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BIOLOGICAL CONTROL

Host Quality of Different Aphid Species for Rearing *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae)

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Abstract

This study aimed to evaluate the quality of the aphid *Myzus persicae* (Sulzer), Lipaphis erysimi (Kaltenbach) and Brevicoryne brassicae (L.) as hosts for the parasitoid *Diaeretiella rapae* (McIntosh). Parasitization by D. rapae was higher on M. persicae than on L. ervsimi and B. brassicae. The time of development of D. rapae from egg to mummy or egg to adult male or female were shorter on M. persicae than on L. ervsimi and B. brassicae. Moreover, D. rapae showed no significant differences in the emergence rate, sex ratio and longevity when reared on the three aphid species. Myzus persicae was the largest aphid host, with B. brassicae and L. erysimi being of intermediate and of small size, respectively. Diaeretiella rapae reared on M. persicae was larger than when reared on L. erysimi and B. brassicae, and females of D. rapae were significantly larger than males on *M. persicae*, but males of *D. rapae* were larger than females when reared on *L. erysimi*. No difference in size was detected between males and females in parasitoids reared on *B. brassicae*. Among the aphid species studied, M. persicae was found to be the most suitable to D. rapae.

Introduction

Aphidiinae endoparasitoids (Braconidae) are the most widely used natural enemies in biological control programs against aphids. In Brazil, studies on aphidiine parasitoids for aphid control were focused on *Aphidius colemani* Viereck a biocontrol agent of *Aphis gossypii* Glover and *Myzus persicae* (Sulzer) (Sampaio et al 2001a,b), *Lysiphlebus testaceipes* (Cresson) (Silva et al 2008a,b) and *Praon volucre* (Haliday) (De Conti et al 2008). It is noteworthy that the country has scarce information about the use of the aphidiine parasitoid *Diaeretiella rapae* (McIntosh) in aphid control. This species is considered a specialist on aphids associated with crucifers (Blande et al 2004), being reported in Brazil as the main parasitoid of *Brevicoryne brassicae* (L.), *Lipaphis erysimi* (Kaltenbach) and *M. persicae* (Sousa &

Bueno 1992, Mussury & Fernandes 2002, Resende *et al* 2006). *Diaeretiella rapae* also occurs associated with other species of aphids in different crops and it is distributed in several states in Brazil (Starý *et al* 2007).

To assess host suitability and to accept a host for oviposition, aphidiine parasitoids use a number of host-derived cues, such as shape, color, odor, taste and movements (Mackauer *et al* 1996, Blande *et al* 2007). Nutritional and physiological characteristics of the host directly affect development, mortality, longevity and fecundity of natural enemies such as parasitoids (Roitberg *et al* 2001). Because immature stages of hymenopteran parasitoids rely on the nutrients present in host insects (Mackauer & Sequeira 1992), parasitoid females oviposit on a particular host when it presents nutritional and physiological requirements for the development of their

offspring. Also, larvae of the parasitoids rely on their mother's ability to access the host quality to ensure their development (Mackauer *et al* 1996).

Several studies have demonstrated the importance of glucosinolates (GS) from crucifers as indicators of the quality of the host plant to herbivorous insects (Moyes et al 2000, Gols et al 2008). These compounds also play a role in the plant defense against insect herbivores (Rask et al 2000) and can indirectly affect predators and parasitoids, causing a cascade effect (Francis et al 2001, Goals et al 2009). Recently, it has been investigated the use of chemical signals from glucosinolates by parasitoids to locate their hosts, habitats and to evaluate host quality (Blande et al 2004, 2007, 2008).

Generalist herbivores produce enzymes that can detoxify a wide range of substrates (Krieger *et al* 1971), whereas specialists evolved an enzymatic system able to detoxify defensive plant compounds (Ratzka *et al* 2002). Thus, generalist herbivores are usually more sensitive to high levels of specific allelochemicals as compared to specialists (Giamoustaris & Mithen 1995). Specialists feeding on brassicaceous plants are adapted to glucosinolates-containing plants, and they can detoxify, excrete, or even sequester these harmful metabolites (Ratzka *et al* 2002, Wittstock *et al* 2004).

The high potential of *D. rapae* as a biological control agent against aphids in various crops, including crucifers, has been already indicated (Lara *et al* 1999, Mussury & Fernandes 2002, Vaz *et al* 2004). But for the establishment of a successful biological control program of aphids through the use of parasitoids, several bioecological aspects of the selected natural enemy, such as, the suitability of host species that are candidates to host for parasitoid mass rearing are required. As little is known on D. rapae in Brazil, this study aimed to evaluate the host suitability of three aphid species that feed on Brassica oleraceae plants, the generalist *M. persicae* and the specialists *L. erysimi* and *B. brassicae*, to *D. rapae*.

Material and Methods

Rearing the aphid hosts

Myzus persicae, L. erysimi and B. brassicae were collected from Brassicaceae plants in the region of Jaboticabal (21°15′22″S, 48°18′58″W), state of São Paulo. Aphids were reared on *Brassica oleracea* var. acephala cultivar Georgia in 500 ml pots in wooden framed cages (45 x 50 x 70 cm) coverage by cheesecloth, and maintained in a greenhouse. Adult females from the stock colonies were used to obtain nymphs for the experiments.

Rearing the parasitoid D. rapae

Aphid mummies of M. persicae, L. erysimi and B.

brassicae were collected in Brassicaceae plants at the same localities as the aphids. The mummies were individualized in glass tubes (80 x 19 mm) and kept at $25 \pm 1^{\circ}$ C, $60 \pm 10\%$ RH and 12h photophase. Adults of *D. rapae* were sexed and released inside wooden cages (described earlier) containing potted plants of *B. oleracea* var. *acephala* cultivar Georgia individually infested with *M. persicae*, *L. erysimi* or *B. brassicae*. Stock colonies of *D. rapae* were maintained on each aphid host species studied. Honey was offered daily as a food source for adult parasitoids.

Quality of M. persicae, L. erysimi and B. brassicae as hosts of D. rapae

Experiments were conducted in a climate chamber at $25 \pm 1^{\circ}$ C, $60 \pm 10\%$ RH and 12h photophase. Fifteen, 0-24h-old, mated, naive females of *D. rapae* were individually released in Petri dishes (5 cm in diameter) containing 20, 3 d-old nymphs of either *M. persicae*, *B. brassicae* or *L. erysimi* for parasitization. Aphids were offered on a kale leaf disc on a layer of a 1% agar-water solution. Parasitoid females were allowed to parasitize for 60 min afterwards, females were removed and the aphid nymphs were maintained inside the chambers for the complete development of parasitoids.

The development time from egg to mummy and from egg to adult parasitoid was determined. The mummified aphids were counted and individualized in glass tubes (80 x 19 mm) to assess parasitization, emergence, and sex ratio. The longevity of *D. rapae* was evaluated from 15 males and 15 females randomly selected. They were fed daily with honey and water deposited on the walls of the rearing glass tubes.

Host and parasitoid sizes were determined based on the length of their hind tibia, as tibia length is considered an appropriate indicator of the size of aphids (Nicol & Mackauer 1999, Van Emden & Kifle 2002) and aphidiine parasitoids (Nicol & Mackauer 1999). Host and parasitoid sizes were also used as indicators of the suitability of each aphid species as a host to *D. rapae*.

Second-instars of *M. persicae, L. erysimi* and *B. brassicae* were kept in Petri dishes (15 cm in diameter) containing a kale leaf disc laid on top of a layer of a 1% agar-water solution. From a single colony of each aphid species, 15 nymphs were collected and they right posterior tibiae were removed. Other 60 to 80 nymphs were offer to a newly-emerged, mated female of *D. rapae*. for 60 min. Parasitized nymphs were maintained at constant temperature and RH (described earlier) until mummification, when they were transferred to separate glass tubes (80 x 19 mm) for parasitoid emergence. Fifteen males and 15 females of parasitoid adults obtained from each particular host were randomly collected for the removal and measurement of the right hind tibia.

The tibiae of both nymphs and parasitoids were

mounted between a glass slide and a coverslip in 70% ethanol and immediately measured with micrometers adapted to a microscope.

Data analysis

The experiments were conducted in a completely randomized design using a 2×3 factorial design (2 sexes \times 3 hosts). All variables (parasitization, development periods, percentage of adult emergence, sex ratio, longevity and size) were analyzed by ANOVA followed by a Tukey test at 5% significance level, using the softwares SAS v.9.0. and AgroEstat v.1.0.

Results and Discussion

The parasitization of the aphid species studied by D. rapae ranged from 42.5% to 66.5%, and was significantly higher on M. persicae than on L. erysimi and B. brassicae (F = 4.91, P < 0.05) (Table 1). Generally, the highest percentage of parasitism may be an indication of host quality. Aphids with better quality provide better conditions for aphidiine females to lay a large amount of eggs (Silva et al 2008a). This can also be characterized by the largest number of stings with ovipositor made by the female parasitoid at the time of laving the eggs (Sampaio et al 2001a). However, host acceptance does not necessarily correlates with host quality, as demonstrated for other aphidiine species (Chau & Mackauer 2001, Henry et al 2005, Sampaio et al 2008). The developmental time is also an indication of host quality, and development from egg to mummy (F = 28.13, P < 0.0001), from egg to adult-male (F = 12.04, P < 0.0001) and egg to adult-female (F = 3.74, P < 0.0001) of *D. rapae* were consistently shorter in *M. persicae* than in *L. erysimi* and in *B. brassicae* (Table 1).

The percentage of parasitism and the development time of *D. rapae* on *B. brassicae* (Table 1) were similar

to those obtained by Bayhan et al (2007) (40.2% and 11.6 d, respectively), but our data on *M. persicae* and *L*. erysimi contradict those obtained by Blande et al (2004). that reported a higher parasitism rate of *D. rapae* on *L.* erysimi than on M. persicae. This controversy may be related to the genetic variation among populations of *D*. rapae and their hosts from different geographic regions (Antolin et al 2006, Baer et al 2004). Another possible explanation is the use of different host plants (Bayhan et al 2007), as Blande et al (2004) reared M. persicae and L. erysimi on turnip, Brassica rapa var rapifera cv Tokyo Cross. The differential effect of host plants on host and parasitoid fitness may also be related to their glucosinolate composition, as the glucosinolates produced by Brassicaceae are hydrolyzed by the enzyme myrosinase under herbivore attack, leading to the production of isothiocyanates, thiocyanates and nitriles, which play a defensive role against herbivory (Rask et al 2000, Fahey et al 2002, Halkier & Gershenzon 2006). It is noteworthy that the profile of glucosinolates in *Brassica* varies significantly among and within populations (Moyes et al 2000, Newton et al 2009). Glucosinolates are known to affect the performance of insect herbivores and the third trophic level depending on their degree of specialization (Gols et al 2008).

Specialist crucifer-feeding aphids, such as *L. erysimi* and *B. brassicae*, can synthesize the enzyme myrosinase (MacGibbon & Beuzenberg 1978, Jones *et al* 2001), which is compartmentalized into microbodies within muscles in the thorax and head of aphids (Bridges *et al* 2002). Therefore, *L. erysimi* and *B. brassicae* have developed the ability to sequester, accumulate and hydrolyze glucosinolates, avoiding their toxic effects, utilizing them to their own benefit as chemical defenses against natural enemies (Husebye *et al* 2005). On the other hand, the generalist aphid *M. persicae* can only tolerate the negative effects of glucosinolates, which are quickly eliminated

Table 1 Average value (\pm standard error) of the biological variables of *Diaeretiella rapae* on different aphid hosts ($25 \pm 1^{\circ}$ C, $60 \pm 10\%$ RH and 12h photophase).

Biological variables -		Hosts		
		Myzus persicae	Lipaphis erysimi	Brevicoryne brassicae
% Parasitism		66.5 ± 5.37 a	42.5 ± 4.51 b	48.2 ± 6.80 ab
Development	Egg-mummy	6.5 ± 0.10 b	7.3 ± 0.08 a	7.2 ± 0.07 a
	Egg-adult (male)	10.5 ± 0.11 bA	11.3 ± 0.16 aA	11.5 ± 0.19 aA
	Egg-adult (female)	10.6 ± 0.10 bA	11.3 ± 0.15 aA	11.5 ± 0.15 aA
% Emergence	•	81.5 ± 4.89 a	91.8 ± 3.02 a	78.8 ± 5.24 a
Sex ratio		0.59 ± 0.06 a	0.53 ± 0.09 a	0.55 ± 0.09 a
Longevity	Male	7.5 ± 0.74 aA	7.0 ± 0.93 aA	5.9 ± 0.36 aA
	Female	7.7 ± 0.96 aA	8.9 ± 0.73 aA	7.4 ± 1.00 aA

Means followed by same lowercase letter horizontally and uppercase vertically, do not differ by Tukey test at 5% probability.

through the gut and excreted with the honeydew (Weber et al 1986). These strategies to handle glucosinolates from the host plant may explain the better performance of *D. rapae* when reared on *M. persicae* as opposed to *L. erysimi* and *B. brassicae*, suggesting a possible influence of glucosinolates in the immature stages of parasitoids.

Male and female of *D. rapae* had similar development time from egg to adult regardless the host aphid species (*M. persicae* - F = 0.20, P > 0.05; *L. erysimi* - F = 0.00, P > 0.05; *B. brassicae* - F = 0.01, P > 0.05) (Table 1). There were no significant differences in immature survivalship (F = 2.33, P > 0.05), sex ratio (F = 0.12, P > 0.05) and adult longevity (males - F = 1.30, P > 0.05; females - F = 0.74, P > 0.05) of *D. rapae* among the three host aphid species (Table 1). Also, the development time and longevity of males and females of *D. rapae* did not differ one from another in each host species (*M. persicae* - F = 0.01, P > 0.05; *L. erysimi* - F = 2.51, P > 0.05; *B. brassicae* - F = 1.89, P > 0.05) (Table 1).

The host size was positively correlated with the parasitoid size, as observed for other aphidiine parasitoids (Sampaio et~al~2008, Silva et~al~2008b, De Conti et~al~2008). Myzus~persicae was the largest aphid (0.454 mm), followed by B.~brassicae (0.327 mm) and L.~erysimi (0.296 mm) (F = 154.16, P < 0.0001) (Table 2), as observed elsewhere (Sampaio et~al~2008). Myzus~persicae~yielded the largest adults of D.~rapae, with males as large as 0.546 mm (F = 12.87, P < 0.0001) and females as large as 0.582 mm (F = 23.00 P < 0.0001), as given by their tibial length. Parasitoids obtained in here were much larger than those reported by Blande et~al~(2004) by rearing D.~rapae on the same host aphid species (males = 0.49 mm; females = 0.51 mm).

Males and females of *D. rapae* showed no significant differences in their tibial length when reared on *B. brassicae* (F = 0.51, P > 0.05) (Table 2). However, males reared from *L. erysimi* were larger than females (F = 8.09, P < 0.01), while females reared from *M. persicae* were larger than males (F = 10.19, P < 0.01) (Table 2). The fact that the smallest aphid host *L. erysimi* yielded larger males than females differs from the pattern observed in aphidiine parasitoid species, in which females are

generally larger than males (Sequeira & Mackauer 1993, Mackauer 1996). Female aphidiine parasitoids can obtain and accumulate more resources per unit time than males, achieving, thus, larger sizes in smaller hosts (Sampaio et al 2008). In this study the opposite was observed, as males of D. rapae were more successful than females to allocate resources, reaching larger sizes in the smaller host L. erysimi (Table 2). Size variation between males and females of aphidiine parasitoids are usually more conspicuous when these parasitoids are reared in smaller hosts and tends to decrease as the host size increases (Sequeira & Mackauer 1993, Mackauer 1996, Sampaio et al 2008). This finding was not observed in this study because males and females of *D. rapae* showed a significant difference in size when reared on the largest and smallest hosts (Table 2).

Diaeretiella rapae reared on *M. persicae* showed a higher percentage of parasitism, the shortest period of development and a larger size. Generally, larger hosts are preferred and provide better quality for aphidiine parasitoids, leading to the development of larger individuals, which have a competitive advantage over those that developed in smaller hosts (Cloutier *et al* 2000, Chau & Mackauer 2001). However, host size may not necessarily correlate with host quality for parasitoid development. Certain biological traits of parasitoids, as their reproductive capacity and longevity, are not directly related to host size, but with the ability of larvae to obtain the available resources from the host (Sequeira & Mackauer 1992, 1994).

Our data on *D. rapae* showed that the host aphid species *M. persicae*, *L. erysimi* and *B. brassicae* were nutritionally and physiologically suitable for parasitoid development, but *M. persicae* was considered the best host among the species tested (larger adults, higher parasitization capacity, shorter development time).

It should be emphasized the need for new studies to clarify the influence of glucosinolates on *D. rapae* biology. The available information refers to the indirect effects of these compounds as chemical signals, mainly in the foraging behavior of *D. rapae* female. Our data suggest that glucosinolates can act not only indirectly on

Table 2 Average (± standard error) tibia size of aphid hosts and the parasitoid *Diaeretiella rapae* (25 ± 1°C, 60 ±10% RH and 12h photophase).

	Tibia length (mm)			
Aphids	Hosts –	D. rapae		
		Males	Females	
Myzus persicae	0.454 ± 0.004 a	0.546 ± 0.009 aB	0.582 ± 0.006 aA	
Brevicoryne brassicae	0.327 ± 0.010 b	0.487 ± 0.008 bA	0.499 ± 0.013 bA	
Lipaphis erysimi	0.296 ± 0.004 c	0.530 ± 0.008 aA	0.494 ± 0.010 bB	

Means followed by same lowercase letter vertically, and uppercase, horizontally do not differ by Tukey test at 5% probability.

the parasitoid, but also directly, increasing the defense mechanisms of crucifer-feeding specialists aphids through bioaccumulation, or influencing the quality of aphids to parasitoids.

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