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Systemic plant pathogen *Botrytis cinerea* Association with Aphids *Myzus persicae* Influence the Growth of Host Plant

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ABSTRACT

Plant pathogens and insect herbivores attaching and surviving on plant and their produce can associate indirectly with each other and interact directly or indirectly resulting in serious economic loss to the growers. In this experiment the influence of co-interaction between systemic plant pathogen *Botrytis cinerea* Pears Fr (Helotiales Sclerotiniaceae) and herbivorous insect green peach aphid *Myzus persicae* on the growth of lettuce *Lactuca sativa* (Asteraceae: Compositae) was investigated. Studies showed that the co- interaction between *Botrytis cinerea* and *Myzus persicae* causes stress on the lettuce plants resulting in economic loss. The studies found that co-interaction between *B. cinerea* and *M. persicae* resulted in lower lesion of *B. cinerea* and *M. persicae* counts when both were present on same host plants. In addition the stress resulting from co-interaction affected the growth of lettuce plant resulting in a significant reduction in plant height, internode length, leaf size and also significant reduction of fresh shoot and root weight.

Keywords: *Botrytis cinerea*, Co-interaction, Plant growth, *Myzus persicae*

INTRODUCTION

Myzus persicae and *Botrytis cinerea* an herbivorous insect and plant pathogenic fungus respectively are of great economic importance due to their ability to spread diseases causing loss of plants and its produce [1-3]. Therefore, the ability of *Botrytis cinerea* and *Myzus persicae* to survive on the same plant host makes them to exhibit a co-interaction.

Myzus persicae Sulzer (Hemiptera: Aphididae), the common green peach potato aphid, is found throughout the world, where it is principally regarded as a serious pest of many important agricultural crops because of its ability to transmit plant viruses [4]. It feeds on crops such as peaches, potatoes, sugar beet, tobacco and various ornamental crops grown in landscapes and in glasshouses and most lettuce plants [4,5]. A high population of *M. persicae* **(Figure 1)** on a crop causes injury by removing large volumes of sap from the plants and depleting them of needed nutrients [4]. In addition, they also cause indirect injury by the production of sugary honeydew which makes the leaves susceptible to microbial attack, which then reduces leaf quality [6].

Botrytis cinerea is a systemic plant pathogen with a wide host range of more than 500 plants [7]. It is the causal agent of grey-mold disease and causes greater economic losses, during both pre and post-harvest phase of plant growth, more than any other disease [2,8], it is also responsible for about 20% losses of affected crops worldwide [2]. It is reported to cause annual crop losses worldwide ranging from \$10-\$100 billion [7]. Plant and their produce stored for weeks or months at temperatures ranging from 0-10°C and transported fruit, small fruits, ornamental flowers and bulbs, as well as forest seedlings are all attacked and destroyed by *B. cinerea* [9-11]. Therefore, Williamson et al. [12] concluded that *B. cinerea* as the most widely distributed disease of vegetables, ornamental fruits and field crops throughout the world. The pathogen produces clear or grey conidia on branched conidiophores which are dispersed by humid air currents, splashing water, tools and clothing; the conidiophores initiate a new infection on healthy plants [2,13]. The conidia of the fungus can infect the seedlings, flowers, stems or leaves through wounded or senescing tissues and directly through the epidermis [2,14]. The symptoms of the pathogen may sometimes appear very

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quickly or an infection may remain quiescent and may appear later when the tissues age or during storage [15-17]. The fungus overwinters either as mycelium in decaying plant debris or as sclerotia which are considered to be the main survival structure of *B. cinerea* in the soil [12].

The lettuce *Lactuca sativa* L. (Asteraceae (Compositae)) is a significant horticultural crop, widely used as source of food [18-20]. Many researchers have reported studies of *Botrytis cinerea* on crops such as strawberries, kiwifruits, raspberries, grapes and others [15]. Lettuces seem to have received little attention with respect to their infection by *B. cinerea* and yet they share the devastating effect caused by *B. cinerea* under favorable conditions, especially in protected crops. The aphids affecting lettuce crops are a problem worldwide causing serious economic losses [21,22]. Higher number of aphids may result in stunted plant growth, but, the most damaging effect of aphid attack is wilting and head contamination, which reduce the market value of the lettuce [21]. Despite the fact that lettuce is attack by several species of aphids however, the most common aphid pest of lettuce is the green peach-potato aphid (*Myzus persicae* Sulzer) [4,21].

The co-interactional interaction of insect herbivores and pathogenic fungi inhabiting the same host plant occur in a situation where the first attacker alters the fitness of the plant host in a way that it affects the second attacker, while in return the second attacker induces the plant host to synthesised chemicals which reduces the growth of the first attacker all at the expense of other important plant processes [23-26]. However, the success of these types of interaction is dependent on the type and behavior of insect herbivore and fungal pathogen involved [27,28]. Therefore two hypotheses were tested which are: (i) Decrease in the spread and expression of *B. cinerea* and lower growth rate of aphids will occur following stress on the host lettuce plant by the co-interaction of systemic pathogen *B. cinerea* and aphids *M. persicae.* (ii) Reduction of plant height and dry mass of the plant will occur as a result of stress on the plant host due to co-interaction of systemic plant pathogens and aphids.

MATERIALS AND METHODS

Study plants

Sterilized seed of lettuce was sown in 80, 15 cm diameter pots filled with a vermiculite growing medium in a controlled environment room (18-20°C, ambient humidity and 12-14 h L: D). Half of the plants (40, plants) were grown from clean uninfected seeds while the remaining half were grown from seed systemically infected with *B. cinerea.*

Plant infestation with the *Myzus persicae*

One month after germination all the experimental plants were infested with ten nymphs of aphids *Myzus persicae* Sulzer (Hemiptera Aphididae). The *M. persicae* were reared for three generation for proper effect of telescoping of generation before being used in the experiment [29]. Infestation was done by placing the aphids on the reverse side of the leaves (20 infected and 20 uninfected plants) using moist brush. In order to prevent the escape of the aphids immediately after infestation plants were covered with a vented plastic container. The remaining uninfested plants (20 infected and 20 uninfected) served as controls.

Population size of *Myzus persicae*

The population size of *M. persicae* was obtained by counting the number of aphids on the plants. The counting was done once a week for twenty weeks, starting four weeks after infestation. Also visual examination was used to assess the appearance of *B. cinerea* infection on the plants.

Plant height

Plant height was taken from all 80 plants in the four treatments. Height of the plants was measured before harvest using measuring tape.

Leaf size

Leaf size was taken from all 80 plants in the four treatments. Before harvest leaf size was measured from the two fully expanded middle leaves using measuring tape.

Internode length

Before harvest Internode length was taken from all the 80 plants in the four treatments. Before harvest length of the internode was measured from all the plants by using measuring tape.

Dry shoot weight (g)

Harvested shoots were removed from all the plants and washed under running tap water and allowed to dry in an oven for one hour. Thereafter, dry shoot weight was taken from all the plants using an electronic balance (Kern scale Technic, 440-21N).

Dry root weight (g)

The roots were washed under running tap water and allowed to dry in an oven for 1 h. The measurements were taken using an electronic balance (Kern scale Technic, 440-21N).

STATISTICAL ANALYSIS

All data were analysed using ANOVA with post-hoc Tukey tests [30]. As the data from plant height, internode length, leaf size, fresh shoot and root weight did not meet assumptions of normality, a Box-Cox approach was used to determine the correct transformation prior to analysis. The data from plant height and internode length were log transformed while data from leaf size and fresh shoot weight was square root transformed before the analysis. Root weight was Ln transformed. Number of aphid colony survivorship between infected and uninfected plants was analysed using two ways ANOVA [31].

RESULTS

Expression of systemic *B. cinerea* lesion on *M. persicae* infested and uninfected plants

More lesion of *B. cinerea* was recorded on plants uninfected by aphids than on infested plants. However, uninfected plants were free of lesions. Therefore, the presence of *B. cinerea* significantly reduces the plant height, leaf size, internode length and plant dry weight **(Table 1)**.

Number of *Myzus persicae* on infected and uninfected plants by systemic *B. cinerea*

The increase in the number of *M. persicae* colonies was very slow on both infected and uninfected plants. Therefore, the rate of survival of *M. persicae* colonies was not affected significantly by plant infection status ($F_{1,19}$=0.43, P=0.363). Higher number of *M. persicae* was counted on uninfected plants, while the lowest count was recorded on infected plants. The effect of aphid infestation significantly decreases the plant height, leaf size, internode length and fresh weight of shoot and root of lettuce plants **(Table 1)**.

Table 1. Influence of *B. cinerea* and aphid infestation on plant height, leaf size, internode length and dry weight of lettuce plants.

Parameters	Plant treatment	Test statistics
Plant height	*B. cinerea* infection	F1,38=55.63, P<0.001
	Aphid infestation	F1,38=53.54, P<0.001
	Interaction term	F1,38=4.57, P<0.361
Leaf size	*B. cinerea* infection	F1,39=52.53, P<0.001
	Aphid infestation	F1,38=45.46, P<0.001
	Interaction term	F1,38=51.12, P<0.641
Internode length	*B. cinerea* infection	F1,38=33.43, P<0.001
	Aphid infestation	F1, 38=44.51, P<0.039
	Interaction term	F1, 38=0.15, P=0.476
Dry shoot weight	*B. cinerea* infection	F1,38=3.21, P<0.001
	Aphid infestation	F1,38=43.51, P<0.001
	Interaction term	F1,38=0.13, P<0.431
Dry root weight	*B. cinerea* infection	F1,39=63.52, P<0.001
	Aphid infestation	F1,38=52.63, P<0.001
	Interaction term	F1,38=34.38, P<0.001

Size of plant parts

Influence of systemic *B. cinerea* and *M. persicae* on plant height: There was a significant effect of aphid infestation status and *B. cinerea* infection status on the height of the plant was obtained from the experimental plants in which the presence of either resulted in a significant reduction in plant height. The interaction term was not significant **(Figure 1)**.

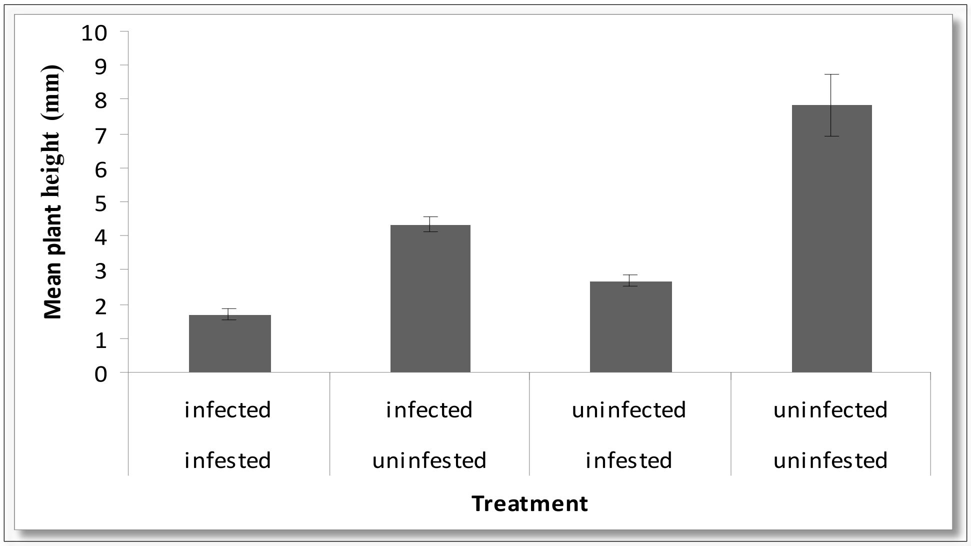

Figure 1. Plant height (mm) for experimental plants in the presence and/or absence of *B. cinerea* and *M. persicae*.

Knowledge of students about lawns in the campus environment

Influence of systemic *B. cinerea* and *M. persicae* on leaf size: There was a significant effect of aphid infestation status and *B. cinerea* infection status on the leaf size of the experimental plants **(Table 1)** where the presence of either resulted in a significant reduction in leaf size. The interaction term was not significant **(Figure 2)**.

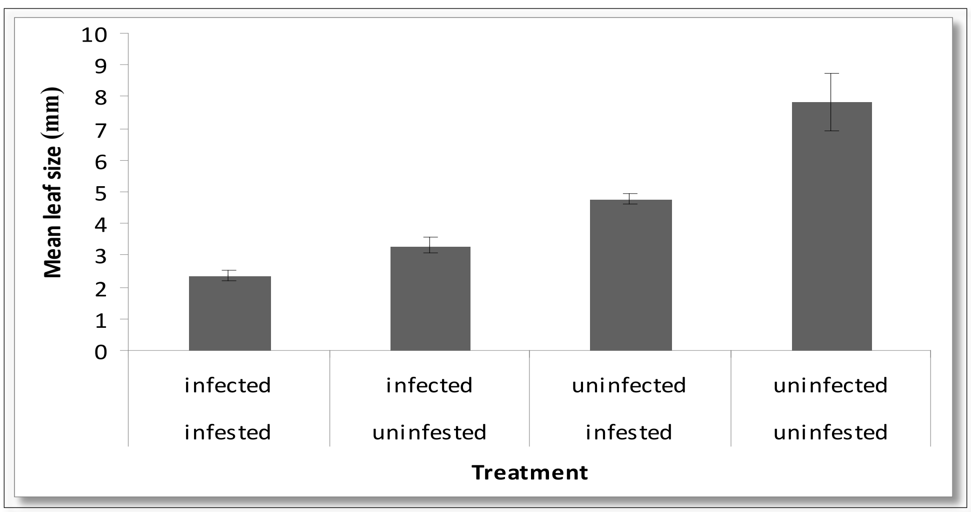

Figure 2. Leaf size (mm) for experimental plants in the presence and/or absence of *B. cinerea* and *M. persicae.*

Influence of systemic *B. cinerea* and *M. persicae* on internode length: There was a significant effect of aphid infestation status and level of *B. cinerea* infection on the internode length of the experimental plants **(Table 1)** where the presence of either resulted in a significant reduction in leaf size. The interaction term was not significant **(Figure 3)**.

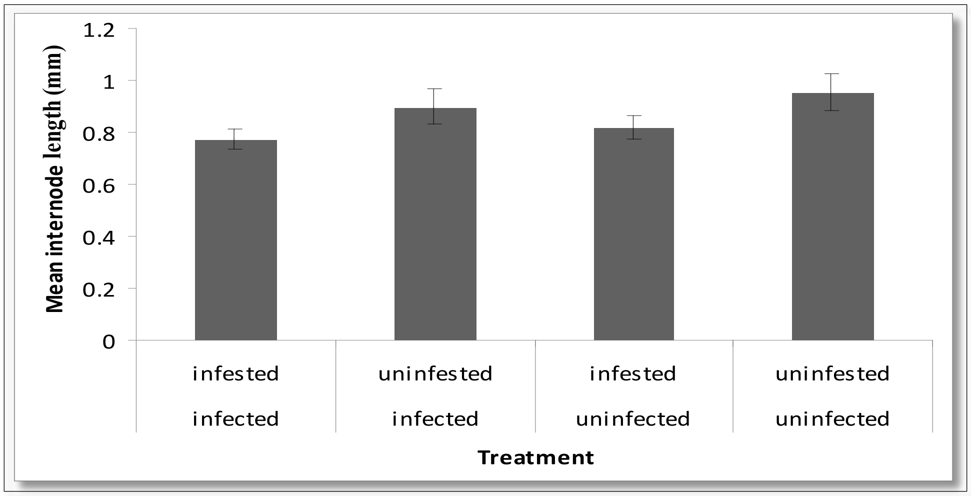

Figure 3. Internode length (mm) for experimental plants in the presence and/or absence of *B. cinerea* and *M. persicae.*

Plant dry weight

Influence of systemic *B. cinerea* and *M. persicae* on dry shoot weight: A significant effect of aphid infestation status and *B. cinerea* infection status on the dry shoot weight of the experimental plants **(Table 1)** where the presence of either resulted in a significant reduction in dry shoot weight. However, the interaction term was not significant **(Figure 4)**.

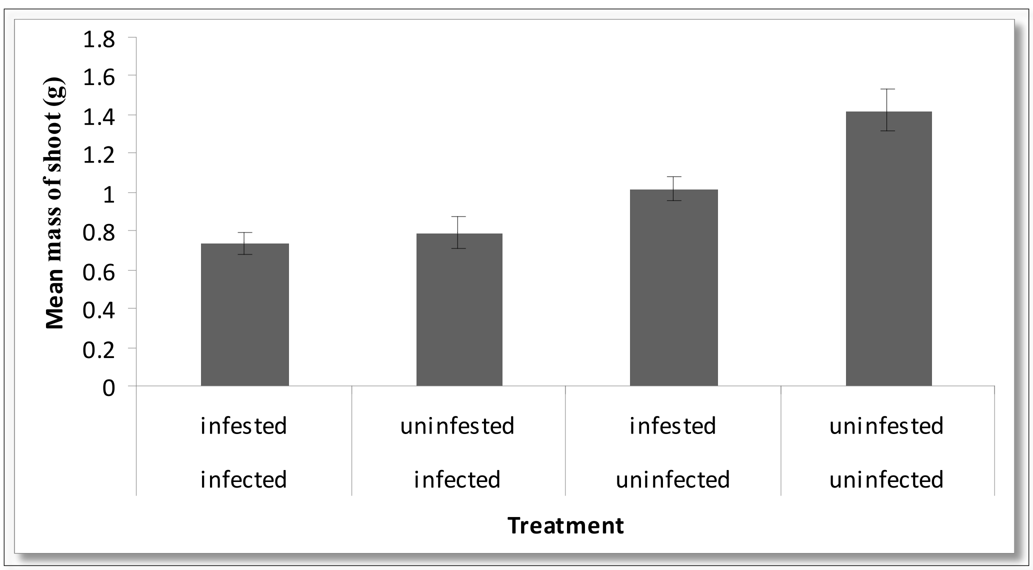

Figure 4. Mass (g) of shoots for experimental plants in the presence and/or absence of *B. cinerea* and *M. persicae*.

Influence of systemic *B. cinerea* and *M. persicae* on dry root weight: *Myzus persicae* infestation or infection by *B. cinerea* courses a significant decrease in the dry root weight of the plants **(Table 1)**. Because the primary influence of either natural enemy was not additive, the interaction was significant **(Figure 5)**.

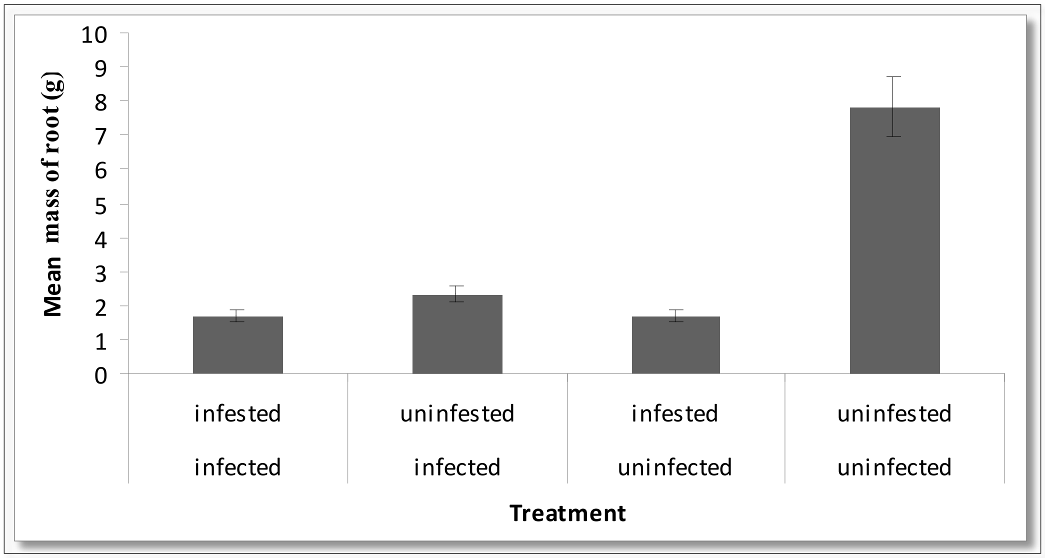

Figure 5. Mass of root (g) for experimental plants in the presence and/or absence of *B. cinerea* and *M. persicae*.

DISCUSSION AND CONCLUSION

The result of the experiment showed that the dynamics of the two natural enemies have an influencing factor on one another. While lettuce plants infested with ten *M. persicae* died within ten weeks of initial infestation, but *B. cinerea* infection did not result in such mortality. Infection by *B. cinerea* and infestation by *M. persicae* reduced plant height, leaf size, internode length, plant dry weight. The rates of expression of *B. cinerea* lesions were however lower on aphid infested plants and aphid numbers were lower on plants infected by *B. cinerea*. In general it appears that the effect of aphid attack was greater than that of *B. cinerea* infection.

In a report by Anna et al. [32] showed that following aphid's infestation wastage of plants and their produce occur as a result of injection of phytotoxin during feeding, causing disintegration of chloroplast which decreases food production there by slowing rate of plant growth. Despite the fact that the mechanism by which aphids affect plant metabolism is not fully understood however, studies by Heil and Bostock [33], Swarbrick et al. [34] and Golawska et al. [35] reported that the induction of defence is costly, causing more need of assimilates by the plant. Also the ability of the herbivore to manipulate the plant's carbohydrate metabolism for its own use deprives the plant sufficient carbohydrate for metabolic activities [36]. From the result of the present study it is clear that plant height, internode length, leaf size and dry shoot and root weights were significantly reduced by the attack of *M. persicae* and *B. cinerea*. Although the interaction of aphid and *B. cinerea* significantly reduced the root weight of the lettuce plants, the interaction of aphid and *B. cinerea* did not significantly affect plant height, internode length, leaf size, shoot weight but their effect is exert independently of one another.

Despite the resource quality of the lettuce plant was not measured in this study. The result of the present study has found that the effects of systemic *B. cinerea* are detrimental to the increasing aphid population in lettuce plants. Infection by systemic *B. cinerea* which spreads into the plant tissues as the plant grows lowers the amount of nutrients available, making the plant less favourable for the attacking aphid. In a similar study on the effect of *B. cinerea* and aphids (*Rhodobium porosum* Sanderson) on rose plants (*Rosa hybrida* L.). Mouttet et al. [37] reported that *B. cinerea* induced the plant to synthesise secondary metabolites which either have toxic effects, aversive and/or anti-feedant effects on aphids which may subsequently attack. Such a negative relationship causes a reduction in the reproduction rate of *M. persicae*, thereby lowering its population size. This showed that co- interactions has the ability to change the ecological interactions and spatial distribution of the insect herbivore [37-41] in particular with regards to its interactions with its natural enemies.

Result of the study showed that expression of *B. cinerea* was drastically reduced after infestation with *M. persicae* when compared to non-infested plants. In a similar experiment Mouttet et al. [37] found that infestation with aphids (*Rhodobium porosum* Sanderson) on *B. cinerea* infected plant result in lower expression of *B. cinerea* and the infestation triggers the induction of Salicylic acid (SA)-dependent pathway around the infection site which kill cells of *B. cinerea* and stop it growth. But, continuous feeding by the aphids on the cell contents triggers the plant to induce the wound-response pathways, (JA) and (ET) dependent pathways which reduce the population of aphids [42-45].

In a related study by Delucchi [46] on the effect of *M. persicae* on the growth of its plant host, Brussels sprouts. A general reduction in dry and fresh weight, height, internode length, leaf number and size was reported, where the most striking observation was reduction in dry root weight which was partly a due to the removal of assimilates, which were otherwise available for storage. Brussels sprouts which were subjected to probing and salivation by *M. persicae* and on which continued feeding by the aphid was prevented showed exactly a similar reduction accompanied by increased respiration of the plant.

Therefore, Delucchi [46] as well as Mackauer and Way [47] concluded that the increased respiration of the plant, a response to wounding and/or aphid salivation, contributed considerably to a reduction in plant height, leaf size and dry weight. However, finding by Heng-Moss et al. [48] reported that the reduction in the plant fresh and dry weight occurs due to the reduction of photosynthesis in leaves which have been injured by interaction of aphid infestation and pathogen attack due to an increased synthesis of defensive chemicals in response to the attack [49].

The present research has confirmed that co-interactional relationship between a systemic pathogen and an insect herbivore attacking lettuce plants reduces growth of the lettuce plant [50,51]. Therefore, the results of the experiment shows that *B. cinerea* affect aphid and also aphid affect *B. cinerea* and each of them stress the lettuce plant. In addition, the experiment confirmed the existence of a negative relationship between *M. persicae* and *B. cinerea* where they independently stress the host plant, and in addition to the reduction of the population growth rate of each other, they also reduce the growth rate of the host plant, probably by triggering the induction of defence chemicals by the plant at the expense of other important vital functions [52-55]. This shows that *B. cinerea* may have far-reaching effects on coexisting insect herbivores. Therefore this research will serve as a valuable pointer in increasing our understanding of the ecological consequences of a ubiquitous but hitherto understudied interaction [56,57].

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