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Research Article

DOES LEARNING BY PARASITOID INFLUENCE HOST CHOICE AND HANDLING TIME?

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ABSTRACT
Parasitoids showed associative learning and the interaction between innate, conditioned and learned behavioural responses due to reinforced practice and conditioning, through which the parasitoid acquires the ability of responding to stimulus with a reflex reaction to another stimulus. The behaviour of these species, which are at different trophic levels, is altered by the presence of a non-visible infection of the host plant. This associative learning may be very valuable in the study of interaction at higher trophic level. Experiment was carried out to determine the influence of learning by the aphid *Myzus persicae* (Hemiptera: Aphididae) and its common Parasitoid *Aphidius colemani* (Hymenoptera: Braconidae). The choice of host by female parasitoid is associated with the experience acquired either during emergence or as a result of brief encounter with the host. It was found that *M. persicae* grown on uninfected plant when given choice shows significant preference to the uninfected plant. However, when grown on infected plants then given choice did not discriminate between infected and uninfected plants. The inexperience source parasitoid (Naïve Parasitoid) do not show any preference between aphids grown on infected and uninfected plants host when given a choice, but this behaviour change with experience. The offspring that emerged showed a strong preference for their host similar to their natal host. However, after emergence when the parasitoid were allowed to gain experience on another host different from their natal host and later given choice show preference to the host they encounter last. This shows that learning is very important in host choice.

Keywords: *Aphidius Colemani, Botrytis Cinerea, Host Learning, Host Choice*

INTRODUCTION
Many researches on insect hosts have shown that in their effort to improve fitness and decrease mortality female parasitoids are expected to oviposit in the most suitable host when provided with a choice (Milner *et al.,* 1984; Poprawski *et al.,* 1992; Frasen and van Lenteren, 1994; Fellowes *et al.,* 1998; Singer *et al.,* 2004). When choosing host by female parasitoid the eggs deposited on lower quality hosts results in lower quality adults with higher mortality rates while eggs deposited on higher quality host result in higher quality and fittest adult (Rehman 1999; Morris and Fellowes 2002; Einat *et al.,* 2006; Rehman and Powell 2010).
Although character of the host play a role in host choice, however, studies have shown that host selection by the parasitoid is control by learning by conditioning the females to the host on which they develop and/or gain experience attacking (Chau and Mackauer, 2001; Morris and Fellowes, 2002). Previous researches have shown that parasitoids showed associative learning and the interaction between innate, conditioned and learned behavioural responses (Morris and Fellowes, 2002; Poppy and Powell, 2004). Rehman and Powell (2010) agreed that learning by parasitoid occurs from reinforced practice and conditioning, in which the parasitoid acquires the ability of responding to stimulus with a reflex reaction to another stimulus.
In a related study investigating the aphid parasitoid host choice Rehman and Powell (2010) have shown that host choice by aphid parasitoids is dependent on the fitness, size, age and the stage of host development, but in addition it is also dependent on both host and the species of parasitoid involved. The quality of the plant host influences the quality of the aphid which in turn influences the quality of the parasitoids (Krues, 2002; Ode *et al.,* 2005; Rehman and Powell, 2010).

Results of experiments by Charnov *et al.,* (1981); Sequeira and Mackauer (1993b); Godfray (1994); Joyce *et al.,* (2002) all reported that in hymenopteran parasitoids sex is determined by haplodiploidy. The haplodiploidy assist the females to control fertilization and determine the sex allocation of her offsprings. The fertilized eggs (diploid) in high quality host develop into females while unfertilized eggs (haploid) in low quality host develop into males. However, Chau and Mackauer (2001) and Henry *et al.*, (2005) argue that host quality is not the most important factor controlling host choice, because in some instance the higher quality hosts may not necessary be the preferred host. In support of this hypothesis Karamauna and Copland (2000), Chau and Mackauer (2001), Morris and Fellowes (2002) and Einat *et al.,* (2006), concluded that during emergence parasitoids learn cues associated with the host. This associative learning conditioned the parasitoids to that host from where they learn and gain experience and this influences future host choice.

This paper describes an experiment aimed at investigating the influence of learning on host choice by parasitoid *Aphidius colemani* (Hymenoptera: Braconidae) on aphid host (the peach potato aphid *Myzus persicae*) grown on infected and uninfected lettuce plant Asteraceae: Compositae) by systemic *Botrytis cinerea*. The study investigates whether host choice by the aphid was influenced by gaining experience on the host. Three hypotheses were tested. First, if the host on which the parasitoid *A. colemani* emerged influenced host preference. Second, whether learning acquired during emergence would influence the host choice for feeding and oviposition decision of parasitoids. Third, that host choice is better influence by learning than host quality.

MATERIALS AND METHODS

Experimental Plants

Lettuce (*Lactuca sativa*) seeds were sown individually in one hundred and forty 20cm diameter pots filled with a vermiculite-based growing medium in a controlled environment room (18-20^{o}C, ambient humidity and 12:12 h L:D). Eighty plants were grown from clean seed without *B. cinerea* infection, while the remaining eighty plants were grown from systemically infected seed collected from plants inoculated at the flower stage and tested by plating on *Botrytis* selective media (BSM) plates.

Peach Potato Aphid Myzus Persicae

The aphid species *Myzus persicae* Sulzer (Hemiptera: Aphididae) used was reared on lettuce plants infected and uninfected with *B. cinerea* for six generations to allow for possible effects of telescoping of generations before use in the experiment (Dixon, 1985).

Choice of Plant Host by the Aphid

Different tests were carried out each with new set of plants and aphids (i) with aphids reared on infected plants (ii) with aphids reared on uninfected plants. In each test, two plants (one infected and one uninfected) were placed into an insect cage (50 X 50 cm). Twenty adult aphids *M. persicae* were place in a Petri dish then the Petri dish containing the aphids was place inside the cage equidistant between the two plants and left for two hours.

The plants were reared in pots and readily accessible to the aphids. This was replicated ten times each time with new set of plants and aphids. The number of aphids on each plant was counted to determine the choice of infected or uninfected plant by the aphids.

Source Parasitoid Host Preference

Two female *Aphidius colemani* as provided by Koppert were given a choice of host in a cage between (i) an infected plant infested with second instar *M. persicae,* reared on infected plant and (ii) an uninfected plant infested with second instar of *M. persicae* reared on uninfected plant. In each test, one infected and one uninfected plant each infested with 30 second instar aphids was placed into a cage (50 X 50 cm). Two *Aphidius colemani* were released into the cage and left for three hours. This was replicated ten times each time with a new set of plants, aphids and parasitoids. Thereafter, the plants were removed and covered with a vented plastic container and kept in a controlled environmental room for one week. The plants were monitored daily for the appearance of mummies. The numbers of mummies and adult aphids were counted one week after the test.

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Influence of Natal Host on Parasitoid Host Preference

Two tests were carried out (i) with second instar *M. persicae* grown on infected and uninfected plants and *Aphidius colemani* reared on aphids grown on infected plants (ii) second instar *M. persicae* grown on infected and uninfected plants and natal *Aphidius colemani* reared on aphid hosts grown on uninfected plants. In each test one infected and one uninfected plant infested with 30 second instar aphids was placed into a cage (50 X 50 cm). Two female *Aphidius colemani* were released into the cage and left for three hours. This was replicated ten times each time with a new set of plants, parasitoids and aphids. Thereafter, the plants were removed and covered and kept in a controlled environment room for one week. The plants were monitored daily for the growth of mummies. The numbers of mummies were counted one week after the test.

Influence of Experience on Parasitoid Host Preference

The parasitoids that emerged from aphid hosts which were either grown on infected or uninfected plants were first given no choice in attacking hosts which were either reared on infected plants or uninfected plants before they were then given a choice. Ten infected and ten uninfected plants each infested with 30 second instar *M. persicae* were individually placed in a separate cage and two female *Aphidius colemani* reared on aphids grown on infected plants were released in each cage and left for three hours (no choice). After three hours the parasitoids were removed and given a choice between infected and uninfected plants infested with 30 second instar *M. persicae* which had been reared on infected and uninfected plants respectively. Another ten infected and ten uninfected plants each infested with 30 second instar *M. persicae* were individually placed into a cage (50 X 50 cm) and attacked for three hours with two female *A. colemani* reared on aphid grown in uninfected plant that emerged from naïve hosts. After three hours, the parasitoids were removed and given a choice of infected and uninfected plants infested with 30 second instar *M. persicae* in a cage for three hours. After the choice test plants were removed from the cage and covered with vented plastic container and placed in a controlled environment room for one week. The resulting mummies and adult aphids were counted.

Statistical Analysis

The proportion data from aphid preference to infected and uninfected plants was acrsine square-root transformed prior to analysis by paired T-test. In analysing the data from parasitoid choice experiment, the proportion (expressed as proportion of *M. persicae* nymphs attacked out of the available nymphs by the parasitoid *A. colemani*) data were arcsine square-root transformed prior to analysis (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION

Result

Choice of Host by Infected and Uninfected Aphid

Aphids grown on uninfected plants when given host choice show significant preference to the uninfected plants (Figure 1, $t_{,9}$= 12.55, P < 0.001). When reared on infected plants and then given choice aphids do not discriminate between infected and uninfected plant ($t_{,9}$= 1.22, P = 0.254).

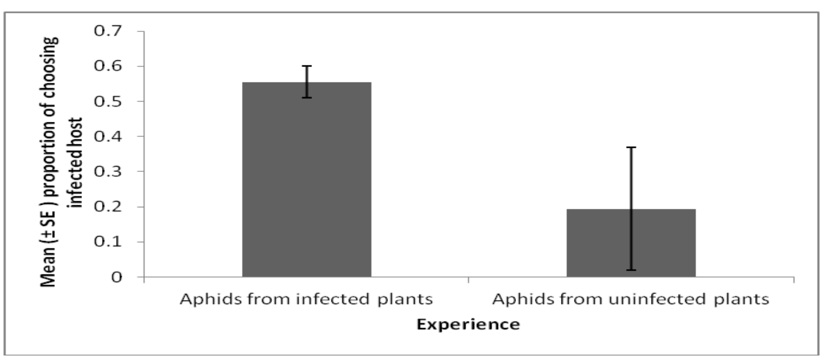

Figure 1: Host plant preference of aphids which had been reared on uninfected or *B. cinerea* infected host plants when given a choice between and infected and uninfected plants

Source Parasitoid Host Preference (Naïve)

Source parasitoids (parasitoid which was not exposed to lettuce or reared on *Myzus persicae*, as they are provided by Koppert) when they were given a choice between *M. persicae* grown on infected and uninfected plants do not show any significant preference ($F_{1,9}$ = 0.74, P = 0.527) with back transformed mean of 0.219.

Influence of Natal Host on Host Choice

Female *A. colemani* that emerged from infected hosts when given choice did not show significant preference to the host from both infected and uninfected plant (Figure 2; $F_{1,39}$ = 1.00, P = 0.323).

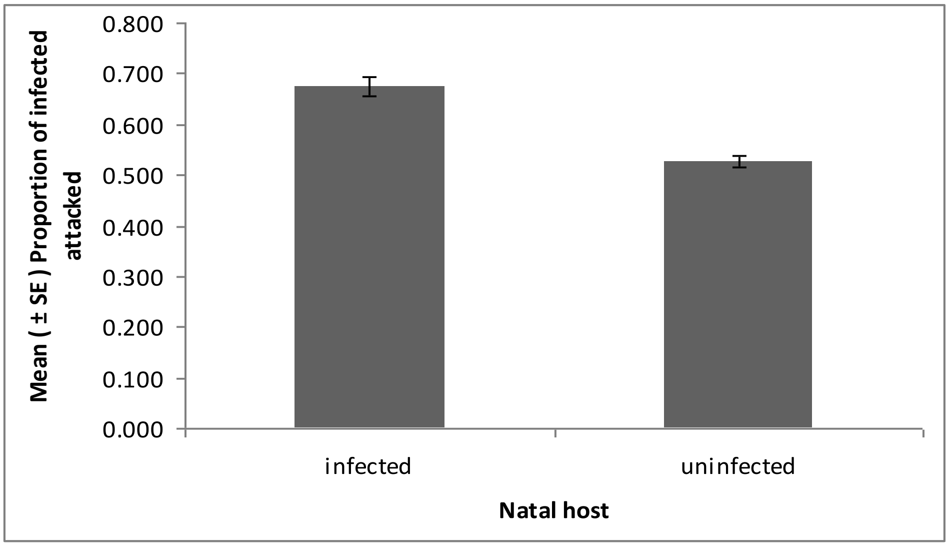

Figure 2: Mean (± SE) proportion *M. persicae* nymphs attacked by *A. Colemani* which had emerged from hosts reared on infected or uninfected plants

Influence of Natal Host and Experience on Host Preference

The natal host has do not affect host choice, the total number of aphid nymph attacked was not related to the natal host (Figure 2; $F_{1,39}$= 1.00, P = 0.323).

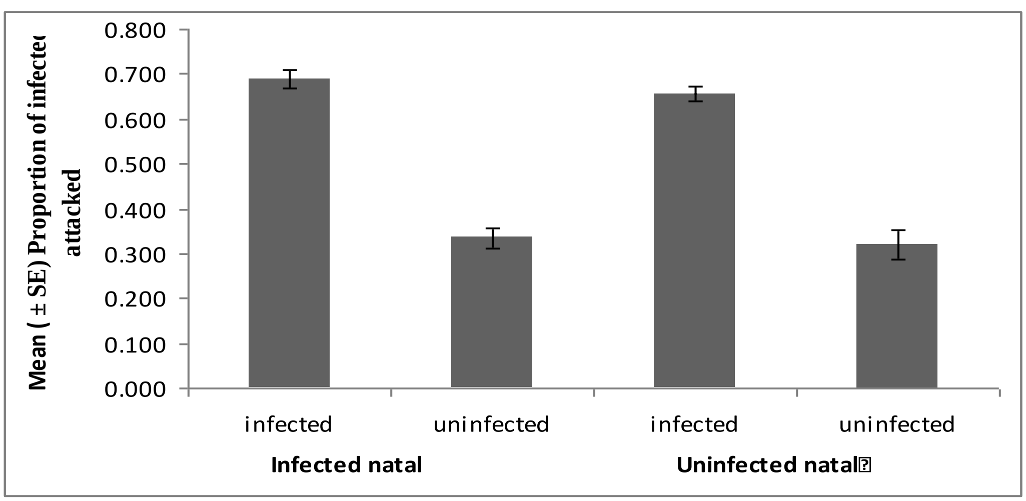

Figure 3: Mean (± SE) proportion of *M. persicae* attacked in choice experiment by *A. colemani* female from infected host with experience attacking infected and uninfectedhost; and female from uninfected host with experience attacking infected and uninfected host

There was no significant interaction between parasitoid type and prior experience (Figure 6.3; $F_{1,\ 39}$ = 0.18, P = 0.677). However, this pattrern changes with experience. Female *A. colemani* show the influence of learning by showing significant preference to the host they were allowed to gain experience before they were given choice (Figure 3; $F_{1,39}$ = 194.16, P < 0.001). *Aphidius colemani* from infected and uninfected natal host when allowed to gain experience on infected host showed a strong preference to infected host when given choice. While *A. colemani* from infected and uninfected natal host when allowed to gain experience on uninfected plant and later given choice shows strong but marginal preference to the host from uninfected plant.

Discussion

The results show that the aphid *M. persicae* reared on uninfected plant when give choice shows significant preference to the uninfected plant. However, aphid reared on infected plants when given choice did not show preference to both infected and uninfected plants. Naïve *A. colemani* do not have any preference between aphids reared on infected and uninfected host plants when given a choice, but experience changes this pattern. The offspring that emerged showed a strong preference for their host similar to their natal host. Experiments showed that learning has an influence on host choice and this is affected by host plant infection status. *Aphidius colemani* grown on aphid reared on uninfected plants when given choice prefer to oviposit on aphid grown on uninfected plants. *Aphidius colemani* reared on aphids grown on infected plants when given choice showed a preference for aphids grown on infected plants. Therefore, the behaviour of both host and parasitoid is affected by experience, and with the parasitoids, that learning can alter host preference behaviour. The behaviour of these species, which are at different trophic levels, is altered by the presence of a non-visible infection of the host plant.

When given a host choice the female parasitoid respond to the preferred attributes which differentiate hosts of varying quality (Kouame and Mackauer, 1991). The female parasitoids when given choice select hosts that have more food and which maximized their rate of foraging value/handling time (Kouame and Mackauer, 1991; Fellowes *et al.,* 2005; Yahaya and Fellowes, 2013). The results of this study suggest that female parasitoids uses experience from different combinations of physiological characteristics to assess host quality for feeding and oviposition.

The results of this study showed that source parasitoids (Naïve parasitoids) when allowed selecting host between infected and uninfected host do not show preference for any of the host; however, this pattern changes with experience. It was found that after emergence parasitoids may learn cues associated with their host and this learned behaviour can be helpful in future host choice of the natal parasitoids. Such cues are learned either during emergence of the offspring or as a result of the parasitoid biting the host exoskeleton or by antennation of the host mummy after emergence (Chau and Mackauer, 2001; Morris and Fellowes, 2002; Einat *et al.,* 2006). It was observed that the parasitoids become associated to the host where they emerge and this increases the possibility of choosing a suitable host (Karamauna and Copland, 2000; Chau and Mackauer, 2001; Morris and Fellowes, 2002; Fellowes *et al.,* 2005; Einat *et al.,* 2006). The ability of the female parasitoids to make a choice between hosts of higher and lower quality depends on their experience. The parasitoid with more experienced on the host are expected to make better host choices that will ensure the survival of their offspring than inexperience naïve ones (Fellowes *et al.,* 2005; Einat *et al.,* 2006; Harri *et al.,* 2008).

The result of this study indicates that learning is affected by host quality where the foraging parasitoids learn cues associated with the host. Lewis *et al.,* (1990) reported that learning food odours share a number of characteristics with learning host odours in the parasitoids, and it is likely that foraging strategies for both are similar. The female parasitoids may find high quality food or hosts as results of their innate response to specific cues or by random searching. Once the females find these quality resources they are able to associatively learn the physical or chemical characteristics and thereafter improve their searching efficiency by using these characteristics as searching cues (Lewis *et al.,* 1990). Turling *et al.,* (1993) reported that the searching behaviour of parasitoid for a quality host is affected by experience at various stages of their life cycle; sometimes cues may be learned at early stages which can manifest in the responses at the adulthood stage however, associative learning during adult stage shows a greater effect on the insect response and contribute to foraging success. Report by Vet and Groenewold (1990) shows that learning during the adult stage is more important in host choice than learning acquired during immature stages. They found that female parasitoids learn to respond to a novel odour, which they may subsequently use during host choice and this assist them to discriminate between higher and lower quality host (Vet and Groenewold, 1990; Turling *et al.*, 1993). The result of the present study shows a strong support for this view the female parasitoids that emerged from hosts grown on infected plant, then exposed to the hosts reared on uninfected plants for few hours without choice and later given choice shows more preference to the host grown on uninfected plant which they encounter last. This shows that learning is associative and requires very short contact with the specific innately recognized stimuli (Morris and Fellowes, 2002; Einat *et al.*, 2006).

Therefore, this study has shown that learning and host choice can influence interactions at multiple trophic levels, and that these behavioural traits are affected by infection status of the host plant (Lewis *et al.*, 1990). Previous studies on influence of learning in parasitoid by Lewis *et al.*, (1990); Turling *et al.*, (1993); Morris and Fellowes (2002); Einat *et al.*, (2006) all support that learning plays a role in strengthening the response to a single stimulus either by promoting the speed or efficiency of the response, or strengthening preference for the stimulus over other stimuli conditioning, or joining two or more different stimulus that occur together the associated learning. The influence of learning on host choice behaviour is therefore the most important factor used by parasitoids during host choice. Learning therefore has the potential to change the outcome of the plant-herbivore and host-parasitoid interactions.

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