Biological Control and Crop Protection

Parasitism rate of *Myzus persicae* (Sulzer) by *Diaeretiella rapae* (McIntosh) in the presence of an alternative, resistant host

Samira Evangelista Ferreira, Marcus Vinicius Sampaio, Reinaldo Silva de Oliveira, Heraldo Luís de Vasconcelos

**A R T I C L E   I N F O**

Article history:
Received 19 March 2017
Accepted 18 October 2017
Available online 13 November 2017
Associate Editor: Lucas Kaminski

**Keywords:** 
Aphid
Aphididae
Aphidiinae
Competition mediated by parasitoid
Indirect resistance

**A B S T R A C T**

The aphids *Lipaphis pseudobrassicae* (Davis) and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) are important Brassicaceae pests, occurring worldwide and causing significant damage to crops. Interspecific variations in the resistance to natural enemies can potentially impact the interaction among aphid populations. Here we evaluated the hypothesis of associational resistance by determining if the presence of resistant aphids (*L. pseudobrassicae*) reduces the rate of parasitism by *Diaeretiella rapae* (McIntosh) on non-resistant aphids (*M. persicae*). The experiment was conducted using collard green plants infested with *M. persicae* and *L. pseudobrassicae* either resistant or susceptible to *D. rapae*. The percentage of parasitism by *D. rapae* was greater on *L. pseudobrassicae* in the susceptible than in the resistant treatment, but parasitism rates on *M. persicae* did not differ between the treatments. There was no difference in average growth rate between *M. persicae* and susceptible *L. pseudobrassicae* populations, but resistant *L. pseudobrassicae* had greater growth rate than *M. persicae*. These results suggest that over a short period of time the presence of resistant *L. pseudobrassicae* does not affect the rate of parasitism by *D. rapae* on *M. persicae*.

© 2017 Sociedade Brasileira de Entomologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

**Introduction**

Interactions involving a shared and limited resource may result in exploitative competition, when individuals use available resources and thus deprive competitors of these resources, or in interference competition, in which individuals inflict damage to each other through direct contact, thus depriving competitors of access to the resource (Price et al., 2011; Schoener, 1983).

When two species do not compete for a resource, their population can be changed by a shared predator, parasite, or pathogen – a phenomenon known as “competition mediated by parasites or natural enemies” or “apparent competition”. This type of competition was defined as a reduction in population of a species when the population of a second species increases through its interaction with a third species of a higher trophic level (Chaneton and Bonsall, 2000; Holt, 1977; Holt and Lawton, 1994). Competition mediated by parasites and natural enemies are very common in nature and can be considered one of the main types of interaction in ecological systems (Bhattachai et al., 2017; Kaplan and Denno, 2007; Price et al., 1986).

There are several cases where the parasitoid species are the same for two hosts, but the impact of this natural enemy is different in each one (Frost et al., 2016; Holmes, 1982; Rice and Westoby, 1982). For instance, a host species with a large population may sustain a large parasitoid population and, thus, indirectly affect the parasitism rate of a second host species (Asgari et al., 1998; Blumberg, 1997; Price et al., 1986; van Veen et al., 2006).

On the other hand, the theory of optimal foraging predicts that parasitoids should oviposit preferentially in hosts of better quality for the development of their offspring (Emlen, 1966; Fellowes et al., 2007; MacArthur and Pianka, 1966; MacArthur and Wilson, 1967). However, some aphid parasitoids (Bracidae: Aphidiinae) may not be able to recognize the best hosts (Henry et al., 2005; Sampaio et al., 2008) or may even oviposit in hosts unsuitable for the development of their offspring (Sampaio et al., 2008; Starý, 1989). As a result, eggs are likely to be deposited in both, resistant and susceptible hosts (Ferrari et al., 2001; Oliver et al., 2003; Oliveira et al., 2013).
Some parasitoid species are able to recognize the resistant hosts (Oliver et al., 2012). Diaeretiella rapae (McIntosh) (Hymenoptera: Braconidae: Aphidiinae), however, deposit its eggs in resistant hosts at the same frequency as it does in susceptible ones, indicating that this parasitoid species cannot recognize the resistant ones (Oliveira et al., 2013). This way, the presence of a resistant host can potentially reduce the parasitoid population, thus favoring the susceptible aphid competitor through indirect resistance (Meisner et al., 2007), which may lead to serious consequences for the biological control of aphid pests that share the same parasitoid.

The aphids Lipaphis pseudobrassicae (Davis) and Myzus persicae (Sulzer) (Hemiptera: Aphididae) are important Brassicaceae pests, occurring worldwide and causing significant damage to crops (Blackman and Eastop, 2007). Studies reveal that the population of L. pseudobrassicae in Uberlândia, southeastern Brazil, has individuals resistant to D. rapae (Oliveira et al., 2013), which is the main parasitoid present in Brassica in the region (Sampaio et al., 2017).

The cause of the resistance of L. pseudobrassicae to D. rapae is still under investigation, but resistant individuals are more abundant than susceptible ones in Uberlândia’s population, which makes D. rapae ineffective in controlling L. pseudobrassicae (Oliveira et al., 2013; Sampaio et al., 2017). In contrast, the parasitoid D. rapae has been found as the main natural enemy of L. pseudobrassicae in regions where the aphid is susceptible, with high rates of parasitism in the laboratory (Silva et al., 2011) and in field and greenhouse conditions (Desh and Chand, 1998; Jeon et al., 2005).

Here we evaluated if resistant L. pseudobrassicae populations can change the parasitism rate of a second host species, M. persicae, through indirect resistance to their shared parasitoid (D. rapae).

Material and methods

Host plant material

Collard green seedlings (Brassica oleracea var. acephala L., cultivar Manteiga da Georgia) were grown in a greenhouse in plastic pots containing the commercial organic substrate Bioplant®. Plants used for rearing the aphids and parasitoids were kept in 3-L plastic pots (15 cm tall and 20 cm diameter), whereas plants used for the experiment were kept in 14.5-L plastic pots (27 cm tall and 30 cm diameter). Leaves were detached from plants and taken to the laboratory to obtain leaf discs, which were then placed on Petri dishes with agar solution to rear the aphids. Thirty five days old seedlings, with six expanded leaves, were covered with an anti-aphid cage for the experiment. The experiment was conducted in a greenhouse from November 10 to December 1, 2012, with an average temperature of 28 °C, and the minimum and maximum averages of 19 °C and 37 °C, respectively.

Aphid rearing

Two clones of L. pseudobrassicae, B3 (resistant) and B6 (susceptible), were collected in collard green plants, selected and rearing for five months in laboratory prior to the experiment (Ferreira, 2013). Aphids were grown on collard green leaf disks placed on 1% agar/water solution in Petri dishes (10 cm diameter). The Petri dishes were maintained in a climate controlled chamber (23 ± 1 °C, 12 h photoperiod, 50–55% humidity) and aphids were transferred to new Petri dishes containing fresh foliage every four days to ensure a good quality of the host plant.

To confirm the resistance of the clone B3 in comparison with the susceptible B6, 60 second instar nymphs of L. pseudobrassicae of each clone were placed individually in Petri dishes with a collard green leaf disk. A mated D. rapae female was released on every Petri dish until it oviposited on six L. pseudobrassicae nymphs. A total of ten D. rapae females were used to parasitize 60 aphids of each clone. Subsequently, these nymphs were kept in a climatic controlled chamber and observed daily. None of the B3 nymphs parasitized by D. rapae mummified, indicating that all individuals were resistant. In contrast, 40 out of the 60 parasitized B6 nymphs were mummified, confirming the susceptibility of B6.

The resistant clone B3 was then reared in the laboratory for five months and used to obtain susceptible individuals within its population. Resistance to parasitoids in aphids, associated with the presence of secondary symbionts, may be lost in some individuals even if they are clones from a single resistant female. This happens because secondary symbionts associated with aphids are transmitted via mother’s ovaries to its offspring; however, this transmission is not perfect, which results in resistance loss by some individuals (Chen and Purcell, 1997; Fukatsu et al., 2000; Oliver et al., 2010; Dykstra et al., 2014). Even though the cause of the resistance of L. pseudobrassicae to D. rapae is still unknown, it was possible to obtain susceptible individuals from the clone B3. To obtain susceptible individuals, 60 nymphs of L. pseudobrassicae of fourth instar were placed individually in Petri dishes with a collard green leaf disk and were subjected to a single oviposition of D. rapae (as previously described above). Parasitized L. pseudobrassicae nymphs of fourth instar are able to reproduce before dying, even the susceptible ones. When the parasitized aphid (four aphids) developed to a regular mummy (normal development of the parasitoid), her offspring was selected and kept for colony formation of susceptible L. pseudobrassicae. Similarly, nymphs produced by a parasitized aphid whose parasitoid did not develop (49 aphids) were used to form colonies of resistant L. pseudobrassicae. After the test, the B3 offspring population was divided between those that lost resistance and those that were still resistant. These two groups were maintained in Petri dishes (100 mm) to be used in the experiment.

Myzus persicae aphids were collected in collard green plants in a greenhouse of the Federal University of Uberlândia and reared in Petri dishes, as previously described. A colony of approximately 50 individuals of various instars was kept in each Petri dish. Thirty Petri dishes with M. persicae were used in the experiment and two dishes of this aphid species were used for rearing D. rapae.

Parasitoid rearing

The parasitoids were obtained from Brevicoryne brassicae L. mummies collected in a commercial collard green crop at the Federal University of Uberlândia. The mummies were taken to the laboratory, placed individually in Eppendorf tubes and kept at 23 °C and photoperiod of 12 h. Once parasitoids emerged, they were fed with 50% honey and water. Two Petri dishes with approximately 50 individuals of M. persicae were used for rearing D. rapae. One mated female of D. rapae was released in each Petri dish and maintained for 24 h for oviposition. After this period, the females were removed and parasitized aphids kept at 23 ± 1 °C, 50–55% RH and photophase of 12 h. The mummified aphids were placed in Eppendorf tubes and, after emergence, the parasitoids fed and mated. After mating was observed, males were removed from the tubes and the females were used in the experiment 24 h after emergence.

Experimental protocol

On the first day of the experiment, the aphid colonies were reduced to 30 individuals of several instars. To ensure a uniform stand, smaller nymphs (1st and 2nd instar) were removed, leaving only the largest nymphs (3rd and 4th instars) and adults. The leaf disk containing the aphids was removed from the Petri dish and placed on a plant inside an anti-aphid cage. Two leaves in opposite positions were infested per plant. Each plant received a leaf
disk containing 30 resistant or 30 susceptible *L. pseudobrassicae* on a leaf and a leaf disk with 30 *M. persicae* on another leaf, totaling 60 aphids per plant. The leaf discs with the aphids were placed on fully developed leaves located in the median region of the plants because this is the preferred leaf position of both aphid species in field conditions (Cividanes and Souza, 2004; Sampaio et al., 2017). The parasitoids were released in the cages 48 h after infesting the plants with aphids.

Parasitoid release took place as follows: two females were released per cage, one in the third and another in the fourth day after the beginning of the experiment; both were released later in the day (milder temperatures) to facilitate the acclimation of the parasitoid. Females were left inside the cages until their natural death.

Observations were made daily, in order to monitor the development of the parasitoids and thus determine the duration of the experiment. The first mummies were formed after seven days and F1 adults emerged 10 days after the release of the first *D. rapae* female. Thus, the mummies of the F2 generation of *D. rapae* began to be formed 17 days after the release of the first female parasitoid. In the 21st day after the beginning of the experiment (19 days after the release of the first *D. rapae* female) plants were taken to the laboratory for evaluation, with sufficient time to complete the mummy’s formation of the F2 generation. The number of live and parasitized aphids from three fully developed leaves (located in the middle region of each collard green) were counted using a stereoscopic microscope.

Plants from two cages, one from each treatment, were lost due to the high aphid population and thus these were removed from the analyses. Thus, in total, there were nine replicates of resistant and 11 of susceptible *L. pseudobrassicae*.

**Data analysis**

Normality of residuals was evaluated by Shapiro Wilk’s test and homogeneity of variance by Levene’s test, using SPSS 16.0 software at five percent probability. The proportion of parasitized individuals was calculated as the ratio between the number of parasitized individuals and the total number of individuals on each plant 21 days after the beginning of the experiment. The relative growth rate of each aphid population was calculated as (T1 – T0)/T0, where: T1 is the number of non-parasitized aphids present on each plant after 21 days and T0 is the initial number of individuals placed in each plant (30 individuals). We compared the proportion of parasitism and the growth rates of *M. persicae* growing in plants with or without resistant *L. pseudobrassicae* populations using the t-test for two independent samples. The same test was used to compare the resistant and susceptible populations of *L. pseudobrassicae*. In order to meet the assumptions of the t-test, we used logarithm (Zar, 1984) and logit (Warton and Hui, 2011) transformation, respectively, for data on relative growth rates and proportion of parasitism. We also compared the proportion of parasitism on *M. persicae* growing in plants with or without resistant *L. pseudobrassicae* using Analysis of Covariance in which the number of parasitized individuals of *L. pseudobrassicae* (log transformed) was treated as the covariate. Growth rate of the two aphid species was compared using paired t-test, and separate tests were performed for plants with the susceptible and resistant populations of *L. pseudobrassicae*. All analyses were conducted using Systat 10.2 (SPSS, 2000).

**Results**

The percentage of parasitism by *D. rapae* on *M. persicae* varied more than 10-fold in our experimental plants (range = 4.5–62.2%), and there was no difference in the percentage of parasitism on *M. persicae* between plants with susceptible or resistant *L. pseudobrassicae* (*t* = 0.64, df = 18, *p* = 0.53) (Fig. 1A). The percentage of parasitism in *L. pseudobrassicae* varied from 0.9 to 40%. Parasitism by *D. rapae* was greater in the susceptible than in the resistant population of *L. pseudobrassicae* (*t* = 4.67, df = 18, *p* < 0.001). On average, parasitism in the susceptible population was 3.5 times greater than in the resistant one (mean ± SD: susceptible = 23.3 ± 9.7%; resistant = 6.6 ± 5.9%) (Fig. 1B).

When we used the absolute number of *L. pseudobrassicae* parasitized in each plant as covariate in the analysis we did not detect any differences in parasitism on *M. persicae* between plants with susceptible or resistant *L. pseudobrassicae* (*F*1,16 = 1.63, *p* = 0.22). Furthermore, there was no effect of the covariate (*F*1,16 = 2.75, *p* = 0.12), i.e. the percentage of parasitism on *M. persicae* in a given plant was not related to the number of parasitized *L. pseudobrassicae* in that same plant (Fig. 2).

The relative growth rate of *L. pseudobrassicae* resistant to *D. rapae* was greater than that of *M. persicae* (paired t-test, *t* = 2.42, df = 8, *p* < 0.041) (Fig. 3A), whereas the relative growth rate of *L. pseudobrassicae* susceptible to *D. rapae* was not different from that of *M. persicae* (paired t-test, *t* = 1.37, df = 10, *p* = 0.20) (Fig. 3B). Also, when comparing the growth rate of the same aphid species in plants with resistant or susceptible populations we found no differences for *M. persicae* (*t* = 0.10, df = 18, *p* = 0.93) nor for *L. pseudobrassicae* (*t* = 1.41, df = 18, *p* = 0.18).

![Fig. 1.](image-url) (A) Proportion of *M. persicae* parasitized by *D. rapae* in plants with resistant or susceptible *L. pseudobrassicae* populations. (B) Proportion of resistant or susceptible *L. pseudobrassicae* parasitized by *D. rapae* in plants with *M. persicae*. 
Fig. 2. Relationship between the absolute number of *L. psedobrassicae* parasitized by *D. rapae* and the percentage of parasitism on *M. persicae*. Each symbol represents a different plant.

**Discussion**

There was no difference in the percentage of parasitism of *M. persicae* by *D. rapae* between plants with susceptible or resistant *L. pseudobrassicae*. An indirect resistance of *M. persicae* was expected in the presence of resistant *L. pseudobrassicae* due to the reduction in parasitoid population by two effects: a reduction in parasitoid foraging capability and greater parasitoid mortality.

The presence of a non-susceptible species can reduce the foraging capability of the parasitoid. The parasitoid *Aphidius ervi* Haliday, for example, oviposits on *Therioaphis maculata* (Buckton), and the presence of this aphid decreases the foraging capacity of the parasitoid on a suitable host, *Acythosiphon pisum* (Harris), reducing the population of the parasitoid (*Meinsner et al., 2007*). As *D. rapae* oviposits at equal rates when *M. persicae* and resistant *L. pseudobrassicae* are simultaneously exposed to the parasitoid (*Oliveira et al., 2013*), a decrease in the parasitoid population was expected, because resistant aphids prevent the development of the parasitoid eggs (*Oliveira et al., 2013*). However, the parasitism of *M. persicae* was not changed in the presence of resistant *L. pseudobrassicae*. Therefore, we did not find support to the indirect resistance hypothesis.

Over short time periods, the presence of a competitor of greater potential growth rate can decrease the effect of the parasitoid on the species with the lower growth potential rate due to the wide range of hosts (*González et al., 2002; Holt and Kotler, 1987*). However, this effect tends to be reversed over time, since a larger population of the parasitoid will be formed (*Holt and Kotler, 1987*). In fact, the presence of another host species may increase the control efficiency of the parasitoid on both aphid species (*Langer and Hance, 2004*).

Parasitism rates were lower in the resistant than in the susceptible population of *L. pseudobrassicae*. Parasitism in the resistant population of *L. pseudobrassicae* can be explained due to the loss of resistance in the offspring, thus parasitized individuals in this population were susceptible offspring of the original resistant clone (*Oliver et al., 2010*). In addition, because parasitoids were confined within a limited space together with the resistant hosts, the chance of finding a previously parasitized host increase, leading to “superparasitism” (*van Lenteren and Bakker, 1976; Kant et al., 2011*). Furthermore, the greater the number of ovipositions in a given resistant host, the greater the chance of breaking the resistance (*Oliver et al., 2012*).

In the present study, the resistant population of *L. pseudobrassicae* showed a greater growth rate than *M. persicae*, whereas the susceptible one did not. Studies indicate that, in the absence of competitors and natural enemies, the population growth potential of *L. pseudobrassicae* on collard green is greater than that of *M. persicae* (*Cividanes and Souza, 2003; Godoy and Cividanes, 2002*). The lower parasitism rate found in resistant *L. pseudobrassicae* may have been the factor for the greater population growth of this aphid species. Typically, when parasitized during the second instar, aphids become mummies and die before reproducing, and even parasitized aphids that survive until adulthood do not reproduce. Therefore, susceptible aphids when parasitized during early instars do not contribute to the growth of the population (*Sampaio et al., 2007; van Steenis and El-Kawass, 1995*), and resistant *L. pseudobrassicae* populations have a positive growth rate even when parasitized during the second instar (*Oliveira et al., 2013*).

However, the presence of the parasitoid can be the reason why we found no differences in growth rates between resistant and susceptible populations of *L. pseudobrassicae*. Although being able to reproduce, the intrinsic rate of population increase (*r*) of resistant *L. pseudobrassicae* parasitized by *D. rapae* is only 63% that of non-parasitized aphids (*Oliveira et al., 2013*). Therefore, the parasitoid can reduce the population growth rate of both, resistant and susceptible populations of *L. pseudobrassicae*.

The populations of *L. pseudobrassicae* and *M. persicae* do compete since both have a preference for feeding on collard green leaves in the intermediate and lower regions of the plant (*Cividanes and Souza, 2004; Sampaio et al., 2017*). As the aphids do not have a mechanism to remove the competitor from the favorite spots, the species with the greatest population growth potential, and that is able to occupy these places first, will probably have a competitive advantage.

![Graph](image.png)

**Fig. 3.** (A) Relative growth rates of *M. persicae* and resistant *L. pseudobrassicae* populations. (B) Relative growth rates of *M. persicae* and susceptible *L. pseudobrassicae* populations.
advantage (Crawley, 1983; Denno et al., 1995; Petersen and Hunter, 2001; Schoener, 1983). Even when this competition is mediated by parasitoids, insects that have a greater potential of population growth tend to support a larger population of parasitoids and gain competitive advantage over species with a lower growth potential (Holt and Lawton, 1994).

This is the first study evaluating the influence of a resistant aphid population on the parasitism of its susceptible competitor. Although additional, long-term studies are needed to rule out the hypothesis of indirect resistance, we showed that over a short period of time the presence of resistant L. pseudobrassicae does not affect the rate of parasitism by D. rapae on M. persicae.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

We thank the Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) for the financial support (Project APQ-1744-12). We also thank the National Council for Scientific and Technological Development (CNPq) and the São Paulo State Research Foundation (FAPESP) for providing financial support to theINCT-HYMPAR/SUDESTE and Coordination for Improvement of Higher Education Personnel (CAPES) for the graduate scholarship for Samira Evangelista Ferreira and Reinaldo Silva de Oliveira.

References

Asgari, S., Theepold, U., Welby, C., Schmidt, O., 1998. A protein with protective prop-
erties against the cellular defense reactions in insects. P.N.A.S. 95, 3690–3695.
Blumberg, D., 1997. Parasitoid encapsulation as a defense mechanism in the Coc-
cocoea (Homoptera) and its importance in biological control. Biol. Control 8, 225–236.
Chen, D.Q., Purcell, A.H., 1997. Occurrence and transmission of facultative endosymb-
Cividanes, F.J., Souza, V.P., 2004. Distribuição vertical de pulgões (Homoptera: Aphi-
well Scientific Publications, London, UK.
drecht, pp. 1–71.
Ferreira, S.E., 2013. Causa da resistência de Lipaphis pseudobrassicae (Dias, 1914) ao parasitismo Diatieraella rapae (McIntosh, 1855) e sua influência sobre o par-
asitismo de Myzus persicae (Sulzer, 1776). Dissertations, Institute of Agricultural Science, Postgraduate Program of Agronomy, Federal University of Uberlândia, Brazil.