

INFLUENCE OF *Botrytis cinerea* ON PARASITOID ATTACK RATIO IN THE FIELD

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ABSTRACT

Effects of infection during parasitoids host choice are ubiquitous, yet we have little understanding of how the presence of a systemic pathogen *Botrytis cinerea* may affect field interactions at higher trophic levels. Interactions between the peach potato aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) and its common parasitoid, *Aphidius colemani* Vierek (Hymenoptera: Braconidae), were investigated in the field which is less controlled environment to determine if these are influence by infection of the host aphids (lettuce *Lactuca sativa*, *L. asteraceae*: Compositae) by the widespread plant pathogen *Botrytis cinerea*. While field experiment showed that females 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 were recorded on uninfected plants than infected ones which were similarly reported in the controlled environment. The field experiment showed the effect of infection on parasitoid sex ratio ($F_{1, 49} = 77.533, P < 0.001$) as a greater proportion of the *A. ervi* collected from hosts reared on uninfected plants were female, while same ratio was found from infected plants with overall offspring sex ratio of 62.2% females and 37.7% males. 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

Despite the wide range of plants in the natural environment the preference of herbivores for host plants may be affected by learning, the presence or absence of diseases, host nutritional quality, induced chemicals produced for

resistance and other factors such as the presence or absence of natural enemies (Johnson *et al.* 2003). A typical example is that pathogen infection can change nutritional qualities and plant defence such as the secretion of secondary metabolites or the triggering of defence-related compounds such as phytohormones, which may ultimately affect the distribution and feeding of herbivores (Stout *et al.* 2006; Vincenzo *et al.* 2006; Roder *et al.* 2011).

The behavioural plasticity of aphid natural enemies is strongly related to the variability of the environment in space and time (Vet *et al.* 1995). The natural enemies adapt to the changing availability of hosts on different plant species. After contacting host the behavioural response of the natural enemies toward the experience stimuli is often changed considerably (Turlings *et al.* 1993). The natural enemy will be attracted by the host odour which was learned during oviposition (Vet and Groenewold 1990) or due to sensitization or associative learning (Turlings *et al.* 1993).

In the field the distribution and efficiency of aphid natural enemies, such as parasitoids, are affected by farming practices, climatic and other environmental factors, each of which have the tendency of disrupting the association between aphids and their natural enemies resulting in aphid dispersal from the plant host (Landis *et al.* 2000; Rehman and Powell, 2010).

Botrytis cinerea, which can grow systemically without any host specificity, poses a serious threat to the survival of both field and glasshouse crops (Williamson *et al.* 2007). This systemic fungus negatively affects the performance of the plant, resulting in unexpected post-harvest problems as produce may decay without any prior warning (Agrios, 2005; Williamson *et al.* 2007). This may result in serious economic losses, during both short and long term storage and subsequent shipment of most types of horticultural produce (Agrios 2005).

However, in an experiment carried out in a controlled environment room, it was found that *B. cinerea* interacts indirectly with the aphid *Myzus persicae*, where they both reduce the growth of each other, resulting in fewer *B. cinerea* lesions and a lower aphid population size when both co-infect the same plant, also the presence of *B. cinerea* and *M. persicae* on the plants did not show a significant effect on the rate of chlorophyll fluorescence. However, their presence significantly decreases rate of photosynthesis, root and shoot weight. It is therefore desirable to study this interaction in a natural setting, which will promote understanding of the ecological processes which will in turn assist in

design of adequate crop management strategies (Moultet *et al.* 2011). In order to understand if results found under controlled conditions are found in the field the experiment needs to be replicated. Therefore, we tested three hypotheses. First, that systemic *B. cinerea* will interact negatively with the phytophagous insect aphid *M. persicae* resulting in fewer *B. cinerea* lesions. Second, preventing access of natural enemies will significantly stress the plant, resulting in the reduction of root and shoot weight. Third, that parasitoids would preferentially oviposit in aphids growing on uninfected plants.

MATERIAL AND METHODS

Experimental Plants

One hundred four-week-old plants grown from infected and uninfected seeds (50 of each) in a Controlled Environment room were transplanted into an open experimental field consisting of two plots each measuring 3x3m long on 1st May, 2013 and grown for seven weeks. The spacing in the field was 25 cm between plants and 40 cm between rows.

Plant Infestation with *Myzus persicae*

Three weeks after transplanting all plants were infested with ten adult *M. persicae* by gently 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, which prevent aphids from attack by aphid natural enemies (Fig. 1).



Fig. 1: Lettuce Plants, Uninfected and Infected by *B. cinerea*, Infested with Aphids, Covered and Uncovered with Insect Nets in the Experimental Field

Measurement of the Rate of Photosynthesis

To obtain a measure of the degree of plant stress induced by aphids, the rate of photosynthesis was measured in each lettuce plant before harvest.

Photosynthesis rate was determined as the amount of CO₂ assimilated per m² leaf surface by intact leaves, using a Red Gas Analyser (ADC Bioscientific LCI Analyser No. 31109) equipped with standard broadleaf chamber measuring an area of 6.5cm². The level of CO₂ in the analyser varied between 400-655µmol CO₂/mol determined by position of the inlet of the analyser outside the controlled environment. Leaves were allowed 2-4 minutes in the chamber to reach equilibrium before the readings were recorded.

Measurement of the Rate of Chlorophyll Fluorescence

The rate of chlorophyll fluorescence of the experimental plants was determined as amount of re-emitted light from the leaf, using a Handy Pea Data Chlorophyll Fluorometer (Hansatech Instrument Ltd. Pea plus version: 1.02). Leaves were placed in the chamber for 20 minutes before taking the measurement. The rate of chlorophyll fluorescence was determined as photochemical efficiency Fv/Fm (where Fv is the maximal variable chlorophyll fluorescence, and Fm is maximal chlorophyll fluorescence).

Measurement of Shoot Weight

Measurement of shoot weight was taken from all 40 plants in the four treatments. Harvested shoots were removed from all of the plants and washed under running tap water, then dried at room temperature on the laboratory bench for two hours. Weight was measured using an electronic balance (Kern scale Technic, 440-21N).

Measurement of Root Weight

Measurement of root weight was taken from all the experimental plants after harvest. Plants were sectioned into roots, stems and leaves. Roots were washed under running tap water and allowed to dry at room temperature on the laboratory bench for two hours before taking the measurements using an electronic balance (Kern scale Technic, 440-21N).

Parasitoid and Attack Rate Size

After harvest, plants which were not covered with a net were examined for the presence of mummified aphids. The mummies were counted and collected into vials 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 determined using a micrometric eye piece attached to a microscope Nikon (New York Microscope Company) at x 50 magnification as described by Nickolas *et al.* (2013).

Statistical Analysis

The data was analysed using analysis of variance and generalised linear models. As the data met assumptions of normality the data for chlorophyll fluorescence, photosynthesis, and dry mass of the plant data were not transformed prior to analysis. The data from the count of number of mummies was log transformed and analysed using generalised linear model with binomial errors (Crawley, 2005). All the analysis was performed using MINITAB (Minitab 2009) or R (Crawley, 2005).

RESULTS

Effect of Plant Covering with Insect Net on the Growth of *B. cinerea* and *M. persicae*

Photosynthesis: Net photosynthesis measured as DCO_2 was not affected by infection of *B. cinerea* which were both infested with aphids (Fig. 4; $F_{1, 99} = 0.95$, $P = 0.333$). The plant covered with an insect net had a significantly reduced rate of photosynthesis ($F_{1, 99} = 3.94$, $P = 0.05$). Infected/covered plants showed much lower rate than infected/uncovered, while no difference was found between covered infected and uninfected plants. The effect of interaction between covered infected and uninfected plants. The effect of interaction between infection and plant cover was not significant on the rate of photosynthesis ($F_{1, 99} = 2.60$, $P = 0.106$).

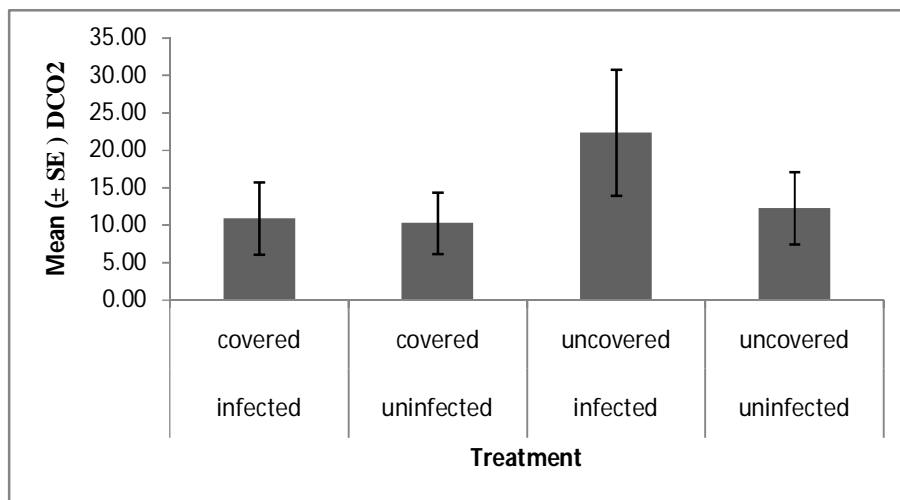


Fig. 4: Effect of Systemic *Botrytis cinerea* Infection and Covering with Insect Net on the Rate of Photosynthesis of Lettuce Plants in the Field

Chlorophyll Fluorescence: The rate of chlorophyll fluorescence FV/FM was not affected by *B. cinerea* infection on infected and uninfected plants which both were infested with aphids (Fig. 5, $F_{1, 99} = 1.01$, $P = 0.317$). 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 interaction between plant cover and infection was not significant ($F_{1, 99} = 2.74, P = 0.101$).

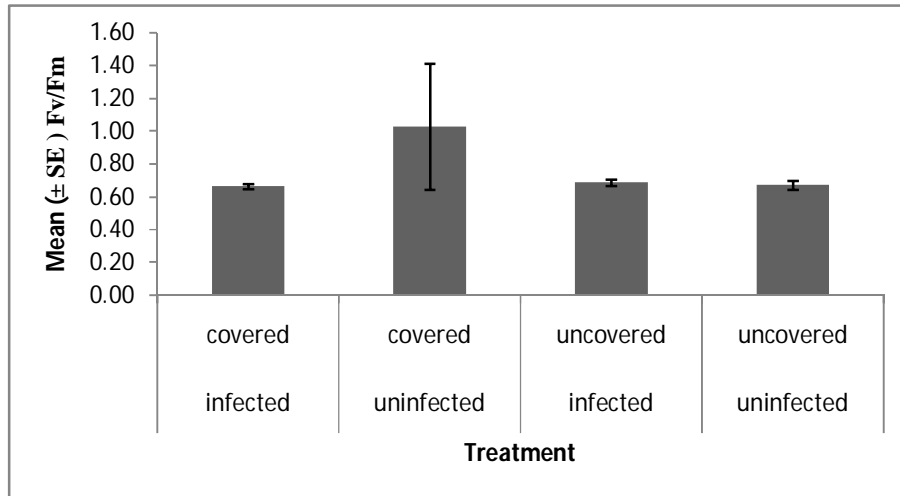


Fig. 5: Effect of Systemic *Botrytis cinerea* Infection and Covering with Insect Net on the Rate of Chlorophyll Fluorescence of Lettuce Plants in the Field

Plant Weight

Shoot weight

Plants infected by *B. cinerea* showed a significantly reduced shoot weight. (Fig. 6; $F_{1, 99} = 93.17, P < 0.001$). A significant difference was found between plants covered with an insect net and those which were not covered ($F_{1, 99} = 28.99, P < 0.001$).

There was a significant interaction effect on shoot weight between covered and uncovered uninfected plants ($F_{1,99} = 23.58, P < 0.001$). While there was no difference in shoot weight between covered and uncovered infected plants, there was a significant change for uninfected plants, where uncovered plants were much larger than covered.

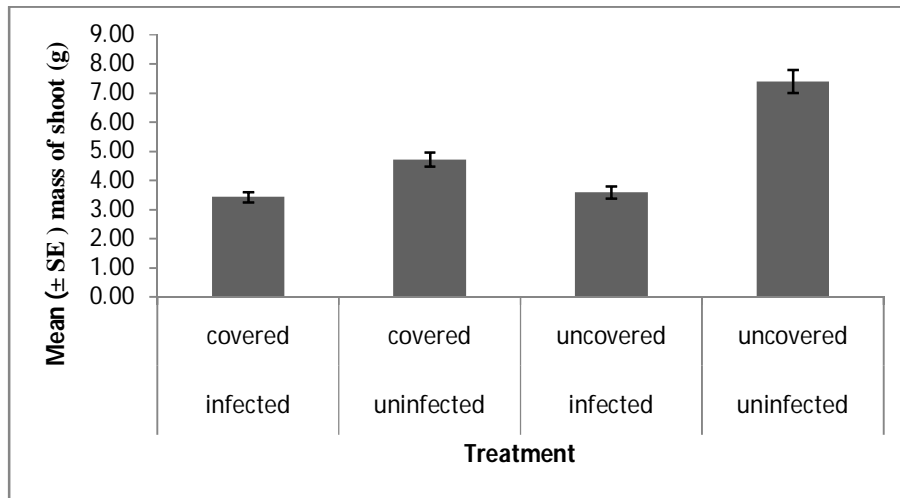


Fig. 6: Effect of Systemic *Botrytis cinerea* Infection and Covering with Insect Net on the Shoot Weight of Lettuce Plants in the Field

Root Weight There was a significant reduction in the dry root weight between infected and uninfected plants, where uninfected plants were much larger than infected ones (Fig. 7, $F_{1, 99} = 80.95$, $P < 0.001$). A significant difference was found between plants covered with an insect net with those which were not covered ($F_{1, 99} = 12.51$, $P < 0.001$). However, the combined effects of *B. cinerea* infection and plant cover with insect net was not significant on the dry root weight ($F_{1, 99} = 2.48$, $P = 0.118$).

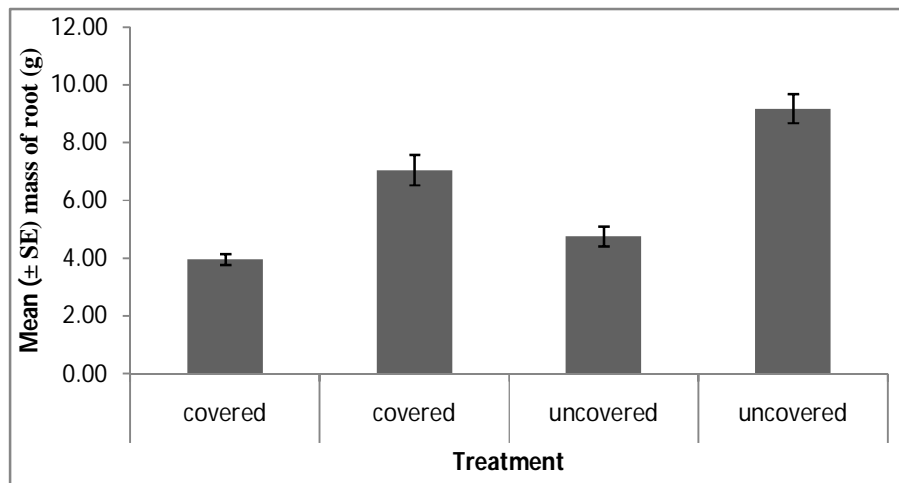


Fig 7: Effect of Systemic *Botrytis cinerea* Infection and Covering with Insect Net on the Root Weight of Lettuce Plants in the Field

Parasitisation of Aphids on *Botrytis cinerea* Infected and Uninfected Plants by *Aphidius ervi*

More *M. persicae* mummies were found on uninfected plants than on infected plants (Fig 8, $F_{1,49} = 67.58$, $P = 0.001$).

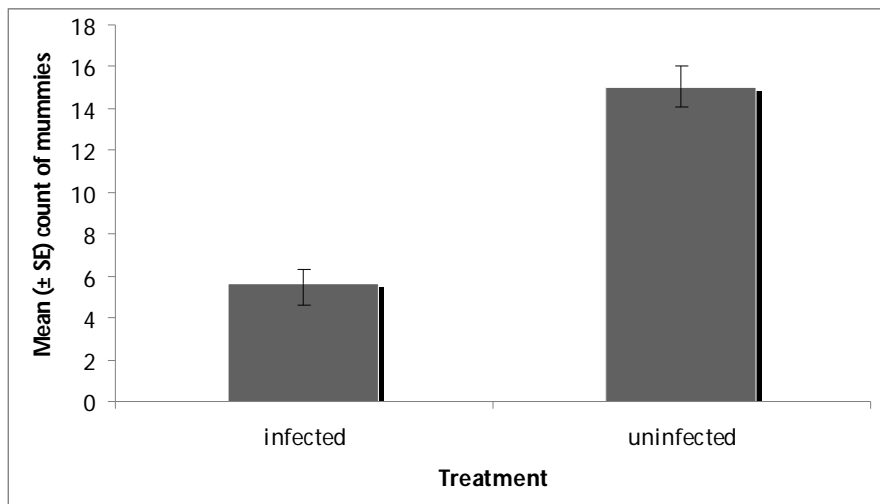


Fig. 8: Mean Count of Aphid Mummies Counted on Uninfected Plants and Plants Infected by *B. cinerea*

***Aphidius ervi* Size**

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 9; $F_{1,49} = 77.53$, $P < 0.001$). Overall female *Aphidius ervi* were significantly larger than males, ($F_{1,49} = 511.70$, $P < 0.001$). However, the interaction between infection and sex was not significant ($F_{1,49} = 1.43$, $P = 0.235$) 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.

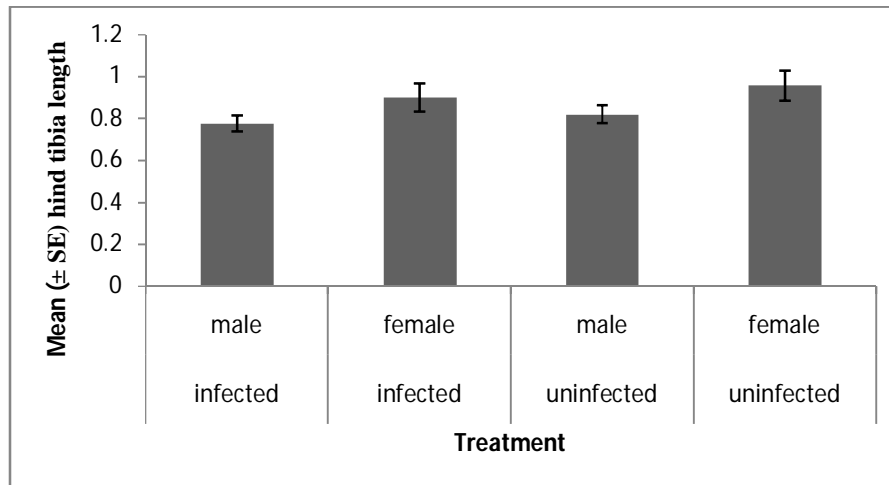


Figure 9: Mean Hind Tibia Length of Parasitoids Emerging from Hosts Reared on Plants Infected and Uninfected by *B. cinerea*

DISCUSSION

Infection by *B. cinerea* had no effect on the rate of chlorophyll fluorescence or the rate of photosynthesis, but did significantly affect the root and shoot weight. Covering the plants with an insect net had no effect on the rate of chlorophyll fluorescence but significantly affected the rate of photosynthesis and also dry mass of the plants. More aphid mummies were counted on uninfected plants than on plants infected by *B. cinerea*, and emerging parasitoids (all *Aphidius ervi*) were larger when they emerged from hosts reared on uninfected plants.

Females parasitoids are generally larger than males, and this may be the results of sex-specific allocation of offspring to host of higher and lower food value and the sex specific exploitation of host resources. In agreement with the present field study Mackauer (1976) and Sequeira and Mackauer (1993b) found that the sex ratio in field populations of many species of aphidiines tends to be female biased. Sequeira and Mackauer (1992) reported that, in hosts of equal size, females of *A. ervi* had a higher growth rate than males, growing to a larger size without a corresponding increase in developmental time, suggesting that larvae may exploit host resources in a sex specific manner.

Aphids were not counted in the field. The delicate nature of the lettuce leaves and the feeding location of the aphids meant that any systematic attempt to census the aphids would have caused significant injury to the leaves and disturbed the aphid colonies, preventing accurate assessment of aphid population size.

In the field *B. cinerea* infection did not affect the rate of photosynthesis, which is in contrast to the results in our laboratory study where this was significantly reduced by infection. However, infection did significantly reduced root and shoot weight of the plants and had no significant effect on chlorophyll florescence, which was found in the laboratory study. Shannag and Ababneh (2007) found that a reduction of the plant weight occurred due to a reduction in food synthesis resulting from a reduction in the rate of photosynthesis by the infected plant. However, Heng-Moss *et al.* (2010) reported that the loss of plant mass occurs due to the increased synthesis of defensive chemicals by the plants in response to injury caused by the pathogen and also by aphid attack. In the controlled environmental room experiments, lesions of *B. cinerea* were visible on the infected plants as the plants grew although it was suppressed by the presence of aphids.

More parasitoid mummies were recorded on uninfected plants than infected ones and this was similarly reported Yahaya and Fellowes (2013). Therefore the lower number of mummies recorded on infected plants in both controlled environment and field experiments may be an indication that *B. cinerea* exerts an unfavourable effect on the tritrophic interaction which lowers the quality and fitness of the plant host by reducing their food value to the parasitoids' hosts.

Contrary to the results of experiment carried out in controlled environment, results from the present field experiment shows the effect of infection on parasitoid sex ratio ($F_{1, 49} = 77.533, P < 0.001$) as a greater proportion of the *A. ervi* collected from hosts reared on uninfected plants were female, while same ratio was found from infected plants with overall offspring sex ratio of 62.2% females and 37.7% males. Similarly, Sequeira and Mackauer (1993b) found a female-biased offspring sex ratio in *A. ervi* with a seasonal mean of 65.7% female which occurred due to maternal manipulation in response to host size, not because of differences in the larval mortality.

Overall the findings of this study were similar to most of the results obtained under controlled conditions. More importantly, the observations of the experiment provide further evidence that hidden, systemic host plant infection by *B. cinerea* influences the interaction between aphids and their parasitoids. Results of the field experiment shows that; more parasitoid mummies were counted on uninfected plants, which resulted in larger parasitoids with a female

based sex ratio; this suggests that *B. cinerea* has a significant effect on the interactions between aphids and their enemies in the field.

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