

# Tritropic interaction between *Myzus persicae* and its natural enemy *Aphidius ervi* grown on lettuce plant infected with systemic pathogen *Botrytis cinerea* under natural environmental condition

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## Abstract

Effects of interactions between the aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) and its common parasitoid, *Aphidius ervi* Viereck (Hymenoptera: Braconidae), were investigated in the field which is less controlled environment to determine if these are influenced by infection of the host aphids reared on lettuce *Lactuca sativa*, L. (Asteraceae: Compositae) by the widespread plant pathogen *Botrytis cinerea*. The field experiment showed that female parasitoids are generally larger than males, which may be due to the results of sex-specific allocation of offspring to host of higher and lower food value and the sex specific exploitation of host resources. More parasitoid mummies *A. ervi* were recorded on uninfected plants than infected ones ( $F_{1,48} = 66.57$ ,  $P = 0.001$ ). Most of the parasitoids reared on host grown on uninfected plants give rise to female parasitoids while parasitoids reared on host grown on infected plants gave rise to equal proportion. Overall 62.2% females were recorded against 37.7% male parasitoids. Female *Aphidius ervi* were larger than males but the sizes were not significantly different ( $F_{1,49} = 2.13$ ,  $P = 0.136$ ) when reared on both infected and uninfected plants. However, the interaction between sex and infection was significant ( $F_{1,99} = 17.21$ ,  $P < 0.001$ ). Also infection of *B. cinerea* has no effect on the rate of chlorophyll fluorescence and the rate of photosynthesis but significantly affects the dry mass of the plant. Although, covering the plants with an insect net did not affect the rate of chlorophyll fluorescence but significantly affected the rate of photosynthesis, internode length, dry root and shoot weight of the plants. Until harvest no sign of *B. cinerea* lesion was seen in any of the experimental plants. Therefore, the observations of the field experiment provide further evidence that hidden, systemic host plant infection by *B. cinerea* influences the interaction between aphids and their parasitoids.

**Keywords:** *Aphidius ervi*, *Botrytis cinerea*, *Myzus persicae*, interaction

## Introduction

Many varieties of plant species available under natural settings make it easy for the foraging herbivorous insect to decide where to feed and deposit egg (Maris *et al.* 2004; Stout *et al.* 2006; Yahaya *et al.* 2015) [12, 16]. However, in many cases choice of food by herbivorous insect is determined by experience. Studies by Johnson *et al.* (2003) [4] and Maris *et al.* (2004) show that the experience may be either presence or absence of diseases, host nutritional quality, induced chemical produced for resistance and other factors like the presence or absence of natural enemies. In addition, the changes that can be induced by the pathogens may play an important influence in the ecological relationship with other insect herbivores. An example of this type of interaction is where pathogen infection can change the nutritional qualities and plant defense like triggering of important defense-related compounds such as phytohormones or by inducing the plant to secrete secondary metabolites, which may definitely interfere with the feeding and distribution of herbivores of a particular species.

Under natural environmental condition the distribution and efficiency of parasitoids the aphid natural enemies, are adversely affected and may sometimes become less efficient due to the style of farming practices like application of pesticides, climatic and other environmental factors which

have the ability of disrupting the association between aphids and their natural enemies which may lead to their dispersal from the plant host (Landis and Wratten 2000; Rehman and Powell, 2010; Nickolas *et al.* 2013) [5, 9, 8]. Although, diversity of the vegetation augments the natural enemies by provision of additional resources, such as additional shelter, nectar, pollen, more prey species and an improved microclimate for the aphid natural enemies (Rakhshani *et al.* 2010).

The systemic and necrotrophic fungal pathogen *Botrytis cinerea*, can grow systemically without showing symptom and without any host specificity, and this poses a serious threat to the survival of both glasshouse and field crops (Agrios 2005; Williamson 2007) [1]. The pathogenic fungi affect the performance of the plant negatively, and may be very extensive and may cause delay in the secretion of defensive mechanisms which could offset the plant fitness, leading to unexpected post-harvest losses as produce may decay without any prior warning (Johnston *et al.* 2006; Yahaya *et al.* 2015) [16]. This may ultimately lead to economic losses, during both short- and long-term storage and subsequent shipment of most types of horticultural produce (Agrios 2005) [1]. However, in the previous study carried out in a controlled environmental room (Yahaya and Fellowes 2013; Yahaya *et al.* 2015) [15, 16] found that indirect interaction between *B. cinerea* and aphid

(*M. persicae*), reduce the growth of each other resulting in lower *B. cinerea* lesion and lower aphid population. Therefore, it will be desirable to understand this relationship under a natural environmental condition, which will enhance further understanding of the ecological processes which will assist in design of adequate crop management strategies.

Previous study in the controlled environmental room (Yahaya and Fellowes 2013) [15] showed that the aphid natural enemy parasitoids have preference on the aphid population and, unless they are food starved, they show more preference for aphids grown on uninfected plants which are healthy, more tasty and palatable being grown from a good source of food than aphids grown on infected plants (Pell *et al.* 1991). In another study Hatano *et al.* (2008) reported that in many instances volatiles produced by herbivores in the natural environment provide reliable information on the presence of prey; however, the volatiles are produced in low concentrations in the natural environment due to the low biomass of aphids. Although plant volatiles are easily detected because of their high biomass, but they are less reliable since plants may or may not necessarily harbour herbivorous insects. Under natural environment predators use host cues to decide whether to lay eggs or not at a given site. However, residues left by the aphid's natural enemies in the previous visits to the plants such as "larval tracks" may deter oviposition by the same or other species. This behaviour was shown by ladybirds including *C. septempunctata*.

Experiment of Yahaya and Fellowes (2013) [15] in controlled environmental room showed that the presence of *B. cinerea* and infestation with *M. persicae* significantly affects photosynthesis and dry mass of the plant (internode length, root and shoot weight). However, the presence of *B. cinerea* and infestation with model insect aphid *M. persicae* has no significant effect on the rate of chlorophyll fluorescence. Result, from another study (Yahaya *et al.* 2015) [16] showed that size is an indicator of host quality and aphids natural enemy showed more preference to the aphid host grown on uninfected plant which are large with high quality food than aphids grown on infected plants which are small with a significantly male-biased offspring sex ratio due to high mortality of females in smaller than larger aphids. In another study Yahaya *et al.* (2015) [16] shows that the aphid natural enemy the parasitoids became conditioned to the host from which they emerged and they learnt cues associated with the host which assisted them in future host preference. It is therefore very important to replicate the experiments carried out under controlled environmental conditions on a larger more complex and realistic scale where the environmental conditions are not controlled (natural environmental condition). This may allows many direct/or indirect biological phenotypes such as infected or uninfected, infested or uninfested by pathogens and herbivores to show whether more preference between high food quality and less food quality plants can affect the distribution of herbivores natural enemy.

Therefore, four hypotheses were tested. First, bi-directional interaction will occur between herbivorous insect aphid *M. and Systemic B. cinerea* sharing the same host plant resulting in low aphid count and lower *B. cinerea* lesion. Second, the interaction of *B. cinerea* and aphid will significantly stress the plant causing a reduction in the rate of photosynthesis, chlorophyll fluorescence and dry mass of the plant. Third, the

feeding and egg deposition by the aphid natural enemy will be affected by the infection status of the plant resulting in fewer offspring on infected plants. Fourth, learning acquired as result of experience will influence host choice and the foraging parasitoid would show more preference to the aphid host from where they emerged and gained experience.

## Materials and Methods

### Plants materials

Four weeks old plants grown from infected and uninfected seeds in a controlled environmental room were transplanted into the open experimental field. Out of the plants, fifty plants were grown from clean seed without *B. cinerea* infection, while the remaining fifty plants were grown from systemically infected seed. Temperature and day length of the controlled environment varied between 18-20°C, ambient humidity and 12-14 h L: D, respectively, during the growth. The spacing in the field was 15 cm between plants and 40 cm between rows (Fig 1 & 2).



**Fig 1:** Transplanted infected and uninfected Lettuce plant growing in two plots and ten rows in the field



**Fig 2:** Three weeks old Transplanted infected and uninfected lettuce plant in rows one week before infestation with *M. persicae*

### Infestation of the experimental plants with Aphids *Myzus persicae*

Exactly, three weeks after transplanting all the experimental plants were infested each with ten adult aphids *M. persicae* (Fig 3) by placing them on the reverse side of the leaves. Immediately after infestation half of the plants from each of the two treatments (twenty-five) were separately covered with an insect net, to prevent their attack by the aphid natural enemies (Fig 4).



**Fig 3:** Nymphs of the green peach aphid *Myzus persicae* (Sulzer) prior to infestation of lettuce plants



**Fig 4:** Lettuce plants infected and uninfected infested with aphids, covered and uncovered with insect nets in the experimental field

#### Determination of photosynthesis rate

The rate of photosynthesis was determined as the amount of CO<sub>2</sub> assimilated per m<sup>2</sup> leaf surface area using a Red Gas Analyser (ADC Bioscientific LCI Analyser No. 31109) equipped with a standard broadleaf chamber measuring an area of 6.5cm<sup>2</sup>.

#### Determination of Chlorophyll Fluorescence

The rate of chlorophyll fluorescence was determined as the amount of re-emitted light from the leaf, measured using a Handy Pea Data Chlorophyll fluorometer (Hansatech Instrument Ltd. Pea plus version: 1.02).

#### Measurement of internode length

Measurement of internode length was taken from all the experimental plants were taken after harvest using a graduated meter ruler.

#### Measurement of dry shoot weight

Dry shoot weight was taken from all the experimental plants after harvest using an electronic balance (Kern scale Technic, 440-21N).

#### Measurement of dry root weight

Dry root weight was taken from all the experimental plant after harvest using an electronic balance (Kern scale Technic, 440-21N).

#### Determination of hind tibia length and number of emerging parasitoids mummies

The mummies were counted and collected into vials from the plants which were not covered and kept on the laboratory bench for the growth of the parasitoids and their subsequent identification. The length of the hind tibia of all of the parasitoids was measured using a micrometric eye piece attached to a microscope (Leica) at x 50 magnification.

#### Experimental design and statistical analysis

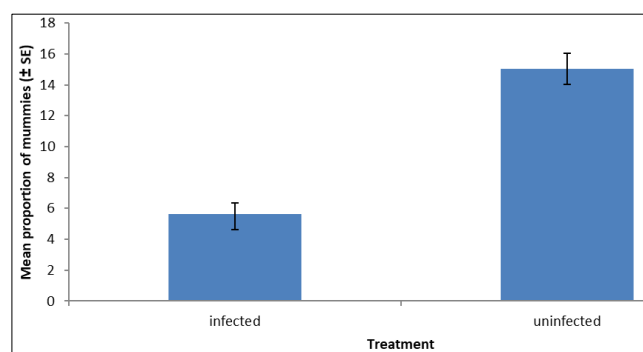
Four weeks old lettuce seedlings, fifty each from infected and uninfected seeds were transplanted at random into the two plots. Infestation of the plants with adult aphid *M. persicae* was done three weeks after transplanting. Following infestation with aphid, half of the plants were covered with an insect net to prevent their attack by natural enemies. At the end of the experiment, eight weeks after transplanting and before harvest, stress in all the plants was determined by the measurement of the rate of photosynthesis and chlorophyll fluorescence. After harvest, all of the plants which were not covered were

examined for the presence of mummies. All the mummies were identified, counted and collected into vials to allow for the growth of parasitoids. The data collected was analyzed by Anova using statistical software (Minitab 2009) [6].

#### Results

##### *Aphidius ervi* feeding on aphids reared on infected and uninfected plants

Parasitoids feeding on aphid *M. persicae* reared on uninfected plants was significantly higher than in infected plants (Fig 4,  $F_{1,48} = 66.57$   $P = 0.001$ ). In addition higher number of mummified aphids was obtained from the uninfected plants (360, 71.8%) then on infected plants (143, 26.1%).

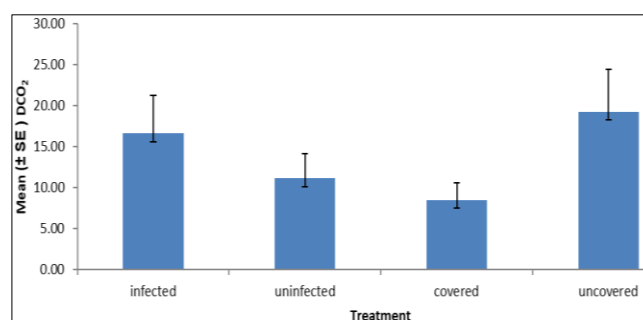


**Fig 4:** Mean (± SE) count of mummies emerging from hosts reared on infected and uninfected plants

##### Effect of plant cover on infected and uninfected plants infested with aphid

###### a) Photosynthesis

The rate of photosynthesis DCO<sub>2</sub> was not affected by *B. cinerea* on infected and uninfected plants which were both infested with aphids (Fig. 5,  $F_{1,99} = 0.94$ ,  $P = 0.321$ ). However, the plant covered with an insect net had a significantly reduced rate of photosynthesis ( $F_{1,99} = 3.94$ ,  $P = 0.050$ ). Moreover, the combined effect of infection and plant cover was not significant on the rate of photosynthesis ( $F_{1,99} = 2.63$ ,  $P = 0.135$ ).

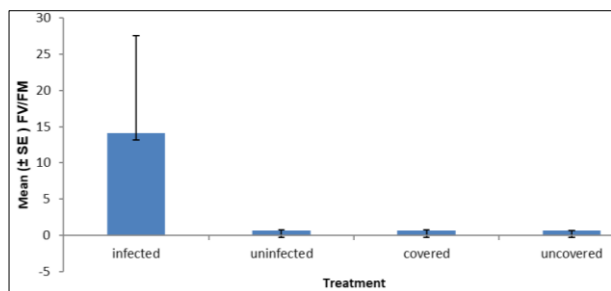


**Fig 5:** Rate of photosynthesis of plants in the four treatment

###### b) Chlorophyll fluorescence

The rate of chlorophyll fluorescence FV/FM was not significantly affected by *B. cinerea* infection on infected and uninfected plants which were infested with aphids (Fig. 6,  $F_{1,99} = 1.02$ ,  $P = 0.315$ ). Likewise, covering the plants, with a net had no significant effect on the rate of chlorophyll fluorescence ( $F_{1,99} = 0.52$ ,  $P = 0.471$ ). Also the combined effect of plant cover and infection was not significant on the rate of chlorophyll fluorescence ( $F_{1,99} = 2.643$   $P = 0.101$ ).



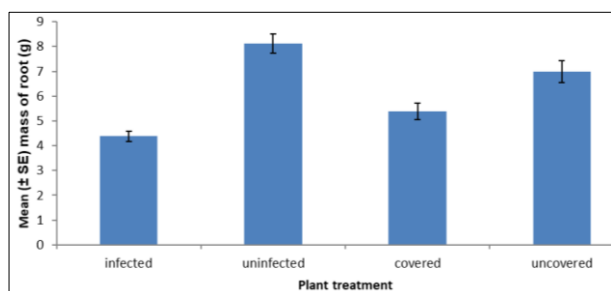


**Fig 6:** Rate of chlorophyll fluorescence of plants in the four treatments

### c) Dry mass of the plant

#### i. Internode length

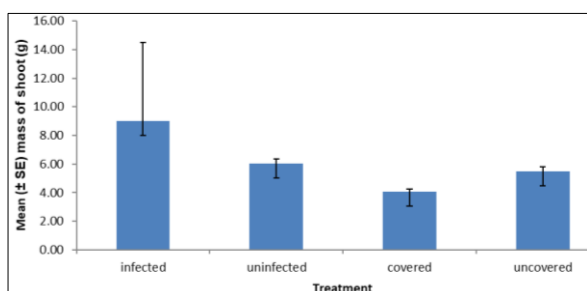
There was a significant difference in the internode length between *B. cinerea* infected and uninfected plants which were infested with aphids (Fig. 7,  $F_{1,99} = 94.14$ ,  $P < 0.001$ ). A significant difference was found between plants covered with an insect net and those which were not covered ( $F_{1,99} = 25.96$ ,  $P < 0.001$ ). This indicates that infection of *B. cinerea* on aphid infested plants or covering the plants with an insect net can cause stress to the plant resulting in a decrease in internode length. Also the combined effects of *B. cinerea* infection of infested plants and covering with an insect net also significantly reduces the internode length ( $F_{1,99} = 24.231$ ,  $P < 0.001$ ).



**Fig 7:** Mean (± SE) internode length of plants in the four treatment

#### ii. Dry shoot weight

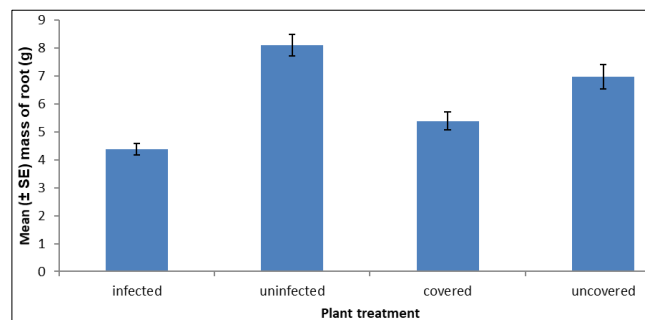
There was a significant difference in the dry shoot weight between *B. cinerea* infected and uninfected plants which were infested with aphids (Fig. 8,  $F_{1,99} = 92.15$ ,  $P < 0.001$ ). Also a significant difference was found between plants covered with an insect net and those which were not covered ( $F_{1,99} = 26.97$ ,  $P < 0.001$ ). This indicates that infection of *B. cinerea* on aphid infested plants or covering the plants with an insect net can cause stress to the plant resulting in a decrease in dry shoot weight. Moreover, the combined effects of *B. cinerea* infection of infested plants and covering with an insect net significantly reduces the dry shoot weight of the plants ( $F_{1,99} = 25.222$ ,  $P < 0.001$ ).



**Fig 8:** Mean (± SE) dry shoot weight of plants in the four treatment

#### iii. Dry root weight

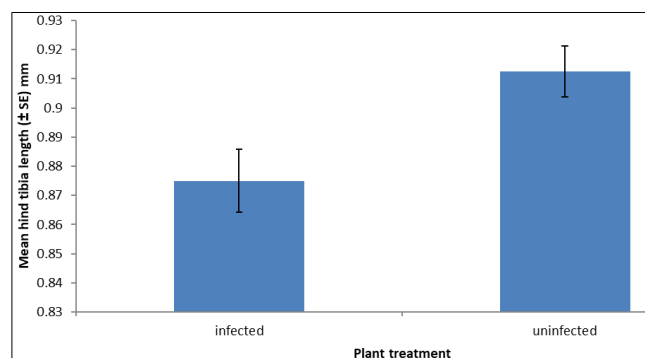
There was a significant difference in the dry root weight between infected and uninfected plants which were both infested with aphids (Fig. 9,  $F_{1,99} = 82.65$ ,  $P < 0.001$ ). Also a significant difference was found between plants covered with an insect net with those which were not covered ( $F_{1,99} = 14.52$ ,  $P < 0.001$ ). This shows that aphid infestation or infection with *B. cinerea* can cause stress to the plant resulting in a decrease in the dry root weight. However, the combined effects of *B. cinerea* infection and plant cover with insect net was not significant on the dry root weight ( $F_{1,99} = 2.36$ ,  $P = 0.117$ ).



**Fig 9:** Mean (± SE) dry root weight of plants in the four treatments

#### Size of parasitoid identified on the experimental plants *Aphidius ervi*

The hind tibia length of *Aphidius ervi* that emerged from aphids reared on uninfected plants was significantly longer than parasitoids reared on aphids feeding on infected plants (Fig 10,  $F_{1,49} = 32.07$ ,  $P < 0.001$ ). Although female *Aphidius ervi* were larger than males but the sizes was not significantly different ( $F_{1,49} = 2.13$ ,  $P = 0.136$ ) when reared on both infected and uninfected plants. However, the interaction between sex and infection was significant ( $F_{1,99} = 17.21$ ,  $P < 0.001$ ). Which is the evidence for this.



**Fig 10:** Mean (± SE) hind tibia length of mummies emerging from hosts reared on plants infected and uninfected by *B. cinerea*

#### Discussion

Findings from the study show that first higher number of mummified aphids was counted from uninfected plants than infected ones and most of the parasitoids reared on host grown on uninfected plants give rise to female parasitoids while parasitoids reared on host grown on infected plants gave rise to equal proportion. Second infection of *B. cinerea* has no effect on the rate of chlorophyll fluorescence and the rate of photosynthesis but significantly affects the dry mass of the plant. Third covering the plants with an insect net did not effect on the rate of chlorophyll fluorescence but significantly

affected the rate of photosynthesis, internode length, dry root and shoot weight of the plants. Fourth the interaction of *B. cinerea* infection and plant cover has no effect on the rate of photosynthesis, chlorophyll fluorescence and dry root weight but significantly affects internode length and the dry shoot weight of the field plants. Until harvest no sign of *B. cinerea* lesion was seen in any of the experimental plants. *Aphidius ervi* was identified as the only parasitoid species found foraging the aphids on the plants throughout the duration of the experiment. The present study has confirmed the results of the previous studies in a controlled environmental room that showed the occurrence of an indirect interaction between Systemic *B. cinerea* and *M. persicae*. The indirect interaction results in the complete absence of *B. cinerea* lesions in infected plants throughout the duration of the experiment and, like the first test experiment carried out in the same field aphid population was quiet low and could not be counted. The feeding habit of the aphids in addition to the soft and delicate nature of the lettuce leaves make it difficult to record an honest count; therefore an attempt to count the aphids may have caused injury to the lettuce leaves which will otherwise give way for the growth of *B. cinerea* which will have otherwise, disrupted the quality of the experiment. In another studies Rakhshani *et al.* (2010) reported that aphid parasitoids were mainly observed in May-June and in September-October coincidentally with wild aphids. They reported that the population density of the parasitoids was almost zero in July and August when air temperature increased and lowered aphid populations. Our study was carried out in the field between April- June and was repeated exactly the same time the following year and that was when the population of aphid parasitoids was expected to be high, however, because of the uncertainty of the weather condition witnessed in the UK our study could not confirm high populations of both aphid and parasitoids between May-June as hypothesised by Godfrey (1994) and Rakhshani *et al.* (2010).

The result of this field study agrees with the result of experiments carried out in a controlled environmental room to determine the effects on plant stress following interaction of *B. cinerea* and aphids under controlled condition. In the field experiment it was clear that *B. cinerea* infection and aphid infestation significantly affect the dry mass of the plant but did not affect the rate of photosynthesis and chlorophyll fluorescence. Similarly, the results from the controlled environmental room showed that infection of *B. cinerea* and aphid infestation significantly affect the rate of photosynthesis and the dry mass of the plant but has no effect on the rate of chlorophyll fluorescence. This finding was similarly reported by Shannag and Ababneh (2007) [11] who found that reduction of the plant dry mass occurred due to a reduction in food synthesis resulting from a reduction in the rate of photosynthesis by the plant due to inhibition of electron transport. However, Heng-Moss *et al.* (2010) relate the loss of dry mass of the plant to the increased synthesis of defensive chemicals by the plants in response to injury by pathogen and aphid attack. In the controlled environmental room experiments, lesion of *B. cinerea* was evidently visible on the infected plants as the plants grew although it was suppressed by the presence of aphid. However, a complete absence of lesion of *B. cinerea* seen on the field plants suggesting that

plant defence can change in space and time following multiple attacks; this was similarly reported by Mouttet *et al.* (2011) [7]. The result of the present field study shows that higher numbers of parasitoid mummies were recorded on uninfected plants than infected ones in the field and this was similarly reported in the experiment carried out in the controlled environmental room (Yahaya and Fellowes 2013) [15]. In another field experiment Heng-Moss *et al.* (2010) carried out to determine the soybean aphid (*Aphis glycines* Matsumura) preference between soybean infected with soybean cyst nematodes and uninfected control soybean plants, they found that the preference of soybean aphids for uninfected plants was significantly higher than for soybean infected with soybean cyst nematodes (*Heterodera glycines* Ichinohe). Therefore the lower number of mummies recorded on infected plants in the both controlled environmental room and field experiments is an indication that *B. cinerea* exerts an unfavourable effect on the tritrophic interaction which lower the quality and fitness of the plant host by reducing their food value. The lower, food values of the infected plant negatively affected the aphid that grows on it making them lower quality host for the foraging parasitoids (Godfrey 1994; Krues 2002; Mouttet *et al.* 2011) [2, 7]. Pell *et al.* (1996) found that pea aphids (*Acyrtosiphon pisum*), infected with the fungal pathogen *Erynia neoaphidis* takes a longer time to consume by the predator ladybirds *Coccinella septempunctata* as compared with the time taken for the consumption of living or dead uninfected aphids. They concluded that ladybirds find the infected aphids to be less palatable than uninfected ones; in addition the ladybirds encounter physical difficulty in feeding on infected aphids compared to uninfected ones.

However, host quality is not the only factor influencing host choice by the foraging parasitoids. The results from both the controlled environmental room and field showed that parasitoids may be conditioned to the host from which they emerge and they may learn cues associated with the host which assist them in future host preference. Similar results were reported by Morris and Fellowes (2002), Poppy and Powell (2004), Rehman and Powell (2010) [9], Tasin *et al.* (2011 and 2012). In addition, Tasin *et al.* (2012) showed that under different settings cues from short and long range play a significant role in the choosing of a better quality host by parasitoids, likewise sometimes aphids use stylets to determine the palatability or unpalatability of the plant.

In agreement with the results of controlled environmental room *A. ervi* emerging from larger aphids showed a more female biased offspring sex ratio. Sequeira and Mackauer (1993b) [10] found that the female-biased offspring sex ratio occurred due to maternal manipulation in response to host size, not because of differences in the larval mortality. Henry *et al.* (2005) reported that parasitoids use host size as an index for host quality and in Koinobiotic species, where the larvae continue to feed as the host is developing, the growth of host parasitoid larval development in *A. ervi* results in development of size fitness relationship which varies with the host instar, rather than by size at parasitisation.

It is therefore clear from the present study that aphid parasitoids have considerable potential as biological control agents but their efficiency is dependent upon their presence in the right place at the right time and at the right host. Understanding parasitoid behaviour, together with identification of physical

and chemical cues regulating the behaviour, will provide exciting opportunities for manipulation of parasitoids in the field, either as natural populations or as populations introduced through inundative releases. In addition, the mechanisms underlying behavioural plasticity in parasitoids and the genetic basis of parasitoid behaviour provide opportunities for mass production of parasitoid strains suitable for use in specific crop/pest situations. The parasitoids can be selectively bred to attack specific hosts and then primed to appropriate plant volatiles as foraging cues before release and could be used in inundative releases (vincenzo and Angela 2006; Rehman and Powell, 2010) <sup>[14, 9]</sup>. Also, parasitoids can be used more effectively by developing strategies to conserve and manipulate their populations in agricultural ecosystems, which include crops and semi natural habitats (Turlings *et al* 1993; Powell, 1986) <sup>[13]</sup>. Therefore, our prediction is that populations of natural enemies would be greater in diversified habitats due to increased availability of alternative hosts and food sources. The parasitoids behaviour of attacking alternative aphid hosts would ensure its population stability in the field and this would ensure sound biological control.

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