INFLUENCE OF EXPERIENCE ON THE FORAGING BEHAVIOUR OF *APHELINUS ABDOMINALIS*

By

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Influence of experience on the ~~foraging~~ foraging behaviour of *Aphelinus abdominalis*

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Abstract

Parasitic wasps have been shown to learn to associate various odors with their hosts. As a result, there are concerns as to how this learning ability can best be exploited in pest management. I evaluated this phenomenon with regard to biological control of aphids in greenhouses. Females of the aphid parasitoid *Aphelinus abdominalis* Dalm. (Hymenoptera: Aphelinidae) were given various oviposition experiences and then tested for their response to the odours of the foxglove aphid (*Aulacorthum solani*) + pepper plant complex and the cereal aphid (*Sitobion fragariae*) + wheat plant complex in a dual choice Y-tube olfactometer. The treatment groups were as follows: no oviposition experience (reared on *Myzus persicae*), oviposition on pepper plant complex only, oviposition on wheat complex only, oviposition on pepper plant complex and then wheat complex, oviposition on wheat complex and then pepper plant complex, no oviposition (reared on *A. solani*) and no oviposition (reared on *S. fragariae*). There were no significant differences ($P > 0.05$) found in the odour choices of any of treatment groups. A computer model was also used to simulate the population dynamics of *A. solani* on pepper plants in a greenhouse that uses *A. abdominalis* in conjunction with banker plants. The following types of learning were tested in the model: no learning, no interaction between learning events, transfer, proactive interference and retroactive interference. No significant differences were found between any of these types of learning at any of the three learning strengths tested (10%, 20% and 30%). The model was also used to look at the effect of using different numbers of banker plants within the greenhouse. It was found that using 2 banker plants reduced the population of foxglove aphids significantly more than using only 1 banker plant ($P < 0.0001$).

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Introduction

Parasitoid Foraging Behaviour

Parasitoids are insects that have a parasitic phase of their life cycle while they are immature. During this parasitic phase they rely on their insect host for survival but as the relationship continues they kill their host. Due to the one sided benefits of the parasitoid – host relationship there is strong selection pressure on hosts to avoid detection by parasitoids while there is equally strong pressure on parasitoids to develop methods of finding their hosts efficiently. It has been known for some time that parasitoids do not simply search for hosts in a random manner. Vinson (1976) broke down the process of host foraging into 5 categories: host habitat location, host location, host acceptance, host suitability, and host regulation. It has been found that volatile chemicals play a major role in all of these steps (Lewis and Martin 1990). These volatiles are generally derived from either the host themselves, the plants on which the host lives or, most commonly, a combination of the two. Parasitoids use these volatiles to locate their sometimes cryptic hosts.

Importance of Semiochemicals

Parasitoids interact with their hosts in a tritrophic context. The parasitoid itself constitutes the third trophic level, the host is the second level and the plant on which the host feeds is the first level. Parasitoids use semiochemicals from the first and second trophic levels to gain information about where to forage for hosts (Vet and Dicke 1992). Semiochemicals are the chemicals that mediate interactions between organisms (Nordlund & Lewis 1976). Semiochemicals can be subdivided into two categories, pheromones and allelochemicals. Pheromones are chemicals used in intraspecific interactions and allelochemicals are chemicals used in interspecific interactions (Nordlund & Lewis 1976). Allelochemicals can be further broken down into three subcategories depending on whether the emitter or the receiver of the chemical benefits from the information it conveys. Allomones are allelochemicals that benefit the emitter, kairomones benefit the receiver and synomones benefit both the emitter and receiver (Nordlund & Lewis 1976). It is important to note that these terms are context specific not

chemical specific (Vet & Dicke 1992). For example a chemical that is used as a pheromone between herbivores of a single species may in turn be used by a parasitoid as a kairomone.

The vast majority of parasitoid host foraging studies have looked at some aspect of how parasitoids use semiochemicals to guide their search for hosts (Du et al. 1996, Rao 1999, also see reviews: Lewis and Martin 1990, Turlings et al. 1993, Vet and Dicke 1992, Vinson 1976). Chemicals from the first trophic level (i.e. plant volatiles) are generally the most abundant, and as a result tend to guide host foraging, especially at longer distances. In contrast, chemicals from the second trophic level (i.e. host pheromones) tend to be less abundant, and thus only guide host foraging at very close range (Vet & Dicke 1992). However, since most hosts can feed on more than one plant species, and on different structures of the same plant, many different plant volatiles could indicate the presence of a host. As a result, these volatiles, while plentiful, are not necessarily reliable indicators of host presence (Vet et al. 1995). Thus it would not be advantageous for a parasitoid to have an innate response to all of these volatiles. Instead parasitoids use learning to narrow the number of volatiles they respond to and utilize only those compounds that their hosts are presently associated with. This allows the parasitoids to search for hosts more efficiently (Turlings et al. 1993, Vet et al. 1995).

Plant volatiles have been found to be so important to parasitoid host foraging that some researchers hypothesize that plants actively call parasitoids and predators when they are under attack by a herbivore (Turlings et al. 1990). Different plant species attacked by the same herbivore species emit different volatile blends and the same plant species attacked by different herbivore species also emit different volatile blends (De Moraes et al. 1998, Dicke et al. 1990, Du et al. 1998 and Turlings et al. 1993). Du et al. (1998) found that *Aphidius ervi* was most responsive to the chemical 6-methyl-5-hepten-2-one. They also found that this chemical is released from broad bean plants, *Vicia faba*, that have been damaged by host aphids, *Acyrthosiphon pisum*, but is not released when the plants have been damaged by nonhost aphids, *Aphis fabae*. Additionally, De Moraes et al. (1998) found that tobacco, cotton and maize plants all produce different blends of volatiles in response to damage by two closely related insect herbivores, *Heliothis virescens* and *Helicoverpa zea*. Furthermore, the parasitoid *Cardiochiles nigriceps*, which parasitizes *H. virescens* but not *H. zea*, is able to distinguish between these volatile blends and can use these cues to find its host. This specificity in the volatile cues allows for more information to be gleaned by the parasitoid; instead of the volatiles simply telling a

parasitoid that a plant is under attack the parasitoid is able to discern what species of plant is under attack by what species of herbivore. By using this information parasitoids are able narrow their search to plants that are infested with appropriate hosts. In this context the plant volatiles act as synamones by benefiting both the emitter (the plant) and the receiver (the parasitoid). This signaling role of plant volatiles has most likely evolved secondarily from defense responses of plants that produce toxins and deterrents to herbivores (Turlings et al. 1995).

Visual Cues

In addition to olfactory cues parasitoids have also been found to utilize visual cues when foraging for hosts (Turlings et al. 1993). Parasitoids can utilize visual cues from their hosts and the host habitat. Because visual cues are not altered by wind currents, unlike olfactory cues, they may in some ways be a more reliable indicator of the direction and distance of the host. However, the major problem with visual cues is that they are easily hidden by physical barriers that may restrict their use by parasitoids foraging for hosts (Turlings et al. 1993). Nevertheless it has been demonstrated that parasitoids can learn both the color of rewarding host microhabitats (Oliai and King 2000, Schmidt et al. 1993, Stireman 2002, Wardle 1990) and their form (Wardle and Borden 1990). There is a distinct lack of research in this area of parasitoid host foraging and much more work needs to be done before we truly understand the role that visual cues play foraging behaviour and how these cues are used in conjunction with chemical cues.

Parasitoid Learning

Individual variation has been found in response of parasitoids to host-related cues and this variation has been attributed to a number of genetic, environmental, physiological and experiential factors (Poppy et al. 1997). While all these factors are no doubt important this review focuses on the experiential factors. Experience that alters a parasitoid's host-foraging behaviour can broadly be referred to as learning (Stephens 1993). Papaj & Prokopy (1989) recommended the use of 3 criteria to define learning: 1. Behaviour changes in a repeatable way as the result of experience. 2. Changes in behaviour are gradual, with continued experience, up to an asymptote. 3. Learned responses wane in the absence of continued experience or as the result of a new experience. Unfortunately these criteria, while useful in defining most learning events, can sometimes exclude events that are indeed examples of learning. For instance the single-trial learning that is often characteristic of parasitoids would be excluded if these criteria

were used rigidly (Vet et al. 1995). Also, according to this definition, behavior must change when learning occurs. An animal that has learned something about its environment but chooses not to alter its behavior would be defined as having not learned. As a result Papaj and Prokopy (1989) suggest using the criteria with caution and to date no one has proposed a more agreeable definition for parasitoid learning.

If we put the debate on defining learning aside there is an important distinction to be made between 4 separate phenomena, all of which result from previous experience but only one involves actual learning. These four phenomena are habituation, sensitization, priming and preference learning. Habituation refers to the waning of a response to a stimulus due to repeated exposure to the stimulus (Papaj and Prokopy 1989). Sensitization refers to an increase in response to a stimulus with repeated exposure but it does not require that the stimulus be paired with another unconditioned stimulus (Papaj and Prokopy 1989) Priming refers to the process where individuals contact an innately recognized stimulus and immediately become more receptive to other cues that were not necessarily present during the experience (Turlings et al. 1993). And finally, preference learning refers to the process by which individuals increase their responsiveness to the specific stimulus or stimuli that were present during the experience (Turlings et al. 1993). Of these four phenomena it is preference learning that has commanded a great deal of the attention of behavioural ecologists studying parasitoid foraging behaviour.

Associative Learning

Preference learning in parasitoids is accomplished via associative learning whereby an association between two stimuli is established through experience (Turlings et al. 1993). For this to occur an unconditioned stimulus (US) that elicits an unconditioned response (UR) is temporally and spatially paired with a conditioned stimulus (CS). The CS does not initially evoke a response resembling the UR but after repeated experience it acquires the capacity to evoke a conditioned response (CR) that is similar to the UR.

Over the past 20 years many parasitoid species have been shown to be capable of associative learning (see reviews by Turlings et al. 1993, Vet et al. 1995). Parasitoids use associative learning to pair stimuli that they innately recognize (US), generally host derived stimuli, with environmental stimuli that they encounter at that time and location, to which they show little or no innate response (CS). After detecting the environmental stimuli in conjunction

with the host derived stimuli the parasitoid learns to respond to the environmental stimuli as it would the host-derived stimuli. Thus the parasitoid can use the environmental stimuli in subsequent host foraging expeditions. As mentioned earlier there is strong selection pressure on hosts to avoid detection by parasitoids. As a result highly reliable host derived cues tend to be hard for the parasitoid to detect. However, associative learning can be used to pair hard-to-detect but highly reliable cues with less reliable but highly detectable cues, thereby making the less reliable cues more reliable (Vet et al. 1995).

When Does Learning Occur?

Given that many parasitoids are capable of associative learning one can ask the question, "When does learning occur?". Hopkins (1917) was the first to note that insects preferred oviposition sites that were similar to the sites that they had been reared on. It was hypothesized that this preference was the result of larval experience with the host plant and that this experience was retained and its effects displayed in the adult insect. This idea became known as Hopkins' host selection principle. Thorpe and Jones (1937) also demonstrated this type of larval learning, which they called pre-imaginal conditioning, in the parasitoid *Venturia (Nemeritis) canescens* (Grav.). Since then, a number of studies have shown *prima facie* evidence for larval learning, however none have proven it exists (Kaiser et al. 1989b and review by Turlings et al. 1993). Corbet (1985) hypothesized that the reorganization of the nervous system, which occurs during the pupal stage, would make the retention of larval experiences unlikely. Instead it was proposed that the learning of host cues most likely would take place in the adult insect as it comes into contact with host cues upon emergence. This type of early adult learning could easily occur in aphid parasitoids, as they emerge by chewing their way out of the dead host and as a result could come into contact with host cues. *Aphidius rophalosiphi*, a parasitoid of cereal aphids, has been shown to prefer the wheat variety on which it has developed over other wheat varieties (Wickremasinghe and van Emden 1992). However, when *A. rophalosiphi* pupae were dissected out of their hosts and the adults allowed to emerge in the complete absence of host products they showed no preference for the wheat variety on which they had been reared (van Emden et al. 1996). A similar result was found in *Aphytis melinus*, a parasitoid of California red scale, *Aonidiella aurantii* (Hare 1996). This indicates that the parasitoids learn the cues of the host they have been reared in at the time of adult emergence, which supports the hypothesis made by Corbet (1985). As a result, earlier studies that appeared to support Hopkins' host selection

hypothesis of larval learning were likely not controlling adequately for adult learning at the time of emergence and thus really demonstrated early adult learning instead.

By ruling out larval learning we are left to assume that, unless undisputable evidence emerges, all the parasitoid learning that has been demonstrated thus far has occurred in the adult stage. Indeed the bulk of parasitoid learning research has focused on demonstrating learning in the adult by pairing a conditioned stimulus with oviposition as the reinforcing stimulus (Bjorksten and Hoffmann 1998, Du et al. 1997, Molck et al. 2000, Nurindah 1999, Papaj and Vet 1990, Perez-Maluf and Kaiser 1998, Turlings et al. 1993, Vet et al. 1995). It is not surprising that the phenomenon of learning seems to be widespread in adult parasitoids, but virtually nonexistent in the larval stage, if we look at learning in an ecological context. Parasitoids learn in order to make use of the most current information about where suitable hosts can be found and any information they would learn as an adult would be more relevant than information learned as a larva.

The experience treatments used in parasitoid learning studies have been quite varied. While many studies have used actual ovipositions as the experience (Bjorksten and Hoffmann 1998, Du et al. 1997, Molck et al. 2000, Nurindah 1999, Papaj and Vet 1990, Perez-Maluf and Kaiser 1998) others have shown that learning can occur without any physical contact with the host by simply exposing the parasitoid to host products such as frass (Lewis & Martin 1990, Lewis & Tumlinson 1988, Turlings et al. 1993 and Vet & Groenewold 1990). Additionally, learning can occur very rapidly and has been demonstrated after a single oviposition (Poolman Simons et al. 1992) or just a 20 second experience with a plant-host complex (Turlings et al. 1989).

Retention of Learned Response

While many parasitoid learning studies have demonstrated that learning occurs few studies have looked at how long the learned response persists. Learning of host-specific stimuli was shown to persist, without reinforcement, for up to 7 days in *Eupelmus vuilleti* (Cortesero et al. 1995) and up to 5 days in *Trichogramma* nr. *brassicae* (Bjorksten & Hoffmann 1998) but learning has also been shown to be very short lived in *Trichogramma maidis*, lasting less than 10 minutes (Kaiser et al. 1989a). Other studies have found that without periodic reinforcement parasitoids tend to 'forget' what they have learned (Papaj and Vet 1990). Additionally, it has

been shown that unrewarding experiences, such as failing to find suitable hosts in a previously rewarding habitat, can reverse the effects of learning in the Drosophila parasitoid, *Leptopilina heterotoma* (Papaj et al. 1994). This type of negative experience has also been found to play a role in the learning of odours associated with food by the larval parasitoid *Microplitis croceipes*, which can learn odours associated with food but when it subsequently experiences these odours in the absence of food the response to the learned odour ceases (Takasu and Lewis 1996). The waning of a learned response, in the absence of continued experience or as the result of negative experience, is part of Papaj and Prokopy's (1989) definition of learning. If we think about parasitoid learning in an ecological context waning of learned responses is a logical part of the learning process as parasitoids must learn cues that lead them to their current hosts but should cease to respond to the same cues when that host is no longer available.

Memory

In addition to the lack of information on the retention of learned responses there is also very little known about the dynamics involved when a parasitoid must learn more than one thing. Cortesero et al. (1995) demonstrated that *Eupelmus vuilleti*, a parasitoid of Bruchidae larvae which develop in the seeds of Leguminosae, can learn the cues of the host they are reared from while they are still in the host larval chamber, just prior to emergence. The response to these odors are retained even when the parasitoid experiences a subsequent oviposition on a different species of host living in a different seed species. Additionally, Bjorksten and Hoffmann (1998) demonstrated that the egg parasitoid *Trichogramma* nr. *brassicae* could retain the memory of two species of host when given successive oviposition experiences in each. The ability of parasitoids to remember cues for more than one species has also been found to apply to learning of odors associated with food sources (Takasu and Lewis 1996). Despite these findings it is likely that there are constraints on parasitoid memory resulting in a limited ability to recall past experiences, as is the case with honey bees foraging for nectar (Menzel et al. 1993). It is possible that parasitoids may only be capable of retaining in their memory a finite number of items and if more items are learned then the parasitoid will be forced to 'forget' a previously learned item.

There has been some research that has investigated the memory of pollinators foraging for nectar in flowers, in an attempt to determine why pollinators tend to display flower constancy (Stanton 1984, Laverty 1994, Woodward and Laverty 1992, Waser 1986). Flower constancy is

the tendency for a pollinator to restrict the flowers it forages on to one species or type even when other rewarding flowers are available (Waser 1986). Darwin (as referenced in Waser 1986) was the first to hypothesize that flower constancy was due to the cost of learning how to handle new flowers. Since then a number of researchers have tested this hypothesis. Lewis (1986) showed that the cabbage butterfly, *Pieris rapae*, was able to learn how to extract nectar from flowers and that learning how to handle a second species of flower interfered with the recall of the previously learned species. Similarly, Waser (1986) found that in bumblebees, flower constancy increased with an increasing difference in flower morphology, which indicates that the cost of learning how to forage on a new type of flower is high enough to make it profitable for a bee to restrict its visits to the type of flower it has already learned. Woodward and Laverty (1992) and Laverty (1994) found that learning how to forage on a new species of flower did indeed interfere with a bee's ability to recall how to forage on previously learned flower species. In both cases, however, the interference effects were small and unlikely to be the sole cause of flower constancy. Dukas (1995) demonstrated that bumblebees foraging on artificial flowers initially showed a reduction in performance when required to switch from foraging on rewarding flowers of one color to another. This interference disappeared, however, when the bees were allowed to switch back and forth between the two rewarding flower types. Dukas (1995) points out that his experiment lacks the dimension of difficulty; all the flowers were artificial and required the same amount of skill in handling. It is possible that interference may be correlated to handling skill and as a result more interference may be seen when bees handle increasingly difficult flowers. Flower complexity has been shown to affect bumblebee performance (Laverty 1994) so perhaps with the added dimension of difficulty interference may indeed reduce the ability of bees to perform sequential switching. While it is still unclear whether flower constancy is due to constraints on learning or some other processes it is evident that there are complex dynamics involved when a foraging insect must learn information about more than one species.

There are two ways in which interference could affect memory. Retroactive interference refers to the case where the learning of new information interferes with the recall of old information and proactive interference refers to the case where the memory of old information interferes with the learning of new information (Spear and Riccio 1994). Depending upon the magnitude of their effect both these types of interference could make it too costly for an individual to forage for more than one species at a time (Dukas 1998).

Opposite to interference is the idea that learning information about one experience could make it easier for an individual to learn information about another, related, experience (Dukas 1995, Singley and Anderson 1989). Honeybees, *Apis mellifera*, have been shown to be capable of this type of transfer of learning when they are given a pre-training session with stimuli that are less similar and then required to learn to discriminate between more similar stimuli that vary in the same dimension (Walker et al. 1990, Zhang and Srinivasan 1994). However transfer was not found in bumblebees, *Bombus occidentalis*, when they were required to learn to distinguish rewarding from non-rewarding artificial flowers on the basis of color (Dukas 1995). If insects are capable of transfer of learning this could effectively reduce the cost of learning new cues and thus make foraging more efficient.

Who Should Learn and Why?

Although learning of host cues seems to be the rule rather than the exception for insect parasitoids (Turlings et al. 1993) the fact that, in some cases, learning has not been found (Potting et al. 1997) begs the question: Who should learn and why? It was once believed that learning should be more common in generalist parasitoids than specialists (Kaiser et al. 1989b). The idea behind this thinking was that generalists would benefit from learning to concentrate on the most rewarding habitats while specialists would have such a narrow host range that it would be more beneficial for them to have innate responses to their hosts and host habitat. It has, however, been found that some specialist parasitoids do indeed possess the capacity to learn (De Moraes et al. 1998, Poolman Simons et al. 1992). Poolman Simons et al. (1992) conducted a rigorous study comparing the learning abilities of 3 closely related eucoilid parasitoids, one of which was a generalist (*Leptopilina heterotoma*) and the other two were specialists (*Leptopilina boulardi* and *Leptopilina fimbriata*). They found that the ability to learn was present in all three species but that the way in which learning was used was different. Previous oviposition experience increased patch residence times in the generalist parasitoid. However, for the specialists previous experience only altered patch times on the alternative (less preferred) substrate and these patch times never exceeded those found on the natural (most preferred) substrate. These findings lead the researchers to hypothesize that generalists may use learning to broaden the types of habitats they forage in while specialists use learning to temporarily divert their foraging efforts to less preferred habitats when there are no other options.

Given that both generalist and specialist parasitoids can learn, we are still left with the question of who should learn and why. Two key factors play a major role in determining whether or not it is adaptive for parasitoids to use learning in host foraging. These factors are the frequency of foraging decisions (Roitberg et al. 1993) and the predictability of the foraging environment (Stephens 1993). Roitberg et al. (1993) used a model to demonstrate that learning was most important to organisms that are required to make many foraging decisions each of which have a relatively minor impact on lifetime fitness while learning is less important to organisms that are only required to make a few foraging decisions each of which have a large impact on lifetime fitness. Their model showed a large fitness cost associated with a short memory, as opposed to both a long memory and no memory, and this cost was most pronounced in those individuals making a few large foraging decisions. Thus the cost of evolving a short memory would likely prohibit species that make only a few large foraging decisions from evolving the ability to learn. Stephens (1993) also used a model to determine the effects of environmental predictability on the adaptive value of learning. He found that learning was not adaptive in highly predictable environments nor was it adaptive in highly unpredictable environments, instead it was adaptive in environments that were moderately predictable. In fact his model broke down environmental predictability into between-generation and within-generation predictability and found that learning was most adaptive when the environment changes between generations but remains relatively constant within a generation. These theoretical studies are supported by two empirical studies which found learning to be absent from the parasitoid, *Cotesia flavipes* (Potting et al. 1997) and the butterfly, *Euphydryas editha* (Parmesan et al. 1995), both of which live in predictable environments and make few foraging decisions.

Modeling of Parasitoid Foraging Behaviour

In addition to the models mentioned above there has been a lot of theoretical work done to try and elucidate some of the criteria used by parasitoids to make decisions about where and when to forage for hosts (see review by Godfray 1994 and van Alphen et al. 2003). Early models of parasitoid host foraging behaviour were rate maximization models (Charnov and Stephens 1988). These models were based on optimal foraging theory and the marginal value theorem (Charnov 1976). They essentially assumed that parasitoids behave in a way that maximizes their fitness and treated this behaviour as fixed. The models then varied the attributes of the patches

within which a parasitoid could forage for hosts and made predictions about acceptance of different host types and timing of patch departure. The problem with these models is that they did not take into consideration the changing state of the parasitoid, with respect to egg load, mortality, age, experience, etc.

More recent models have used a dynamic-state-variable approach to make predictions about parasitoid foraging behaviour (Mangel 1989). These models take into consideration the changing state of the parasitoid and use this information to determine the dynamics of things such as host acceptance and patch residence time. In these models parasitoids are assumed to make optimal foraging decisions in order to maximize their lifetime fitness and these decisions are flexible and can change depending on factors such as current egg load, mortality risk, age, experience, etc. By approaching foraging decisions in this dynamic way one can predict how a parasitoid's foraging behaviour will change in various circumstances.

One of the biggest pitfalls of the rate maximization models is that they assume host acceptance by a parasitoid is absolute: parasitoids should either always accept a particular host type or never accept it (Mangel 1989). The dynamic-state-variable models overcome this problem by allowing for partial host preferences. This means that under some conditions a particular host type will be accepted but under different conditions it will be rejected. Partial host preferences are influenced by the state variables included in the model, such as egg load, mortality risk and even information state (Li et al.1993 and Mangel 1989). This dynamic approach to the modeling of parasitoid foraging behaviour allows for much more realistic predictions to be made about parasitoid foraging decisions.

Over the past few years many models of parasitoid foraging behaviour have been built but few of these models have been applied to practical situations (Krebs and Kacelnik 1991 and Roitberg (in review)). Many problems in biological control could be first approached by building a theoretical model of the system and then testing the predictions made by the model empirically. Doing this would provide a strong basis of knowledge with which to not only choose the most appropriate biological control but also apply it in the most effective manner.

Application of Parasitoid Learning to Pest Management

Most parasitoids used in biological control programs are mass reared. It is of some concern that these mass reared insects may learn the host cues associated with the artificial

rearing environment and thus be less effective when released in the natural environment (Prokopy and Lewis 1993). In fact Wardle and Borden (1986, 1991) showed that prior experience of the parasitoid *Exoristis roborator* with an artificial rearing environment decreased its ability to find hosts in the natural environment. By contrast, it has been suggested mass reared parasitoids could be given some type of experience with the target host, either exposure to host derived products or a complete oviposition experience, prior to release in order to reverse any effects of the artificial rearing environment and increase the parasitoid's efficiency in the natural environment (Gross et al. 1981, Prokopy and Lewis 1993). Although this type of training of mass reared parasitoids has been suggested by many researchers it has not to my knowledge been tested extensively in the field, so it remains unknown what type of effect it may have on the efficacy of biological controls in real life situations.

The effectiveness of biological control programs is undoubtedly influenced by the environment into which the parasitoids are released. Recently, Perfecto and Vet (2003) looked at how parasitoid learning and the presence of nonhost plants in a diverse agroecosystem might effect the foraging ability of two parasitoid species, *Cotesia glomerata* and *Cotesia rubecula*. *C. glomerata*, a generalist parasitoid, initially showed a decrease in its foraging efficiency in the diculture as compared to the monoculture. However, this decrease disappeared when the parasitoid had previously experienced the diculture system. This implies that the parasitoid is able to use learning to enhance its ability to discriminate between the nonhost plant and host plant odours. In contrast, *C. rubecula*, a specialist parasistoid, showed an increased foraging efficiency in the diculture system and this increase was not effected by experience. This research indicates that learning may enhance the foraging efficiency of generalist parasitoids in diverse agroecosystems but perhaps not that of specialist parasitoids. Information about a parasitoid's foraging behaviour in different types of environments is important to growers who are contemplating using biological controls as well as cultural controls such as intercropping, which is the practice of planting more than one crop in the same area (Prokopy and Lewis 1993).

Currently pest management practices are carried out without much regard to the impact parasitoid learning may have on them. Generally this oversight has not been a problem as most pest management practices that are routinely used are effective, otherwise they would not be employed. However, it is possible that with further knowledge of parasitoid learning and how it

pertains to pest management we could improve the efficacy of current practices and possibly develop new ways in which to control various pest species.

Integrated Pest Management of Aphids in Greenhouse Vegetable Crops

Overview of the Greenhouse Industry

Greenhouse production covers about 300,000 ha of land worldwide, with 195,000 ha of this land producing vegetables and 105,000 ha producing ornamentals (van Lenteren 2000). Greenhouse agriculture is a highly productive growing system capable of producing large quantities of top quality produce on very little area. For example, in The Netherlands only 0.5% of the total agricultural land is occupied by greenhouses but this area accounts for approximately 20% of the country's total agricultural production (van Lenteren 1995). Because the greenhouse growing system is so intense production costs are relatively high, as compared to field crops. As a result, greenhouse growers are unable to tolerate even low amounts of crop damage due to pests. Consequently, any pest management strategy that is used must be reliably effective.

Until the 1960s pest management tactics in the greenhouse were largely chemically based (van Lenteren 2000). However, the occurrence of pesticide resistance in the 1960s, 70s and 80s lead growers to consider other control options (van Lenteren 1995). The emerging pest control strategy at the time was integrated pest management (IPM). IPM has been defined in many ways but perhaps one of the most broad definitions would be that it is a pest management strategy that uses all suitable techniques in an integrated way to reduce and maintain pest populations below the level at which they cause economic injury (van Lenteren 1995). This shift from mainly chemical control strategies to a broader IPM strategy was quickly adopted by the greenhouse industry.

One of the cornerstones of virtually any IPM strategy is biological control. Biological control can be very broadly defined as the use of living organisms, or their products, as pest control agents (Dent 1995). The idea of biological control was not new to the greenhouse industry. In the 1920s the whitefly parasite, *Encarsia formosa*, was discovered in Great Britain and within a few years of its discovery it was being used by several hundred greenhouses in Great Britain to control the greenhouse whitefly, *Trialeurodes vaporariorum* (van Lenteren

1995). Use of *E. formosa* as a biological control of the greenhouse whitefly spread from Great Britain to other parts of the globe and was used until the 1930s when it was replaced by the new and easy to use chemical insecticides that were coming onto market (van Lenteren 1995). Perhaps it was this long forgotten legacy of biological control that has enabled greenhouse growers and researchers alike to embrace biological control more quickly than most other cropping systems.

The greenhouse environment is, in many ways, ideally suited for the use of biological controls (see van Lenteren 2000). The isolated nature of the greenhouse unit means that fewer pest species are able to immigrate into the crop and any biological control agents released in the crop will be more or less contained. In conventional growing systems pesticides can drift from neighboring fields and reduce the efficacy of biological controls but in the greenhouse environment this problem is virtually non-existent. Greenhouses are routinely cleaned at the end of the growing season, which reduces the amount of pest carryover from one season to the next. And the growing environment in the greenhouse is tightly controlled, with respect to temperature, light and humidity, which can greatly aid in the establishment and survival of biological control agents. These factors, combined with the lack of a better alternative, have lead to widespread acceptance of biological control as a key part of IPM in the greenhouse industry. Today, The Netherlands leads the way in greenhouse IPM with more than 90% of its greenhouse vegetable production following some sort of IPM strategy (van Lenteren 2000). The rest of the world lags behind, with only 5% of the total worldwide greenhouse area using IPM. However, with development of new biological control agents for this industry and with the rapid increase in biological control suppliers this percentage will likely increase dramatically in the next few years.

Aphid Pests in Greenhouses: Biology and Ecology

Aphids are one of the biggest pest problems in the greenhouse vegetable industry (Rabasse and Wyatt 1985). Aphids are small, soft-bodied insects that feed on the phloem sap of plants. Most aphid species reproduce by parthenogenesis during the summer months and then switch to a different host plant in the winter where they reproduce sexually, for at least one generation (Dixon 1998). However, in the greenhouse aphids never switch to their winter host and, as a result, continue to reproduce by parthenogenesis year round (Rabasse and Wyatt 1985). Aphids are somewhat unique among insects in that they give birth to live young. These live

young develop quickly and usually reach adulthood within about 1 week of being born (Dixon 1998). Since parthenogenic females do not require fertilization of their eggs development starts immediately after ovulation. As a result, a nymph can have embryos developing within itself and these embryos can also have embryos developing within themselves. This telescoping of generations provides aphids with an extremely high reproductive rate (Dixon 1998).

Aphids are also well adapted for dispersal. Adult aphids can be found in two different forms, or morphs. The wingless morphs, apterae, disperse over short distances, such as to another part of the same plant or other nearby plants, by walking. The winged morphs, alatae, disperse over large distances, such as to a new plant or field, by flying (Dixon 1998). When a colony first becomes established more apterae then alatae are produced. However, as soon as the colony starts to become crowded alatae are produced in higher numbers. The decision to produce apterae or alatae is made either just as the embryo is released from the ovariole or early on in the postnatal development of the nymph, depending on the aphid species (Dixon 1998). In either case the decision is made in response to factors such as crowding, decreased food quality or a reduction in day length. Since aphids develop so quickly this change in morph frequency can occur quite rapidly. This ability to disperse, combined with a very high reproductive rate, make aphids a difficult pest to control.

There are four aphids species that are common pests in greenhouses. They are: *Aphis gossypii*, *Myzus persicae*, *Macrosiphum euphorbiae* and *Aulacorthum solani* (van Schelt 1994). These aphids can enter the greenhouse by coming in on propagation material or directly from the outside environment via the vents that are opened and closed to maintain temperature and humidity (Bethke and Paine 1991). Once inside the greenhouse aphids multiply rapidly.

High densities of aphids can suppress plant growth and thereby cause a reduction in crop yield. Aphids are also a vector for a number of plant viruses. However, the biggest problem with aphid infestations in the greenhouse is due to aphid honeydew, the sticky excreta produced by aphids feeding on phloem sap. This honeydew drips onto the lower parts of the crop where it promotes the growth of sooty mold (Rabasse and Wyatt 1985). Honeydew can also contaminate the fruit making it necessary for the fruit to be washed before it is packaged and shipped which causes an increase in production cost.

History of Aphid Control in the Greenhouse

Controlling aphids in the greenhouse has always been problematic due to their extremely high rate of reproduction and the favourable environmental conditions that the greenhouse provides. In the 1950s aphids were managed primarily through the use of broad-spectrum insecticides (van Lenteren 2000). However, with the introduction of biological control for the two spotted spider mite, *Tetranychus urticae*, and the greenhouse whitefly, *Trialenrodes vaporariorum*, in the late 1960s and early 1970s there became an increasing need to control aphids without the use of the broad-spectrum insecticides (Ramakers 1989). This need was filled in the early 1970s by the registration of pirimicarb, a carbamate insecticide that is highly selective for aphids (Ramakers 1989). However, due to the frequent applications of this insecticide that were needed to keep the aphid population under control resistance began to appear in the late 1980s (Furk and Hines 1993). At this point growers were once again faced with the dilemma of having to use a broad-spectrum insecticide to control aphids while at the same time using biological controls to manage other pest problems. This dilemma led to a great deal of research into potential biological controls for aphids in the greenhouse.

Widespread use of aphid biological control began in The Netherlands in 1988 with the introduction of the predatory midge, *Aphidoletes aphidimyza* (van Schelt 1994). While the results with *A. aphidimyza* were promising, adequate control was not always achieved, especially at low pest densities. By the early 1990s growers began to use the aphid parasitoids, *Aphidius matricariae* and *Aphidius colemani*, in addition to *A. aphidimyza* (Jacobson and Croft 1998). This strategy of using both a predator and a parasitoid to control aphids was successful and is still popular today (Portree 1996).

Biological Controls

Aphidoletes aphidimyza

Aphidoletes aphidimyza is a small midge whose larval stage is predatory on a wide range of aphid species. Adult *A. aphidimyza* are small flies approximately 2.5 mm long with long thin legs. These flies are nocturnal and feed on aphid honeydew (Nijveldt 1988). Once mated, females spend most of their time laying eggs on the underside of aphid-infested leaves. Females preferentially select aphid-infested plants over aphid-free plants to oviposit on as the aphids are a food source for the developing larvae. One study actually found that adult *A. aphidimyza* were

capable of detecting a single aphid-infested *Brassica* plant out of 75 non-infested plants within a greenhouse (as cited in Nijveldt 1988). Aphid honeydew appears to be the cue that A. aphidimyza uses to locate aphid infestations (ManYoung et al. In Review). Female *A. aphidimyza* live for about 1 week and in that time they can lay up to about 55 eggs (Harizanova and Ekbom 1997).

The egg stage generally lasts about 3 days after which the first instar larvae hatch and begin to search for aphids to feed on (Markkula and Tiitanen 1985). *A. aphidimyza* larvae attack aphids by biting their leg joints and excreting a toxin, which paralyzes and then kills the aphid (Markkula and Tiitanen 1985). The toxin also acts to dissolve the contents of the aphid, which *A. aphidimyza* then sucks out. This attack is so sudden, and the toxin is so effective, that aphids generally do not have time to pull their proboscis out of the plant before becoming immobilized and thus remain attached to the plant even after they have died and their contents sucked dry (Nijveldt 1988). *A. aphidimyza* spend about 5 – 6 days in the larval stage during which time a single larva may kill an average of 24 aphids (Harizanova and Ekbom 1997). Interestingly, when aphid densities are very high it has been found that *A. aphidimyza* will kill more aphids than they can eat (Nijveldt 1988).

Once *A. aphidimyza* larvae have nearly completed development they crawl or drop to the ground where they burrow down about 3 cm and build a cocoon within which to pupate (Markkula and Tiitanen 1985). Pupation takes about 12 days (Harizanova and Ekbom 1997). When adults are about to emerge the pupae exit their cocoons and crawl to the surface of the soil. Adults take approximately 2 – 3 minutes to emerge and can fly within 10 minutes (Markkula and Tiitanen 1985). Adults then mate and the cycle begins again. In the greenhouse it is important to provide *A. aphidimyza* with adequate pupation sites in order to get continued production of these natural enemies. This can be done by spreading a thin layer of peat, sand or straw on the ground between the rows of plants (Portree 1996).

In their natural habitat *A. aphidimyza* larvae overwinter by diapausing in their cocoon in late autumn or early winter and emerging as adults in the spring (Markkula and Tiitanen 1985). In the greenhouse diapause generally begins to occur in the fall in response to the shorter days (Costello et al. 1992). Diapause can cause a breakdown of aphid control if alternate measures are not taken. Diapause can be prevented by providing *A. aphidimyza* larvae with extra light during the winter months (Costello et al. 1992). The current recommendation for preventing

diapause is to add 4 watts / m^2 of artificial light to increase the photoperiod to 16 or more hours per day during the winter months (Portree 1996).

A. aphidimyza is currently used for aphid control in greenhouses worldwide (Nijveldt 1988). Growers can buy these midges from suppliers of beneficial insects. Generally *A. aphidimyza* is shipped while still in the pupation stage as it is somewhat protected while in the cocoon. To release *A. aphidimyza* growers can either sprinkle the cocoons, which usually come mixed with vermiculite as a carrier, on the damp rock wool at the base of plants (Portree 1996) or they can simply open the shipping bottle and place it within the crop and allow the adults to emerge directly from the bottle (van Schelt and Mulder 2000). By allowing adults to emerge directly from the shipping bottle the cocoons are somewhat protected and have less of a chance of drying out before emergence which can cause significant mortality.

Although *A. aphidimyza* is usually effective at controlling aphids in the greenhouse there are sometimes unexplained failures. It is thought that these failures might be due, in part, to intraguild predation (van Schelt and Mulder 2000). The eggs and young larvae of *A. aphidimyza* are susceptible to predation by a number of other predators that are often used in greenhouse biological control, such as the spotted ladybird beetle (*Coleomegilla maculata lengi*) (Lucas 1998), lacewings (*Chrysoperla rufilabris*) (Lucas 1998), and the predatory mites *Amblyseius cucumeris* and *Amblyseius degenerans* (van Schelt and Mulder 2000). To date little is known about the dynamics of such intraguild predation by various biological control agents (Rosenheim 1998), however, it is likely that intraguild predators may affect biological control efficacy in some way. Despite this possible problem *A. aphidimyza* is generally able to complete all stages of its life cycle in the greenhouse and thus many generations can be maintained. This, combined with its relatively high reproductive rate, makes it particularly attractive as a biological control agent.

Parasitoids

There are a number of parasitoid wasps that can be used in conjunction with *Aphidoletes aphidimyza* to control aphids in the greenhouse. The most commonly used aphid parasitoids in the greenhouse system are *Aphidius matricariae*, *Aphidius colemani* and *Aphelinus abdominalis* (van Schelt 1994). All three of these parasitoids are solitary wasps that lay eggs singly in a wide variety of aphid species.

Aphidius matricariae and *Aphidius colemani* are the most popular aphid parasitoids used in the greenhouse system (Portree 1996). Adults feed on nectar from flowers or aphid honeydew. Once mated females spend much of their time searching for aphids in which to lay their eggs. Both species have very broad host ranges, however, they do exhibit host preferences (van Schelt 1994). *A. colemani* will parasitize both *Myzus persicae* and *Aphis gossypii* equally well but is reluctant to parasitize *Macrosiphum euphorbiae* and *Aulacorthum solani*. Whereas *A. matricariae* will parasitize *M. persicae* but is somewhat reluctant to parasitize *A. gossypii* and is even more reluctant to parasitize *M. euphorbiae* and *A. solani*. Both of these parasitoid species live as adults for 2 – 3 weeks and are capable of parasitizing several hundred aphids in that time (Stary 1988).

Aphelinus abdominalis is less commonly used in greenhouse aphid control than the *Aphidius* spp. because it has a lower rate of reproduction and it does not disperse well (van Schelt 1994). Females will parasitize all aphid instars and have a wide host range (Wahab 1985). Females live for about 24 days and have a mean total fecundity of 182 adults of the next generation per female (Jarosik et al. 1996). *A. abdominalis* is thought to be time limited rather than egg limited with females generally senescing with eggs remaining in their ovaries (Wahab 1985). Females are able to determine the sex ratio of the next generation by controlling whether they lay a haploid, unfertilized male egg or a diploid, fertilized female egg with female eggs generally being laid in larger hosts (Honek et al. 1998). Like most aphid parasitoids *A. abdominalis* adults feed on aphid honeydew and the nectar of flowers, however, they also feed on aphids (Stary 1988). They do this by inserting their ovipositor into the aphid and paralyzing it. While the aphid is paralyzed the wasp removes the ovipositor and drinks the haemolymph that leaks out of the puncture site (Stary 1988).

Females of all three of the parasitoid species search for hosts by crawling, hopping and flying around on the leaves, stems, and branches of plants. When a potential host is encountered the wasp assesses its suitability by tapping it with its antennae and probing it with its ovipositor (Stary 1988). If the host is deemed to be suitable the parasitoid then inserts its ovipositor into the aphid and injects a single egg. The egg hatches inside the aphid and the larva begins to develop and as it does so it consumes the internal organs of the aphid eventually killing the aphid. After the aphid dies it takes on a papery appearance and is called an aphid mummy. The parasitoid

larva continues to develop within the aphid mummy for several days. Eventually it pupates and the adult parasitoid chews its way out of the aphid body. Larval development usually takes about 12 – 14 days from the time of parasitization to emergence of the adult parasitoid (Harizanova and Ekbom 1997). Once the adult parasitoid emerges it starts the whole cycle over again.

Growers can purchase parasitoids from a growing number of beneficial insect suppliers. Parasitoids are generally shipped as larvae within the aphid mummy as they are somewhat protected in this stage. Growers then shake the mummies onto the crop where they will remain until the adult parasitoid emerges. Application rates for parasitoids are in the range of 150 – 500 mummies per 1000 m^2 every week for 2 – 3 weeks (Portree 1996).

Parasitoids that are used in greenhouses to control aphids are vulnerable to attack by hyperparasitoids that enter the greenhouse from the outside environment in late summer (Portree 1996). Hyperparasitoids are wasps that parasitize and kill other parasitic wasps. These hyperparasitoids can be quite a problem in the greenhouse in late summer and are often the cause of parasitoid failure (Fernandez and Nentwig 1997). At the moment there is little that can be done about hyperparasitoids other than release more parasitoids to make up for the ones lost due to parasitism or switch to an emphasis on predators.

Others

There are a number of other biological controls for aphids that are used on a small scale in some greenhouses. These include ladybird beetles, lacewings, and syrphid flies (Lee 1994). The efficacy of these controls in the greenhouse environment has been disappointing (see Lee 1994). As a result, they may be used on a small scale to augment other biological control practices but they cannot be used as primary control agents within the greenhouse.

Release Mechanisms for Biological Controls

There are a number of different strategies for releasing biological controls in the greenhouse. These include inundative releases, seasonal inoculative release, trickle application and banker plant systems (van Lenteren et al. 1997). There are advantages and disadvantages to each strategy and one must take into consideration not only biological factors but also the economics of each system before deciding which method is most appropriate to use.

Inundative Release

Inundative release of biological control is the term given to periodic release of large numbers of natural enemies to quickly bring a pest population under control, without the aim of building up permanent population of natural enemies (van Lenteren et al. 1997). This type of release mechanism is often used to bring large pest outbreaks under control much in the same way the application of a pesticide does. For example, since aphids can reproduce so quickly their numbers often get out of control and inundative releases of parasitoids and/or *Aphidoletes aphidimyza* are required (van Lenteren et al. 1997). Although inundative release of biological controls is effective in that it can reduce pest populations quickly, it is also very expensive, as growers must obtain a large number of natural enemies from the supplier every time there is a pest outbreak.

Seasonal Inoculative Release

Seasonal inoculative release differs from the inundative release mechanism in that its aim is to build a lasting population of natural enemies that will continue to control the pest population over the growing season (see van Lenteren et al. 1997). In this system large numbers of natural enemies are obtained and released into the greenhouse at the beginning of the growing season. It is hoped that these natural enemies will not only bring the immediate pest population under control but they will also establish themselves within the greenhouse and continue to control the pest throughout the growing season. In order for this to work the natural enemies must be able to reproduce well in the greenhouse environment. While this system is definitely more economical than the inundative release system few natural enemies reproduce well enough in the greenhouse for it to be effective.

Trickle Application

Since aphids have a huge reproductive rate, biological control generally works best if the natural enemies are introduced into the greenhouse when the aphid population is still very low (van Lenteren 2000). This presents a problem because at these low pest densities natural enemies are unlikely to find enough resources to become sufficiently established in the greenhouse, which rules out the possibility of seasonal inoculative release. On the other hand there is no large pest population that must be brought quickly under control, which rules out inundative release. As an alternative, Jacobson and Croft (1998) tested a method of trickle

application for *Aphidius colemani*. In this system low levels of the natural enemy are released periodically before the pest appears on the crop. While this may seem counter intuitive it is often more effective and more economical than waiting until the pest population explodes and applying natural enemies via an inundative release (Jacobson and Croft 1998).

Banker Plants

Another way of getting around the problem of having natural enemies in the greenhouse before the pest arrives is to provide the natural enemies with an alternate source of food or hosts. This is accomplished through the use of a banker plant system (Bennison and Corless 1993, Jacobson and Croft 1998, Kuhne 1998 and van Lenteren et al. 1997). Banker plants are essentially pots of sprouted wheat or barley that have been infested with a cereal aphid. The cereal aphids are unable to feed on the crop so they do not pose a threat to it, however, the aphid parasitoids can use them as an alternate host when the population of pest aphids in the crop is low. The concept of banker plant is not limited to cereal plants. In theory, any plant that harbors non-threatening alternative hosts should work. By using a banker plant system growers can establish and maintain a parasitoid population before the pest even arrives in the crop. As a result parasitoids are ready to start controlling the pest as soon as it invades the greenhouse. This system has been shown to be more effective at controlling aphids than frequent inundative releases of parasitoids and is generally more economical (Bennison and Corless 1993 and Jacobson and Croft 1998).

Chemical Control

With the widespread use of biological control in the greenhouse fewer chemical controls are being used (van Lenteren 2000). This is due mainly to the fact that the use of chemical control, especially the use of broad-spectrum insecticides, can disrupt concurrent biological control programs by killing natural enemies as well as pests. In the greenhouse, where the bulk of pest control is achieved through the use of biological controls, this disruption cannot be tolerated. However, under extreme circumstances, such as massive pest outbreaks, the application of chemical insecticides may be warranted even within an IPM plan. As of 1996, the following chemicals were registered for use in Canada for aphid control in greenhouse vegetable crops: endosulfan, parathion, nicotine smoke, basudin, malathion, thiodan and insecticidal soap (Portree 1996). With the increasing use of biological controls and the decreasing list of

registered chemical pesticides it is predicted that we will see greenhouse vegetable production without chemical pesticides sometime in the near future (van Lenteren 2000).

Areas for Future Research

Despite the fact that biological control is so effective in the greenhouse industry there is always room for improvement. A standardization of application procedures, complete with application rates, would help take much of the guesswork out of using biological control agents. Currently, the beneficial insect supply companies usually provide application instructions. These instructions can be quite varied and are open to interpretation. Since correct application is vital to the successful establishment of most biological controls instruction is an area that needs improvement.

Additionally, there is quite a bit of variability in the quality of natural enemies that are produced via mass rearing by biological control companies (Fernandez and Nentwig 1997). This variability can be due to the rearing, storage, and/or shipping procedures used by the company. As a result, the International Organization for Biological Control has developed a number of guidelines for testing the quality of biological control agents (van Lenteren 2000). These guidelines can be used by the supply companies to ensure their product meets a certain quality standard. Despite these guidelines the quality of beneficial insects produced by suppliers remains varied. In fact, Fernandez and Nentwig (1997) found that the quality of products from the same producer often differed as much as the quality of products from different producers. This clearly shows a need for improved quality control procedures.

Biological control has clearly been very successful in the greenhouse vegetable industry. In fact it has been so successful that it now provides the framework on which greenhouse IPM is built (van Lenteren 2000). Every year more biological controls become available to greenhouse growers, not only for aphids but for all other pests as well. As a result, fewer chemical controls are being used. One of the major reasons biological control has been so successfully adopted in the greenhouse vegetable industry is that it has been shown to be economical in addition to being effective (van Lenteren 2000).

The Question

There is a wide body of literature on parasitoid learning, however very little of this research has investigated how parasitoid learning might be applied to pest management practices. The prevalent use of banker plants in conjunction with aphid parasitoids in the greenhouse system raises interesting questions about the dynamics that are involved when a parasitoid is required to learn information about more than one species of host. For example, does learning the cues for the cereal aphid in the banker plant interfere with the parasitoid's ability to locate and parasitize pest aphids in the crop? Or, does learning the cues for the cereal aphid in the banker plant make it easier for the parasitoid to then learn the cues of the pest aphid in the crop? Parasitoid learning could also affect how the banker plants are used in terms of density and placement within the greenhouse. By answering these questions it may be possible to improve the efficacy of biological controls used in the greenhouse.

A. abdominalis has been used in greenhouses to control aphid outbreaks since 1990 (Haardt and Holler 1992). It is generally recommended for the control of the potato aphid, *Macrosiphum euphorbiae* (Molck and Wyss 2001) but it will also parasitize *Myzus persicae* and *Aulacorthum solani* (personal obervations) which are common greenhouse pests. In Canada *A. abdominalis* is generally used in sweet pepper crops to control a variety of aphid species. These parasitoids are purchased as live adults that are released into the greenhouse either directly on the aphid infestation or on banker plants before the aphid outbreak occurs. Since *A. abdominalis* is likely to come into contact with more than one species of host aphid, living on more than one species of plant, it is relevant to ask the question: What happens when a parasitoid is required to learn about more than one species of host? It has recently been shown that *A. abdominalis* is capable of learning odours of its host-plant complex (Molck et al. 1999, Molck et al. 2000). It is the aim of this thesis project to confirm the ability of *A. abdominalis* to learn host cues and to look at 5 possible types of learning interactions that may occur when a parasitoid is required to learn the cues of two different aphid species (Table 1).

Table 1 – Types of learning interactions investigated.

Type of Learning Interaction	Definition	Possible implications to the greenhouse system
No Learning	No learning occurs and parasitoids rely on their innate responses to stimuli	This could make it harder for the parasitoid to control the pest aphid depending on what their innate responses are to both the pest aphid and the banker plant aphid.
No Interaction	Learning occurs but there is no interaction between two successive learning events.	This would neither improve nor hinder the ability of the parasitoid to control pest aphids.
Transfer	Learning one set of stimuli enhances the learning of a second set of related stimuli.	This would improve the ability of the parasitoid to control pest aphids.
Proactive Interference	Learning one set of stimuli makes it harder to learn a second set of stimuli.	This could hinder the ability of the parasitoid to control pest aphids.
Retroactive Interference	Learning a second set of stimuli makes it harder to recall the first set of stimuli.	This could hinder the ability of the parasitoid to switch between foraging for pest aphids and banker plant aphids.

Materials and Methods

Rearing

Sweet pepper (*Capsicum annuum*, cv. 'Staddon's Select') and wheat (*Triticum aestivum*) were grown at the Simon Fraser University greenhouse. All plants were seeded in sterile potting mix (Sunshine Mix # 10). Plants were watered daily and fertilized weekly.

Aphid colonies of *Myzus persicae* and *Aulacorthum solani* were reared separately on sweet pepper seedlings. Aphid-infested seedlings were kept in cup cages. Cup cages were made from two 12 oz. clear plastic drinking cups, one cup inverted on top of the other. Cages were sealed using parafilm and the top cup had a hole (2 cm diameter) cut out of it that was covered with a fine mesh to reduce humidity within the cage. Aphid colonies of *Sitobion fragariae* were reared on potted wheat plants that were housed in plexiglas cages. All aphid colonies were reared under a 16L: 8D photoperiod at a temperature of 20°C (+/- 5°C) with a relative humidity of about 30%.

Aphelinus abdominalis adults used in treatment groups 1 – 5 (see below) were reared on *Myzus persicae* feeding on sweet pepper plants. *A. abdominalis* adults used in treatment group 6 were reared on *S. fragariae* feeding on wheat. And *A. abdominalis* adults used in treatment group 7 were reared on *A. solani* feeding on sweet pepper plants.

Adult parasitoids were released into large plexiglass cages and supplied with aphid-infested plants. New plants and new aphids were periodically placed in the cage to provide the parasitoids with a continual supply of hosts. Plants in the parasitoid cages were routinely checked for aphid mummies. All aphid mummies were carefully removed from the plants and placed in 16 oz. paper Dixie cups fitted with petri dish lids. Each cup contained a supply of 50% honey water for parasitoids to feed on upon emergence. Cups were checked daily for newly emerged adult parasitoids. Adults used for rearing were released into rearing cages and adults used for experiments were kept in the Dixie cups with honey water until needed for experiments

Experimental Plants

4 – 5 week old pepper plants and 1-week-old wheat plants were inoculated with 20 *A. solani* and 20 *S. fragariae* respectively. Aphids used for the inoculations were of a mixed age class. Inoculated plants were held in cup cages for approximately 24 hours before use in the experiments.

Parasitoid Experience

Parasitoids were left in emergence cages for 3 days prior to use in experiments to assure mating had occurred. At this point the parasitoids had never contacted a host, except for the aphid mummy from which they emerged, and had never contacted a plant and thus were considered naïve. These 3-day-old mated females were then subjected to one of 7 treatment groups. The treatment groups were as follows.

Group 1: Naïve females reared on *M. persicae* were held, singly, for 2 days in cup cages with no access to plants or aphids. Parasitoids were provided with 50% honey water.

Group 2: Individual parasitoids reared on *M. persicae* were placed, singly, on experimental pepper plants and allowed to forage on that plant for 24 hours after which time they were removed and placed on a new experimental pepper plant and allowed to forage for another 24 hours. A source of 50% honey water was available at all times.

Group 3: Individual parasitoids reared on *M. persicae* were placed, singly, on experimental wheat plants and allowed to forage on that plant for 24 hours after which time they were removed and placed on new experimental wheat plants and allowed to forage for another 24 hours. A source of 50% honey water was available at all times.

Group 4: Individual parasitoids reared on *M. persicae* were placed, singly, on experimental pepper plants and allowed to forage on those plants for 24 hours after which time they were removed and placed on experimental wheat plants and allowed to forage for another 24 hours. A source of 50% honey water was available at all times.

Group 5: Individual parasitoids reared on *M. persicae* were placed, singly, on experimental wheat plants and allowed to forage on those plants for 24 hours after which time they were

removed and placed on experimental pepper plants and allowed to forage for another 24 hours. A source of 50% honey water was available at all times.

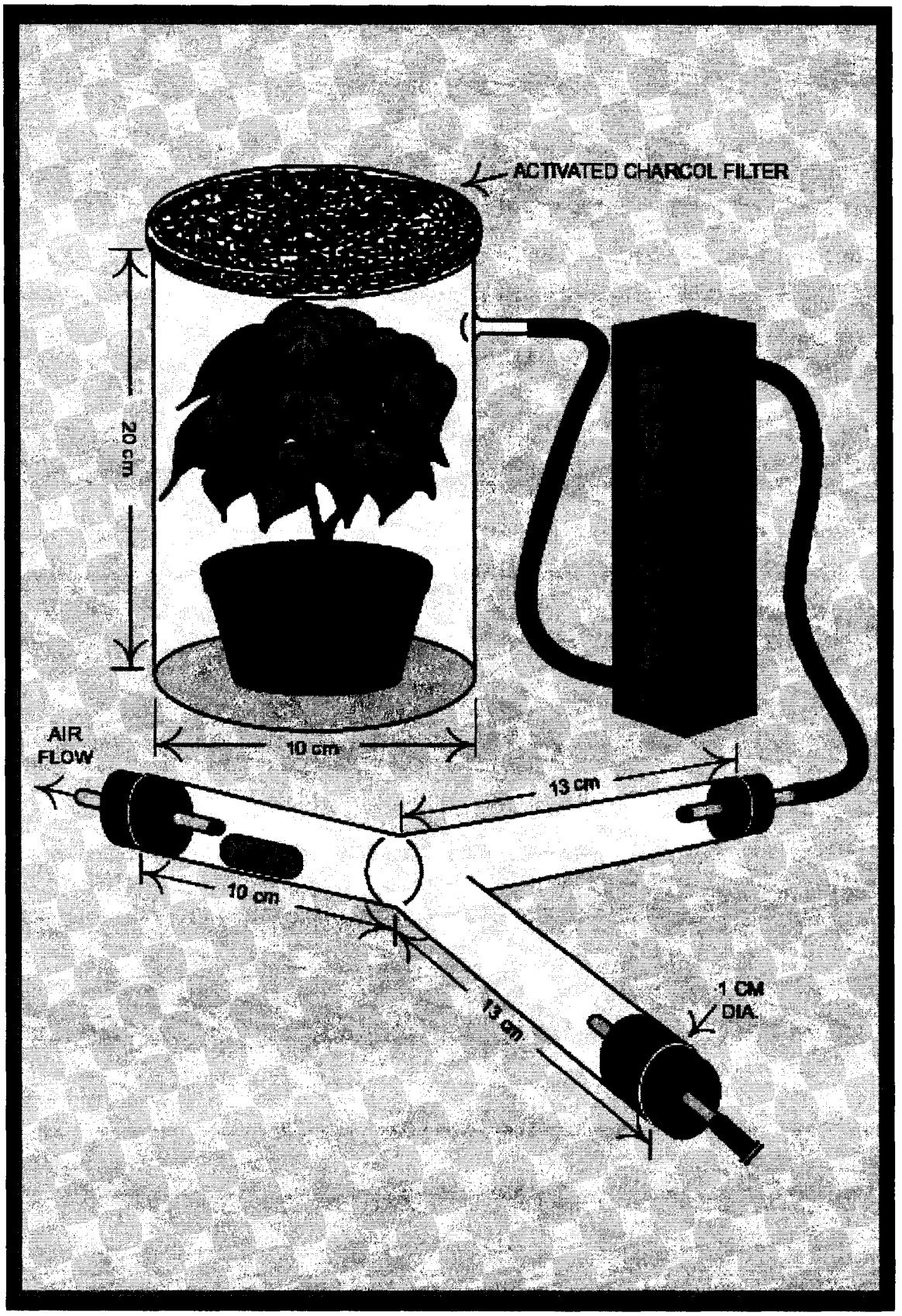
Group 6: Naïve females reared on *A. solani* were held, singly, for 2 days in cup cages with no access to plants or aphids. Parasitoids were provided with 50% honey water.

Group 7: Naïve females reared on *S. fragariae* were held, singly, for 2 days in cup cages with no access to plants or aphids. Parasitoids were provided with 50% honey water.

Y-Tube Olfactometer

Bioassays were performed in a Y-tube olfactometer (figure 1). The basal arm of the Y tube was 10.0 cm long and the distal arms were both 13.5 cm long. The diameter of the Y-tube was 1.0 cm. Air was pulled through the olfactometer via a vacuum pump aspirator (Nalgene Cat. No. 6140-0010) attached to a laboratory faucet. Air entering the olfactometer was passed through a charcoal filter before entering the bait containers. The bait containers were 20.0 cm high and 10.0 cm in diameter with a 1.5 cm diameter opening 10 cm from the bottom of the container. One bait container contained an experimental pepper plant while the other contained an experimental wheat plant. The speed of the air entering each arm of the Y-tube was measured using acrylic block flowmeters (Key Instruments Model # FR2A13BVBN). Air flow in each arm was maintained at 0.6 LPM (600 ml/min). To avoid contamination each flowmeter was designated as either a pepper flowmeter or a wheat flowmeter and were used with those specific baits for the duration of the bioassays. Light was diffused above the Y-tube using a piece of light cotton material so that the light in each arm of the olfactometer was even. A single point source of light was placed at an equal distance between the two distal arms of the Y-tube 13.0 cm from the junction of the distal and basal arms to encourage the parasitoids to initiate an upwind oriented walk.

Figure 1 - Diagram of Y-tube olfactometer apparatus.


ACTIVATED CHARCOL FILTER
20 cm
10 cm
AIR
FLOW
13 cm
10 cm
13 cm
1 CM
DIA.

Experimental Procedure

In all bioassays the parasitoids were transferred individually from the experience cup cages to the Y-tube via small gelatin capsules. At the beginning of each bioassay the parasitoid being tested was gently released into the basal arm of the Y-tube which was then connected to the aspirator. To test whether previous experience with a plant/host complex alters the parasitoid's preference for the same plant/host complex parasitoids were required to make a choice between walking toward either a pepper plant + *A. solani* odor source or a wheat plant + *S. fragariae* odor source. Each parasitoid was given a maximum of 5 minutes to respond by showing an oriented upwind walk, after which they were deemed unresponsive. Once the parasitoid began to respond the bioassay was run for 5 minutes. Parasitoids were allowed to change their selected arms as many times as they wished within the 5-minute bioassay period. The arm or arms chosen by each parasitoid were recorded as was the cumulative time spent in each arm.

Between each bioassay the Y-tube was thoroughly washed with detergent and acetone to prevent contamination of the apparatus by odours possibly left behind by previously tested parasitoids. Additionally, the arms that the odours were presented in were switched between each run of the bioassay so that any preference for one side of the Y-tube or the other would be controlled for. The experimental plants were replaced with new experimental plants after each bioassay.

Statistical Analysis

A χ^2 test with a Bonferroni correction ($\alpha = 0.007$) was used to determine whether there were significant differences in the numbers of parasitoids choosing different odor sources and different sides of the Y-tube between the 7 treatment groups. One-way analysis of variance with a Bonferroni correction ($\alpha = 0.007$) was used to determine whether there were significant differences in the mean time spent in the Y-tube arms between the 7 treatment groups. All statistics analyses were done using the JMP-In ^{TM} statistical software.

Description of Model

The following is a description of the model that was used to simulate the population dynamics of the foxglove aphid, *Aulacorthum solani*, on pepper plants in a greenhouse that uses the parasitoid *Aphelinus abdominalis* in conjunction with banker plants, of barley and *Sitobion fragariae*, as alternate host reservoirs. The model looked at 5 different interactions that might occur when a parasitoid is required to learn more than one different type of host. The possible interactions between two consecutive learning events were as follows: no learning, no interaction between learning events, transfer, proactive interference, and retroactive interference. The no learning scenario was the null hypothesis where no learning occurred and the parasitoids relied on their innate preferences for hosts and these preferences did not change with experience. In the no interaction scenario parasitoids were able to learn host cues but there is no interaction between consecutive learning events. The transfer scenario assumed that learning one host type enhanced the learning of future host types. Conversely, the proactive interference scenario assumed that learning one host type made it harder to learn a second host type while the retroactive interference scenario assumed that the learning of the second host type interfered with the recall of the previously learned host type.

At the beginning of the simulation a single banker plant, that had been infested with 250 *S. fragariae*, was placed in the greenhouse of 5,000 pepper plants (arranged in 50 rows of 100 plants) and allowed to establish for 5 days. On the 5^{th} day 200 parasitoids were applied directly to the banker plants. On the 20^{th} day a single *A. solani* invaded the greenhouse at a randomly chosen location. The model ran for 50 days following the initial *A. solani* invasion. The various life history parameters included in the model can be found in Appendix 1. As the model ran the amount of aphid damage to the crop was recorded. Aphid damage was measured in aphid days; with each day an aphid was alive equaling one aphid day. In addition to the total amount of aphid damage the total number of pepper plants infested was also recorded.

In the model both aphids and parasitoids were free to emigrate from their current plant but the manner in which they did so differed. For aphids it was assumed that the tendency to leave was a positive function of aphid density at their current plant (equation 1, appendix 1) whereas for the parasitoid it was an inverse function of aphid density (equation 2, appendix 1). A shape parameter was also added to the emigration function so that little emigration would

occur until aphid populations approached their carrying capacity (Dixon 1998). The shape parameter for the parasitoid facilitated emigration at low to moderate aphid densities.

Two types of movement were possible for any *A. abdominalis* that "chose" to emigrate. The majority moved to adjacent pepper plants whereas a smaller proportion randomly alighted on pepper plants. When parasitoids "chose" to emigrate they did so in the following manner. First they examined each and every plant within a 5-plant radius from their current position and determined their attractiveness; the attractive index is a function of aphid colony size and distance from current plant determined by equation 3 (appendix 1). The host plant with the highest index was deemed the most attractive. The index was then compared with the minimum recognizable attraction score, which was set equivalent to a colony of 10 aphids at distance 1. If the index for the most attractive plant exceeded the minimum score then the parasitoid moved to that plant otherwise it would randomly resettle in the greenhouse. The value of the minimum recognizable score was determined by the learning affect, as will be described in further detail below. In other words, parasitoids that had learned a particular aphid-plant odor complex became more sensitive to such odors.

The model followed each parasitoid as it foraged and used a sliding memory window (Mangel and Roitberg 1989) to keep track of the 2 most recent foraging experiences at any given time. For each of these experiences the model assigned a value of 0, 1, or 2, depending on the host that had been encountered. 0 represented no encounter, 1 represented an encounter with *A. solani* on a pepper plant and 2 represented an encounter with *S. fragariae* on the banker plant. The sequence of the two most recent host encounters determined the value of the minimum recognizable score for the next foraging round. In addition to the host encounter sequence a parasitoid's minimum recognizable score also depended on the strength of learning, as set by the user. Table 2 shows an example of the amount learning alters a parasitoid's minimum recognizable score for *A. solani* on pepper plants, for each of the 5 types of learning and for all possible host encounter combinations with the strength of learning set at 20%.

Parasitoids attacked aphids, both *A. solani* and *S. fragariae*, according to a modified version of Holling's functional response (Holling 1966, see also Mondor and Roitberg 2000). The number of aphids and parasitoids, on a per plant basis, was updated once per day in the functional response equation. Aphids that were not attacked by parasitoids reproduced at a daily replacement rate of $R = 1.3$.

The model was run for each of the 5 different learning types with the learning strength set at 10%, 20% and 30% and with one banker plant placed in the middle of the greenhouse. A one-way analysis of variance with a Bonferroni correction (α=0.003) was then used to determine whether there were significant differences in the mean amount of total aphid damage and the mean number of plants infested between each of the 13 treatments. Additionally, the model was run for each learning type with the learning strength set at 20% and 2 banker plants spaced evenly in the greenhouse. A one-way analysis of variance with a Bonferroni correction (α=0.005) was then used to determine whether there were significant differences in the mean amount of total aphid damage and the mean number of plants infested between each of the treatments. For each set of assumptions the model was run 100 times.

Table 2 - Amount that learning enhances the innate ability of the parasitoid to detect a foxglove aphid on a pepper plant for all possible host encounter combinations under each of the 5 models (strength of learning set a 20%).

Possible Combinations of Host Encounters	Default	Default Learning	Transfer	Proactive Interference	Retroactive Interference
0,0	0	0	0	0	0
0,1	0	↑20%	↑20%	↑20	↑20
1,0	0	↑20%	↑20%	↑20	↑20
1,1	0	↑30	↑30%	↑30	↑30
0,2	0	0	0	0	0
2,0	0	0	0	0	0
2,2	0	0	0	0	0
1,2	0	↑20%	↑20%	↑20	0
2,1	0	↑20%	↑30%	0	↑20

Results

Y-Tube Experiment

All parasitoids were allowed to make as many arm choices as they wished in the 5 minute bioassay time period, however, most parasitoids remained in the first arm they chose (Table 3). Odour source choice results from both the first choice (Figure 2) and the final choice (Figure 3) were analyzed. No significant differences ($P > 0.007$) were found between the choices made for any of the treatment groups. Side choice results from both the first choice (Figure 4) and the final choice (Figure 5) were analyzed. No significant differences were found between the treatment groups for both the side of first choice ($P > 0.007$) and the side of final choice ($P > 0.007$). The mean times spent in the pepper arm (Figure 6) and wheat arm (Figure 7) of the Y-tube were also analyzed. No significant differences ($P > 0.007$) were found.

Table 3 - Number odour choices made by parasitoids in the 5 minute bioassay period.

Treatment Group	Number of parasitoids making only one odour choice.	Number of parasitoids making more than one odour choice.
Naïve (reared on *M. persicae*)	22	6
Pepper + A. solani	24	2
Wheat + S. fragariae	17	4
Pepper + *A. solani* then Wheat + *S. fragariae*	19	2
Wheat + *S. fragariae* then Pepper + *A. solani*	22	3
Naïve (reared on *A. solani*)	11	12
Naïve (reared on *S. fragariae*)	17	2

Figure 2 - Proportion of *Aphelinus abdominalis* females choosing either pepper + *A. solani* or wheat + *S. fragariae* as their first choice in the Y-tube olfactometer. χ^2, $P = 0.3501$.

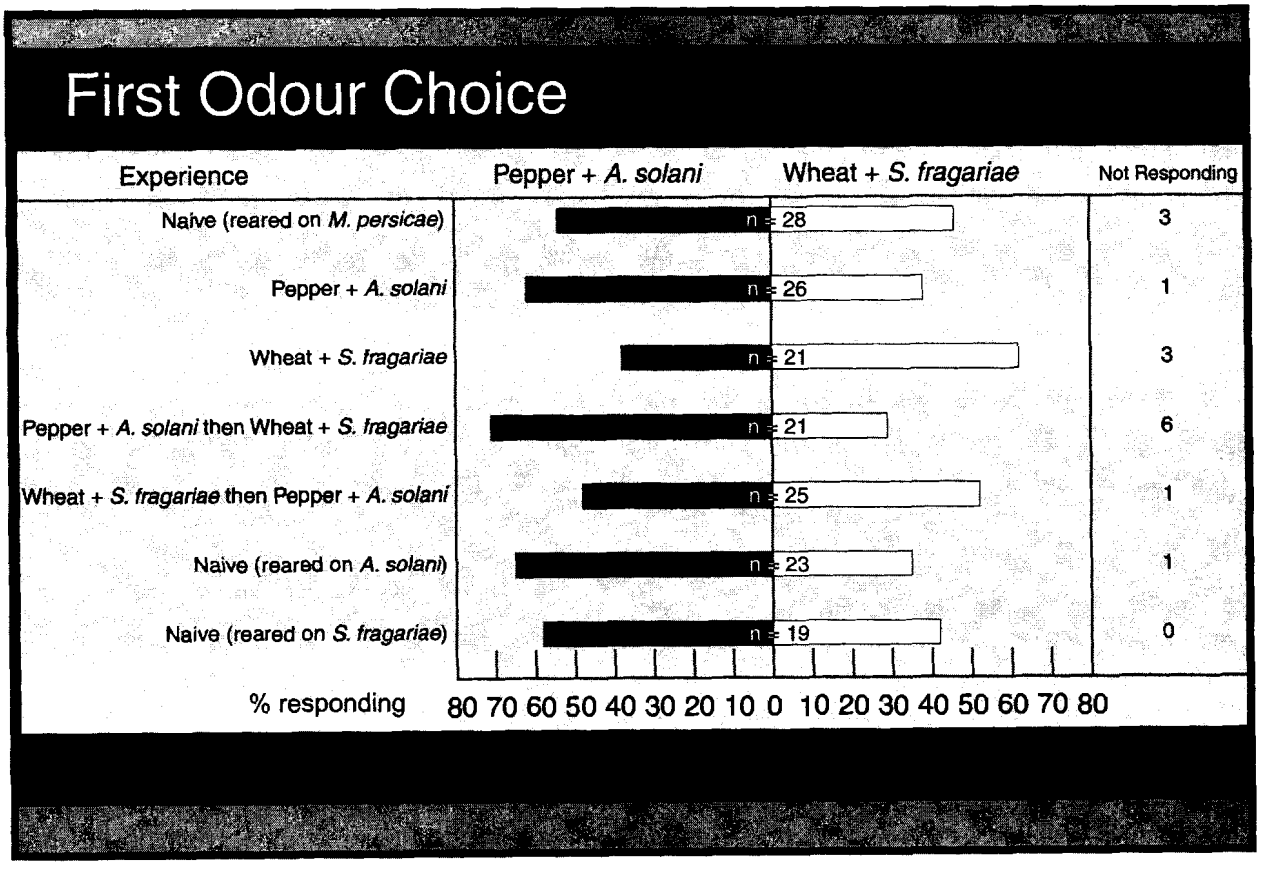

First Odour Choice
Experience
Pepper + A. solani
Wheat + S. fragariae
Not Responding
Naive (reared on M. persicae)
n = 28
3
Pepper + A. solani
n = 26
1
Wheat + S. fragariae
n = 21
3
Pepper + A. solani then Wheat + S. fragariae
n = 21
6
Wheat + S. fragariae then Pepper + A. solani
n = 25
1
Naive (reared on A. solani)
n = 23
1
Naive (reared on S. fragariae)
n = 19
0
% responding
80 70 60 50 40 30 20 10 0 10 20 30 40 50 60 70 80

Figure 3 - Proportion of *Aphelinus abdominalis* females choosing either pepper + *A. solani* or wheat + *S. fragariae* as their final choice in the Y-tube olfactometer. χ^2, $P = 0.8824$.

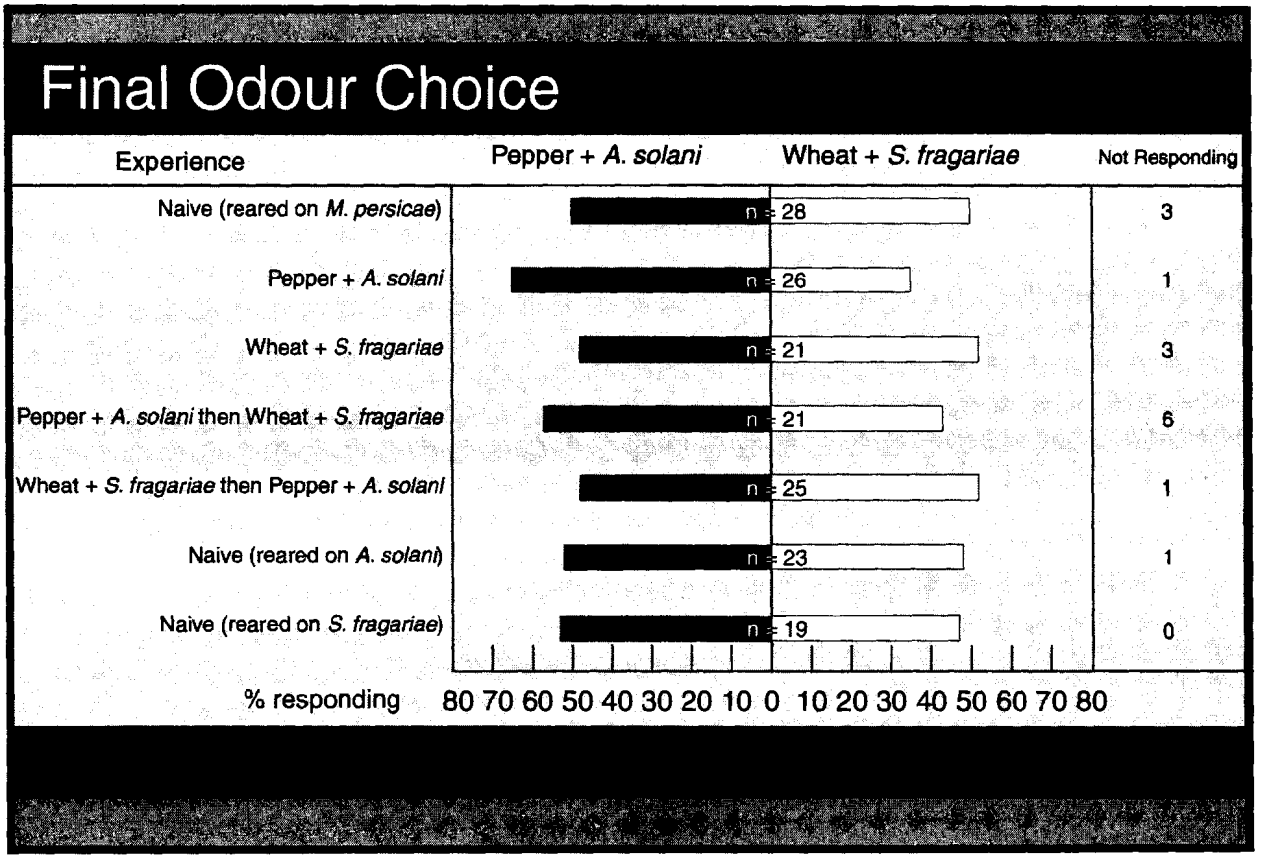

Final Odour Choice
Experience
Pepper + A. solani
Wheat + S. fragariae
Not Responding
Naive (reared on M. persicae)
n = 28
3
Pepper + A. solani
n = 26
1
Wheat + S. fragariae
n = 21
3
Pepper + A. solani then Wheat + S. fragariae
n = 21
6
Wheat + S. fragariae then Pepper + A. solani
n = 25
1
Naive (reared on A. solani)
n = 23
1
Naive (reared on S. fragariae)
n = 19
0
% responding
80 70 60 50 40 30 20 10 0 10 20 30 40 50 60 70 80

Figure 4 - Proportion of *Aphelinus abdominalis* females choosing either the left or right side of the Y-tube olfactometer as their first choice. χ^2, $P = 0.0158$.

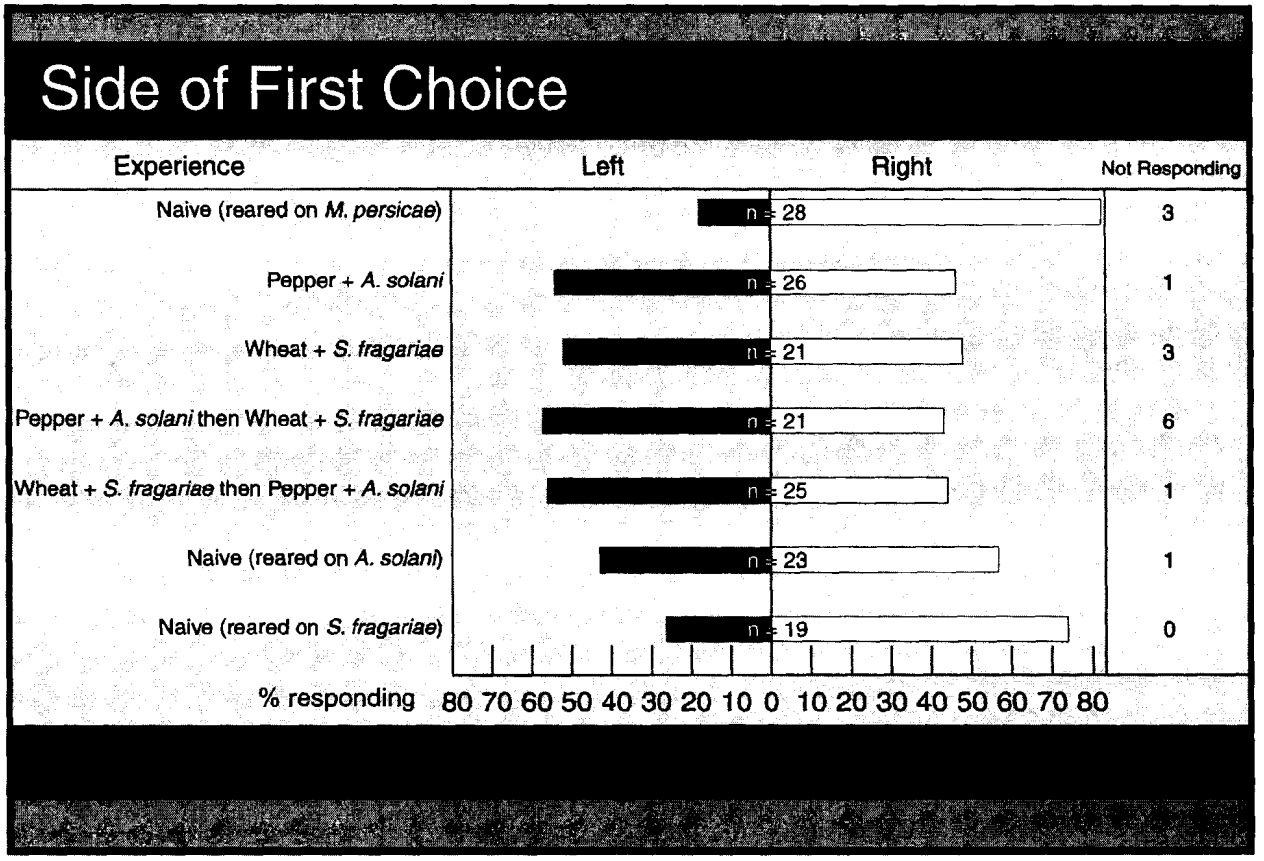

Side of First Choice
Experience
Left
Right
Not Responding
Naive (reared on M. persicae)
n = 28
3
Pepper + A. solani
n = 26
1
Wheat + S. fragariae
n = 21
3
Pepper + A. solani then Wheat + S. fragariae
n = 21
6
Wheat + S. fragariae then Pepper + A. solani
n = 25
1
Naive (reared on A. solani)
n = 23
1
Naive (reared on S. fragariae)
n = 19
0
% responding
80 70 60 50 40 30 20 10 0 10 20 30 40 50 60 70 80

Figure 5 - Proportion of *Aphelinus abdominalis* females choosing either the left or right side of the Y-tube olfactometer as their final choice. χ^2, $P = 0.0138$.

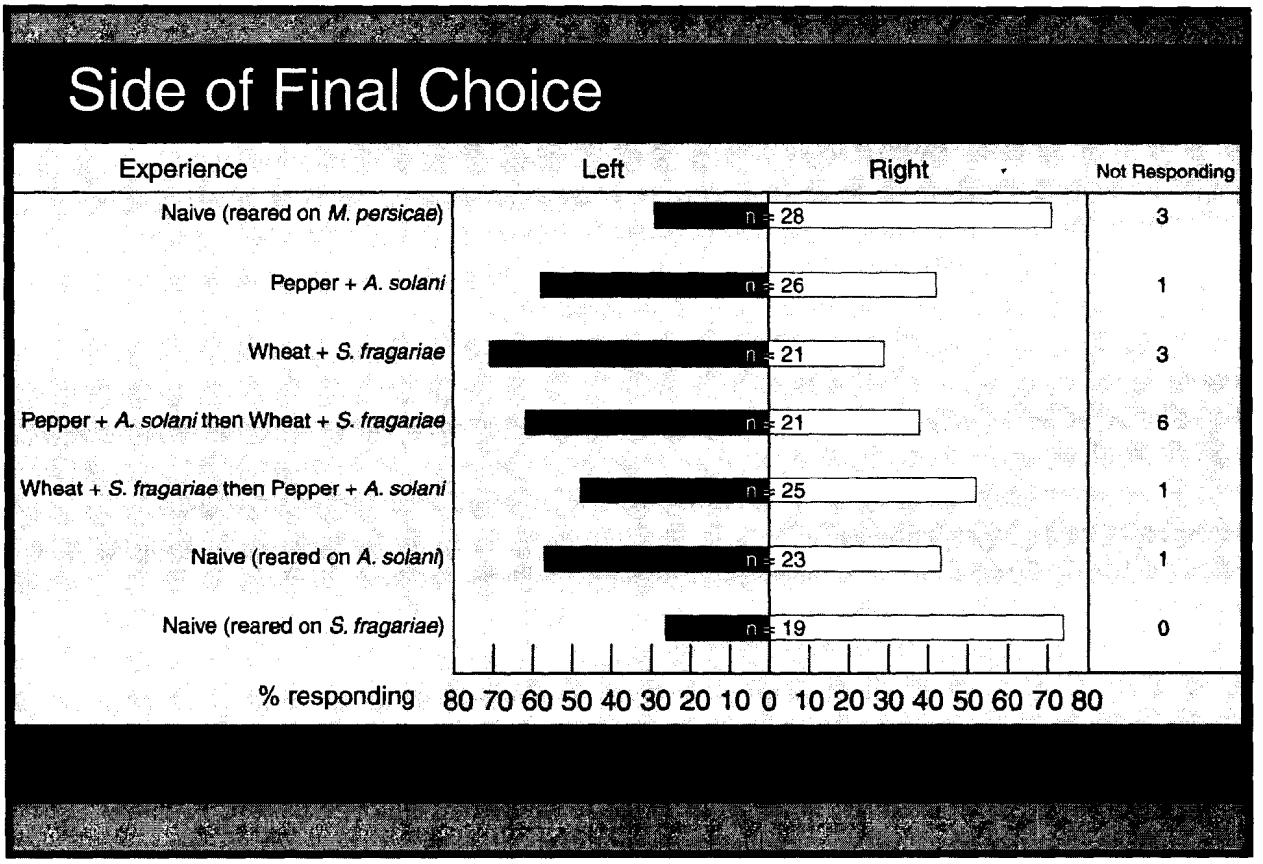

Side of Final Choice
Experience
Left
Right
Not Responding
Naive (reared on M. persicae)
n = 28
3
Pepper + A. solani
n = 26
1
Wheat + S. fragariae
n = 21
3
Pepper + A. solani then Wheat + S. fragariae
n = 21
6
Wheat + S. fragariae then Pepper + A. solani
n = 25
1
Naive (reared on A. solani)
n = 23
1
Naive (reared on S. fragariae)
n = 19
0
% responding
80 70 60 50 40 30 20 10 0 10 20 30 40 50 60 70 80

Figure 6 - Mean amount of time spent in the pepper arm of the Y-tube olfactometer. ANOVA, $P = 0.3934$.

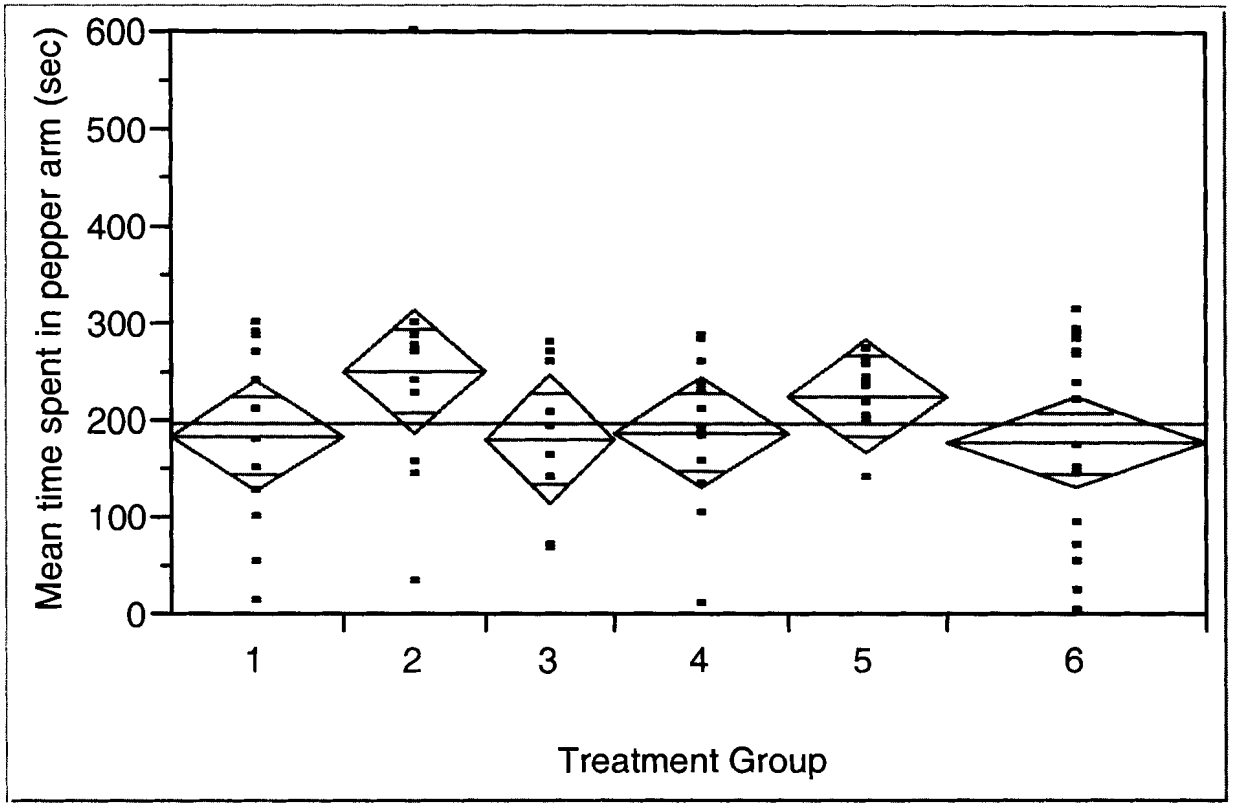
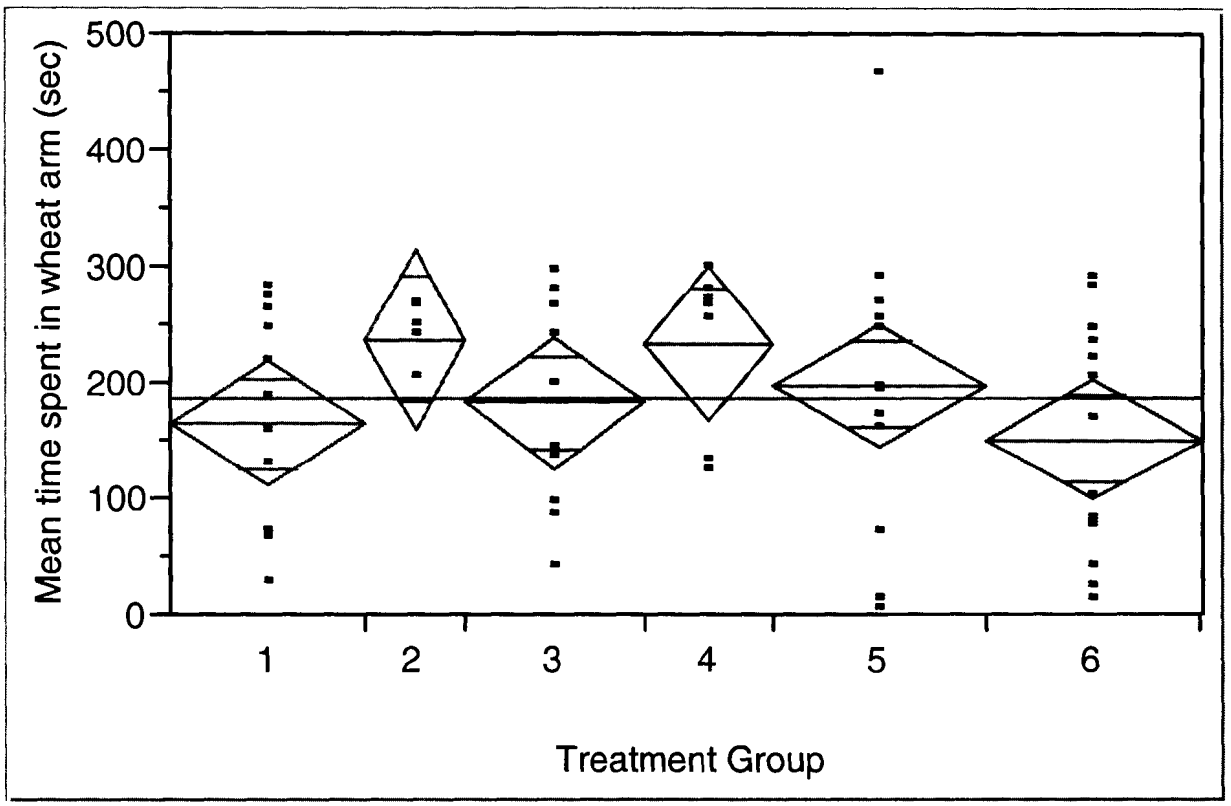

Mean time spent in pepper arm (sec)
600
500
400
300
200
100
0
1
2
3
4
5
6
Treatment Group

Figure 7 - Mean amount of time spent in the wheat arm of the Y-tube olfactometer. ANOVA, $P = 0.2909$.


Mean time spent in wheat arm (sec)
500
400
300
200
100
0
1
2
3
4
5
6
Treatment Group

Model Results

There was no significant difference found in the mean amount of total aphid damage between runs of the model made with the learning strengths set at 10%, 20% and 30% and one banker plant situated in the middle of the greenhouse P=0.1296, (Table 4 and Figure 8). A retrospective power analysis indicates that the power of the test was low, 0.8039, due to a high degree of variability in the data. In order to obtain significant results at least 1,557 repetitions for each set of assumptions would be required. The mean amount of aphid damage was found to be 298,781 aphid days.

There was also no significant difference found in the mean number of plants infested between runs of the model made with the learning strengths set at 10%, 20% and 30% and one banker plant situated in the middle of the greenhouse P=0.2512, (Table 5 and Figure 9). A retrospective power analysis shows that the power of the test was low, 0.7143, due to a high degree of variability in the data. In order to obtain significant results at least 1,846 repetitions for each set of assumptions would be required. The mean number of plants infested was found to be 939 plants.

The mean amount of total aphid damage was significantly less when 2 banker plants were used in the model compared to when only 1 banker plant was used P<0.0001, (Table 6 and Figure 10).

The mean number of plants infested was significantly less when 2 banker plants were used in the model compared to when only 1 banker plant was used P<0.0001, (Table 7 and Figure 11).

Figure 8 - Mean amount of total aphid damage in greenhouse of 5,000 pepper plants and one banker plant at position 2550 with learning strength set at 10%, 20% and 30%. ANOVA, $P = 0.1296$.

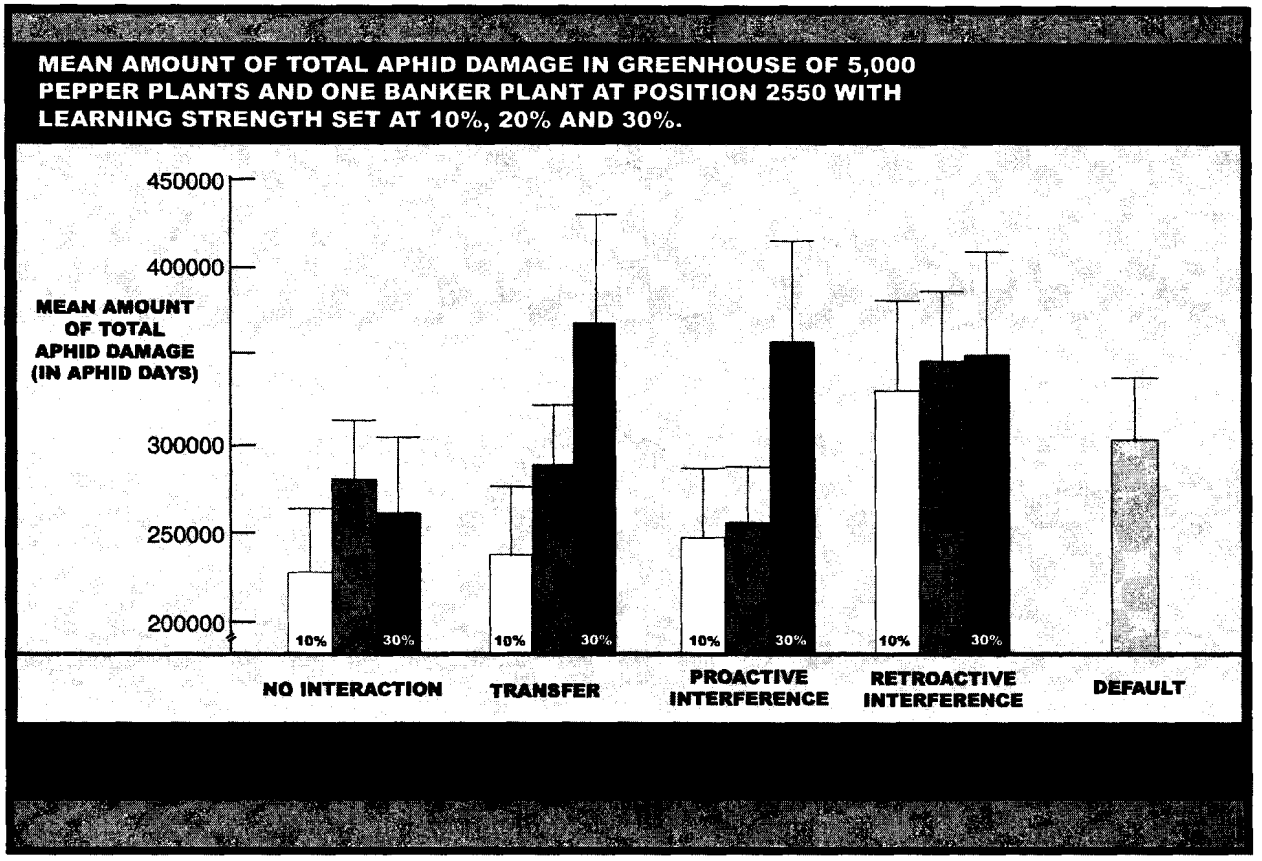

MEAN AMOUNT OF TOTAL APHID DAMAGE IN GREENHOUSE OF 5,000 PEPPER PLANTS AND ONE BANKER PLANT AT POSITION 2550 WITH LEARNING STRENGTH SET AT 10%, 20% AND 30%.
450000
400000
300000
250000
200000
MEAN AMOUNT OF TOTAL APHID DAMAGE (IN APHID DAYS)
10%
30%
10%
30%
10%
30%
10%
30%
NO INTERACTION
TRANSFER
PROACTIVE INTERFERENCE
RETROACTIVE INTERFERENCE
DEFAULT

Figure 9 - Mean number of plants infested in greenhouse of 5,000 pepper plants and one banker plant at position 2550 with learning strength set at 10%, 20% and 30%. ANOVA, $P = 0.2512$.

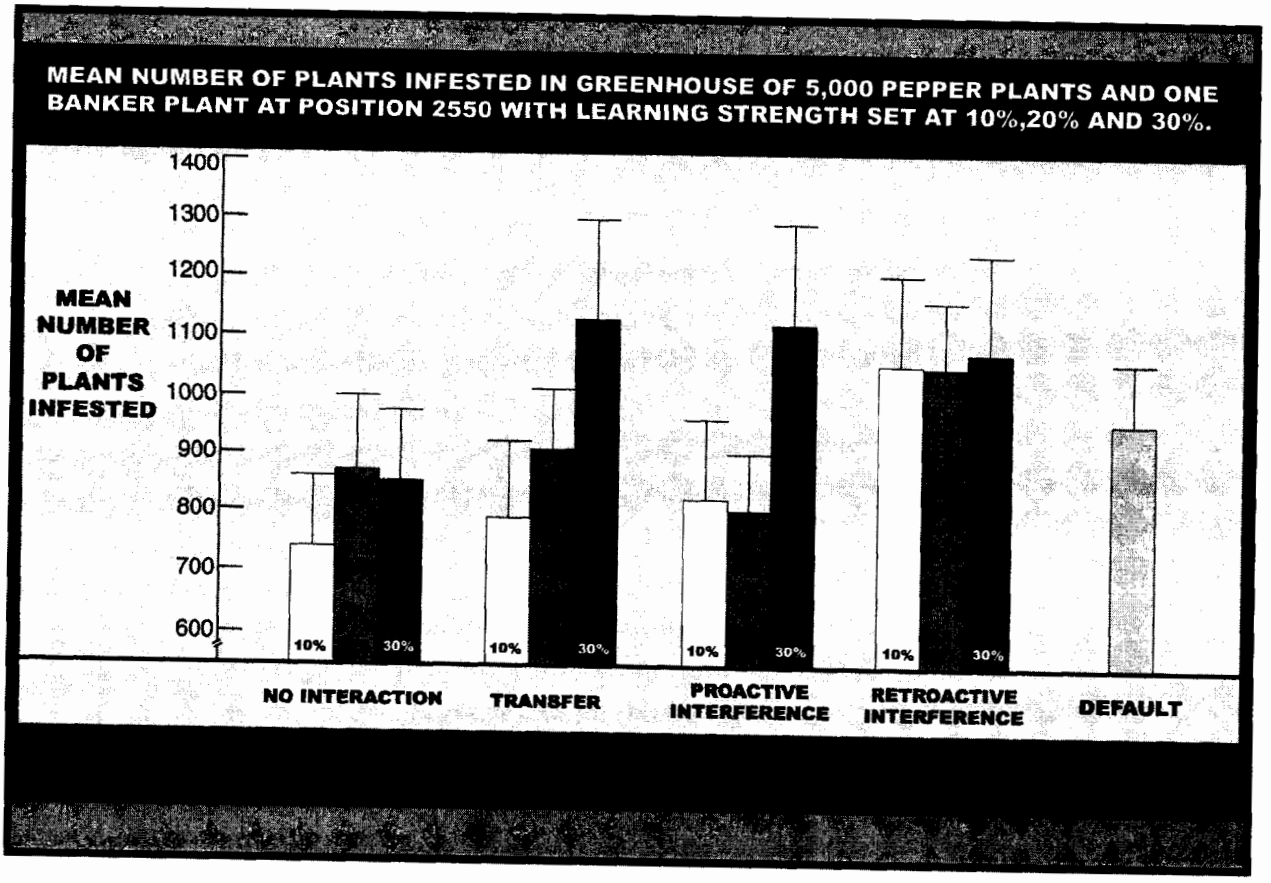

MEAN NUMBER OF PLANTS INFESTED IN GREENHOUSE OF 5,000 PEPPER PLANTS AND ONE BANKER PLANT AT POSITION 2550 WITH LEARNING STRENGTH SET AT 10%,20% AND 30%.
MEAN NUMBER OF PLANTS INFESTED
1400
1300
1200
1100
1000
900
800
700
600
10%
30%
NO INTERACTION
TRANSFER
PROACTIVE INTERFERENCE
RETROACTIVE INTERFERENCE
DEFAULT

Figure 10 - Mean amount of total aphid damage in greenhouse of 5,000 pepper plants with either 1 or 2 banker plants and the learning strength set a 20%. ANOVA, $P < 0.0001$. Bars with the same letter superscript are not significantly different, Tukey-Kramer HSD test.

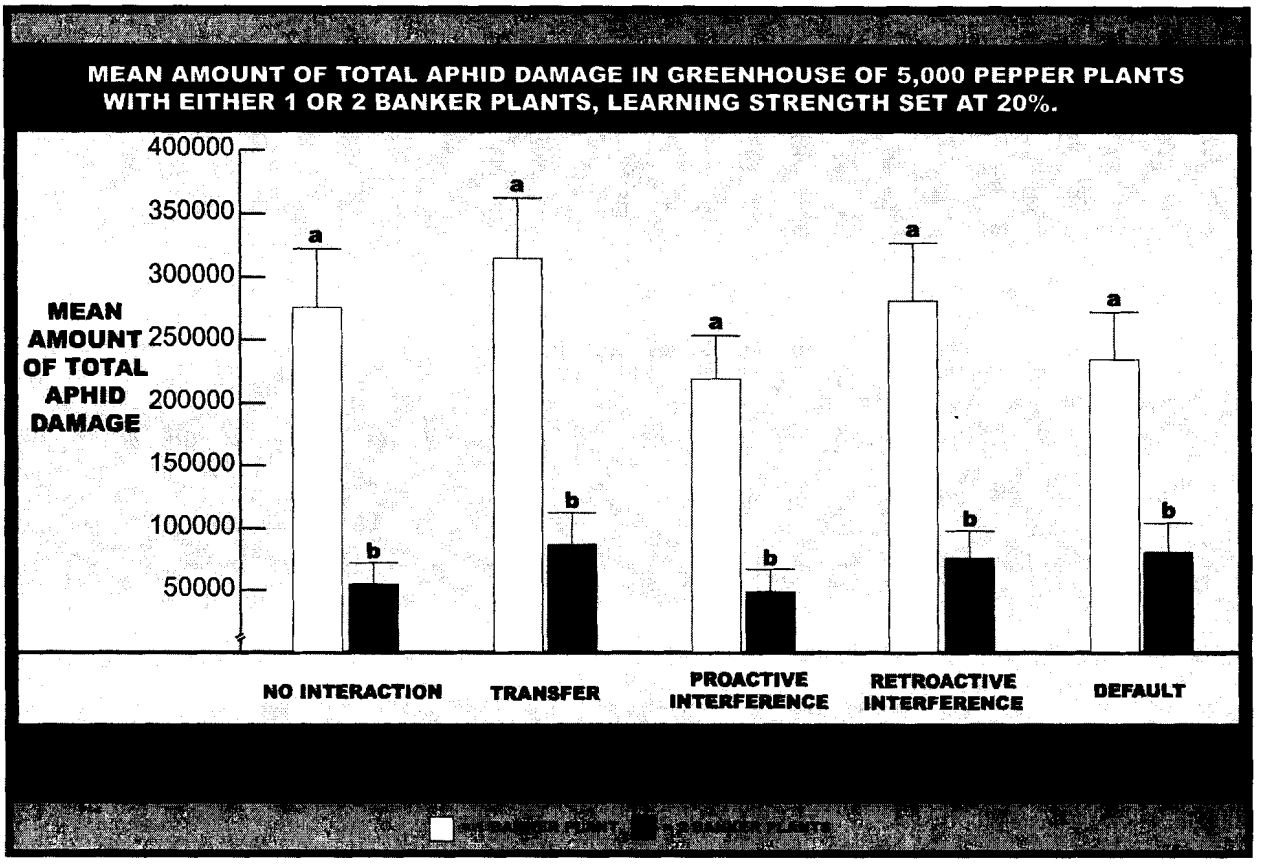

MEAN AMOUNT OF TOTAL APHID DAMAGE IN GREENHOUSE OF 5,000 PEPPER PLANTS WITH EITHER 1 OR 2 BANKER PLANTS, LEARNING STRENGTH SET AT 20%.
MEAN AMOUNT OF TOTAL APHID DAMAGE
400000
350000
300000
250000
200000
150000
100000
50000
a
b
NO INTERACTION
TRANSFER
PROACTIVE INTERFERENCE
RETROACTIVE INTERFERENCE
DEFAULT

Figure 11 - Mean number of plants infested in greenhouse of 5,000 pepper plants with either 1 or 2 banker plants and the learning strength set a 20%. ANOVA, $P < 0.0001$. Bars with the same letter superscript are not significantly different, Tukey-Kramer HSD test.

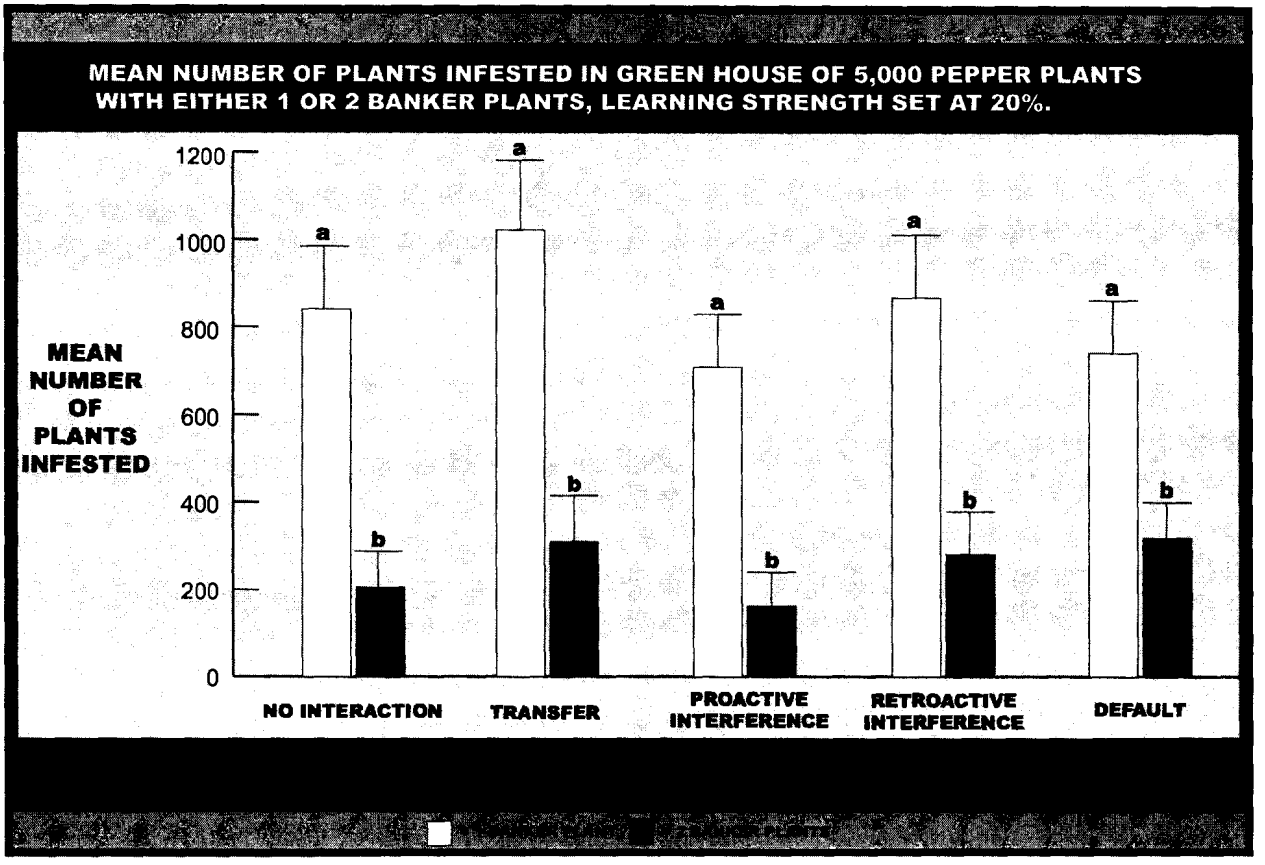

MEAN NUMBER OF PLANTS INFESTED IN GREEN HOUSE OF 5,000 PEPPER PLANTS WITH EITHER 1 OR 2 BANKER PLANTS, LEARNING STRENGTH SET AT 20%.
MEAN NUMBER OF PLANTS INFESTED
1200
1000
800
600
400
200
0
a
b
NO INTERACTION
TRANSFER
PROACTIVE INTERFERENCE
RETROACTIVE INTERFERENCE
DEFAULT

Table 4 - Analysis of variance table for mean amount of aphid damage for 5 different learning types at 3 different learning strengths.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	12	4.29831e12	3.5819e11	1.4679	0.1296
Error	1287	3.14053e14	2.4402e11		
C. Total	1299	3.18351e14			

Table 5 – Analysis of variance table for mean number of plants infested for 5 different learning types at 3 different learning strengths.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	12	32517098.9	2709758	1.2377	0.2512
Error	1287	2817709286	2189362		
C. Total	1299	2850226385			

Table 6 – Analysis of variance table for mean amount of aphid damage with 1 or 2 banker plants and learning strength set at 20%.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	9	1.02268e13	1.1363e12	9.1657	<.0001
Error	990	1.22734e14	1.2397e11		
C. Total	999	1.32961e14			

Table 7 – Analysis of variance table for mean number of plants infested with 1 or 2 banker plants and learning strength set at 20%.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Treatment	9	92718712.2	10302079	7.4368	<.0001
Error	990	1371431528	1385284.4		
C. Total	999	1464150240			

Discussion

The aim of this research was to determine if *Aphelinus abdominalis* has the ability to learn host + plant odours and to investigate the dynamics of this learning, and its future recall, when more than one type of host + plant complex is learned. Although there were no significant differences in odour choice between any of the treatment groups in the Y-tube bioassays we can look at the trends in the data and speculate on what might be going on. Naïve groups appear to show no preference for either the pepper + *A. solani* complex or the wheat + *S. fragariae* complex (figure 2). This indicates that there is no innate preference for one host over the other. The naïve group that was reared on *A. solani* shows a slight preference for the pepper + *A. solani* complex which may be due to learning of host cues during adult emergence.

The first odour choice of both groups given only one type of experience, on either pepper + *A. solani* or wheat + *S. fragariae*, show a slight increase in preference for the complex that was previously experienced (figure 2). This indicates that learning may occur, however, since the results are not significant we cannot make this conclusion with confidence.

The first choice results of the treatment group that received two different experiences, first on the pepper + *A. solani* complex and then on the wheat + *S. fragariae* complex, shows an even greater preference for the pepper + *A. solani* complex than the group that experienced only the pepper + *A. solani* complex (figure 2). This suggests that proactive interference may be occurring, with the learning of the first experience hindering the learning of the second complex. Again, the results are not significant so no conclusions can be drawn. However, if transfer or retroactive interference had occurred we would expect to see an increase in preference for the wheat + *S. fragariae* complex. This is because the learning of the pepper + *A. solani* complex would either have facilitated the learning of the wheat + *S. fragariae* complex (transfer) or subsequent learning wheat + *S. fragariae* complex would have hindered the recall of the pepper + *A. solani* complex (retroactive interference).

The results of the treatment group that experienced the wheat + *S. fragariae* complex first and the pepper + *A. solani* complex second show that there is subsequently no preference for either of the odour sources (figure 2). This result is ambiguous but it may be a sign of either

transfer or retroactive interference as the preference for the wheat + *S. fragariae* complex is less than that of the group that has only experienced the wheat + *S. fragariae* complex.

A Y-tube olfactometer design was chosen for this research because it is relatively easy and inexpensive to construct compared to that of a wind tunnel olfactometer (Du et al. 1996). In addition, the small size and poor flying ability of *A. abdominalis* would make testing it in a wind tunnel olfactometer difficult. Consequently a small Y-tube olfactometer, that requires the parasitoid to show a response by walking upwind towards an odour source, was deemed to be more appropriate for this insect. One of the major arguments against Y-tube olfactometers is that they do not allow the insect to sample the odour sources before making a choice and as a result initial wrong decisions may be recorded (Du et al. 1996). To overcome this problem the protocol set out by Du et al. (1996) was followed. This protocol allows parasitoids to make as many choices as they wish during a given time period and all choices are recorded. This allowed us to analyze not only the initial first choice results but also the final choice results, which would allow the parasitoid to correct wrong initial decisions.

In their research with *Aphidius ervi* Du et al. (1996) found no significant results in the responses of the parasitoids when the first choice was analyzed but there were significant results when the final choice was analyzed. When the final choice results are analyzed in this research it was found that there is even less of a difference between the treatment groups, with all of the groups showing close to no preference for either the pepper + *A. solani* complex or the wheat + *S. fragariae* complex. This would seem to indicate that the parasitoids are not correcting initial wrong decisions. The reason for this may be that the parasitoids that have made correct initial decisions are then unable to locate the source of the odour that they are attracted to. These parasitoids then search in vain for an odour source and hence, in the 5 minute bioassay period, end up in the "wrong" arm of the Y-tube.

In addition to the choice of odour sources made by the parasitoids the time spent in each arm of the Y-tube was also recorded. This was done to see if perhaps the parasitoids displayed their preference for an odour source by concentrating their search time to a particular arm of the Y-tube rather than choosing one arm of the Y-tube before another. Again, however, no significant differences were found between any of the treatment groups (figures 6 & 7).

One of the reasons for the lack of significance found in this experiment could be a problem with the Y-tube olfactometer. Preliminary runs of the bioassays without any odour sources indicated that the parasitoids did not show a preference for one side of the olfactometer over the other. To further control for this possible problem the arms that the odour sources were presented in were switched between every run of the bioassay. However, when all the results were analyzed it was found that the naïve groups, particularly those reared on *M. persicae* and *S. fragariae*, showed a preference for the right side of the olfactometer (figures 4 and 5). The reasons for this preference could be any number of minor physical differences between the sides that are not detectable by the researcher but are detectable by the parasitoid, such as a difference in light, temperature, vibration or color. The reason that the preference is only found in the naïve groups could be due to the naïve parasitoids using something other than odour to orient their search. This would be a reasonable assumption since the naïve groups had no previous exposure to the plant + host complex. This finding would agree with other researchers who have found that *A. abdominalis* females that have not had an oviposition experience tend to fly off and be unable to locate relatively nearby hosts when released in a greenhouse setting (Molck and Wyss 2001).

Another reason that learning may not have been detected in this research could be a problem with the experience protocol. All experienced parasitoids were confined to an experimental plant, that had been infested with the appropriate aphids, for a 48 hour period. This amount of time was ample to allow for parasitization of a number of aphids. In addition the experimental plants were subsequently kept and aphids were checked for mummies. All plants produced at least one aphid mummy indicating that all experienced parasitoids had oviposited at least once. However, it is likely that there was a degree of variation in the number of oviposition experiences each parasitoid had during the 48 hour time period. This variation could have lead to a variation in the subsequent learned responses which, in turn, may have obscured any evidence of learning. This problem could have been solved by actually observing each parasitoid during the oviposition experience to make sure that each parasitoid had experienced the same number of ovipositions. This was not done due to time constraints.

Variation in the parasitoid response may have been due, in part, to day to day variation. Steinberg et al. (1992) found that the parasitoid *Cotesia glomerata* showed a variation in response from day to day that was correlated with the direction of change in barometric pressure.

When the barometric pressure was steadily increasing the parasitoids were more likely to respond compared to times when the barometric pressure was steadily decreasing or fluctuating. To control for this type of variation there was an attempt to run replicates of each of the treatment groups on every day of experimentation. This was not always achieved due to the low number of parasitoids available and to the loss of some parasitoids during the experience phase. Additionally, due to time constraints, it was not possible to run all replicates in a short time period. Instead the replicates were run off and on throughout one year which could have also contributed to the day to day variation given that Burnaby Mountain is more prone to decreasing barometric pressure during fall and winter months than in the spring and summer months.

The inability of this research to demonstrate learning in *A. abdominalis* was surprising given that other researchers have shown *A. abdominalis* is capable of learning (Molck et al. 2000) as are a great number of other hymenopterous parasitoids (Turlings et al. 1993). The parasitoids used in these experiments were reared in a laboratory setting from a population of approximately 300 individuals that were obtained from a commercial insectary. There is known to be genetic variation in the ability of parasitoids to learn (Lewis et al. 2003) and it is has also been known for some time that commercially reared parasitoids may not perform as well in the field setting as their wild counterparts due to genetic changes that occurred through artificial selection pressures in the mass rearing process (Mackauer 1976). Parasitoids massed reared in a commercial insectary environment are provided with a more than ample supply of easily found hosts and as a result associative learning of host cues is not an important characteristic for these parasitoids (van Lenteren 2003). Consequently, it is possible that the parasitoids used in these experiments had lost the ability to learn due to a failure of the artificial rearing environment to select for the learning trait. This possibility warrants further investigation as it could have a negative effect on the ability of *A. abdominalis* to control *A. solani* outbreaks in the greenhouse environment.

In addition to the inability to demonstrate learning in the Y-tube bioassays no significant learning effects were found in any of the sets of assumptions for the model. This lack of significance was likely due to a large degree of variability in the data. This variability may be a consequence of the massive difference in the number of pepper plants compared to the number of banker plants in the greenhouse. All simulations were run in a greenhouse containing 5,000 pepper plants and either 1 or 2 banker plants, to try to approximate the actual situation found in

commercial greenhouses while staying within the confines of what is practical to model. At the beginning of our simulation all parasitoids were placed directly onto the banker plants before any *A. solani* infestations occurred in the pepper crop. At this point, since there were no other hosts, the majority of the parasitoids would stay to forage on the banker plant. Therefore, unless the *A. solani* infestation happened to occur in close proximity to a banker plant, the majority of the parasitoids in the greenhouse would be unable to detect the presence of *A. solani* at the beginning of the infestation. This inability to find the infestation at the outset would then result in a more widespread infestation of the greenhouse, which would be difficult for the parasitoids to control. Conversely, there would be situations were the *A.solani* infestation was initiated in close proximity to a banker plant, or near one of the few parasitoids foraging in the crop, and hence would be quickly found and brought under control. It appears that the dynamics of the random placement of the initial *A.solani* infestation may have overshadowed any learning effects in the model.

The model did detect a significant effect in the density of banker plants within the greenhouse. There was significantly less aphid damage, in terms of aphid days and number of plants infested, when 2 banker plants were used compared to when just 1 banker plant was used (figures 10 and 11). This density effect is the result of there being a greater chance of the initial infestation happening in close proximity to a banker plant and thus being detected by the parasitoids. This strengthens the conclusion made previously that the placement of the initial *A. solani* infestation, in relation to the banker plants, is more important than any effects learning might have on the ability of *A. abdominalis* to control *A. solani* within the greenhouse.

The results of this model suggest that growers should be more concerned with placing banker plants in locations that are likely to receive the initial aphid infestations then with any possible effects of parasitoid learning. Aphid hotspots frequently originate under vents, where aphids can come in from the outside, or near the central corridor of the greenhouse, where workers or machinery can bring in aphids unknowingly. If banker plants were placed near these locations the parasitoids would be able to quickly locate the aphid pests and bring the infestation under control. Due to economic and practical constraints it is not feasible to place banker plants under every vent or at the beginning of every row, as a result, growers must decide where to place banker plants so that they are most effective. It is possible that better control of aphids in

the greenhouse could be achieved with more strategic placement of banker plants and this is an area that would benefit from more research.

Another area that would benefit from more research is the possibility of increasing the retention of *A. abdominalis* in infestation areas by giving them an oviposition experience prior to their release in the greenhouse. The preference of naïve *A. abdominalis* for one side of the Y-tube olfactometer over the other (figures 4 and 5) and the finding, by Molck and Wyss (2001), that naïve *A. abdominalis* tend to be unable to locate nearby hosts in a greenhouse setting suggest that oviposition experience enhances the parasitoid's ability to utilize cues from the plant + host complex. *A. abdominalis* are currently shipped to growers as mated adults with no prior exposure to the plant + host complex. It would be prudent to investigate whether exposing *A. abdominalis* to the plant + host complex, either prior to shipping or upon arrival at the greenhouse, increases their ability to control foxglove aphid infestations.

Conclusion

This research was undertaken with an applied question in mind and the intention that the results generated would inspire further research in a more realistic field setting, such as a research greenhouse. Due to the inability of the results to demonstrate *A. abdominalis* is capable of learning it would be prudent to do further research in the laboratory setting to refine the bioassay procedure before setting out to do learning research in the field. However, the results of the model suggest that growers would likely benefit from field research into the optimal placement and density of banker plants within the greenhouse regardless of what the parasitoids may or may not be able to learn.

Additionally, more research into the training of parasitoids with an oviposition experience prior to their release in the field may enhance the efficacy of current biological control agents. Biological control agents may also be improved by commercial insectaries adopting strict quality control protocols to ensure that genetic traits, such as the ability to learn, are not lost during the mass rearing process.

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Appendix 1

Model Parameters

Distance a parasitoid checks for aphid infestations = 5 plants in any direction.

Cost to parasitoid of flying a long distance to other plants = 0.9

Sex ratio of parasitoids emerging from *Aulacorthum solani* = 50:50

Sex ratio of parasitoids emerging from *Sitobion fragariae* = 50:50

Growth rate of *A. solani* on pepper plants = 0.3

Growth rate of *S. fragariae* on banker plants = 0.3

Carrying capacity of pepper plant = 1500 aphids

Carrying capacity of banker plant = 1500 aphids

Number of *S. fragariae* seeded onto banker plant = 250

Number of parasitoids seeded onto banker plant = 200

Proportion of parasitoids that make long trips after leaving a plant = 0.20

Sensitivity of aphids to crowding = 3.5

Sensitivity of parasitoids to lack of aphids = 0.5

Search efficiency of parasitoid = 0.0006

Oviposition frequency = 0.005

Frequency of rejecting host = 0.005

Rate of decay of odor plume over distance = 0.222

Maturation rate of aphid mummies into adult wasps = 0.14

Daily death probability for parasitoids = 93 (gives a 50% probability of survival to 10 days)

Equation 1:

$$\lambda_x = \left(\frac{\alpha_x}{K_x}\right)^{\gamma\beta}$$

Equation 2:

$$\lambda_x = 1 - \left(\frac{\alpha_x}{K_x}\right)^{\gamma\beta}$$

Where λ_x is the leaving tendency from plant x, α_x is the number of aphids on plant x, K_x is the carrying capacity of plant x and $\gamma\beta$ is the sensitivity to aphid colony size for species β.

Equation 3:

$$\phi_x = \left(\frac{\alpha_x}{K_x}\right)^{-\delta\pi}$$

Where ϕ_x is the attractive index for colony at plant x, α_x is the number of aphids on plant x, K_x is the carrying capacity of plant x, δ is the distance from the current host plant and π is the rate at which the aphid-plant odor complex concentration declines.