals taken per beetle per day or eggs laid per female beetle per day. These data were tested for homogeneity of variances across treatments using a Bartlett's test. Where heteroscedasticity remained, median values were compared between treatments using appropriate non-parametric tests at $P < 0.05$.

The location of beetles (marked versus unmarked individuals) was recorded at various times following their introduction, and analysed as follows, using combined data from the three repeats. If the proportion of those beetles originally released on plant species *a* which have remained there is p_{aa}, while the proportion of those beetles originally released on plant species *b* which have moved to species *a* is p_{ba}, then the total proportion of beetles on species *a* will be:

$$p_a = (p_{aa} + p_{ba}) / 2.$$

Similarly,

$$p_b = (p_{bb} + p_{ab}) / 2.$$

The difference between p_a and p_b was then used as a measure of the preferential movement by the beetles between the two plant species,

$$p_a - p_b = (p_{aa} + p_{ba} - p_{ab} - p_{bb}) / 2.$$

Using variances and covariances from the multinomial distribution, an approximate standard error for this difference was obtained as follows:

$$s.e.(p_a - p_b) \approx \sqrt{\{(p_{aa}[1-p_{aa}] + p_{ba}[1-p_{ba}] + p_{ab}[1-p_{ab}] + p_{bb}[1-p_{bb}] + 2p_{aa}p_{ab} + 2p_{ba}p_{bb})/(4n)\}}$$

where n is the number of beetles originally released on each species. A z-score was then used as an approximate test of the statistical significance of the difference from zero,

$$Z = (p_a - p_b) / s.e.(p_a - p_b)$$

This procedure was used to test the hypothesis that the beetles expressed a preference for parthenium over Noogoora burr as a substrate, reflected in their location in the cage, at each assessment period. Similar procedures were used to compare the effects of treatments on plant species preferences, and on the proportion of beetles that were not located on either plant species.

Experiment 2: No-Choice Sequential Assays

Sequential no-choice trials were conducted to examine the potential effects of time-dependent changes in responsiveness as well as feeding experience on the acceptance of non-target plants. Knowing that acceptance of Noogoora burr, and also of sunflower, increases with extreme food deprivation, we tested *Z. bicolorata* in a no-choice sequential assay with a long exposure time (5 days) on non-target plants to see whether we could induce either false negative or false positive results.

Adult *Z. bicolorata* were collected from annual ragweed in Brisbane and maintained on parthenium for two weeks prior to testing. Tests were conducted under glasshouse conditions (25± 3°C) with supplementary halogen lighting used to create a photoperiod of 14:10 L:D.

Sixteen clear plastic cylinders (25 cm diam x 32 cm H) were filled with soil to a depth of 25 cm. A black gauze sleeve (25 cm diam x 110 cm L) was suspended above each cylinder and fitted tightly to prevent the escape of the insects. A single, potted, vegetative-to-early flowering parthenium plant was placed into each enclosure. The pots were buried to give a smooth soil surface up to the base of the plant stem.

Five mating pairs of adult *Z. bicolorata* were introduced into each cage of the 16 cages between 08:30 and 09:30 hrs on the first experimental day (March 1999). For the next two days at 09:00 hrs, the number of eggs were counted and feeding level noted on each plant. On the third day the 16 sets of insects were randomly allocated to 4 treatments (each with 4 cages): Noogoora burr, sunflower, bean (*Phaseolus vulgaris* L., Fabaceae) or parthenium. For the next 5 days the number of feeding sites (each scalloped area removed from a leaf edge) as well as eggs laid were recorded as accurately as possible, e.g., on parthenium scoring of feeding sites was impaired by the extensively lobed leaf margins and the extensive feeding. After 5 days the insects were transferred to new enclosures containing parthenium plants where feeding and oviposition were again recorded for a 5-day period. This produced a sequential, no-choice trial in which the insects were monitored for two days on the target host (parthenium), then 5 days on one of three test plants or the target-control, and then returned to parthenium for a further 5 days. A clean enclosure sleeve was fitted at the time of each insect transfer to reduce the possibility of cross-contamination of host plant cues.

Results

Experiment 1 – Two Choice Assays

Responsiveness state of the beetles. The results obtained from observations of individual beetles in this study agreed with the findings of earlier experiments (Withers, 1999), on which basis the treatments had been chosen. The sampled beetles were in the expected time-dependent states, with the exception that both the recently-fed beetles and the three hours post-meal beetles responded similarly to host plants. Most recently-fed and three hours post-meal beetles were responsive to parthenium (10/11 fed) but not to Noogoora burr (1/11 fed). This suggested that the recently-fed beetles were best described as being in a discrimination phase rather than a refractory phase. This result has two possible explanations. Either the experimental parthenium plants ranked higher than the ragweed plants on which the beetles had taken their previous meal, or for some beetles, being removed from ragweed following a meal and being held in a container for up to 30 mins in some cases was sufficient to increase their responsiveness when introduced onto parthenium. For this reason we have combined the results of the recently-fed and three hours post-meal categories for all further analyses.

Half (3/6) of the 6-day deprived beetles accepted Noogoora burr. Acceptance of Noogoora burr only occurred after a greater number of test bites were taken (mean of 23) than were taken preceding acceptance of parthenium (mean of 3). This confirms that the 6-day deprived beetles were in the deprivation phase. However, even in this state, the lower ranked plant was not accepted as readily for feeding as the higher ranked plant.

Movements of beetles between plants. In the two-choice tests, almost all recently-fed and 3 hour post-meal beetles released onto parthenium remained on parthenium plants for the entire test. Only in seven instances was a beetle, released onto parthenium, found later for a short time on a Noogoora burr plant. In all deprivation treatments, beetles released onto a Noogoora burr plant moved off the plant and ended up on a parthenium plant. The differences between treatments were in how rapidly this movement from parthenium to Noogoora burr occurred. Over half of the recently-fed and 3 hour post-meal beetles released onto Noogoora burr had left within the first 30 mins. In contrast, half of the 6-day deprived beetles had left Noogoora burr after between 5 and 24 hours (Fig. 1). The proportion of beetles found elsewhere in the cage (on the netting walls, and paper floor) was consistently lower in the 6 day deprived treatment than in the recently-fed/ 3 hour post-meal treatment (Fig. 1).

The difference in the proportion of beetles remaining on parthenium was compared with the proportion of beetles remaining on Noogoora burr at a number of times throughout the test. Significant differences in beetle location, on the basis of a difference in the proportion of beetles leaving the plant species that they were introduced on to, occurred virtually immediately in the recently-fed/ 3 hours deprived treatment. However, in the 6-day deprived treatment a difference in the proportion of beetles showing a location preference for parthenium over Noogoora burr was significant only two hours into the test. The plant preferences shown by beetles on the basis of location were compared directly between treatments and visualised on a logarithmic scale, where movement of beetles within the first 10 hours could be seen more clearly (Fig. 2). Beetles in the 6-day deprived treatment were slower to leave Noogoora burr and move onto parthenium (revealed as a smaller difference), as well as slower to move elsewhere in the cage, in comparison to the recently-fed/3 hours post-meal treatment. The differences between the time-dependent treatments in the proportion of beetles remaining on the plant species onto which they had been introduced, were significant between 30 minutes and 28 hours into the test.

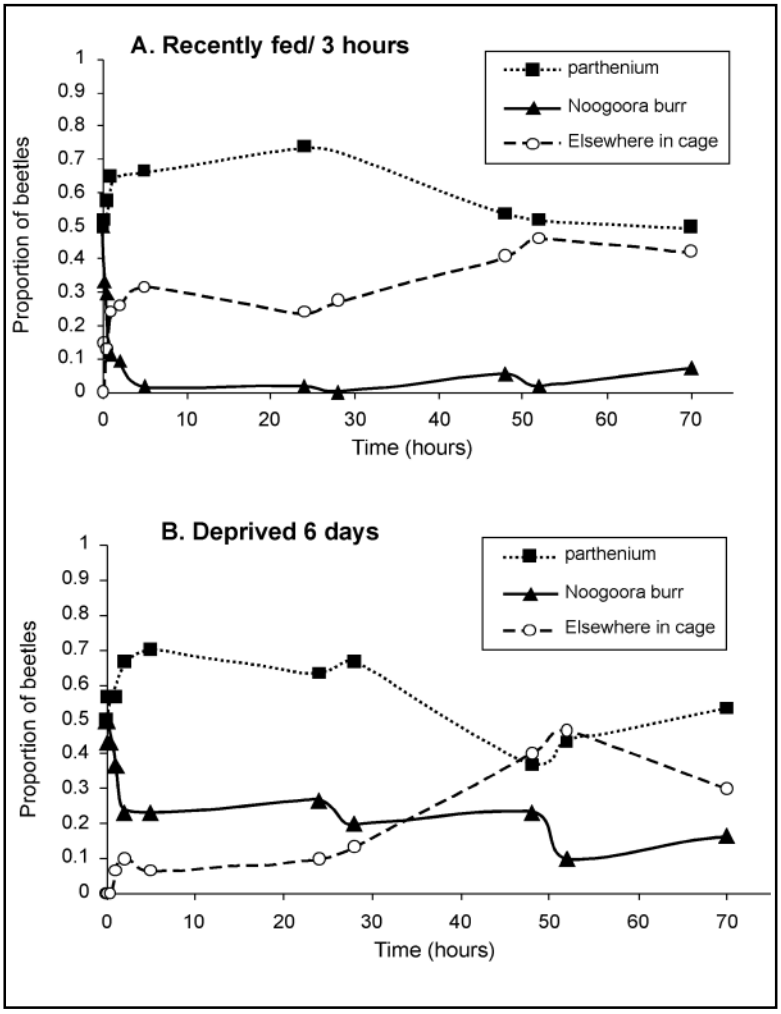

Fig. 1. The influence of initial state of food deprivation on the location of adult *Zygogramma bicolorata* Pallister over the course of two-choice cage tests. The proportion of beetles on a parthenium (*Parthenium hysterophorus* L.) plant, a *Noogoora burr* (*Xanthium occidentale* Bertol.) plant, or elsewhere in the cage, when beetles were introduced (a) recently-fed or 3 hours post-meal (combined data n = 54), or (b) after 6 days of food deprivation (n = 30).

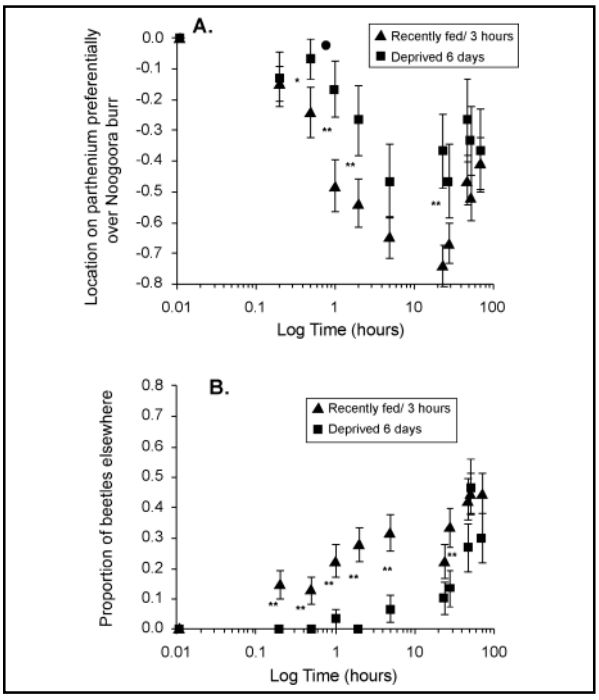

Fig. 2(a-b). The influence of initial state of food deprivation (recently-fed/ 3 hours post-meal compared to 6 days of food deprivation) on the location of adult *Zygogramma bicolorata* Pallister over the course of two-choice cage tests. (a) The difference in the proportion of beetles showing a preference for parthenium (*Parthenium hysterophorus* L.) over Noogoora burr (*Xanthium occidentale* Bertol.) or (b) proportion of beetles found elsewhere in the cage. Note the logarithmic scale. Significant differences in location according to treatment at each time period are indicated (* at P < 0.1, and ** at P < 0.05).

From the second day (28 hours after introduction) initial treatment was no longer influencing location of beetles in the cage (Fig. 2).

Plant consumption. Overall, significantly more meals were taken per beetle from parthenium than from Noogoora burr throughout the two-choice tests (Fig. 3)(Mann-Whitney test $W = 378$, $P < 0.0001$). In the recently-fed/ 3 hours post-meal treatments, virtually no meals were taken from Noogoora burr, whereas a significant number of meals were taken from this plant when beetles had been deprived for 6 days (Fig. 3).

The number of meals taken from Noogoora burr plants was significantly affected by deprivation state of the beetles at the start of the test (Mann-Whitney test W = 182.5, P < 0.0001) (Fig. 3). Most meals taken from Noogoora burr were in the 6-day deprived treatment (mean of 0.7 meals/ beetle/ day), and the least in the recently-fed/three hour post-meal treatments (mean of 0.03 meals/ beetle/ day). There was no significant effect of day of the test on the number of meals per day taken from Noogoora burr (Kruskal-Wallis test H = 1.3, df =2, P > 0.51). Overall the number of meals taken on the Noogoora burr plant onto which beetles had been introduced was positively correlated (+0.64) with the mean proportion of beetles located on that plant on that day. The same correlation was not obtained when the data from both Noogoora burr plants were combined.

The number of meals from both parthenium plants was not significantly influenced by the deprivation state of the beetles at the start of the choice tests (Mann-Whitney test W = 283.0, P > 0.12). Over all three days, the mean number of meals taken from the parthenium plants by beetles that began the tests in a 6-day deprived state appeared lower (6.5 meals/ beetles/ day) than for beetles in the less deprived states (mean of 9.4 meals/ beetle/ day) (Fig. 3). The number of meals from a parthenium plant overall was positively correlated (+0.36) to the mean proportion of beetles located on both parthenium plants that day.

In order to test for a significant difference in preference for parthenium over Noogoora burr caused by degree of time-dependent responsiveness at the start of the two-choice test, the following analyses were carried out. A coefficient of preference (Heard, 1995) for parthenium over Noogoora burr (CP) was calculated using the formula:

$$CP = (P-NB)/(P+NB)$$

where P = mean number of meals taken per beetle from parthenium, and NB= mean number of meals taken per beetle on Noogoora burr. This index varies from –1 (when all meals are taken from Noogoora burr), to 0 (when equal meals are taken from both parthenium and Noogoora burr), to +1 (when all meals are taken from parthenium).

The Coefficient of Preference calculated daily over the combined replicates indicated that two-choice tests with beetles initially 6-day food deprived revealed the lowest preference for parthenium over Noogoora burr on the first 24 hours data. The preference for parthenium over Noogoora burr increased as the days of the two-choice test passed, however it never reached the same level of virtually complete preference for parthenium, as occurred when the test beetles were recently-fed and 3 hours post-meal (Fig. 4).

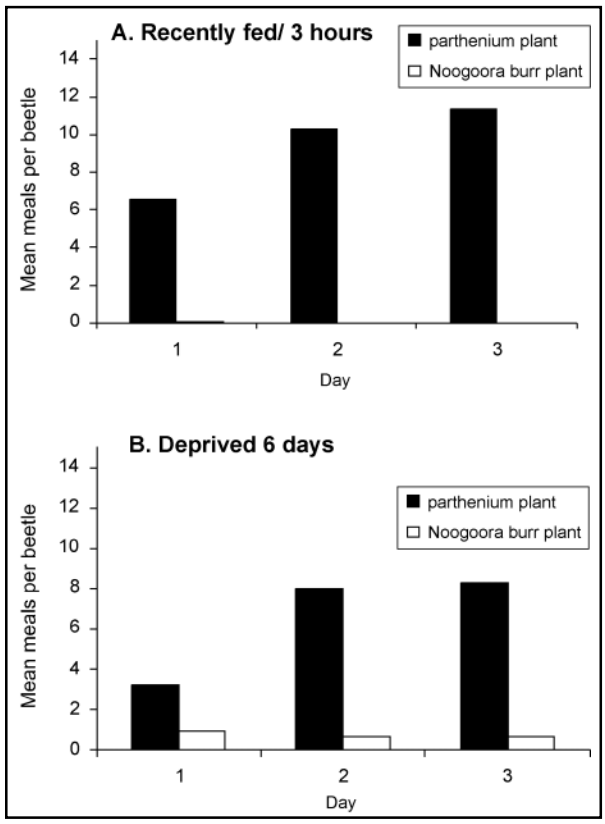

Fig. 3(a-b). The influence of initial state of food deprivation on consumption by adult *Zygogramma bicolorata* Pallister beetles of foliage over the course of two-choice cage tests. The mean number of meals taken per beetle on each of three days of the test from parthenium (*Parthenium hysterophorus* L.) or Noogoora burr (*Xanthium occidentale* Bertol.) when beetles were introduced (a) recently-fed or 3 hours post-meal (combined data n = 54), or (b) after 6 days of food deprivation (n= 30).

Oviposition results. Significantly more eggs were laid on parthenium plants (mean of 6.8 eggs/female/day) than on Noogoora burr plants (mean of 0.15 eggs/female/day) over all the tests (Mann-Whitney test W = 489.5, P < 0.001). This was the case in both the recently-fed/ 3 hours post-meal states, as well as the 6 days deprived state.

The state of deprivation of the beetles significantly influenced the number of eggs laid per female on parthenium plants (Mann-Whitney test $W = 331.0$, $P < 0.0001$) and this was not significantly influenced by the day of the test (Kruskal-Wallis test $H = 2.55$, df = 2, $P > 0.28$). On parthenium, the least eggs were laid when the beetles began the test 6-day deprived (mean of 0.3 eggs per female/ day), and the most eggs were laid when the beetles were in the recently-fed and 3 hours post-meal states (mean 13 eggs per female/ day).

Neither state of deprivation of the beetles (Kruskal-Wallis test $H = 3.6$, df = 1, 26, $P > 0.06$), nor day of the test (Kruskal-Wallis test $H = 0.89$, df = 2, $P > 0.64$), significantly influenced the number of eggs laid on Noogoora burr plants, probably because so few eggs were ever laid on Noogoora burr throughout the tests (range of 0 to 0.3 eggs per female/ day).

Experiment 2: No-Choice Sequential Assays

Feeding was extensive at all times on parthenium, and continuous throughout the sequence used as a control (Fig. 5). Feeding sites were scarce (mean of 0.1 meals per beetle) on the first day of no-choice exposure to Noogoora burr, but increased steadily each day that the no-choice test continued (Fig. 5). There was a significant effect of the day of exposure to Noogoora burr in the no-choice test on the number of meals taken from Noogoora burr (Kruskal-Wallis test $H = 16.5$, df = 4, $P < 0.002$). In three cases, feeding sites on Noogoora burr were too numerous to be counted accurately after the fifth day of the no-choice trial, so were assigned the score of 100. There was no feeding at all on the non-target plants of sunflower and bean (Fig. 5). In all cases, consumption of parthenium after the 5 days exposure to the non-target, returned immediately to pre-non-target levels and continued for the last 5 days of the test (again feeding sites on parthenium were too numerous to be counted).

Oviposition results. During the testing sequence of no-choice exposure to a test plant or a control plant, significantly more eggs were laid per day (Fig. 6) on parthenium (mean of 8 eggs/ female) than on Noogoora burr (mean of 0.72 eggs/ female) (Mann-Whitney test W = 332.5, P < 0.0001). Oviposition in the control cages (continuous access to parthenium plants) differed significantly between days (Kruskal-Wallis test by day: H = 28.5, df = 11, P < 0.003). In particular, egg laying was significantly reduced (oviposition dropped to a mean of 0.7 eggs/ female) on the day that beetles had been handled and moved to another plant (day 3).

A small number of eggs were laid apparently indiscriminately (on the cage walls) in all tests during the no-choice trials. On parthenium significantly more eggs were laid on parthenium

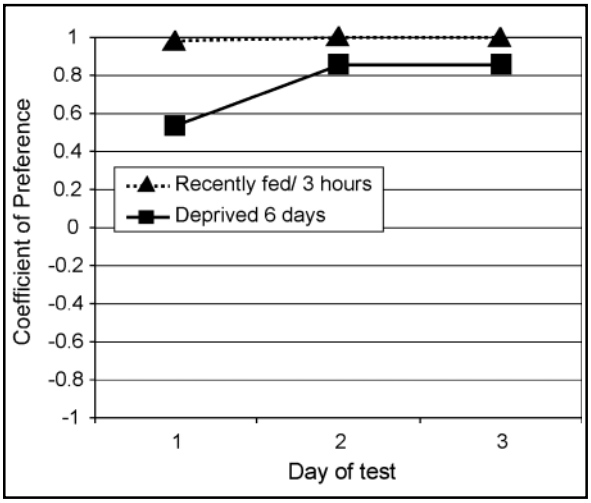

Fig. 4. The influence of initial state of food deprivation on the coefficient of preference shown by adult *Zygogramma bicolorata* Pallister for parthenium (*Parthenium hysterophorus* L.) over Noogoora burr (*Xanthium occidentale* Bertol.) in two-choice tests. The coefficient of preference when beetles were introduced recently-fed and 3 hours post-meal (n = 54) or after 6 days of food deprivation (n = 30). The coefficient of preference varies from +1 to –1, with +1 indicating that all feeding was on parthenium, -1 indicating that all feeding was on Noogoora burr, and 0 indicating no preference.

(mean of 8 eggs/ female) than on the cage (mean of 1.1 eggs/ female) (Mann-Whitney test $W = 530$, $P < 0.016$). However, on Noogoora burr (on which more eggs were laid than on the other test plant 3.7 versus 0.7 eggs/female) ($W = 537.5$, $P < 0.0003$). There were no eggs laid on Noogoora burr on the first day of the test. Despite this, there was no significant effect of day of testing on Noogoora burr on eggs laid on a Noogoora burr plant per day (Kruskal-Wallis test $H = 5.7$, df = 4, $P > 0.2$). On sunflower, more eggs were laid on the cage structures (mean of 1.2 eggs/female) than on the plant itself (mean of 0.16 eggs/female) ($W = 546$, $P < 0.0001$). However, in bean tests, very few eggs were laid (Fig. 6), even on the cage (mean of 0.1 eggs/ female) so there was no significant difference in where the eggs were laid ($W = 422$, $P > 0.3$).

Discussion

Time-Dependence Influencing the Outcome of Host Specificity Assays

In this paper, we have focused on how food or oviposition-site deprivation experienced by an insect (i.e., changes designated as time-dependent by Papaj and Rausher [1983]) might influence the outcome of host specificity tests. Typically, in cage-based choice and no-choice tests, feeding or oviposition is scored at the end of an often arbitrarily selected assay period, with results from non-target plants compared to the target weed. Such host specificity assays usually do not provide an opportunity for examining the behavioral mechanisms responsible for observed outcomes. Theoretical models of host acceptance, particularly the hierarchy-threshold model of individual insect diet (Courtney et al., 1989) and the rolling fulcrum model (Miller and Strickler, 1984), helped us to formulate specific predictions about the influence of time-dependent changes in responsiveness upon the end-points of common types of host range assays.

In relation to choice tests, our predictions included the following outcomes. Commonly, choice tests include both one higher ranked plant species (e.g., the target weed) and at least one lower ranked but acceptable plant species (which may be taxonomically-related or chemically similar to the target weed) (Heard and van Klinken, 1998). We predicted that a non-deprived insect introduced into a test will always find, or be in contact with, the target weed, well before becoming sufficiently deprived to ever accept the lower ranked plant. Thus, we predict that choice tests including the target weed are particularly prone to producing false negative results. However, should the same insect enter the same choice test as described above when deprived

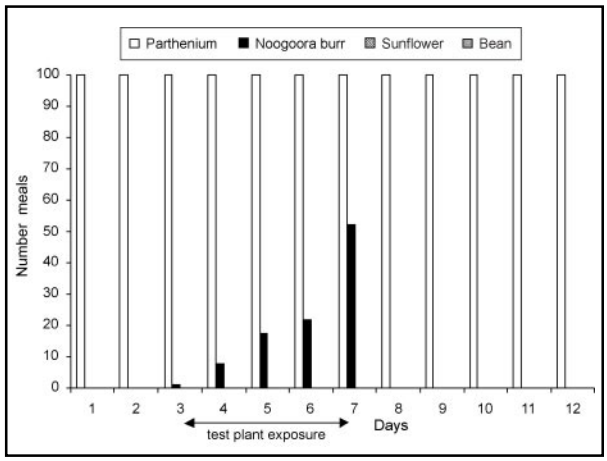

Fig. 5. The mean number of meals taken by five pairs of adult *Zygogramma bicolorata* Pallister per day over a sequential no-choice trial (n = 4 replicates). The first two days were no-choice exposure to the target *Parthenium hysterophorus* L., followed by five days on one of three non-target plants or *P. hysterophorus* (control), followed by another five days on *P. hysterophorus*. A score of 100 meals was assigned when the number of meals was too large to be accurately counted.

of food, its responsiveness to plant cues will be much higher. In this case, we predict that the lower ranked non-target plant may receive eggs or be fed upon early in the choice test, but that the incidence of this will decline as the test proceeds, reducing the likelihood of a false negative result.

Time-dependent changes in responsiveness are also likely to have significant impacts on the measurable outcomes of no-choice tests, particularly when high-ranked and lower ranked plants are presented sequentially to the insect. Insects which have had unlimited access to higher ranked plants (such as is normal when rearing procedures for the insect require continual access to the target weed) before being put in a no-choice assay with non-target plants, will be in a state of low responsiveness. Whether or not the non-target plant is ever accepted for feeding or oviposition, will depend in part upon the duration of the no-choice assay. Only if the duration exceeds that required for responsiveness to reach the acceptance level will feeding or oviposition on the non-target take place. We predicted that short duration no-choice assays have a high potential for producing false negative results.

Predictions on the Outcome of Assays with Z. bicolorata

We were able to design the assays testing the impacts of time-dependence on host acceptance with substantially more knowledge of *Z. bicolorata* than is usually available for biological control agents. Previous experiments (T. Withers, *unpublished data*) had shown that adult female beetles take

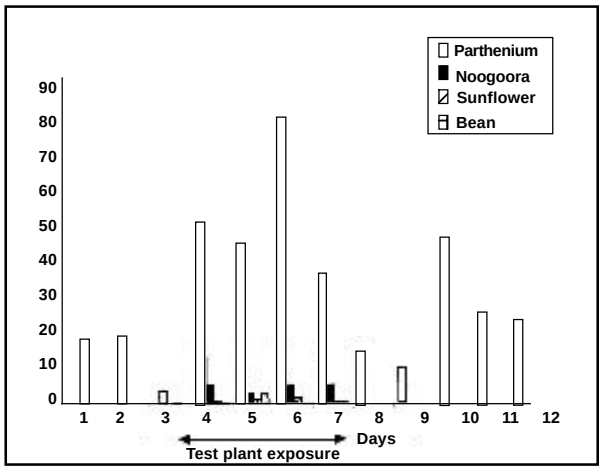

Fig. 6. The mean number of eggs laid on plants per day by five pairs of adult *Zygogramma bicolorata* Pallister over a sequential no-choice trial (n = 4 replicates). The first two days were no-choice exposure to the target *Parthenium hysterophorus* L., followed by five days on one of three non-target plants or *P. hysterophorus* (control), followed by another five days on *P. hysterophorus*.

almost twice as many meals per day (6-7), as males (3-4). Meals on parthenium take 4-6 minutes to complete, and eggs are laid singly on parthenium leaves, generally away from the sites of feeding. This means that beetles that have just completed a meal are unresponsive to plants for feeding. Thereafter, responsiveness to host plant cues increases. Following the normal inter-meal interval of approximately 2.3 hours for female beetles and 4 hours for males, feeding resumes on parthenium (Withers, 1999). If deprived of host plants, adult *Z. bicolorata* become progressively more responsive until lower ranked and non-target plants are accepted for feeding (McFadyen and Heard, 1997) and oviposition (Withers, *unpublished data*). For instance, after 6 days of deprivation, over 50% of adult beetles accept the less acceptable host, Noogoora burr, for feeding (Withers, 1999), whereas none accept it when in a discrimination phase, after they have fed on either parthenium or ragweed. Although a poor host for physiological development for larvae, Noogoora burr has been shown to be a host in the field and under no-choice conditions in the laboratory. Therefore it is important to reiterate that a lack of feeding or a lack of oviposition on Noogoora burr by adult *Z. bicolorata* in cage assays can be considered to be a genuine false negative result (sensu Marohasy, 1998).

It is fortunate that all host plants (Heliantheae: Ambrosiinae) that support development to the adult stage of *Z. bicolorata* in Australia as well as in India are weeds, and not beneficial or native plants. The controversy surrounding adult *Z. bicolorata* causing feeding damage on the leaves of sunflower plants in the field in India (Jayanth et al., 1993; Jayanth and Visalakshy, 1994) can be attributed at least partly to the same behavioral mechanism, i.e., a deprivation-induced increase in responsiveness to plant cues. We did not focus strongly on sunflower in these experiments because this plant does not support larval development, and because of that, some biological control workers would not consider a lack of acceptance of sunflower in laboratory assays necessarily as a false negative result.

Did the results of choice and no-choice sequential assays with *Z. bicolorata* agree with our predictions of their potential to induce false negative results ? We predicted that, in relation to feeding, when adult *Z. bicolorata* were in a refractory (satiated) or

discrimination phase at the commencement of a choice test, the lower ranked host would not be fed upon, thus inducing a false negative result. This was indeed the case (Fig. 3). Based on the two-choice test with recently-fed and 3 hours post-meal adult beetles, Noogoora burr would be considered outside the host range of *Z. bicolorata.* As predicted, two-choice assays with adult beetles that had first been deprived for 6 days of food produced a more accurate estimation of field host range (Noogoora burr has been defoliated by beetles in Queensland, Australia, when growing amongst parthenium weed that has been defoliated by high populations of adult *Z. bicolorata*). Deprived beetles introduced into the two-choice assay were significantly more likely to feed on the plant they had first contacted and were slower to move off either plant. In comparison to less deprived beetles, those introduced onto a Noogoora burr plant were more likely to feed, often took multiple meals, and were much slower at leaving the plant.

Feeding from Noogoora burr plants continued for longer than we predicted, with some beetles remaining on the plant and feeding throughout the three days of the test (Figs. 1 and 2). This finding suggests that the results are being influenced by one or more other behavioral processes that affect responsiveness to plant cues (e.g., some kind of experience-induced changes in responsiveness to host plants), the effect of which interferes with the clear expression of time-dependent changes. It is also possible that ingestion of a meal of Noogoora burr by *Z. bicolorata* does not cause a decrease in the level of responsiveness to plant cues as was anticipated, causing the state of deprivation to continue longer than predicted.

We had predicted that sequential no-choice assays with *Z. bicolorata* would reveal strong time-dependent affects with adult beetles initially rejecting lower ranked hosts after the previous no-choice access to the higher ranked parthenium. We also predicted that feeding on Noogoora burr would increase progressively over the duration of the no-choice test. Indeed, the assays followed this prediction closely. Feeding was negligible on day one of the no-choice test on Noogoora burr (as well as the other two non-target plants tested), and then steadily increased each day over the next four days (Fig. 5). These results can be fully explained by time-dependent increases in responsiveness to a lower ranked host with increasing food deprivation.

The importance of such a result is obvious. If no-choice sequential assays were used when the duration of exposure to the non-target were only one day, or possibly two days, any slight amount of feeding on Noogoora burr may be deemed insignificant or perhaps misinterpreted as "exploratory feeding", thereby producing a false negative result. Whereas a no-choice sequential assay with a duration of exposure to the non-target of four or five days, as was run in this experiment, more accurately predicted the field host range of *Z. bicolorata.* Therefore sequential no-choice assays are capable of producing both genuine and false negative results, according to the duration for which they are run.

Based upon time-dependent changes in responsiveness we made similar predictions in relation to oviposition by *Z. bicolorata*, as those that were made for feeding. We know less about the temporal patterning of oviposition of *Z. bicolorata* than we do about feeding. The time-dependent treatments were designed for replicating food deprivation, and the effect of these treatments on responsiveness for oviposition were uncertain. Nevertheless we counted eggs laid in all experiments. The oviposition data do not agree closely with all time-dependent based predictions. More eggs were laid than was expected on Noogoora burr in the just-fed and 3 hours post-meal treatments in the two-choice tests, although the level was less than one egg per female per day. We had predicted no oviposition on the lower ranked host in the presence of the higher ranked target weed. When beetles were introduced into the two-choice test after six days of food deprivation, oviposition had ceased completely and the first eggs were first laid on parthenium only on day three. Previous experiments have indicated that oocytes are resorbed by female *Z. bicolorata* following 2-3 days of food deprivation (Withers, *unpublished data*). This conclusion has been reinforced by dissection of deprived females (Withers, *unpublished data*).

We obtained further information about the oviposition behavior of *Z. bicolorata* from the no-choice sequential test with parthenium as the control, and eggs counted daily for the full 12 days. This revealed an overall mean egg laying rate of 8 eggs per female per day. Oviposition was apparently affected by external conditions such as handling, e.g., the egg laying rate noticeably reduced following handling of the beetles on days 3 and 8 of the 12 day experiment (Fig. 6). Oviposition was also significantly reduced when beetles were transferred to lower ranked plants. Oviposition on Noogoora burr

followed an almost identical pattern to feeding, with no oviposition on day 1, although egg laying gradually increased over the next 4 days. On all the lower ranked plants some egg laying continued throughout the no-choice test, but more so on the cage, than on the plants themselves. Oviposition steadily increased towards pre-non-target levels, following the return of beetles to parthenium plants. This pattern is explained by the resorption of oocytes by deprived female beetles, and the resumption in oocyte production following two days of feeding on parthenium.

The oviposition results suggest that oviposition behavior by *Z. bicolorata* on cage structures in no-choice tests may occur because the insects ranked some non-target plants even lower than some neutral surfaces (Withers and Barton Browne, 1998). In conclusion, with *Z. bicolorata,* the results of oviposition trials of a duration greater than 24 hours would indicate that Noogoora burr is within the fundamental host range for oviposition (van Klinken, this volume), although it ranks considerably lower than parthenium weed. This conclusion would not be considered as a false result, but an accurate reflection of the situation in the field.

Implications and Recommendations for Host Specificity Testing Protocols

An important issue in host specificity testing is the ability for different tests to accurately predict the likely field host range of an insect. We were fortunate to be able to use hindsight to allow us to compare the outcomes of laboratory assays with field data. In the field, we know that very high population levels of *Z. bicolorata* sometimes occur in combination with a virtual collapse in the availability of their target plant, parthenium weed. This sometimes has a predictable seasonal component, with parthenium weed (and beetles) rapidly appearing following the onset of the rainy season, while parthenium dies soon after the onset of drought. Such was the case with *Z. bicolorata* in India (Narendra, 1990). In this case parthenium weed was completely defoliated and destroyed by large populations of *Z. bicolorata.* This resulted in beetles becoming severely food deprived and some adults accepted sunflower foliage for feeding. A more common event in Australia is oviposition on, and defoliation of, the acceptable but lower ranked weed, Noogoora burr. The ability for biological control researchers to predict such an event from laboratory based assays will always be limited. Our results indicated that, as predicted, it was only when deprived *Z. bicolorata* were placed into choice tests, or sequential no-choice tests were run for greater than two days, that the acceptability of lower ranked non-target plants was revealed.

No-choice tests will always be more effective than choice tests to reveal the acceptability of lower ranked hosts because of the action of time-dependent increases in responsiveness following deprivation from higher ranked hosts. Thus, if a plant is ever to become acceptable to an insect in a naturally occurring time-dependent state, then it is more likely to be expressed during a no-choice test than in any other test. In order to maximize the safety of an introduction of an exotic insect, we recommend therefore that no-choice tests be used to ascertain the maximum range of acceptable hosts. In order to avoid the potentially frustrating occurrence of false positive results (which may be caused by an unrealistic excessive period of deprivation in a no-choice test), choosing an appropriate duration for the no-choice assay is very important. This can only be done after additional information is gathered on the insects natural temporal patterning of feeding and oviposition, their biology, and the effects of time-dependent changes in responsiveness. For instance, significant acceptance of Noogoora burr for feeding occurs after two to three days of food deprivation. This duration is equivalent to the loss of between eight and eighteen normal meals (based on the observation of 4-6 meals per day, depending on beetle age and sex).

Choice tests will continue to be an important test method for ascertaining the relative acceptability of different hosts and to predict which plants will be acceptable under a range of field scenarios (Marohasy, 1998). Our findings reveal that choice tests which include the target do not always reveal the acceptability of lower ranked hosts. On this basis it would be unwise to use multiple choice tests that include any high ranking host plants as the first method to ascertain non-target plants under risk of attack. Reducing the host testing list of plants for subsequent no-choice feeding assays on the basis of such results would be risky. Such an order of testing has a very high potential for producing potentially dangerous, false negative results. In most cases we would recommend that a reduction in the host testing list of plants for more stringent tests be only made on the basis of results from appropriately-designed and run no-choice feeding or oviposition assays.

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Potential Evolution of Host Range in Herbivorous Insects

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Abstract

Many clades of herbivorous insects are remarkably conservative in the plants that they attack, and in many groups, related insects tend to feed on related plants. However, rapid evolution of host range has been documented in several species. Managers who contemplate introducing a host-specific insect for biological control of a weed would like to predict whether or not the species to be introduced poses an appreciable risk that it might evolve rapidly in host range and adapt to non-target plants. Guidelines as to which plants might most readily be incorporated into the insect's diet may be provided, in some cases, by their phylogenetic relationship to the insect's normal host and by the diet of insects closely related to the proposed control agent. The likelihood of rapid evolution of a shift to a non-target plant may be judged to some extent by screening populations of the insect for genetic variation in behavioral responses to and performance on the plant, since genetic variation is the prerequisite for evolutionary change.

I describe a series of studies on species of *Ophraella* Wilcox (Coleoptera: Chrysomelidae) that were intended to assess the likelihood that constraints on genetic variation might make some imaginable host shifts less likely than others, and might, indeed, have influenced the history of evolution of host association in the genus. This history was inferred from mapping host associations onto a phylogeny based on DNA sequence data. Four species of *Ophraella*, host-specific on various Asteraceae, were assayed for evidence of genetic variation in consumption of and larval survival on several hosts of their congeners. Significant variance among full-sib or half-sib families was taken as evidence of probable genetic variation. In about half the beetle-plant combinations in which consumption was assayed, no evidence of genetic variation was found (in some such instances almost no feeding was recorded). Genetic variation for larval survival on non-host plants was discerned in a small minority of cases. Genetic variation was most often displayed in responses to plants that were relatively closely related to the insect's normal host (i.e., in the same tribe of Asteraceae), but genetically variable feeding on more distantly related plants within the Asteraceae was recorded in some cases.

The results indicate that: (1) all these species harbor genetic variation that might enable rapid adaptation to some plants other than their normal host; and (2) the plants most at risk of adaptation are especially but not exclusively those most closely

related to the insect's normal host. Although this study provides evidence that paucity of genetic variation in responses to novel plants could constrain or influence the direction of the evolution of insect diet, the methods used in this study are inadequate to reveal rare alleles that might enable rapid response to natural selection for expansion of diet. In order to judge the likelihood that a proposed weed control agent might adapt to a non-target plant, large samples of insects should be screened, ideally by selection experiments.

Keywords: adaptation, Chrysomelidae, genetic variation, host specificity, novel host plants, *Ophraella*

Introduction

Although management of pest species by integrated pest management or by biological control has clear advantages over chemical control alone, it is not risk-free. Precautions must be taken against introduction of organisms that may attack not only the target, but also crop plants or other economically important organisms. It is perhaps equally important to guard against introducing organisms that will attack elements of the native biota, and an argument has arisen about whether or not there exist adequate safeguards against this potential danger (e.g., Howarth, 1991; DeLoach, 1991; Simberloff and Stiling, 1996). For instance, the predatory snail *Euglandina rosea* (Ferrusac), widely introduced to control the African snail *Achatina fulica* Bowditch, has extinguished many endemic species of tree snails in the Hawaiian and Tahitian archipelagoes, and the weevil *Rhinocyllus conicus* (Frölich), released in the United States to control several Eurasian thistles, is severely reducing seed production of several native thistles as well (Louda et al., 1997). It is well understood that in order to avert such disasters, potential biological control agents must be tested for specificity. The ideal biocontrol agent will attack only the target pest species, and no others.

As an outsider to the field of biological control, I would not presume to prescribe testing procedures, and indeed am not familiar with standard procedures in any detail. I assume that in screening herbivorous arthropods for potential control of weeds, non-target plant species are presented to the arthropod in no-choice tests, since this would resemble the decision-making context for dispersing insects that encounter plants singly. *(Note: No-choice tests are not always used. In some instances, choice tests are used in which plant species are presented concurrently or in sequence; see Heard and van Klinken [1998] for a review of testing procedures used for screening weed biocontrol agents).* Likewise, intraspecific variation in plants may affect the outcome of feeding or oviposition trials. Taking these and other considerations into account, it may be possible to achieve considerable confidence that a non-target species of plant is not acceptable to the arthropod population - at the present time.

There exists, however, the possibility that the control agent, having been released, will adapt to the non-target plant and become, itself, a pest in the future. That is, evolutionary change in the introduced species may alter its specificity, or host range (Roderick, 1992; Simberloff and Stiling,1996).

Evolutionary changes that have transpired within the last century have been documented in hundreds of species of organisms (Bishop and Cooke, 1981; Travis and Futuyma, 1993; Thompson, 1998; Futuyma, 1998). Most such changes have occurred in response to human alterations of a species' environment, or in populations that have been transplanted into new environments. The most conspicuous and familiar examples are the evolution of antibiotic resistance in many bacteria and other disease-causing organisms and of insecticide resistance in more than 500 species of insects and other arthropods (Metcalf and Luckmann, 1994). Other examples of rapid evolutionary change include changes in migration patterns of birds, life history features of fishes, and the ability of insects and other crop pests to attack previously resistant crop varieties (Travis and Futuyma, 1993). Of particular relevance to biological management of weeds are the several well-documented cases in which insects have altered their host range within the last century by adapting to introduced plants (Thompson, 1998). For instance, populations of the checkerspot butterfly *Euphydryas editha* Boisduval have added the plantain *Plantago lanceolata* L. (Plantaginaceae) to their diet (of

several Scrophulariaceae) (Singer et al., 1993); the rhopalid bug *Jadera haematoloma* (Herrich-Schaeffer) has adapted morphologically and physiologically to several introduced Sapindaceae (Carroll et al., 1997); the clouded sulfur *Colias philodice* Godart has added alfalfa (*Medicago sativa* L.) to its diet of native legumes (Tabashnik 1983); and the apple maggot fly (*Rhagoletis pomonella* [Walsh]) adapted to and became a serious pest of apple, on which it has formed a distinct "host race" from the native hawthorn-feeding population, and is evidently becoming a distinct species (Feder et al., 1990; Filchak et al., 1999). It has been clear for some time to evolutionary biologists that populations of most organisms have the potential ability to evolve rapidly in many of their characteristics - so rapidly that their ecological interactions with other species, including humans, may change appreciably on the scale of decades (Thompson, 1998).

Evolution consists of change in the genetic composition of populations: changes in the proportions of different genotypes. In some cases, a prevailing genotype may be completely replaced by another; in other instances, proportions are less fully altered, and no one genotype is fixed (i.e., reaches a frequency of 100%). The change in proportions may result in some instances from genetic drift (i.e., random changes due to accidents of sampling, but adaptive changes result from natural selection, a nonrandom difference in reproductive success between genotypes, due often to their interaction with environmental factors). Because evolution consists of genetic change, it cannot occur unless there exists genetic variation, consisting of two or more alleles at a gene locus, or at some of the several or many loci, that affect a characteristic. Each such allele arises *de novo*, by mutation, at a low rate, so if a population is initially genetically homogeneous, the "waiting time" for genetic variation to arise and enable evolution to occur may be quite long. In most populations, however, mutations at many loci that have arisen in the past persist for a considerable time, so that many characters are genetically variable and can change to at least some degree almost immediately if changes in the environment alter the regime of natural selection, and favor a different character state. Indeed, over the last 50 years, population geneticists have so consistently found genetic variation in the features of diverse organisms that the majority of workers are inclined to think that evolution is seldom constrained by lack of genetic variation (Lewontin, 1974; Barker and Thomas, 1987). Most of the time, characters do not evolve noticeably simply because the environment selects for a stable, quasi-optimal trait, or because the selection regime fluctuates without favoring change consistently in any one direction (Endler, 1986). But a consistent change in the selection regime, as when a population is introduced into a new region that differs in climate and possible food sources, is likely to evoke rapid, often substantial, genetic responses.

Managers who contemplate releasing a species into a new region, such as an insect that promises to control a weed, should assume that the population will undergo some evolutionary changes. (Indeed, if it is so genetically homogeneous that the capacity for evolutionary adaptation is unlikely, the population probably has a dim future.) The question is whether these changes are likely to include expansion of diet to include native plants or crops. Since it is impossible to test the insect against all the plant species it will encounter in its new home – or in places it might disperse to from the site of introduction – it would be useful to judge which plants might be most at risk of becoming included in the insect's diet.

Phylogenetic Patterns

A conspicuous pattern in the diet of many, although not by any means all, groups of herbivorous insects is that related species tend to feed on related plants. That is, species in a higher taxon of insects, such as a genus or subfamily, common feed on taxonomically related plants, often in the same family. This pattern, long known to insect systematists, was the basis of an influential theory of coevolution by Ehrlich and Raven (1964), who proposed that chemical compounds shared by related plants (due to common descent) elicit feeding and egg-laying by specialized insects. Similar responses to chemical stimuli are shared by insect species derived from recent common ancestors. Subsequent research has provided some confirmation of this hypothesis. For instance, iridoid glycosides are feeding and oviposition stimulants for species of *Euphydryas* butterflies (Bowers 1991); these compounds characterize the butterflies' host plants in the Scrophulariaceae, as well as the Plantaginaceae, a lineage of Scrophulariaceae that has become adapted for wind-pollination. That *E. editha* has recently expanded its diet to include *Plantago* is thus readily understandable.

Many classes of insects are remarkably conservative in diet. For example, all species in the butterfly tribe Heliconiini feed on Passifloraceae as larvae; all

tetraopine cerambycid beetles feed on Asclepiadaceae or the closely related Apocynaceae; among the true fruit flies (Tephritidae), the huge subfamily Tephritinae is almost exclusively associated with Asteraceae. Based on the fossil record, biogeography, and levels of DNA sequence divergence, it is clear that many such monophyletic groups are 40 to 60 million years old, or even older (Mitter et al., 1991; Mitter and Farrell, 1991; Farrell and Mitter, 1993). The most parsimonious interpretation is that these lineages have retained much the same host association throughout their long history of diversification while continents have moved, climates have changed drastically, and whole orders of mammals have originated and become extinct. Evolution may be rapid in some respects, yet slow in others: for example, a molecular phylogeny of the aphid clade consisting of the genera *Uroleucon* and *Macrosiphoniella* implies that the 319 described species have evolved in only 5-10 million years, yet they have retained similar host associations, all feeding on Asteraceae or the closely related family Campanulaceae (Moran et al., 1998).

Such examples of very conservative feeding habits strongly suggest that there exist constraints on the ability of these insects to adapt to plants distantly related from their normal hosts. On the other hand, these constraints are not universal among insects. In some clades, related species are host-specific, but on distantly related plants; for example, species of the leaf beetle genus *Tricholochmaea* specialize on willow (Salicaceae), blueberry (Ericaceae), or meadowsweet (Rosaceae), which tend to grow in similar habitats but do not otherwise have obvious traits in common. In some taxa, one or a few species depart far from an otherwise conservative pattern; in the large leaf beetle genus *Trirhabda*, for example, all the species feed on Asteraceae except for two that have Hydrophyllaceae species as hosts. In other cases, many species have broad diets, and their more specialized relatives may occupy a great variety of plant taxa (e.g., the aphid genus *Aphis*).

Nevertheless, a pattern of strong phylogenetic conservatism of diet in a higher taxon that includes a potential biocontrol agent does suggest that the non-target plants at greatest risk of unintended attack are those closely related to the insect's known normal hosts. Even if the insect does not show an immediate ability to feed, survive, and reproduce on a related plant, we may hypothesize that plants closely related to its normal hosts would be more likely to elicit adaptation than distantly related plants. More generally, we might be concerned about adaptation not only to plants that are closely related to the insect's normal host, but also plants that are hosts to the near relatives of the insect. For example, if a species of *Trirhabda* were a candidate for release, one might be concerned about the possibility of its evolving the ability to feed not only on various non-target Asteraceae, but also Hydrophyllaceae.

Plant taxonomy is already used as a guide to screening biocontrol agents for specificity. For instance, the reliably reported host plants of the American leaf beetle *Ophraella communa* LeSage include *Ambrosia* (ragweeds) and several other genera of Ambrosiinae, a subtribe of the tribe Heliantheae, family Asteraceae. Palmer and Goeden (1991) tested this insect's responses to several species of Heliantheae and found that it reproduced and survived successfully on cultivated sunflower, *Helianthus annuus* L. Hence the species was considered unsuitable for introduction into Australia as a control agent for *Ambrosia artemiifolia* L. and two other adventive ambrosiine weeds. It must be noted that the use of plant taxonomy in this way depends on accurate assessment of evolutionary, i.e., phylogenetic, relationships among plant species. If *Ambrosia* had been wrongly classified with sagebrush (*Artemisia*) in the tribe Anthemideae, on the basis of their convergently similar wind-pollinated flowers, Palmer and Goeden might well not have been led to test this beetle's response to sunflower. By the same token, the feeding habits of insect species closely related to a proposed biocontrol agent might legitimately alert us to possibly susceptible non-target plant species only insofar as the phylogenetic relationships among insects are accurately known.

Chemical and Phenetic Similarity of Plants

Dethier (1954), and later Ehrlich and Raven (1964), suggested that insects adapt most readily to plants that share key features with their ancestral hosts, and that this accounts for the association of related insect species with related plant species. The key features, they suggested, are often the "secondary compounds" that characterize higher taxa of plants, such as the glucosinolates of Brassicaceae (mustards) and the cardiac glycosides of Asclepiadaceae (milkweeds). Thus one might propose to ignore phylogenetic relationships among plants, and test the responses of candidate insect species against native or cultivated plants that are chemically similar to the insect's normal hosts.

Although reasonable in principle, this may not always be a practical approach. First, the plant characters that may have acted as "bridges" for the evolution of new host associations seem not to be chemical in all cases, as illustrated by insect taxa whose various host plants have in common their habitat rather than their chemistry (Mitter and Farrell, 1991). Second, plants generally have not one secondary compound but many, often representing several very different chemical families. These compounds affect both insect behavior and post-ingestion physiology by acting as toxins or interfering with digestion. Behavioral and physiological responses of insects to such compounds are often very complex (Rosenthal and Berenbaum, 1992; Bernays and Chapman, 1994). Although in some insects a single compound may elicit feeding or oviposition, and so account for host specificity, it is far more common for these behaviors to be based on a multifactorial response to several or many compounds. Some compounds act as stimulants, others as deterrents, often with synergistic effects. Determination of these effects may require assaying responses to compounds not only singly, but also in combinations. The Colorado potato beetle (*Leptinotarsa decemlineata* [Say]), for instance, is attracted to a complex blend of volatile compounds produced by its *Solanum* hosts, not to any single compound. Its feeding behavior is stimulated not by any identifiable *Solanum*-specific compounds (much less by the steroidal glycoalkaloids that are most characteristic of these plants), but instead by a wide array of compounds that are not host-specific (Mitchell, 1988; Hare, 1990). Thus individual compounds shared by plants (such as the steroidal glycoalkaloids of *Solanum*) may not play any role in the adaptation of insects to new hosts, and the similarity of plant species with respect to their overall chemical profile likewise need not predict host associations, especially in those cases in which a few critical compounds do indeed play a leading role.

Genetic Variation

Another approach may be to screen populations of an insect species (being considered as a biological control agent) for genetic variation in its capacity to oviposit, feed, and develop on non-target plant species. Note that we are not concerned with the simple question of whether or not a small sample of insects will readily attack a plant, which might indicate that the species might immediately include the plant in its diet if released. Rather, we are asking if a plant that is rejected by the majority of the population, or which is unsuitable for development of the majority, may nevertheless become a suitable host plant due to rapid evolution of the introduced insect population. Showing that the population harbors genetic variation in features required to develop on the plant may indicate that the population could readily adapt to the non-target species. The prudent course of action would be to assume that such evolution could occur. However, it is impossible ever to say that an insect species absolutely lacks now and must forever lack the genetic variation that is the prerequisite for such adaptation to occur. I return to this point below.

An Example: *Ophraella* Leaf Beetles and Their Hosts

The approach of screening for genetic variation in an insect species' responses to potential future host plants is illustrated by work in my laboratory on genetic variation in the leaf beetle genus *Ophraella* (Futuyma et al., 1993, 1994, 1995). This research was undertaken in order to determine if the course of historical evolution of host shifts in this genus may have been influenced by genetic constraints on variation. We tested the hypothesis that populations harbor genetic variation in responses to only certain plants, so that adaptation to new hosts has been restricted to a limited number of possibilities. Thus according to this hypothesis, the plant species actually adopted as hosts during the course of evolution of *Ophraella* were more likely to have been adopted, due to genetic constraints, than many other plants that were available. It should surprise no one if species in a genus that feeds on Asteraceae (sunflower family) were to display absolutely no ability at all to feed on, say, ferns, club mosses, or pines. Thus, in order to restrict our analysis to plants that could be regarded as plausible potential hosts, we screened species of *Ophraella* for genetic variation in responses to plants that are hosts of other species of *Ophraella*, species that are either very close or relatively distant relatives of the particular species tested. Thus, if limits on genetic variation had closely guided the evolution of host shifts, we might expect a species to display genetic variation in response to the host of its nearest relative, but with lesser likelihood to the host of a phylogenetically more remote species of *Ophraella*. It might be noted that many or most population geneticists would expect genetic variation to be revealed in a species' responses to any of the plants on which they were tested, since genetic variation has been found in most characters of organisms, when sought.

Ophraella (Coleoptera: Chrysomelidae, Galerucinae) is a North American genus with at least 13 species (LeSage, 1986; Futuyma, 1990, 1991). Both larvae and adults are externally feeding folivores. Oviposition and usually pupation occur on the host plant. Adults overwinter; some species are univoltine, but most appear to be multivoltine, with egg-to-egg generation time (in the laboratory) of a month or slightly more (Futuyma, 1990). The hosts fall into four tribes of the Asteraceae (Compositae). Some species of *Ophraella* have been recorded from only a single host species, but most are known from several congeneric hosts, and *Ophraella communa* LeSage, which is geographically variable in host association, has been found breeding on several genera in two subtribes of the tribe Heliantheae.

Because this work addressed the relation of patterns of genetic variation to the actual history of evolution of host associations of these insects (an issue that would not necessarily arise in screening biocontrol agents), part of the research program consisted of inferring phylogenetic relationships among the species of *Ophraella*, to provide a framework for inferring a most likely history of host associations. First, using morphological and allozyme data, and later DNA sequences (866 base pairs of the mitochondrial cytochrome oxidase I and 16s ribosomal RNA genes), we obtained an estimate of phylogeny, in which most clades are well supported statistically (Funk et al., 1995). The beetle phylogeny does not closely match that of the host plants, and the levels of DNA sequence divergence among species of *Ophraella* strongly suggest that they have originated much more recently than the divergence of the several tribes of Asteraceae that include their host plants. Thus most of the diversity of host plant use has arisen as populations or species have shifted from one host plant to another, rather than by cospeciation and contemporaneous divergence of insect-plant associates. These host shifts, however, have been rather conservative: the phylogeny implies that about eight of the approximately twelve host shifts in the history of the group have been between genera in the same tribe of plants, and only four shifts between tribes have occurred.

We screened four species of *Ophraella* for genetic variation in responses to their normal hosts, as well as to five or six plant species that are hosts of species of *Ophraella* other than the focal species. For example, *O. communa* was tested for responses not only to one of its normal hosts, the common ragweed *Ambrosia artemisiifolia*, but also to *Solidago bicolor* L., *Solidago altissima* L., *Chrysopsis villosa* (Pursh)Nutt. Ex DC., *Eupatorium perfoliatum* L., *Artemisia vulgaris* L. and *Iva frutescens* L., which are the respective hosts of *Ophraella pilosa* LeSage, *O. conferta* (LeConte), *O. bilineata* (Kirby), *O. notata* (Fabricius), *O. artemisiae* Futuyma, and *O. notulata* (Fabricius). (*Artemisia vulgaris* was actually a surrogate for the species of *Artemisia* that *O. artemisiae* normally feeds on.) These species of *Ophraella* span the range from the closest to the most distant relative of *O. communa*, and their host plants are included both in the same tribe as *O. communa's* normal hosts and in the other tribes of Asteraceae. We assayed behavioral (feeding) responses to these plants, and in most cases we assayed larval survival as well. Feeding responses were tested for neonate larvae in 22 *Ophraella*-plant combinations (other than on the species' normal host plant), and for newly eclosed adults in 18 such combinations, by placing insects individually in small petri dishes with discs of leaf tissue of a single plant species (no-choice tests) on moist filter paper. Consumption was measured 24 h later (and in a few cases repeatedly thereafter) by counting squares in an ocular grid in a dissecting microscope. The adults used in consumption tests had been reared on their normal host plant. In tests of larval survival, we maintained individual larvae in dishes with leaf fragments of the test plant, which were replaced every 2 days until death or pupation. Further details are described in the original papers (Futuyma et al., 1993, 1994, 1995).

We assumed that the methods of quantitative genetics (Falconer and Mackay, 1996) are appropriate for assaying genetic variation in phenotypic characters of this kind. Our main interest was in determining whether or not genetic variance could be detected, on the principle that heritable characters display greater variation among than within families (i.e., significant among-family variance). We succeeded in most cases in using a half-sib design, in which each male was mated to two or more virgin females, and a trait (such as consumption of Ambrosia) was scored on several offspring of each female. Significant variance among sires (i.e., among families of half-sibs) is generally taken to indicate additive genetic variance, the kind of genetic variation that enables ready response to natural selection. Significant variance among dams (females) within sires may be attributed not only to additive genetic variance, but also to nonadditive genetic variance (owing to dominance and epistasis), to maternal effects (including both nongenetic effects and

common environment that full sibs often share (e.g., neonate feeding on a leaf on which the eggs were laid, before dispersal or removal). My concern in these experiments was to search for evidence of possible constraints on the genetic variation needed for host shifts to evolve, so any data interpretable as evidence of genetic variation would count against my hypothesis that genetic constraints exist, and in favor of the widespread belief that paucity of genetic variation does not generally affect the direction of evolution. Therefore, instances of significant variance among dams were counted as evidence for genetic variation (even though maternal effects or common environment cannot be entirely ruled out) because doing so provided a bias against my hypothesis. (If I were screening a proposed biological control agent, I might take the same position, on the grounds that it would be prudent to assume that the insect population harbors genetic variation enabling it to adapt to a non-target plant, unless rigorous screening strongly suggested otherwise.)

In most of the assays, we tested progeny of 20 to 40 sires, each mated to 2 virgin dams (cf. Table 1). The parents were taken from one locality or a few sites near each other. All populations displayed genetic variation in feeding responses to at least one plant species, so none of them was lacking in genetic variation due to inbreeding. (Electrophoretic studies and DNA sequence variation have shown that local populations of all *Ophraella* species are typically as highly heterozygous as insect species generally [Futuyma and McCafferty, 1990; Knowles et al., 1999].)

Table 1 presents a sample of the data on variation in larval feeding responses. The original papers (Futuyma et al., 1993, 1994, 1995) may be consulted for the full data, a summary of which is provided by Futuyma et al. (1995). From the point of view of possible genetic constraints, perhaps the most important result of these studies was that in 14 of the 16 tests of larval survival, and in 18 of 39 tests of larval or adult feeding on plants other than the insect's normal host, no genetic variation was demonstrable. In 10 combinations of beetle and plants, no larvae survived to pupation, and in only 2 of the other 6 cases was there evidence of genetic variation, manifested as significant variance among full-sib families (from different mothers), among half-sib families (progenies of different males), or both. These 2 cases involved 2 species that feed on different members of the subtribe Ambrosiinae (*Ambrosia artemisiifolia* and *Iva frutescens*): each displayed genetic variation in survival on the other's host.

Certainly a major cause of larval mortality was failure to feed, although in some instances death occurred after several days of feeding, suggesting that toxic or other post-ingestion effects also played a role. For larval consumption, we found evidence of genetic variation (Table 1) in 15 of the 22 combinations; of the 7 combinations in which no genetic variation was discerned, virtually no feeding occurred in 6: at most, trace feeding was exhibited by a small minority. Of 17 tests of adult consumption, evidence of genetic variation was found in 6; among the other 11 combinations, at least modest feeding occurred in 7, and virtually no feeding in 4. Thus failure to discern evidence of genetic variation in a feeding response was sometimes but not always a result of non-acceptance of a novel plant by the experimental animals.

As already noted, the two cases in which we discerned genetic variation in survival entailed growth on a plant closely related to the insect's normal host. A similar, though less dramatic pattern, was found for feeding response. In 7 of the 21 cases in which genetic variation in feeding response was detected, the test plant was in the same tribe as the insect's normal host, whereas in only 1 case out of 18 was a plant that elicited no genetically variable feeding a member of the same tribe as the normal host. The association is significant by likelihood-ratio test ($P = 0.0373$). Phylogenetic relations among the beetle species, on the other hand, provided no additional prediction of which plants would elicit genetically variable feeding behavior. Hosts of *Ophraella* species in the same major clade as the test species were significantly more likely to elicit genetically variable feeding than hosts of more distantly related species of *Ophraella*, but species in the same major clade of *Ophraella* generally feed on plants in the same tribe.

Interpretations and Implications

These beetles are more likely to display genetically variable feeding responses to plants that are closely related to their normal hosts than to more distantly related plants. However, out of the 31 different combinations in which larval or adult feeding was scored on plants in a different tribe of Asteraceae than the beetle's normal host, 14 showed evidence of genetically variable feeding. Thus, one cannot assume a priori that the feeding response to a relatively distantly related plant could not evolve. Bear in mind, however, that the test plants in this study are all in the same family, and moreover are a highly biased sample: they are all hosts of one or another species of *Ophraella*,

Table 1. Examples of analyses of variance of consumption of test plants by neonate larvae of *Ophraella artemisiae* Futuyma and *O. notulata* (Fabricius)

Plant Species	Source[1]	df	MS	F
*Ophraella artimesae (*natural hosts*: Artemisia ludovicana* Nutt.*, A. carruthii* Wood ex *Carruth.,* [Anthemideae])				
Artemisia vulgaris L.	S	37	0.1869	1.27
(Anthemideae)	D(S)	22	0.1859	1.31
	E	175	0.1418	
Ambrosia artemisiifolia L.	S	37	0.3740	0.66
(Ambrosiinae)	D(S)	22	0.5628	2.42***
	E	177	0.2330	
Eupatorium perfoliatum L.	S	35	0.0881	1.01
(Eupatorieae)	D(S)	21	0.0877	1.55
	E	156	0.0566	
Chrysopsis villosa (Pursh) Nutt. Ex DC.	S	32	0.0637	0.44
(Astereae)	D(S)	19	0.1436	1.97*
	E	149	0.0727	
Ophraella notulata (natural hosts: *Iva frutescens* L., *I. annua* L. [Ambrosiinae])				
Iva frutescens L.	S	28	0.2526	0.88
(Ambrosiinae)	D(S)	29	0.2885	1.43
	E	406	0.2016	
Artemisia vulgaris	S	28	0.2595	0.84
(Anthemideae)	D(S)	28	0.3089	2.61***
	E	395	0.1186	
Eupatorium perfoliatum	S	28	0.4027	2.81**
(Eupatorieae)	D(S)	26	0.1432	1.54*
	E	381	0.0931	
Chrysopsis villosa	S	28	0.2526	0.88
(Astereae)	D(E)	27	0.5810	2.53***
	E	388	0.2293	

[1]The terms in the ANOVAs are sire (S), dam within sire (D[S]), and error (E). Significant S or D(S) terms were taken as evidence of genetic variance in the character scored. (Data from Futuyma et al. 1994, 1995).

*$p < 0.05$

** $p< 0.01$

***$p < 0.0001$

chosen specifically because such plants might be expected to elicit genetically variable reactions, given that at least one species of *Ophraella* has adapted to each of them. This choice was made in order to see if evidence of constraints on genetic variation might come to light even in characters that an orthodox population geneticist might least expect to be genetically invariant. It is certainly likely that many other plants would elicit no feeding response at all, and thus no genetic variation, as was the case in some instances studied. Likewise, even given some feeding, complete larval mortality was observed on some test plants, and this surely would be even more conspicuously true if a wider variety of plants were presented.

For evaluating candidate species for biological control, an important implication of the studies I have described is that even a small sample of an insect population may display genetic variation in some of the features that would enable it to colonize other species of plants than its normal host. For example, *Ophraella notulata* feeds only on two species of *Iva* (tribe Heliantheae, subtribe Ambrosiinae), as far as is known. It displayed genetically variable larval survival (as well as a capacity to oviposit) on *Ambrosia*, as well as genetically variable feeding responses not only to *Ambrosia*, but also to *Solidago bicolor*, *Chrysopsis villosa* (both in tribe Astereae), *Artemisia vulgaris* (tribe Anthemideae), and *Eupatorium perfoliatum* (tribe Eupatorieae). The second implication is more encouraging: survival requires more than feeding, and this measure of performance showed far less evidence of genetic variation. Moreover, feeding response of animals confined in a small space is only one of the several or many traits that may have to change in order for an insect population to adapt to a novel plant. Even the low levels of consumption often observed in these experiments might not occur in the field, where animals may disperse in search of more acceptable hosts, and establishment of viable populations may require changes not only in feeding, but also in postingestive physiological characters and oviposition behavior, to say nothing of factors such as phenology and avoidance of host-associated predators or parasitoids.

It is impossible, however, to prove a negative statement, such as a claim that a species has no genetic variation in a character (e.g., feeding response to a particular plant). Moreover, variation among geographic populations was not included in this study, and the sample sizes were relatively small, because the broad comparative nature of the study required assaying variation in numerous combinations of species, and thus was labor-intensive. Thus genetic variation within the sample would not be discerned if the heritability of a character were very low, and rare alleles would have a high probability of not having been included in the sample. Screening for genetic variation by assaying variance among families may be particularly insensitive if the variation that enables a character to evolve is due to rare mutations at one or a few loci. The limited evidence to date suggests that behavioral responses of herbivorous insects and their performance on different hosts are generally multifactorial, although the effective number of loci may not be great (Jaenike, 1986; Hagen,1990; Thompson et al., 1990; Sheck and Gould, 1996; Jones, 1998). Nevertheless, a polygenic character may respond to selection even if rare alleles at contributing loci are not readily detectable in small samples.

For these reasons, if an arthropod proposed for introduction as a weed-control agent is to be evaluated for its likelihood of adapting to a non-target plant species, the screen for genetic variation should entail much larger samples than those I have employed in my work, and should probably include assays of several geographic populations. If any evidence of growth, survival, or oviposition is found, it may be advisable to investigate further the role that variation in the plant (with respect to age, phenology, growth conditions, or provenance) may play in revealing a latent potential for the insect to adapt to particularly susceptible variants. Perhaps the best method of assay would be to impose mass selection on large experimental populations of the arthropod for adaptation to the plant, in order to determine if survival and other components of fitness increase over the course of 5-10 generations (e.g., Gould, 1979; Wasserman and Futuyma, 1981; Fry, 1990). This has the advantages that all characters that contribute to fitness are exposed to selection and that a response to selection may be obtained even if alleles contributing to the response are too rare to detect easily. Selection experiments may have various practical disadvantages, however, that depend on the species, such as the time required for a multigeneration experiment with univoltine insects, or the sometimes arduous logistics of rearing plant material in large quantity. Arduous as such experiments may be, the more difficult task is in extending such assays to all the species of plants that might plausibly be at risk of some day falling within the insect's range of diet.

The Role of Evolutionary Biology in Pest Management

Evolution is often cited as the single most important unifying principle of biology, but a broad recognition of its pervasive implications and applications is only slowly developing. Evolutionary biologists themselves are only now becoming fully aware of the range of applications of their science, as described in a recent report on "Evolution, Science, and Society" (Futuyma et al., 1999). These applications are particularly conspicuous in agriculture and pest management. The evolution of resistance to chemical pesticides in both arthropods and plants is only one reason for turning to alternative management methods or to integrated pest management. Breeding and genetically engineering plants for disease resistance and other useful traits relies in part on principles of evolutionary genetics. The possibility that beneficent agents of weed control might evolve into noxious plagues of crops or natural ecosystems provides yet another context in which the methods, principles, and data of evolutionary genetics are important. And, as noted earlier, any judgment of which plants might be most at risk depends on an accurate taxonomy and phylogeny of plants and often of the biological agents proposed to control them - and to infer phylogeny is to infer evolutionary history. It is imperative that at least the elementary principles of evolutionary biology be part of the training and awareness of pest managers and other applied ecologists.

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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

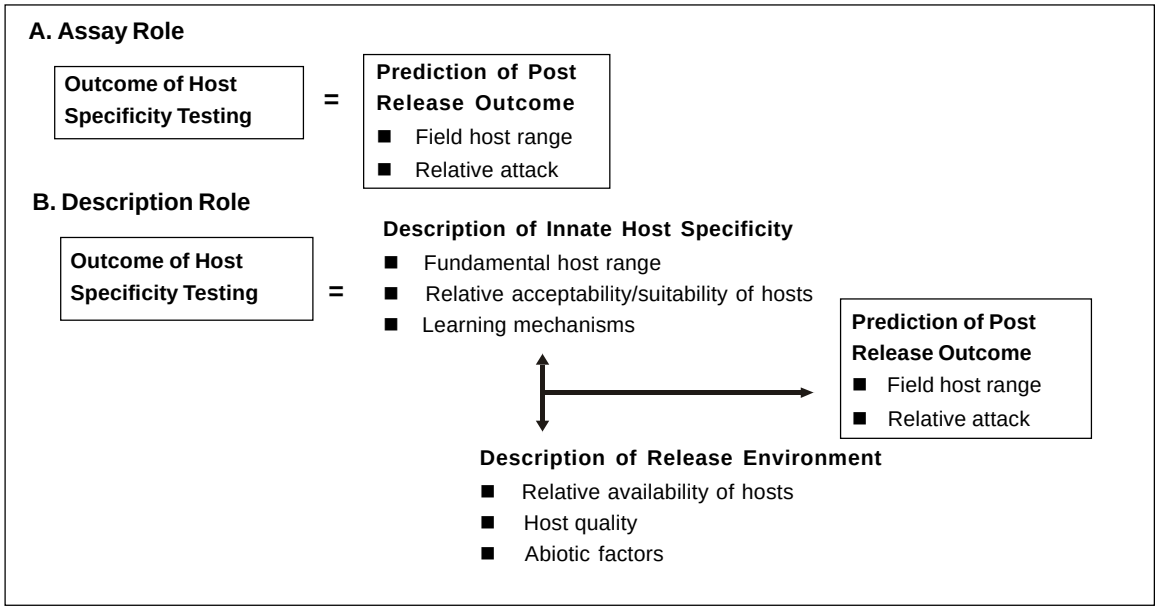

Fig. 1. Diagrammatic representation of the two roles host specificity testing could play in the prediction of relative non-target attack. The first seeks to experimentally simulate field conditions. The second seeks to describe the insect's innate host specificity, and use this description in combination with knowledge of the post-release environment to predict relative non-target attack.

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

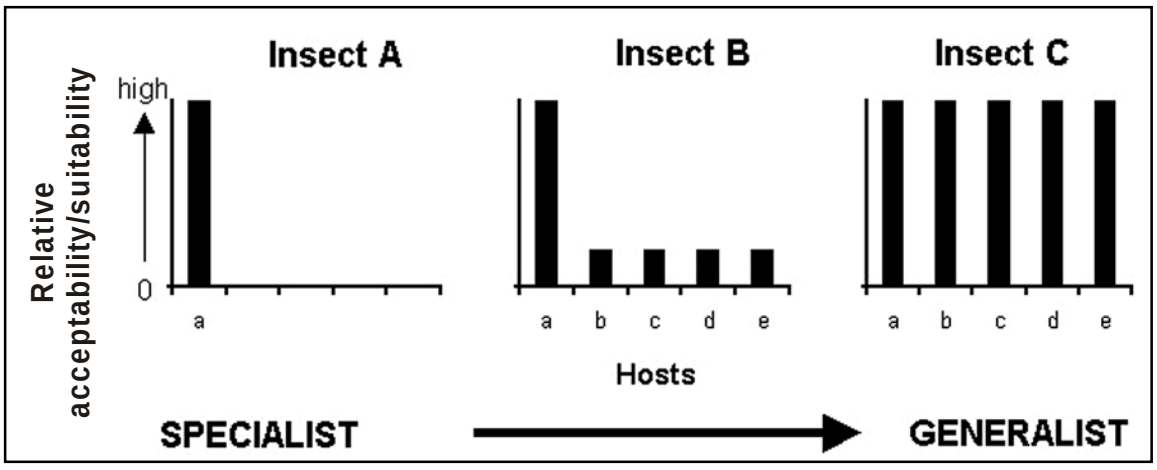

Fig. 2.
A hypothetical gradation of host specificity from specialist to generalist. Host specificity is described with two axes, host range and relative acceptability/suitability of those hosts. Insects B and C have identical host ranges (n=5) but differ in the relative acceptability/suitability of their hosts.

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

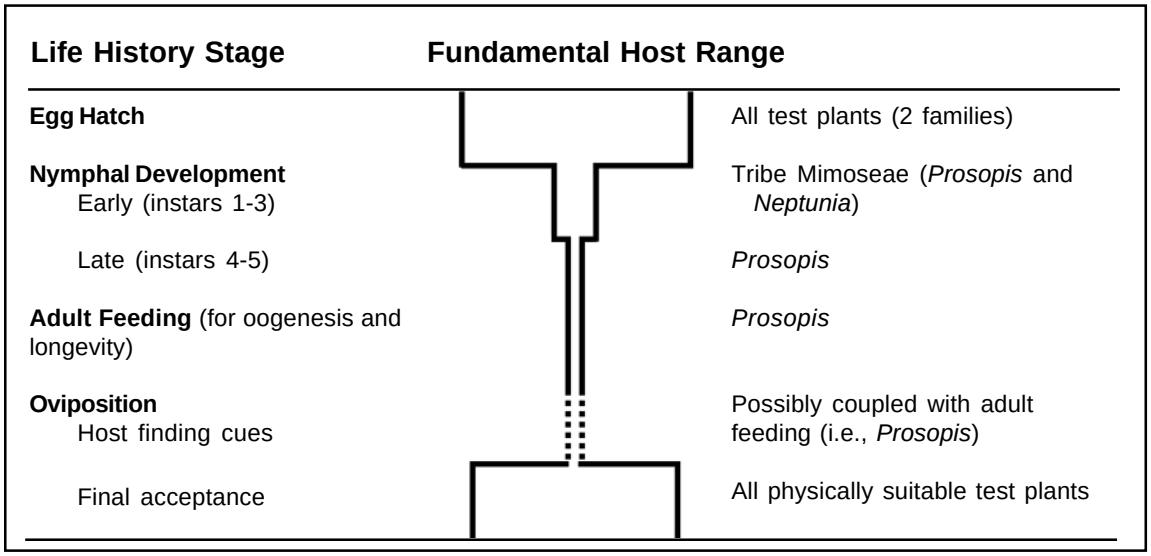

Fig. 3. Fundamental host range estimated for different aspects of the life history of the psyllid, *Prosopidopsylla* flava Burckhardt (van Klinken, *in press*). *P. flava* inserts eggs into plant tissue and oogenesis occurs as adults. Host range breadth is represented schematically.

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 Rodriguez, 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; Olckers, 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 Rodriquez, 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 hepialid 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 behaviours 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[a]

Single test conducted on complete plant list
• Examine limited part of life history (e.g., first feeding instar)
• Choice minus target trials
Compromised test on complete plant list, test assumptions on a subset
• Use experienced individuals
• Limit the duration of tests

**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 naive 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

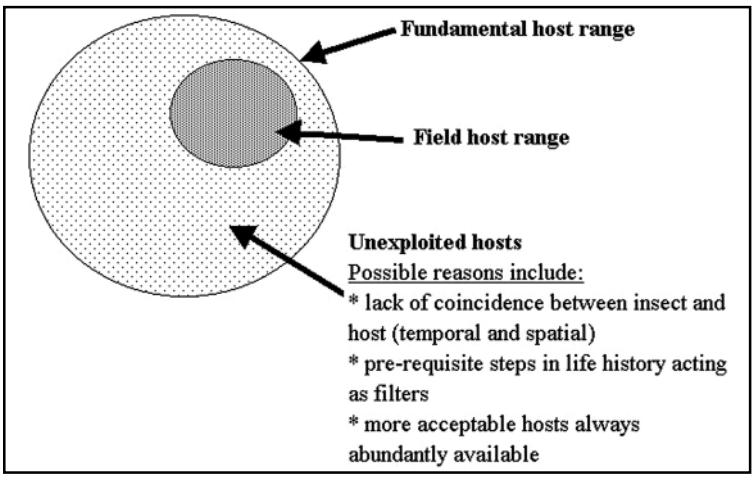

Fig. 4. Possible reasons for the fundamental host range (e.g., for complete larval development or adult feeding) not being fully realized in the field.

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; Wan 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;

Eigenbrode 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, 1991).

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., Blossey 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 Neser, 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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Evaluating the Host Range of Agents for Biological Control of Arthropods: Rationale, Methodology and Interpretation

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Abstract

Before releasing exotic natural enemies for biological control of weeds, host range tests are almost universally required by authorities, to ensure that agents are unlikely to have detrimental impacts on non-target plants. However, for biological control of arthropod pests, tests to determine the potential host range of exotic agents have not been so widely practiced, leading to concerns that agents once established may have undesired impacts on beneficial organisms and native fauna. The rationale for host range tests is similar for weeds and arthropod projects and the centrifugal method for selecting non-target taxa related to a target is applicable to both, but the taxonomic relationships for arthropods are often not as well known as for plants. The number and range of non-target arthropods to be tested with an exotic agent must be selected carefully, since it is impractical to maintain in culture an extensive range of taxa. Non-target beneficial or threatened arthropod taxa may be priorities for testing as potential hosts but their life histories are sometimes unknown or appropriate stages may be difficult to obtain or culture. Tritrophic agent/ host/plant interactions are not uncommon and difficult to evaluate, and predators need special evaluation when compared with parasitoids. Although adults of predacious arthropods are sometimes generalists, their immature stages may be sufficiently specific to be acceptable. Current methods for evaluating the host range of agents for biological control of arthropod pests are discussed, taking into account issues of insect taxonomy and behavior that influence testing procedures, as well as some environmental and faunistic considerations that need to be considered in making decisions relating to safety or risk assessments of potential agents.

Introduction

The selection of non-target plants for testing the host range of agents for biological control of weeds has been implemented for many years (Wapshere, 1974; Harley, 1979). Before weed agents are translocated from another country and released, they are tested to demonstrate that they will not damage: (a) plants of economic importance or ornamentals, or (b) have a significant impact on native flora, particularly rare or threatened species. For parasitoids and predators used for biological control of arthropods, the host range has been considered differently and tests were usually conducted before the release of such species only if they were thought likely to attack beneficial organisms (Ertle, 1993). However, the justification for host range tests on arthropod agents has gained further attention (Van Driesche and Hoddle, 1997), following claims that some undesirable impacts have occurred to non-target organisms (Howarth, 1991). As a result of these concerns, pre-release studies on introduced arthropod agents have been adopted in New Zealand (Barratt et al.,1999) and Australia (Keller, 1999).

For weed projects, guidelines for testing non-target plants have been available and summarized (e.g. Waterhouse, 1991; Harley and Forno, 1992), but due to lack of detailed studies, specific procedures for arthropod agents have not been well developed (Sands and Papacek, 1993). The criteria needed for assessing the host range of arthropod agents may also differ from those applied to weed agents (Sands, 1997), and some of the procedures for predicting host ranges of arthropod agents have recently been discussed (Barratt et al.,1999).

The rationale, methodology, and interpretation of tests for determining the host range of arthropod agents are discussed.

Rationale for Testing the Host Specificity of Arthropod Agents

Biological control agents with narrow host ranges are generally considered to have few, if any, detrimental effects on beneficial or indigenous organisms, even when non-target species are used as hosts (Waterhouse, 1991). However, this may be attributed to the lack of documentation, especially when monitoring for effects in non-target organisms would require sampling in habitats different from those occupied by the target pest (Simberloff and Stiling, 1996). For weeds projects, there is evidence that some narrowly-specific agents have attacked rare or endangered native plants (Louda et al., 1997). Although the impact of these herbivores on the native plant populations has not yet been shown to be definitely detrimental (Herr, 1999), their attacks have been cause for concern (Louda et al., 1998).

In the case of arthropod agents with broad host ranges, their development on non-target taxa has sometimes been considered to be beneficial; such agents are seen as "lying in wait" ready for an opportunity to parasitise or prey on a pest when outbreaks occur (Murdoch et al.,1985). A further extension of this strategy has been to establish agents in readiness for exotic pest incursions. For example, attempts were made in Australia to establish the polyphagous parasitoid *Aphelinus varipes* (Foerster) on the aphid *Rhopalosiphum padi* (L.) in preparation for the possible arrival of the Russian wheat aphid, *Diuraphis noxia* (L.) (Hughes et al., 1994).

High densities of natural enemies maintained by exotic prey species are claimed to have the potential to drive rare non-target species to extinction (Simberloff and Stiling, 1996). Other aspects of the safety of arthropod agents are being debated, and methods to predict their host ranges have recently been reviewed (Barratt et al.,1999). In particular, tests are recommended when the biology of a candidate indicates that it has a wide host range, poses risks to economically-important, endangered, or ecologically significant non-target species (Van Driesche and Hoddle, 1997).

Potential Impacts on Other Biological Control Agents

There is a risk that parasitoids introduced to control a pest might also attack biological control agents of weeds and reduce their efficacy (Table1). This is particularly possible if a target pest is closely related to a beneficial agent. For example, the parasitoids *Tamarixia leucaenae* Boucek and *Psyllaephagus yaseeni* Noyes, used for biological control of the psyllid pest *Heteropsylla cubana* Crawford, were deliberately not introduced into Australia to avoid risk to the effectiveness of another psyllid (*Heteropsylla spinulosa* Muddiman, Hodkinson and Hollis), an agent established for control of the weed *Mimosa invisa* Martius ex Colla (Waterhouse and Norris, 1987). Similarly, pyralid shoot-borers in the genus *Hypsipyla* are serious pests of trees in the family Meliaceae, and biological control agents have been introduced from India into Central America in attempts to control these borers (Rao and Bennett, 1969). In Australia, *Hypsipyla robusta* (Moore) has been considered as a potential target for biological control, since a much wider range of natural enemies are known from elsewhere than currently exist in Australia. However, the phylogeny of the subfamily Phycitinae, to which *Hypsipyla* spp. belongs has not been satisfactorily resolved (M. Horak, *personal communication*). Shaffer et al. (1996) note that *Hypsipyla* Ragonot is related to *Cactoblastis* Ragonot, and belongs to the same tribe, Phycitini. Since *Cactoblastis cactorum* (Bergroth) is an important agent for control of prickly pear (*Opuntia* spp.) in several countries including Australia, it might be an alternative host for

Table 1. Rationale for host range tests

Avoid detrimental effects to:
A. Exotic biological control agents and other beneficial organisms (e.g., parasitoids, predators, pollinators).
B. Native species, especially those which are threatened, rare, and of conservation concern.
C. Organisms of commercial, cultural, or aesthetic significance.

parasitoids introduced for control of *Hypsipyla* spp., unless such parasitoids were narrowly specific to only this genus. Any reduction in efficacy of the beneficial non-target species *C. cactorum* would be unacceptable. This example illustrates the importance of understanding taxonomic relationships in biological control projects.

Native natural enemies play an important role in the control of many pests. Room (1979) for example, listed many natural enemies of *Helicoverpa armigera* (Hübner) in cotton crops in Australia. Predators included the pentatomid bugs *Cermatulus nasalis* (Westwood) and *Oechalia schellembergii* (Guerin-Meneville), both also known to be important in a wide range of other crops. When introducing agents for control of pest pentatomids, effects on these important predators are considered, to avoid a possible decrease in their effectiveness. For example, tests were conducted that showed that neither of these predators would be attacked by the tachinid *Trichopoda giacomellii* (Blanchard) before it was approved for release as a biological control agent for *Nezara viridula* (L.) (Sands and Coombs, 1999).

Potential Impacts on Non-target Indigenous Species

Few detrimental effects have been recorded from deliberately introduced arthropod natural enemies with a broad host range. An egg parasitoid, *Trissolcus basalis* (Wollaston), is said to have had an impact on native Pentatomidae in Hawaii as well as on its exotic target pest, the green vegetable bug, *Nezara viridula* (L.) (Howarth 1991). In Hawaii and other closed geographical populations, non-target organisms are believed to be more susceptible to effects of exotic generalists than those on larger land masses (Howarth and Ramsay, 1991). In Australia, impacts by *T. basalis* leading to decline in abundance of non-target taxa have not been reported, even though the eggs of many native species are parasitized by the parasitoid, including important predatory species (Waterhouse and Norris, 1987).

Although non-specific agents may sometimes develop on native non-target taxa, it is very difficult to predict the levels of attack or possible detrimental impacts before their introduction. For example, the tachinid *Bessa remota* (Aldrich), a generalist parasitoid of moth larvae, was reared from the zygaenid *Amuria catoxantha* (Hampson) and introduced from Malaysia to Fiji to control the zygaenid coconut pest *Levuana iridescens* Bethune-Baker (Tothill et al.,1930), but it also had impacts on non-target species, including the related moth *Heteropan dolens* Druce. These two moths, *L. iridescens* and *H. dolens,* are said to have become extinct in Fiji (Robinson, 1975), but there is some debate as to whether both have disappeared in Fiji, or merely continue to occur at very low densities (Paine, 1994; Sands, 1997). In Hawaii, of the 679 agents deliberately introduced for control of pests between 1890 and 1985, 243 agents became established and 20 have been recorded attacking non-target species (Funasaki et al.,1988). However, these authors considered that only the generalist tachinid *Lespesia archippivora* (Riley), introduced for control of armyworms, may have contributed to the extinction of a non-target species, the noctuid *Agrotis crinigera* (Butler).

In Guam, generalist parasitoids introduced to control lepidopterous pests parasitize the eggs and pupae of indigenous butterflies, including the nymphalids *Hypolymnas anomola* (Wallace) and *Hypolymnas bolina* (L.), but neither species is threatened as a consequence of attacks by these introduced parasitoids (Nafus, 1993). Up to 40 percent of eggs and 25 percent of pupae of *H. bolina* were attacked by the exotic parasitoids, but only the pupal parasitoid was considered to have had an adverse effect on the butterfly by reducing its abundance.

Even when levels of parasitism of a non-target host are higher than parasitism rates on the target species, impacts on the non-target species' population may not occur. For example, the native New Zealand weevil *Irenimus aemulator* (Broun) is parasitized by the exotic parasitoid *Microctonus aethiopoides* Loan at a level equal to or greater than that of the target, *Sitona discoideus* Gyllenhal (Barratt et al.,1996; 1997), but detrimental effects on populations of *I. aemulator* have not been demonstrated.

Potential Impacts on Organisms of Conservation, Commercial and Aesthetic Significance

Non-target taxa known to be rare or threatened may require special consideration, especially if they are taxonomically related to the target (Van Driesche and Hoddle, 1997). However, the biologies of such species are often poorly known or unknown and the logistics of testing them with a potential agent may prove to be impractical. If target species are abundant, their

populations may support large populations of an agent, increasing the chances of "spill over" onto non-target species. This effect may locally depress populations of such rare species or even drive them to extinction (Howarth, 1991), especially at the edge of their range (Cullen, 1997). The reduction of distribution of *Pieris napi oleraceae* Harris in Massachusetts by the braconid *Cotesia glomerata* (L.), (introduced against the still common *Pieris rapae* L.), may be such a case (Benson and Van Driesche, unpublished). Evidence for effects on rare taxa is lacking and it has been suggested that they may not be so prone to impacts of exotic agents in their native habitat, where they avoid attack because of their low numbers (Cullen, 1997).

The need to test potential biological control agents against species of commercial or aesthetic significance is not based only on their relationships to a target pest but rather their perceived value. Testing is justified by the claims that: (a) any impacts on commercially important taxa are unacceptable, and (b) aesthetically important organisms are well known and valued by the public and may be "flagship" species that serve as symbols for invertebrate conservation activities. For example, representatives of the commercially valuable birdwing butterflies (*Ornithoptera* spp., Papilionidae) were tested to ensure that their larvae would not be parasitized by the braconid *Cotesia erionontae* (Wilkinson) before release of this species in Papua New Guinea for biological control of the Asian banana skipper, *Erionota thrax* (L.), was approved (Sands et al., 1993).

Methods and Interpretation

Selecting Non-Target Taxa for Testing with Agents

When conducting specificity tests with weed agents, potted non-target plants can usually be maintained for exposing to agents, but it is impractical to maintain cultures of many non-target arthropods for such testing. The number of non-target taxa that can be tested in an arthropod biological control project cannot, therefore, be as great as the numbers of potted plant species tested against weed control agents. To successfully run such tests, sufficient numbers of the appropriate stages of each non-target species of interest must be available from cultures, or obtained from the field and exposed to the agent in a way that will provide evidence of host suitability, which can then be compared with the suitability of the target host. Collection of suitable stages of non-target species from the field requires careful establishment of identity of the species and evidence that stages collected are not already parasitised or diseased. Risks will remain that unrecognized effects influence host acceptance or development of an exotic natural enemy in a particular non-target species.

Information on the degree of taxonomic relatedness of non-target taxa is important when selecting species for use in centrifugal testing but a major impediment for selecting non-target species is lack of systematic knowledge of insects when compared with plants (Kuhlmann et al.,1998). Species of the same genus as the target, followed by ones in related genera, tribes or subfamilies can be used for appropriate testing (Table 2) (Sands, 1998). Difficulties arise when testing the host range of an agent if little is known of the taxonomic relationships of a target with indigenous fauna. Also, without knowing the phylogenetic relationships between a target species and its relatives, it may be very difficult to select for testing, related non-target taxa in the proposed country of introduction. Use of molecular methods for identifying phylogenetic relationships may be an option when conventional morphological features do not adequately clarify relationships, or when complementary information is required (Maley and Marshall 1998). Misidentifications of agents and their hosts, which sometimes occur in the literature and data attached to specimens, can affect conclusions about an agent's host range, especially if taxonomic studies on the agents and their hosts are lacking.

Difficulties and costs of maintaining cultures of rare or threatened taxa for testing can be serious constraints to evaluating the host range of agents (Kuhlmann et al.,1998). For example, two major pests in Australia,

Table 2. Criteria for selecting non-target taxa for host range tests

A. Phylogenetic centrifugal methods (morphological or molecular) can be used to determine the relationships
B. The inclusion of threatened or other taxa of conservation significance may be desirable even when such species are not closely related to the target organism.
C. Appropriate numbers of specific stages must be available by collecting or culturing.
D. Tests may not be necessary when the host range is known from elsewhere and if taxa closely related to the target (e.g., in the same tribe, genus) are unknown in the receiving country.

Helicoverpa armigera (Hübner) and *Helicoverpa punctigera* (Wallengren), have been targets for classical biological control but the introduction of at least one potential agent, the braconid *Microplitis croceipes* (Cresson), has been deferred in Australia due to difficulties in testing it with related, non-target taxa (D. Murray, *personal communication*). Although *M. croceipes* is specific to *Heliothis* and *Helicoverpa* species, the very rare *Helicoverpa prepodes* (Common) has not been cultured, its life history is unknown, and it cannot therefore be evaluated as a potential host for exotic biological control agents. If *M. croceipes* became an abundant parasitoid as a result of attack on the other two target pest species, the rare *H. prepodes* might be at risk.

Also, the results from laboratory tests in which test species are chosen based on their phylogenetic relationship to the target species may fail to detect distantly related or unrelated potential hosts that are suitable for development by a natural enemy (Van Driesche and Hoddle, 1997). In such cases, the life history, plant hosts, or habitat of the target may be more important in influencing the foraging and selection behavior of a parasitoid than the taxonomic relatedness among potential hosts.

Barratt et al. (1999) suggest that even organisms unrelated to the target should be tested with a potential agent if they occupy a similar ecological niche, for example, species that feed on related plant species, or all develop as leaf miners, or all are seed feeders, grassland dwellers, or canopy feeders. However, the logistics of testing a wide range of organisms on the basis of their similar ecological niche is likely to be impractical. A number of taxa, carefully selected on the basis of their relatedness to a target, their life history and choice of habitat, should, however, provide an indication of the likely degree of safety of an agent. When the hosts of natural enemies closely related to an agent are known, predictions for the agent's host range can sometimes be made. However, if the group of agents include in their host range unrelated taxa, such predictions are of little value (Sands, *in press)*. If a potential agent can be shown to be unlikely to develop on any non-target taxa, or only on exotic pest species (given literature records of known host groups and occurrence of such groups in the fauna of the area targeted for introduction), there may be little need for any formal host specificity testing.

Table 3. Evaluating the host ranges of exotic arthropod agents

A. Information on hosts may be available from an agent's country of origin or where it has already been introduced
B. Laboratory experiments contribute to predicting the likely host range of an agent after its release in a receiving country.
C. Monophagous agents are preferred candidates, but many with "narrow" host ranges are often the most effective agents.
D. Generalist natural enemies with "broad" host ranges are not acceptable unless the benefits outweigh possible risks to non-target species.

Assessing the Degree of Host Specificity of Potential Agents

Information on the host range of potential biological control agents may initially be compiled, based on records from the agent's country of origin (when known), from countries where it has been introduced, or from pre-release studies conducted in a quarantine facility (Table 3). Monophagous agents that complete development and reproduce only on one target species are preferred, but in practice most potential arthropod agents attack more than one species in their native range. While strictly monophagous parasitoids are rare or unknown for some groups (Zwölfer, 1971), some stenophagous species become functionally monophagous if introduced into countries where taxa closely related to the target do not occur.

Host specific biotypes (or races) of agents may be overlooked if specimens reared from samples from different hosts are not distinguished in surveys and subsequent colonization in quarantine. For example, a biotype of the pteromalid egg-predator *Scutellista caerulea* (Fonscolombe), originally from South Africa, developed only on the scale *Ceroplastes destructor* Newstead in the field in Australia, but in the laboratory it also developed on other exotic *Ceroplastes* species (Sands, *in press*). Another biotype of *S. caerulea* (morphologically distinguishable, Sands et al. 1986), developed in the field on several other Coccidae but not on *C. destructor*. Despite the importance of selecting biotypes specific to the target pests, there are no simple ways to detect such biotypes other than by extensive field and laboratory evaluation. The frequency of

parasitoid biotypes in nature indicates that they are likely to be overlooked when agents are collected and pooled only on the basis of their morphology. However, molecular methods may be useful for identifying host specific biotypes and separating them from a polyphagous species.

Multiple Choice and No-Choice Tests to Determine the Host Range

When conducting host range assessments in the laboratory, the pattern and sequence of contact between the agent and test species can affect the response observed. Tests that present each test species separately to an agent (usually naive individuals with no previous host contacts) is a "no choice test". In contrast "choice tests" present several potential hosts to the agent simultaneously (Table 4). Choice tests typically, but not always, include the target pest in the mix of species presented to the agent.

As with the response of herbivores to plants, those of parasitoids or predators to arthropod hosts are affected by the test design. Among possible effects (see Edwards, 1993; Marohasy, 1998 for review) of test design are: (1) false positives, in which non-hosts are used by agents when deprived for long periods from their normal hosts, (2) false positives in which non-hosts are used when in close proximity to the normal host due to transference of stimuli, and (3) false negatives in which valid, but less preferred, hosts are ignored in the presence of a more preferred host. For discussion we refer to these as: (1) "desperation" effects, (2) "spillover" effects, and (3) "diversion" effects.

Neither choice or no-choice testing is universally superior to the other and often there are advantages to running tests of both designs on the same agent. Some thought needs to be given to interpretation of the outcomes of sets of tests of varied designs. We can recognize four cases (Table 4):

Case I. Choice and no-choice tests both suggest that a given species is not a host for an agent. If no attack by an agent occurs in either design on a non target species, it may be assumed to be outside of the host range. Control tests are needed subsequently with the agent and target to confirm the ability of the agent to oviposit or feed, unless the target was included in the choice test.

Case II. Choice and no choice tests both suggest that a given species is a host for an agent. If a potential host is utilized under both choice and no choice designs, the test species may be assumed to be in the host range.

Case III. Choice test is positive, but no choice test is negative. If a species is utilized *only* in a choice design (but not in a no choice design), the positive result in the choice test is likely to be a spillover effect caused by stimuli from presence of the target host. In such cases, the non-target tests species is likely to be outside the fundamental host range.

Table 4. Interpreting test programs in which both choice and no choice designs are employed for the determination of insect host ranges

	Choice Test Results (-/+) and Interpretation Relative to Result (-/+) in No-Choice Test	
	- Result	+ Result
No-Choice Test, - Result	Case I Test species *outside* host range	Case II Test species is outside host range and positive result in Choice Test is likely due to "spillover effect"
No-Choice Test, + result, immediately	Case IV-A Test species is inside of host range and negative result in Choice Test is likely due to "diversion effect"	Case III Test species *inside* host range
No-Choice Test, - result, + result, after several days deprivation	Case IV-B Test species is outside of host range and positive result in No-Choice Test is likely due to "desperation effect"	

Case IV. Choice test is negative, but no-choice test is positive.

- **Subcase A.** A positive response to a test species is present immediately in the no-choice test. In this case the test species is likely to be a valid host and failure to detect it in the choice test is due to the "diversion effect" caused by presence of a more strongly preferred host.

- **Subcase B.** A positive response to a test species in the no-choice test is not initially present, but only develops after extended periods (e.g. several days) of deprivation. In this case, the positive response in the no choice test is likely to be erroneous, due to the "desperation" effect. The negative result is then a reliable indication that the test species is outside the host range.

Effects of Confinement on Natural Enemy/ Host Interactions

Confinement in cages or the laboratory may disrupt the normal behavior of parasitoids or predators and is equally of concern in weed biological control projects (Cullen, 1989). False positive results are commonly experienced when agents encounter non-target species in the laboratory under environmental circumstances that would not occur in the field (Table 5). For example, Field and Darby (1991) found that in choice tests with the target species *Vespula germanica* (Fabricius), the parasitoid *Sphecophaga vesparum* (Curtis) parasitized two non-target species of *Ropalidia,* but in no-choice tests, the non-target species were not attacked. Apparently the parasitoids were stimulated into attacking the non-target species, by the presence of the host or saliva from the larvae of the natural host (Field and Darby, 1991). In another example, a biotype of the pteromalid egg predator *S. caerulea* that was adapted to the soft scale *C. destructor* was easily reared on the related *Ceroplastes sinensis* Del Guercio in the laboratory (when its host *C. destructor* was not available for culture). However, after *S. caerulea* became established in the field, only *C. destructor* was attacked (Sands 1993). In this example, close proximity of agent and non-target apparently disrupted the host recognition, leading to false positive results.

False negatives, in which an agent failed to attack or develop on a species in the laboratory but subsequently did so on the species after release, have not been well documented for arthropod agents. However, Barratt et al. (1997) suggested that cage tests may have underestimated the host range of the braconid *M. aethiopoides*, which failed to attack the weed biological control agent *Rhinocyllus conicus* (Froehlich) in the laboratory but did so after its establishment in the field.

Table 5. Effects of confinement on host range tests with arthropod agents

A. Choice tests are more susceptible to false positive results than no-choice tests when carried out in cages.
B. Cages may inhibit mating and induce false positives for false negative host recognition and acceptance.
C. Tri-trophic interactions and behavior are often disrupted when agents, plants and potential hosts are confined in cages.
D. Habitat specialists may be very difficult to evaluate as habitats often cannot be reproduced in laboratory tests.
E. Cage design or materials may influence agent/target interactions. Controls must be included by presenting agents with suitable targets, to avoid false negatiove results with non-targets taxa (when parasitoids fail to oviposit for physiological reasons).

For weed biological control agents, false positive results seen in choice tests are believed to have been induced by experience-dependent changes in the agents' responsiveness, adsorption of volatile kairomones onto test plants, or indiscriminate behavior of agents when confined in cages (Marohasy, 1998). In the case of arthropod agents, major problems may arise if agents are held in confinement with other organisms that would rarely if ever, be found naturally in close proximity with the host. One or more behavioral phenomena may then lead to acceptance of an organism as a host by a natural enemy under such conditions (e.g., example in Field and Darby, 1991). Moreover, the presence of kairomones from a host in close proximity with another test species may induce a natural enemy to mistakenly recognize the non-target species as a host. Cages used for routine rearing of an agent may not be appropriate for host range testing because the amount of space required for mating and oviposition in a favored host may not be comparable with the requirements for expression of an agent's behavior towards a non-target species. For example, confinement can disrupt diapause in some parasitoids, especially if diapause is regulated by the host physiology. Such circumstances would require a

more detailed study of the natural enemy and its host to avoid misinterpretation of host specificty tests.

The physiological state of a non-target host may change in a laboratory environment and affect parasitoid development. Such effects need to be considered when assessing host range tests. For example, the first instars of the parasitoid *Anicetus communis* (Annecke) only break diapause in the host scale *C. destructor* when the adult scale is in a pre-ovipositional state; otherwise, no development of the parasitoid occurs in hosts for up to 8 months on plants in the field (Sands *et al.,*1986). In the laboratory if the plant host is stressed, diapause in *A. communis* is broken and the parasitoid develops in the pre-ovipositional scale. In such cases, failure of a parasitoid to develop when in diapause in a non-target host on an unstressed plant, could easily be misinterpreted as host unsuitability, rather than being attributed to the predisposing condition of the plant.

Cage design, size, materials, and access to light may all influence the responses of natural enemies to their hosts, and each species of agent and host may require specialized treatments. For example, cage size influenced the levels of parasitism in weevils by the braconid parasitoids *M. aethiopoides* and *Microctonus hyperodae* Loan (Barratt et al., 1999). Food offered to both agents and the target species in the cages is also important. For example, the longevity and fecundity of an agent may be reduced by poor nutrition, affecting responses to non-target species being tested. Overcrowding of agents may inhibit their mating or host recognition. The number of agents and stages of hosts, or their ratios, may require adjustment to avoid anomalous results in both choice and no-choice tests (Barratt et al., 1996). Care must be taken to ensure that the non-target species is not presented on a plant that would not be its natural host under field conditions.

Cage materials, especially synthetic substances, may adsorb kairomones from contact with a target species. In choice and no-choice tests these adsorbed compounds on cages may promote attack by agents on non-target species exposed in affected cages. These errors are most likely to occur in choice tests but can be avoided if necessary by replacing the cage materials after each test. Confinement in cages may also disrupt mating behavior. Sometimes this problem can be corrected by using black materials that transmit light instead of white cage materials, which scatter light. For example, pairs of *Aprostocetus ceroplastae* (Girault), a parasitoid of soft scales, failed to mate in white cages but mated immediately when exposed to sunlight in cages made from black organdy (Sands unpublished). Fine black materials transmit light in a different way than do white or pale colored cage materials, which scatter light, sometimes affecting both mating and ovipositional behavior of parasitoids. Plexiglass may also be useful for replacing white materials.

Superparasitism leading to host mortality frequently results from confinement of several gravid agents with hosts. To avoid crowding effects, the exposure period must sometimes be adjusted so that an individual attracts oviposition by only one agent before it is removed and transferred to its own host plant. This effect was also observed when the braconid *M. aethiopoides* attacked the alfalfa weevil, *Hypera postica* (Gyllenhal), in the laboratory (Neal, 1970). Tests may require withdrawal of a host immediately after exposure and parasitoid oviposition, to ensure that optimal chances are provided for the development of a parasitoid.

Choice tests may exacerbate effects of confinement on selection of hosts by an agent. These problems can sometimes be avoided by no-choice tests using sequential, separate exposures of target host and test species. For example, Sands and Coombs (1999) conducted no-choice tests by exposing gravid females of *T. giacomellii* (Tachinidae) alternatively to the target host, *N. viridula*, for two hours and then to each non-target species, each for two hours, to record oviposition. The number of eggs deposited on the target host were then compared with the number of eggs (if any) deposited on the non-target species, for each two-hour period. In this way, false positive responses due to the "spillover" effect (Table 4) were avoided. Possible effects of conditioning by prior exposure to the target were separately evaluated by exposing gravid naive parasitoids only to the non-target species tested.

Tri-trophic Effects May Influence Host Acceptance

Host range tests can be designed to take into consideration the kinds of tri-trophic interactions that often affect host recognition of agents. Such effects are important especially when a plant host of a target organism is a cue for host location (Table 6). Without a particular plant substrate, the searching activity of some parasitoids may be severely reduced. For example, *Eretmocerus* spp. from Spain and India performed well as a parasitoid for *Bemisia tabaci* (Gennadius) (biotype B) in all crops; however, *Encarsia* sp.

nr *pergandiella* Howard performed well on melons but not on cotton or kale (Goolsby et al.,1998).

Some plants stimulate or inhibit ovipositional responses in parasitoids. For example, *Trichogramma* spp. rarely parasitize eggs of *H. armigera* on pigeon pea (*Cajanus cajan* [L.] Millsp.), but levels of parasitism are much higher if the eggs are deposited on other plants (Romeis et al.,1997). Similarly, the eggs of *Leptocorisa oratorius* (F.) (Hemiptera: Alydidae) on rice were more heavily parasitised by the scelionid *Gryon nixoni* Masner than were eggs deposited on six other plant hosts of the bug (Morrill and Almazon, 1990). Characteristics of pigeon pea that inhibited parasitism included volatile compounds (emitted by leaves and pods) that repelled or deterred the parasitoids, leaf trichomes that inhibited the parasitoid searching behavior, and exudates that trapped the adult parasitoids. Feeding damage on plants may also affect the behavior of parasitoids. For example, Steinberg et al. (1993) demonstrated the attraction of the braconid *Cotesia glomerata* (L.) to cabbage damaged by larvae of its host, *Pieris brassicae* (L.). In host range tests, non-target species should be presented to parasitoids both with and without their associated host plants.

It may be necessary to evaluate an agent with a target on several of the target's plant hosts, and false positives may occur if non-target species are presented on host plants of the target species because the agent may be stimulated to oviposit in the test species by the plant substrate. For example, the egg parasitoid *Ooencyrtus erionotae* Ferriere was introduced from southeast Asia into Guam, Saipan, Mauritius, and Hawaii for control of the banana skipper, *E. thrax* (Waterhouse and Norris, 1987; Sands et al.,1993). When laboratory tests were conducted with *O. erionotae* in Papua New Guinea, the presence of banana leaves affected the species of hosts attacked. Parasitoids, in the presence of leaves, oviposited in eggs of *Cephrenes augiades* (Felder), a species belonging to the same subfamily (Hesperiinae) as *E. thrax* (Sands, 1991) and also attacked other Lepidoptera that were not taxonomically related to the target or attacked in the field (Sands, *unpublished*).

Table 6. Responses of arthropod agents to plants

A. Physical or chemical characteristics of plants may predispose host recognition by an agent.
B. Plants may stimulate, reduce, or prevent host recognition, leading to false positive or negative interpretations of the specificity of agents.
C. Plant damage from feeding by herbivores and their feces may stimulate responses in agents.
D. Plants form part of the habitat specialization of agents.

Using Developmental Parameters to Assess Host Suitability

Differences in the life history parameters of natural enemies have been used as indicators of host suitability and, thus, the likely levels of impact on non-target taxa compared to that in the target host. For example, Wright and Kerr (1988) compared the development of the parasitoid *Encyrtus saliens* Prinsloo and Annecke in two scales. *Pulvinaria delottoi* Gill was shown to be less suitable than *Pulvinaria mesembryanthemi* (Vallot) because: (a) development of *E. saliens* in *P. delottoi* required more thermal units, (b) from the same initial host size, immature parasitoids in *P. delottoi* developed more slowly than *P. mesembryanthemi*, (c) adult parasitoids emerging from *P. delottoi* were smaller and less fecund, and (d) small *P. delottoi* received fewer parasitoid eggs, and these were deposited at a lower rate when compared to oviposition rates on *P. mesembryanthemi*. The authors concluded these results demonstrated that *P. delottoi* was a less suitable host for development and maintenance of *E. saliens* than was *P. mesembryanthemi*. The authors also predicted that *E. saliens* would persist better on *P. mesembryanthemi* and attack *P. delottoi* only when it occurred with the primary host in mixed infestations. Field evaluation of these predictions is lacking, but the approach provides a framework for using laboratory data to predict field outcomes among host and parasitoid populations.

The same approach has been used to infer that a natural enemy might have a greater impact on a target than on non-target species after the natural enemy is established. Sands and Coombs (1999), when evaluating the tachinid *T. giacomellii* for control of *N. viridula*, found that although the parasitic fly laid eggs on six non-target pentatomids, only three supported its immature development. On the three suitable non-target hosts, parasitoid fecundity and longevity were reduced when compared with that on the target species, *N. viridula*.

These findings plus the reduced size of the puparia from these non-target species, indicated that those species were sub-optimal hosts for the parasitoid. If an agent's population size depends on its utilization of sub-optimal hosts, it is likley to have little effect on density. However, if the geographical ranges of abundant, high quality and sub-optimal hosts overlap, population densities of sub-optimal hosts may be reduced via "spillover" of parasitoids deriving from the better host.

The quality and health of the agent, the target species, and non-target organisms need to be monitored when host specificity tests are undertaken, since they affect the developmental parameters (Barratt et al.,1999). Infection with micro-organisms may affect natural enemy/host interactions, and these pathogens must be removed from cultures if meaningful tests are to be carried out. Microsporidia are commonly found in insect cultures and are well known in parasitoids. For example, Sheetz et al. (1997) identified a species of *Nosema* infecting the ovaries of the parasitoid *E.* nr *pergandiella*, that lead to a steady decline in parasitoid fecundity. An antibiotic was used to effectively treat the microsporidium infection in the parasitoid culture, indicating that there may be a place for more routine use of antibiotics in insect cultures, to ensure that host range tests are not biased by the presence of similar infections. Such infections if overlooked, might easily influence the assessment for host suitability of a non-target species.

Effects of Conditioning and Prior Experience

The process of locating a host and confirming its suitability by a parasitoid is a progressive response to environmental and host cues that lead finally to acceptance when oviposition occurs (Vinson, 1976). Although many parasitoid species have both preferred and less favored hosts, adaptive behavior allows parasitoids to focus on those species that have already proven to be appropriate and locally available hosts (Vet, 1985). The individual, prior experience of an agent is known to sometimes affect its behavior towards another host and may affect the outcome of both choice and no-choice tests. Tests with agents can be designed to determine if prior exposure of parasitoids to a target host influences the subsequent acceptance of a non-target species (Sands and Coombs, 1999).

Arthropod Predators and Their Evaluation

Arthropod predators are second only in importance to parasitoids as agents for classical biological control of arthropods. Although many species of exotic predacious arthropods have been established in various countries without host range testing, few examples of detrimental non-target effects are recorded. Two important groups, coccinellids and mites, are often generalists, adapted to groups of hosts or a particular type of habitat. Adaptation to certain habitats or plant hosts may be important when considering the acceptability of species otherwise considered generalist predators.

In some species of predators, adults may be generalists, but immature stages may be more specific in their choice of prey. For these cases, separate tests with the appropriate stages of prey may be needed for the different predator life stage (Table 7). A number of adult coccinellids are generalists, but have immature stages that are much more specific. For example, *Rodolia cardinalis* (Mulsant) is specialized to feed and breed on only a few species of margarodid scales, but adults of *R. cardinalis* can subsist on a wide range of other insects and nectar for up to three months. However, margarodid scales are strictly required for development of the immature stages (V. Brancatini, *unpublished*). Despite the close adaptation of this coccinellid to its prey, the undoubted value of *R. cardinalis* for controlling *Icerya purchasi* Makell might be questioned if only the host range of adults were tested. There is little evidence available from field or laboratory studies on *R. cardinalis,* to demonstrate any preference for *I. purchasi* over *Icerya aegyptiaca* (Douglas), even though on some tropical atolls *R. cardinalis* is unable to maintain control of the latter species (Waterhouse 1993).

Field association of a predator with a target prey is a common means of choosing an agent for introduction against a particular pest. The coccinellid, *Curinus coeruleus* (Mulsant), for example, was imported to Hawaii from Mexico in 1922 for control of the coconut mealybug, *Nipaecoccus nipae* (Maskell) (Waterhouse and Norris, 1987). Such field associations may, however, give misleading impressions about an agent's actual preference among a broader range of potential prey. In the case of *C. coeruleus*, the subsequent invasion of Hawaii by the leucaena psyllid, *Heteropsylla cubana* Crawford, presented *C. coeruleus* an additional prey. A preference by *C. coeruleus* for the psyllid over the mealybug, rapidly became evident as *C. coeruleus* populations, formerly present only at low densities, increased significantly on the psyllid. Such cases argue strongly that predator preferences need to be

Table 7. Evaluating predators

A. Habitat specialization may be a primary cue for prey location.
B. Host ranges of adults may differ from those of immature stages.
C. Phoretic mites may require specific evaluation of intermediate carrier agents.

determined by testing and not merely surmised from field associations.

When separate host range tests are carried out on adult and immature stages, differences in host/prey interactions can be detected. Predators, in common with other natural enemies, might be adapted to prefer to forage under certain environmental conditions or on certain plant substrates. These affinities can be revealed by tests in the laboratory and results used to evaluate the association of a natural enemy with a non-target prey. For example, woody stems of the host plants for the ortheziid scale *Orthezia insignis* Browne were shown to affect the performance of the coccinellid *Hyperaspis pantherina* Fursch when cultured in the laboratory (Booth *et al.,*1995). The coccinellid was easily cultured when its prey was held on plants with woody stems, but on whole infested plants held in large cages, very few larvae of the predator matured due to scarcity of woody stems infested with the host, and their development was impossible to monitor. Such adjustments to selecting a plant substrate to rear prey with its predator in the laboratory need to be developed before meaningful host specificity tests can be applied to non-target species on their own host plants.

Determining the host range of predatory mites, a group of predators cultured for suppression of pest tetranychid mites, poses difficulties. Micro-habitat and tri-trophic cues are likely to be important to consider when testing their responses to prey, and species adapted to forage in host plants of a target may provide an appropriate choice. Phoretic mites, such as those used for biological control of dung-breeding flies, may require that we also evaluate the suitability of the micro-habitats and of symbiotic carrier agents.

Risk Assessment: Acceptance or Rejection of a Potential Agent

What is the breadth of host range that should be considered unacceptable? Should agents that develop on non-target host or prey of a particular taxonomic level of relatedness to target be excluded? For example, should an agent be considered unacceptable if it feeds on other members of the same subfamily, tribe or genus as the target? Most difficulties arise when making decisions about agents that complete development on a limited number of non-target taxa in the laboratory. Such tests demonstrate the potential to develop on non-target taxa even though the agent may fail to do so in the field. Agents may be considered to be acceptable if they complete development and reproduce only on non-target species that are closely related (same genus or tribe) to the target pest. However, if more distantly related (different genus or tribe), unrelated (e.g., different family or order), or beneficial organisms are shown to be suitable hosts, the potential for detrimental effects on unrecognized organisms should be considered before release of an agent.

When an agent develops on one or more non-target taxa, the benefits need to be carefully weighed against any risks of undesirable effects. Such risk assessment aims to reduce risks, but not to completely eliminate them (Bourchier and McCarty 1995). The likely benefits, i.e., effective control of the target pest and associated benefits, need to be compared against possible declines or extinctions of the non-target species that might be attacked. Estimates of host ranges of potential agents that are based on results of tests carried out in the laboratory influence governmental decisions about whether or not to release the agents. For example, four egg parasitoids in the same genus *Ooencyrtus* were not released in the United States for control of *N. viridula* because they were shown to attack at least 20 species of unrelated native Hemiptera. The decision not to release them was based on their wide host ranges and lack of evidence that they were effective in suppressing the target pest in their native ranges (Jones, 1988).

Information on the range of habitats used by an agent in the country of origin may provide evidence to suggest that a non-target taxon that only occurs in different habitats would not be likely to be at risk. If an agent and target are known to be adapted to an environment different from that in a receiving country where a non-target species is present, it can be argued that the risks of the agent adapting to that environment are minimal.

Environmental criteria were used to evaluate non-target risks after the release of *Cotesia flavipes* Cameron, a braconid wasp released in Kenya against the stem borer *Chilo partellus* (Swinhoe). Host searching of *C. flavipes* was limited to plant

communities of long-stemmed grasses in natural and agricultural habitats, where the only acceptable hosts present were lepidopteran stem borers. Natural grasslands were occupied by several native parasitoids, including *Cotesia sesamiae* (Cameron), which was possibly at risk of displacement by *C. flavipes* in the agricultural habitats. Displacement of *C. sesamiae* was less likely in the grasslands since its response to native grasses was stronger than to sorghum (Overholt et al., 1994). It was suggested that some displacement of *C. sesamiae* by *C. flavipes* might occur where its habitat overlaps with that of *C. partellus*, but that *C. sesamiae* would persist in native habitats where the dominant host species were unsuitable for *C. flavipes.*

Assuming tests can demonstrate the host range of potential agents and that only agents with "narrow" host ranges are candidates, the process of risk assessment begins with making a decision whether or not some complete development of an agent on a non-target species is acceptable, as few parasitoids are strictly monophagous. Secondly, the likelihood and nature of any detrimental effects (e.g., decline in density, extinction) need to be balanced with the benefits of controlling a pest. For agents proposed for release that are not narrowly specific, more comprehensive assessments of potential impacts and benefits are needed before reaching decisions. Polyphagous agents, while often undesirable, may be necessary and beneficial in specific contexts.

Discussion

Assessment of the host range of potential arthropod parasitoids and predators before they are introduced from another country is usually necessary to reduce risk of harm to related non-target organisms. For some agents, laboratory host range tests may not be required if appropriate information is available from overseas and when there are no species related to the target in the receiving country. In other cases, tests with non-target organisms may be required to estimate the likely host range before introduction of the agent. Some host range tests are easily implemented, but others are difficult to conduct or evaluate. In some cases, it may be impossible to approve the release of potentially valuable agents because non-target taxa or their appropriate stages needed for testing are not available, or because there are anomalies in the behavior of an agent when confined in cages or when tri-trophic responses and other difficulties affect interpretation of results.

The interactions of parasitoids with plants need to be considered when designing host range tests. For example, the effects of different food plants used by target or non-target species may influence conclusions about performance of an agent, when it, a potential host and the host's food plant are brought together. It may be necessary to evaluate performance of an agent with the target species on its various plant hosts before comparative studies are initiated with non-target species.

Some instances of development of introduced agents on non-target taxa must be considered acceptable if classical biological control of arthropods is to continue, since mono-specific agents are few and often are not available. If the use of host range tests is to be realized, they must be limited to a few representative non-target taxa or those of special conservation significance. While each assessment will be made on a case by case basis (Barratt et al.,1999), a framework for testing procedures exists that could be adopted as the basis for such testing protocols. Caution will be needed when agents are shown in the laboratory to complete development on beneficial and other non-target taxa (Van Driesche and Hoddle, 1997). There is a need to review case histories where non-target taxa support development of exotic natural enemies to determine the nature and dynamics of impact on their populations. Excellent examples are available for case studies to quantify impacts on non-target taxa. For example, *T. basalis* has been introduced to control *N. viridula* in many countries, in some of which it also develops on eggs of many unrelated pod-sucking bugs. Its actual impact on these non-target species has not been fully investigated.

While biological control is the most cost effective and safe alternative to pesticides and genetically modified plants for the management of pest arthropods, resources for prolonged detailed studies on the interactions of agents and non-target species are not readily available. In cases where non-target species are shown by laboratory evaluation to be attacked by an agent, the likely benefits of pest control must be weighed against the possibility of some detrimental effects. Without neglecting the importance of protecting non-target taxa, more evidence of detrimental impacts is required before the release of only mono-specific species becomes a priority and a major limiting factor for arthropod biological control.

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Host Specificity Assessment of European *Peristenus* Parasitoids for Classical Biological Control of Native *Lygus* species in North America: Use of Field Host Surveys to Predict Natural Enemy Habitat and Host Ranges

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Abstract

The *Lygus* bug complex (Het., Miridae) at many sites in North America causes economic damage to a wide variety of agricultural crops and is the focus of numerous research projects. *Lygus* can be best suppressed using parasitoids in unsprayed non-cropping situations as well as in crops where pollination is important for maximizing yields. The European parasitoid *Peristenus digoneutis* Loan (Hym., Braconidae), which attacks several species in the genera *Lygus* and *Adelphocoris* in Europe, has been established in the eastern USA to control the native pest *Lygus lineolatus* (Palisot de Beauvois) in alfalfa. The success of this project has stimulated interest into the potential for the establishment of additional European species for biological control of pest *Lygus* bugs in several regions of North America. The research of this ongoing case study concentrates on assessing strategies and methods for host specificity testing of these parasitoids in relation to Europe and North America. Predicting the impact of European parasitoids by using existing and new knowledge of the host range and habitats in the area of origin will aid in choosing *Peristenus* species for further release in other regions and will have important implications for the practice of classical biological control.

Introduction

Need to Estimate Host Ranges of Entomophagous Biological Control Agents

Concerns about reliance on chemical pesticides in crop protection worldwide have led to the development of integrated pest management strategies, which depend on natural enemy manipulations as alternatives to exclusive use of chemicals. Classical biological control, the introduction of exotic biological control agents to permanently suppress exotic pests, is one such approach. It is practiced ever more widely as successes accumulate and pest invasions follow trade liberalization.

A high level of host specificity in the introduced natural enemies is desirable and should be sought during foreign exploration (Nechols et al., 1992). Potential environmental risks of arthropod biological control agents have usually seemed negligible, but recent concern about the impact of alien species on biodiversity and natural ecosystems has caused this belief to be reconsidered.

The potential risks of arthropod biological control to native non-target arthropods are being examined for some cases (i.e., Goldson and Phillips, 1990; Barratt et al., 1998, 1999). While there are no recorded examples of *monophagous* or *narrowly oligophagous* agents changing their host range to cause damage to non-target insects (Waterhouse, 1991), the use of *polyphagous* insects as biological control agents may reduce populations of non-target hosts or cause their extinction (Horn, 1991; Howarth, 1991; Samways, 1994). However very little quantitative information is available

on the influence of these agents on the population densities of these non-target populations.

Regulatory agencies that oversee biological control introductions are responding to concerns of potential non-target impacts by requiring more rigorous testing and demonstration of a high degree of host specificity by candidate arthropod biological control agents before granting permission for release. However, because of an earlier lack of concern for non-target arthropods, methodologies for host specificity screening of arthropod natural enemies are much less developed than for testing of herbivorous insects imported for weed biological control. Recent papers discuss methods to evaluate the potential impact of parasitoids of arthropod pests on non-target hosts before and after release of parasitoids (i.e., Sands, 1997, Sands and Van Driesche, 2000; Van Driesche and Hoddle, 1997; Hopper, 1998). Two categories of data can help in estimating the host ranges of insects: (1) host-natural enemy associations as seen in the published literature or in specially conducted surveys; and (2) laboratory testing in which candidate species are presented in cages to natural enemies, whose oviposition and immature development are then observed. Because insect faunas are often very large, study of host-natural enemy associations by field surveys are often important in choosing species for testing in the laboratory.

Approaches to Host Ranges Estimation for Parasitoids: Field Surveys vs. Laboratory Testing for Large Faunas

Traditionally, host association records from the literature provide the first estimate of the host range of an entomophagous biological control agent. Such records are especially useful for distinguishing candidates with obviously broad host ranges from those that might have suitably narrow ones. Misidentifications, especially in the older literature, often cause spurious host records. However, some of the problems posed by such errors may be avoided by considering the quality of data, taking into account such aspects as numbers of individuals examined, percent parasitism observed, spatial and temporal extent of studies, and confirmation of identity by a specialist. In this way, observations of attacks on non-hosts, especially those based on single or few specimens and not properly identified are less likely to be incorrectly entered into the host record list. This will also ensure that actual alternate hosts, occasionally used, are reported.

The laboratory methods used to assess the host ranges of herbivorous insects being introduced as weed biological control agents are often suggested as a model for efforts to assess host specificity of entomophagous insects. However, several difficulties exist in applying this approach. A significant problem is the major difference in the status of systematic knowledge of plants and insects. Whereas the taxonomy and phylogenetic relationships of plant species are relatively well known, far less is known about these relationships for insects. The uncertainty of the relatedness of insects within families and tribes makes the choice of species for testing uncertain. Secondly, the large number of species in some insect groups often easily exceeds the number of plants in similar level taxa by an order of magnitude. This often precludes testing of more than a tiny subsample of species in related groups. If for example, the target insect's family contains 5000 species (a common possibility), most of which have biologies and distributions that are poorly known, choosing a list of species for host range testing is extremely difficult. Consequently, systematics is a critical part of host specificity testing programs for projects of biological control targeting insect pests. Reconstructions of phylogenies may be needed to guide the host testing and agent selection process. Finally, lack of information about the biology and rearing methods for many insects makes it impractical to assemble sets of target species for laboratory testing in the conventional manner used to test herbivory on plants (which may be collected and stored as seeds until needed in most cases).

Once a species test list has been defined, uncertainties exist on how to conduct and interpret laboratory host range tests for entomophagous species. Sands (1993) identified several difficulties in interpreting results of such laboratory tests on the physiological (=fundamental, see Van Klinken, 2000) host ranges of entomophagous insects. It is extremely difficult to reproduce accurately the cues and stimuli that influence the host searching and assessment behavior of a parasitoid in a natural environment. In laboratory tests, entomophagous insects often accept a broader range of hosts than in nature, over-estimating field host range (Loan and Holdaway, 1961). In laboratory trials, patterns of oviposition, feeding, or development in arthropod hosts are typically assessed in small containers. Sands and Papacek (1993) reported that restricted space often leads to an inaccurate assessment

of host specificity by disrupting the processes governing host recognition and acceptance. For example, parasitoids in small cages may oviposit in hosts that normally do not support development of the parasitoid, or parasitoids may oviposit in hosts that normally are not accepted in the field. The physiological host range measured in the laboratory and the realized host range in the field thus might differ.

Field studies of parasitoid-host complexes in the area of the pest's origin (or for new association projects, the area where parasitoids are to be collected) provide the basis for correctly interpreting host range estimation made via laboratory testing. This is important because in the laboratory there are inherent problems related to altered behavior of entomophagous agents and rearing of potential hosts. These field studies can be designed to gain insight into such important issues as the range of habitats in which a candidate agent might forage if it were to be released and the level of attack (observed as the level of parasitism) achieved in various field habitats on various hosts. In general, it is believed that such field data on the host ranges of candidate biological control agents in the area of origin are a reasonably good predictor of what the realized host range will be in the area of introduction.

A set of field surveys of parasitoids of mirid bugs is being conducted in Europe to guide the choice of parasitoid species for possible introduction to North America, for suppression of pest mirids of the genus *Lygus*. These mirid host-parasitoid surveys are being done in a variety of different habitats in the area of origin over several years to assess the ecological host ranges of several European *Peristenus* species under field conditions. Data from the survey will be compared to results from laboratory experiments to estimate the physiological host range of the candidate species chosen for introduction. These findings will help improve the design of testing methods used to study potential risks of European parasitoids that become candidates for introduction to North America. The host range survey in Europe will also provide information that can be used to determine which species of North American mirids should be included in the laboratory host range tests.

Biological Control of *Lygus* Plant Bugs: A Case Study

Species of *Lygus* Hahn plant bugs (Heteroptera: Miridae) cause economic damage to a wide variety of agricultural crops in various parts of North America and have been the focus of numerous research projects. Parasitoids are best able to suppress *Lygus* species in unsprayed non-cropping situations and crops in which pollination is important for maximizing yields. Nymphal parasitoids of the subfamily Euphorinae (Hymenoptera: Braconidae) belonging to the genera *Leiophron* Nees and *Peristenus* Foerster are known to be associated with plant bugs of the family Miridae in Europe and North America (Loan, 1974a, 1980; Loan and Shaw, 1987). Analysis of Nearctic and Palaearctic *Peristenus* species attacking species of *Lygus* revealed that a larger number of European species exist compared to indigenous species in North America. Biological control practitioners, therefore, became interested in the possibility of reducing pest plant bug numbers in North America by introducing additional exotic parasitoids, choosing species that were more successful in attacking all generations of the Mirini genera *Lygus* and *Adelphocoris* Reuter.

North American efforts at biological control of *Lygus* species began in the 1960s and have been summarized for the United States (Coulson, 1987) and Canada (Craig and Loan, 1987; Carl and Mason, 1996). Surveys were conducted to search for exotic parasitoids in Europe, Turkey, Iran, Pakistan, India, Indonesia, and eastern and southern Africa. Several *Peristenus* species have been introduced and released in Canada and the United States, with some success (Craig and Loan, 1984; Hedlund and Graham, 1987; Day, 1996). The European species *Peristenus digoneutis* Loan (Hymenoptera: Braconidae), a parasitoid of *Lygus* and *Adelphocoris* species, has been established in the northeastern United States to control the native pest *Lygus lineolaris* (Palisot de Beauvois) (Day, 1996, 1999). Day *et al.* (1992, 1998) reported that an additional species, *Peristenus conradi* Marsh, probably of Palaearctic origin, has also established, but this parasitoid was introduced accidentally.

The success of the introduction of *P. digoneutis* has stimulated interest in research into the potential for the establishment of additional European species of *Peristenus* for biological control of pest *Lygus* species in several regions in North America. Accordingly, Kuhlmann et al. (1998) outlined an approach for the assessment of potential risks for introducing additional European *Peristenus* species as biological control agents of native *Lygus* species in North America. Kuhlmann et al. (1998) concluded that research is required on the taxonomy of *Lygus* and *Peristenus*, the biology and realized host range of candidate biological control

agents, the development of rearing techniques for target and non-target host species as well as *Peristenus* species, and the development of suitable techniques for evaluating the physiological host range of biological control agents in the quarantine laboratory.

This paper concentrates on assessing strategies to predict the impact of European *Peristenus* species on potential non-target mirid populations by using existing and new knowledge of the phylogenetics of *Lygus,* the systematics and biology of *Peristenus*, and on strategies to assess the realized host ranges of *Peristenus* species in Europe in different habitats.

Pest Status of North American Lygus Plant Bugs

Schwartz and Foottit (1992) reported that the most important *Lygus* pests in North America are the tarnished plant bug, *L. lineolaris*; the western tarnished plant bug, *Lygus hesperus* Knight; the pale legume bug, *Lygus elisus* van Duzee; and *Lygus borealis* (Kelton*). Lygus lineolaris* is distributed continent-wide and is the only species causing economic damage in eastern North America, affecting seed alfalfa (lucerne), cotton, vegetables, and fruit crops such as apples and strawberries. In western North America, *L. hesperus* occupies approximately the same ecological niche as *L. lineolaris* in the East, but is more abundant, being especially common on alfalfa and cotton (Day, 1987). *Lygus elisus* and *L. borealis* are serious pests of canola in the western United States and the Canadian Prairie Provinces (Butts and Lamb, 1991ab). *Lygus borealis*, a well-known pest of seed alfalfa, became a pest of canola (Butts and Lamb, 1991b) as the acreage of that crop increased (Lamb, 1989). This is an example of how development of new crops can lead to new associations that result in the emergence of new pests. All species of *Lygus* feed preferentially on either the developing reproductive organs (buds, flowers, and developing seed) or on the apical meristematic and leaf primordial tissue (Strong, 1970). It is the concentration of feeding on reproductive parts that makes *Lygus* species some of the most insidious pests of seed crops (Schwartz and Foottit, 1992).

Phylogenetics of Nearctic and Palaearctic Lygus Species

Discussions of safety issues in *Lygus* biological control projects have identified a need for better identification tools for *Lygus* species, for both the adult and immature stages. Many species of *Lygus* are morphologically similar, taxonomic character differences are subtle, and in some species there is a wide range of ontogenetic and geographic variation (Schwartz and Foottit, 1992). This variation may reflect recent adaptations and evolution in changing natural and agricultural environments in North America. Additionally, the genus itself and its relationships to other related genera have been difficult to define.

Much of the recent taxonomic research on *Lygus* has being synthesized in a revision of the Nearctic species (Schwartz and Foottit, 1998), which includes a review of and key to the Palaearctic species. The latter are important as hosts of potential biological control agents for the Nearctic species. The revision includes illustrations, descriptions, and keys to the species of *Lygus*, data on species relationships, distributions, biogeography, and the range of plant species attacked. Although much information is provided by this comprehensive systematic study of *Lygus*, involving classical and molecular approaches, there is still a need to determine the genetic variability among populations within various *Lygus* species to better understand their relationships.

Taxonomic characters of *Lygus* were classified by Schwartz and Foottit (1998) as primitive (ancestral) or advanced (derived) and were used to produce a cladogram, which represents the hypothetical relationships among the species. Fig. 1 shows a maximum fit cladogram of *Lygus* and outgroup taxa adapted from Schwartz and Foottit (1998), based on morphological and molecular characters, with biogeographic placement. These cladograms have aided in the recognition of natural groups of *Lygus* species. Cladograms also provide practical information, as for example, that the major North American pest species *L. lineolaris* is not necessarily closely related to other North American pest species such as *L. elisus*, *L. hesperus*, and *L. borealis*. Understanding these relationships is important when searching for candidate biological control agents in the area of origin for suppression of *Lygus* in North America (Foottit and Schwartz, 1996). The cladogram also provides information about the relatedness of North American and European species (e.g., *Lygus rugulipennis* Poppius and *Lygus pratensis* [L.]) from which *Peristenus* cocoons are collected for shipments to North America. Fig. 1 demonstrates that the European *L. pratensis* is most closely related to the North American pest *L. hesperus*, closely related to *L. elisus* and *L. borealis* but only distantly related to *L. lineolaris*. Interestingly, *L. rugulipennis*, widely

distributed in Europe and the major host source for *Peristenus* cocoons, has been recently recognized as a senior synonym of *Lygus perplexus* Kelton (Schwartz and Scudder, 1998), a species confined to the montane and northern regions of western North America. Thus, *L. rugulipennis* is Holarctic (Schwartz and Scudder, 1998; Schwartz and Foottit, 1998).

The evolution of host plant associations and biogeography in other plant bug taxa has been investigated by fitting extrinsic data (host plant or areas of endemism) to phylogenetic hypotheses based on structural characters (Stonedahl, 1990; Schuh, 1991). Schwartz and Foottit (1998) applied this method to the maximum fit cladogram of proposed relationships for *Lygus,* but no clear patterns of either host-plant association or biogeography were found because of the non-specific host plant utilization and the widespread distribution of many species. However, Schwartz and Foottit (1998) generalized that *Lygus* is composed of species that feed on a wide range of host plants and, with the exception of the *Lygus striatus* Knight, *Lygus bradleyi* Knight, and *Lygus lupini* Schwartz clade, the *Lygus* species that do feed on plants of the same family are not closely related. Therefore Schwartz and Foottit (1998) considered that the major *Lygus* pest species, *L. hesperus*, *L. elisus*, *L. borealis*, *L. rugulipennis*, and *L. lineolaris* do not form a monophyletic group.

European Peristenus Parasitoids

Systematics of Peristenus. Comprehensive revisions to species of *Leiophron* and *Peristenus* are available for Europe (Loan and Bilewicz-Pawinska, 1973; Loan 1974a) and North America (Loan, 1974b). New name combinations were provided by Loan (1974b) because of the separation of *Peristenus* from *Leiophron* (Loan and Bilewicz-Pawinska, 1973). In Europe, 17 *Peristenus* species are described (Loan 1974a, 1976; 1979) and of those, *P. digoneutis*, *Peristenus stygicus* Loan, *Peristenus rubricollis* (Thomson), *Peristenus adelphocoridis* Loan, and *Peristenus pallipes* (Curtis) (Holarctic) are known to parasitize species of *Lygus* and/or species of *Adelphocoris*. Day et al. (1992) described an additional *Peristenus* species for Europe, *P. conradi*, reared from *Adelphocoris lineolatus* (Goeze), and he considered that this species was introduced, unknowingly, with other European *Peristenus* species at the beginning of the 1980s. *Peristenus adelphocoridis* and *P. pallipes* are sibling species that are

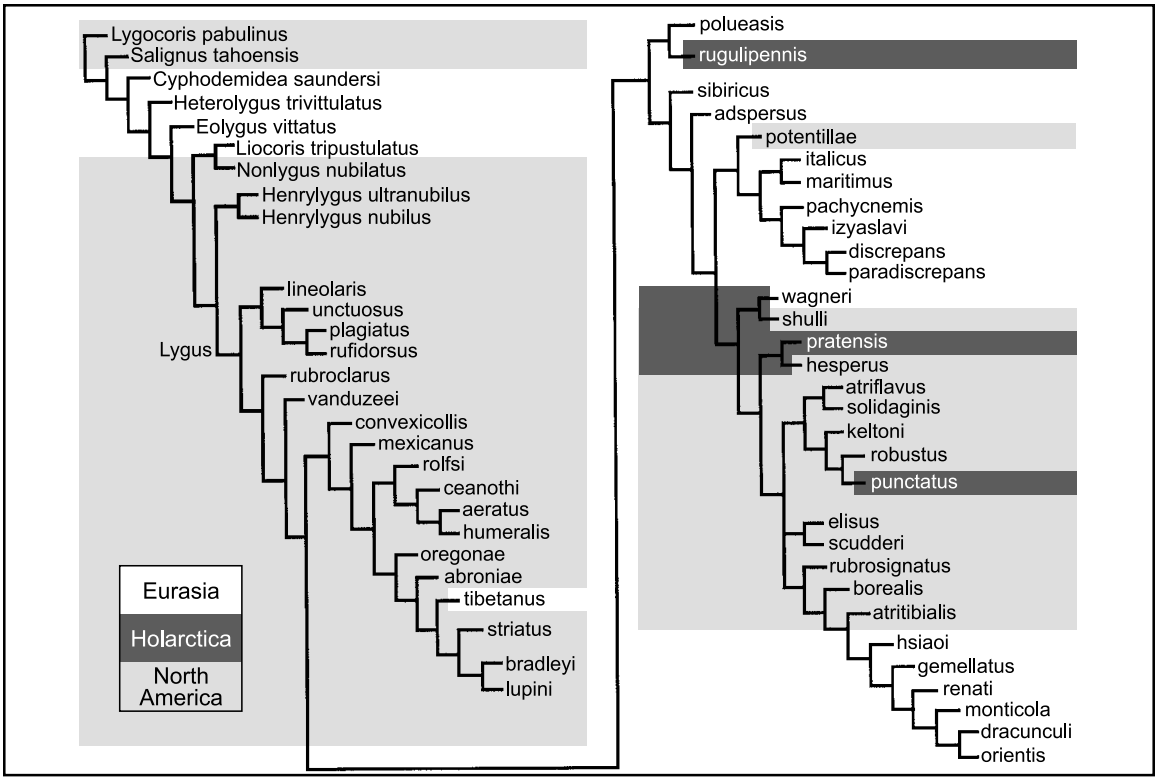

Fig. 1. Maximum fit cladogram of Lygus and outgroup taxa based on morphological and molecular characters with biogeographic placement (adapted from Schwartz and Foottit 1998).

morphologically difficult to separate (Loan, 1979), and this species complex also includes the Nearctic *Peristenus pseudopallipes* (Loan) (Loan and Shaw, 1987). Although *P. pseudopallipes* is nearly inseparable morphologically from *P. pallipes*, its temporal separation is evidence of species isolation (Loan, 1970*). Peristenus digoneutis*, *P. rubricollis*, and *P. conradi* belong to another complex of species that are difficult to separate morphologically (Loan and Bilewicz-Pawinska, 1973; Day et al., 1992), but *P. conradi* differs because it is deuterotokous or nearly thelytokous (Day et al., 1992). Goulet (pers. comm., 1999) reported that subsequent studies are confirming Loan's species concept for several *Peristenus* species, but numerous difficulties occurred when Loan's keys to species in Europe and North America were used by biological control experts and taxonomists. Recent studies by Goulet (*personal communication,* 1999) suggest that the European species *P. pallipes* could be a complex of up to 10 sibling species.

Biology of Peristenus. In general, *Peristenus* species attack mirid nymphs of the second or third instar and emerge from the fifth (last) nymphal instar or occasionally from the newly emerged (teneral) mirid adult. There are probably six *Peristenus* species that attack nymphs of *Lygus* and/or *Adelphocoris* species in Europe. Of these *P. digoneutis* and *P. stygicus* are known to be bivoltine (Bilewicz-Pawinska, 1982). *Peristenus rubricollis* is strictly univoltine, and *P. adelphocoris* appears to be univoltine (Carl and Mason, 1996). *Peristenus rubricollis* appears not to be well synchronized with either the first or the second generation of *A. lineolatus*; the peak of adult abundance probably falls between the two generations. Carl and Mason (1996) suggested that *P. rubricollis* might be better adapted to the trivoltine *L. rugulipennis* in Europe which may in fact be its major host. *Peristenus conradi* is univoltine and attacks *A. lineolatus* in northeastern United States (Day *et al.,* 1992). This species is considered to belong to the Palaearctic, but its distribution and area of origin is still obscure. The Holarctic species *P. pallipes* is considered to be univoltine in Europe as it attacks first generation plant bug nymphs (i.e., *A. lineolatus*) in early summer.

European Host-Peristenus Associations. A comprehensive study was carried out in Poland from 1966 to 1971 and continued from 1973 to 1977 (Coulson, 1987) to examine the role of braconid parasitoids in the regulation of populations of *L. rugulipennis* and other *Lygus* species, primarily in cereal crops. A complex of braconid parasitoid species was identified and reliable information was obtained on their mirid hosts in different habitats. Taxonomic studies in Canada carried out by Loan clarified the identity of *Peristenus* species in Europe (Loan and Bilewicz-Pawinska, 1973; Loan 1974a). Results of these studies are well documented in several publications by Bilewicz-Pawinska (1969; 1974a; 1974b; 1975; 1977; 1982). According to information in the CAB Pest CD (1973 – May 1999) there have not been any recently published studies on parasitoids of *Lygus* plant bugs in Europe, despite recent European work on *Lygus* parasitoids as documented in internal reports. Additionally, the most recent catalogue of parasitoids and predators of heteropteran hosts (Herting, 1971) has no information about *Peristenus-Lygus* relationships (results of the Polish study were published later). However, these catalogues might also contain errors by the original authors and the records require critical appraisal to distinguish reliable reports from doubtful or erroneous ones.

In Table 1, host associations of six European *Peristenus* spp. determined by analysis of reliable published information, are presented. Included are the *Peristenus* species, host species, reference, habitat, and country of record. According to the literature reviewed, the host ranges of the selected *Peristenus* parasitoids seem to be specific and are probably restricted to the family Miridae. Previously, Loan (1980) concluded that all of the *Peristenus* species for which host records exist have restricted host ranges. Some are monophagous and others attack a few Miridae species on the same plant or type of plant growth. In addition, Loan and Shaw (1987) characterized species in the genus *Peristenus* as specific to particular or few species in the Miridae. In the northeastern United States, Day (1999) recently demonstrated that the introduced European *P. digoneutis* parasitized principally only the first and second generation of *L. lineolaris. Peristenus digoneutis* was also found parasitizing small numbers of the Miridae *Trigonotylus coelestialium* (Kirkaldy) and *Leptopterna dolobrata* (L.) which have a Holarctic distribution (Day 1999).

Realized Host Range Evaluation in the Area of Origin

Information is often lacking on the realized host ranges of parasitoids in their area of origin. This is important to predict the potential risks, such as competition between introduced and native parasitoids, to non-target host species in the area of introduction. Knowledge of the hosts attacked by a candidate biological control agent (and its near relatives) in the area of origin can be used to select

Table 1. European *Peristenus*-Miridae host associations based on published information.

European *Peristenus*	Host (Miridae)	Reference (Habitat and Country)
Peristenus digoneutis	***Lygus rugulipennis***	Loan and Bilewicz-Pawinska, 1973 (Poland); Bilewicz-Pawinska, 1976 (potato, Poland); Bilewicz-Pawinska, 1977 (alfalfa, Poland); Bilewicz-Pawinska, 1982 (rye, barley, wheat, alfalfa, potatoes, clover, maize, Poland); Carl and Mason, 1996 (alfalfa, Switzerland and Germany)
	Lygus pratensis	**?***
	Adelphocoris lineolatus	Carl and Mason, 1996 (alfalfa, Switzerland and Germany)
Peristenus stygicus	***Lygus rugulipennis***	Bilewicz-Pawinska, 1977 (alfalfa, Poland); Bilewicz-Pawinska, 1976 (potato, Poland); Bilewicz-Pawinska, 1982 (rye, barley, wheat, oats, alfalfa, potatoes, clover, maize, Poland); Carl and Mason, 1996 (Switzerland, Germany)
	Lygus pratensis	**?***
	Adelphocoris lineolatus	Carl and Mason, 1996 (alfalfa, Switzerland and Germany)
	Polymerus unifasciatus	Drea et al., 1973 (asparagus, Turkey**)**
	Trigonotylus coelestialium	Bilewicz-Pawinska, 1982 (barley, wild growing grasses, Poland)
Peristenus rubricollis	***Lygus rugulipennis***	Bilewicz-Pawinska, 1977 (alfalfa, Poland); Bilewicz-Pawinska, 1982 (rye, barley, wheat, oats, alfalfa, Poland); Carl and Mason, 1996 (alfalfa, Switzerland and Germany)
	Lygus pratensis	**?***
	Adelphocoris lineolatus	Bilewicz-Pawinska, 1977 (alfalfa, Poland); Bilewicz-Pawinska, 1982 (alfalfa, Poland); Craig and Loan, 1987 (alfalfa, Austria);Carl and Mason, 1996 (alfalfa, Switzerland and Germany)
Peristenus conradi	***Adelphocoris lineolatus***	Day et al., 1992 (alfalfa, USA, but not in Europe so far)
Peristenus adelphocoridis	***Adelphocoris* sp.**	Loan, 1979 (France, Denmark)
	Adelphocoris lineolatus	Craig and Loan, 1987 (alfalfa, Austria); Carl and Mason, 1996 (alfalfa, Switzerland and Germany)
Peristenus pallipes	***Adelphocoris lineolatus***	Bilewicz-Pawinska, 1977 (alfalfa, Poland); Bilewicz-Pawinska, 1982 (alfalfa, Poland)
	Leptopterna dolobrata	Bilewicz-Pawinska, 1982 (rye, Poland)
	Trigonotylus coelestialium	Bilewicz-Pawinska, 1982 (wild growing grasses, Poland)
	Closterotomus norvegicus	Brindley, 1939 (UK) Bilewicz-Pawinska, 1982 (wild growing grasses, Poland)

**In studies by Bilewicz-Pawinska, occasional individuals* of *Lygus pratensis* (L.) *occurred among the larger number of L. rugulipennis but not in sufficient numbers to permit sampling for incidence of parasitoids.*

species from the area of proposed introduction for host specificity testing in quarantine. The *Lygus* case study discussed here includes research on developing strategies and testing methods for realized host range evaluation in the area of origin. To obtain such information, long-term surveys in the area of origin are needed to assess the effects of European *Peristenus* species on target and non-target mirid hosts in their native habitats. Such studies can define, at least qualitatively, the realized host ranges of candidate European *Peristenus* species.

Most of the central European species of Heteroptera belong to the family Miridae, which has 8 subfamilies. There are about 10,000 species worldwide (Kerzhner and Josifov 1999), of which 1,500 species occur in an area from northern France east to Russia to beyond the Black Sea, south to the middle of the Red Sea, and west to Spanish Sahara (Wagner, 1971). Therefore some simplifying assumptions were made to identify species of greatest relevance for field sampling to determine the realized host ranges of candidate *Peristenus* species. We assumed that:

- Host ranges of *Peristenus* species are restricted to the family Miridae, based on an analysis of the literature.
- Rare or endangered species of Miridae are not present in the area of origin.
- To be at potential risk, non-target mirids in the areas of origin and introduction must have similar climatic affinities, regional and habitat distributions, as the target pest species.
- European *Peristenus* species search for hosts belonging to Miridae in cultivated habitats (i.e., alfalfa, red clover, barley, etc.) but might also search for other mirids in non-cultivated habitats and that such habitats may contain a rich assortment of mirid species of interest; assessment of the insect complexes in various specific habitats allows evaluation of the diversity of species at potential risk, from which species should be chosen for testing.
- Phylogenetic relatedness can be used to select test species more likely or less likely to be attacked.
- Biological characteristics of non-target and target mirids are similar enough so that the host/parasitoid synchrony will provide opportunities for introduced *Peristenus* species to encounter non-target species.
- Non-target mirids chosen for field sampling should be common so that adequate numbers in replicated samples can be obtained to accurately measure parasitism.

Using these assumptions, appropriate mirid species can be selected for host specificity studies of European *Peristenus* species in the areas of origin and introduction. A clear understanding of the taxonomic identity (especially mirid nymphs) and basic life histories of the hosts and their associated *Peristenus* species is needed before valid host specificity tests can be carried out in the field. For some potential test species, the lack of such biological and ecological information may prevent the correct timing of collection of test species or may prevent the development of effective rearing methods needed for emergence of parasitoid adults (needed for identification).

Selection of *Lygus* spp. for testing is based on review of the literature. Schwartz and Foottit (1998) recognized 20 Palaearctic species and two Holarctic species of *Lygus*. Of these, only five Palaearctic (*Lygus wagneri* Remane, *L. pratensis*, *Lygus gemellatus* [Herrich-Schaeffer], *Lygus maritimus* Wagner, and *Lygus adspersus* [Schilling]), and two Holarctic species (*L. rugulipennis* and *Lygus punctatus* [Zetterstedt]), occur in the study area of central Europe. The European species *L. pratensis* and *L. wagneri* are closely related to the North American pest species *L. hesperus* (occurring in crop habitats as alfalfa and cotton) and *Lygus shulli* Knight (occurring in crop habitats as alfalfa, clover, potato, and several vegetables), respectively. These two European species, *L. pratensis* and *L. wagneri,* should be collected in their area of origin to determine if their parasitoids might be suitable candidate biological control agents based on the high degree of host relatedness and similarity of habitat. In addition, the Holarctic *L. rugulipennis* is common in European crop habitats and, therefore, shares habitat preferences with the North American key target *L. lineolatus* (occurring in crops as alfalfa, cotton, canola, vegetables, and fruits). None of the European *Lygus* species is closely related to the North American target species *L. lineolaris*.

Cultivated and non-cultivated habitats selected for surveying must be chosen based on ecological characteristics (e.g., field crops, mountain meadow, grassy fallow field) and the dominant flowering plants in each habitat must be identified. Samples of mirid nymphs and adults must be sorted to morphologically similar types, authoritatively identified, parasitoid cocoons obtained by rearing host nymphs, and the parasitoid cocoons reared to

obtain adult wasps for identification.

In Europe, field surveys were carried out in cultivated habitats, crops such as alfalfa, red clover, barley, and mustard as well as in non-cultivated habitats such as Swiss Mountain meadow (up to 1000 m) and fallow field (study by H. White, University of Manitoba, Canada, in collaboration with CABI Bioscience, Switzerland). During these surveys a total of 27 mirid species were found and studied for *Peristenus* parasitoid occurrence. Miridae collected belonged to 21 genera in 5 subfamilies: *Lygus*, *Adelphocoris, Polymerus, Leptopterna, Megaloceraea, Stenodema, Notostira, Calocoris, Closterotomus, Stenotus, Megalocoleus, Capsus, Lygocoris, Pithanus* (Mirinae), *Lepidargyrus, Amblytylus, Plagiognathus* (Phylinae), *Heterotoma, Orthocephalus* (Orthotylinae), *Dicyphus* (Dicyphinae), and *Deraeocoris* (Deraeocorinae). Mirid species richness was high with 21 species in the Swiss Mountain meadow, followed by fallow field and red clover with twelve mirid species each, alfalfa with eleven, seven in mustard, and four in barley. Interestingly, the number of *Peristenus* species reared from host mirids was not related to the mirid species richness/habitat or habitat. For example, *Peristenus* was reared from five host species in a non-cultivated habitat where 21 mirid species occurred, but three out of four mirid species collected in a cultivated habitat were parasitized by *Peristenus.* Individuals of the following five mirid genera are parasitized by three *Peristenus* species: *Lygus*, *Adelphocoris* by *P. digoneutis*, *Stenodema* by *P. stenodema*, *Closterotomus* and *Leptopterna* by *P. pallipes*, but the remaining 16 mirid genera are not attacked by *Peristenus* species.

European field surveys demonstrated that some mirid species, *L. pratensis*, *L. rugulipennis*, and *A. lineolatus,* are common and present in all habitats sampled. These mirid species are parasitized only by *P. digoneutis* and this parasitoid was not reared from other host mirid species collected in these habitats even when a high mirid diversity is present. It can be concluded that although the biological control agent *P. digoneutis* occurs in several different European habitats studied it appears to be specific, parasitizing only *L. pratensis*, *L. rugulipennis*, and *A. lineolatus.*

European Field Surveys Provide Basis for Laboratory Host Range Testing

Sampling mirids and their parasitoids in Europe provides an opportunity to obtain information about the diversity of mirids that live in different kinds of habitats and to assess the host ranges of their associated parasitoids. This information can then reveal much about the degree of host specificity that particular European mirid parasitoids might have. This information, which would be logistically very difficult to obtain by laboratory testing, can provide initial guidance about which *Peristenus* parasitoids might be specific enough to consider using as biological control agents for introduction into North America. Such field collecting, as a means to assess host ranges, might be useful for other studies in arthropod classical biological control. This approach narrows the host test list, avoiding the need to maintain a large number of potential host species often making testing programs impractical. It also resolves such potential practical problems related to test species as uncertainty about their identity, lack of information about their biology, or difficulty in their rearing.

Phylogenetic hypotheses for host and parasitoid groups can provide valuable insight when interpreting the significance of host range data. However, practical application of these methods for selection of appropriate non-target species for physiological host range testing of entomophagous biological control agents is not useful. This approach may result in overestimating host range because, as shown above, parasitoids may attack a few species that are not closely related even though they occur in the same habitat. Thus, good biological control agents would not be considered for further evaluations. Use of ecological information obtained in European habitats will reduce the number of mirid species that must be tested. Field surveys for target and non-target mirids are necessary to assess the diversity of mirid species present in the proposed area of importation so that only appropriate non-target species will be collected in selected habitats and reared for testing in the laboratory. In conclusion, the realized host range of *Peristenus* parasitoids is a reasonably good predictor of host range in the area of introduction and should be always considered as an important step in classical arthropod biological control projects.

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