Seasonal abundance and mutual interference of *Diaeretiella rapae* (McIntosh) on *Brevicoryne brassicae*

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ABSTRACT: *Brevicoryne brassicae* (Hemiptera: Aphididae), known as cabbage aphid, is cosmopolitan in distribution that infests cruciferous crops such as broccoli, cabbage, cauliflower, and mustard. *Brevicoryne brassicae* is suppressed by various species of natural enemies, the most important and abundant being *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae). The present study was carried out at Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni Solan, Himachal Pradesh, on the seasonal abundance and mutual interference of the parasitoid, *D. rapae* during the years 2020-2021. The study showed the peak population of both host (221.36 aphids/plant) and parasitoid (27.28 mummified aphids/plant) with 12.32 per cent parasitization on the 10th Standard Meteorological Week (SMW). On the 14th SMW of 2021, the aphid population was lowest (2.72 aphids/plant) and parasitization by *D. rapae* was highest (47.05%). The *D. rapae* when foraged at densities of 2, 4, 6 and 8 parasitoids per 30 and 50 constant host nymphs, the third-instar host nymphs had a higher mutual interference coefficient than fourth instar host nymphs. Therefore, *D. rapae* can be utilized as an important biocontrol agent in the management programme of a cabbage aphid (*B. brassicae*).

KEYWORDS: *Diaeretiella rapae,* mutual interference, parasitoid, per cent parasitization, seasonal abundance

INTRODUCTION

The cabbage aphid, *Brevicoryne brassicae* (L.) is a notable pest of cruciferous plants. This insect species poses a significant threat to crops due to its feeding behavior, which involves the extraction of plant sap, as well as its ability to transmit plant viruses (Ellis *et al*., 1998; Blackman and Eastop, 2000; Capinera, 2001; Desneux *et al*., 2006).

Aphid abundance is determined by migration. Aphid colony expansion results in the development of winged morphs that fly and infest new plants (Way and Cammell, 1970), and the winged aphids migrate into the field (Zhang and Hassan, 2003).

This phenomenon may be attributed to the positive response of aphids and the cabbage white butterfly *(Pieris rapae)* to volatile compounds emitted by the host plants, as well as the honeydew excreted by aphids, which serves as a kairomone for their natural enemies (Bundemberg, 1990; Brown *et al*., 1970; Dicke and Sabelis, 1988). In the context of biological control, aphid parasitoids have proven to be effective tools in integrated pest management programs implemented in both polyhouses and open fields (Boivin *et al*., 2012). Natural enemy's ecological and biological characteristics can be used to predict their value in a biological control system (Zahiri *et al.,* 2014). The most important behavioural traits to determine the effectiveness of parasitoids for the biological control of aphids are host stage preference and mutual interference (Luck, 1990). At a given host density, mutual interference between foraging parasitoids can reduce per capita search efficiency (Hassell and Varley, 1969; Godfray and Pacala, 1992; Cronin and Strong, 1993). The groundbreaking research conducted by Hassell and Varley in 1969 laid the foundation for studying mutual interference in parasitoids. They observed that there exists an inverse relationship between the searching efficiency of parasitoids and their density. This relationship is expected because as the density of parasitoids increases, more time is wasted by individuals in encountering and interacting with other members of the same species.

Diaeretiella rapae is well-known for its ability to control cabbage aphids naturally (Read *et al.,* 1970; Mackauer and Kambhampati, 1984; Elliot *et al.,* 1994; Pike *et al.,* 1999; Jankowska and Wiech, 2003; Boivin *et al.,* 2012). Several studies have also demonstrated significant mutual interference among female *D. rapae* parasitizing *L. erysimi,* including the works of Pandey *et al.* (1986), Abidi *et al.* (1989), and Shukla *et al.* (1997). So, the present investigations have been taken up to study the seasonal abundance and mutual interference of *D. rapae* were studied on the 3rd and 4th nymphal instars of *B. brassicae* at constant density of 30 and 50 host nymphs.

MATERIALS AND METHODS

Maintenance of *B. brassicae* culture

The cabbage aphid, *B. brassicae,* was kept in the Biocontrol Research Laboratory of the Department of Entomology, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP). For this cabbage aphids were colelcted from the field and released onto pot-raised cauliflower plants kept in the laboratory. Also, naturally infested cauliflower leaves were brought to the laboratory and released on potted cauliflower plants kept in screened cages. To ensure a continuous supply of the cabbage aphid for the experiments, the exhausted and dried cauliflower plants were replaced on a regular basis with fresh seedlings/plants.

Maintenance of culture of *D. rapae*

Mummified aphids were collected from cauliflower plants and placed inside a glass tube to allow parasitoid adults to emerge. Fine streaks of honey were placed on the sides of the glass tube as food for the newly emerged parasitoid adults. These adults were then placed in a 45x45x45 cm wooden cage containing 3-4 aphid-infested cauliflower plants in pots. The honey streaks on the cotton swab were provided as a food source for the parasitoid. The parasitized aphids were gently removed after mummification and placed in glass vials for adult emergence.

Mutual interference in varied densities of the parasitoid, *D. rapae* at constant density of *B. brassicae*

For mutual interference among *D. rapae* adults, fixed density (30 or 50 nymphs) of cabbage aphid, *B. brassicae* was exposed separately to the varied densities (2, 4, 6 and 8) of *D. rapae* females. Each set was replicated five times.

Following a 24-hr period of parasitization, the aphids were carefully maintained for mummification and subsequently reared individually to facilitate the emergence of the parasitoids, following the established protocol. The number of hosts that were successfully parasitized by the parasitoids was recorded for data analysis and further investigation.

Data analysis

Hassell and Varley (1969) empirical model was fitted to calculate the parameters of mutual interference as under:

$$\text{Log E} = \log Q - m \log P$$

where,

E = Area of discovery / searching efficiency

Q = Quest constant (value of E when P = 1)

m = Mutual interference coefficient

P = Parasitoid density

The searching efficiency (E), which represents the per capita parasitization under conditions of mutual interference among parasitoid individuals within the same searching arena, was computed using the formula

$$E = 1/P \log_e [N/(N - Na)].$$

Here, N denotes the density of available hosts, while Na refers to the number of host larvae that have been successfully parasitized. This equation was derived from the work of Varley *et al.* (1973).

RESULTS AND DISCUSSION

Seasonal abundance of *D. rapae* parasitizing *B. brassicae*

The perusal of data in Table 1 showed that the aphid population persisted throughout the cropping period on cauliflower, whereas the parasitoid appeared four weeks later than the cabbage aphid and then remained throughout the crop growing period. The first sighting of *B. brassicae* (2.10 nymphs/plant) on cauliflower was in the 52nd SMW, i.e., the last week of December, 2020. There was no parasitism of the cabbage aphid by the parasitoid, *D. rapae*, during this time period. The cabbage aphid population exploded reaching a peak of 319.48 nymphs per plant on the 9th SMW, or the first week of March, 2021. Later, the population of *B. brassicae* started falling progressively , and on the second week of April 2021 (14th SMW), it reached its lowest population of 2.72 nymphs/plant. While the first record of the parasitoid *D. rapae* (3.68 per cent parasitization) was made during the fourth standard week (the last week of January 2021), with 2.58 mummified aphids per plant. The parasitization gradually rose and peaked on the 10th SMW i.e. or the second week of March 2021 (27.28 mummified aphids/plant; 12.32% parasitization). The parasitoid population then gradually fell until it reached its lowest point, or 1.28 mummified aphids per plant, on the 14th SMW, or the second week of April 2021. It is evident from the data that aphid population started to decline after 9th standard week whereas, the parasitoid population declined from 11th standard week onwards. But the parasitization rate kept on increasing gradually after

6th standard week reaching to its peak of 47.05% on 14th standard week. This may have happened due the fact that the population decline was much sharper in host population as compared to the parasitoid population. The observation on mutual interference of parasitoid at different density variables (2, 4, 6 and 8) showed that there was a gradual increase in number of nymphs parasitized with increasing parasitoid density, whereas a gradual decline in number of nymphs parasitized per female as shown in Table 1.

In the present study, the aphid population started declining after attaining its peak in the 9th standard week, and the aphid parasitization increased slowly with the aphid density and favourable environmental conditions. Both the aphid and the parasitoid remained active and persisted till end of the cropping period. These findings were in accordance with the findings of Verma *et al.* (2019), who reported that both *B. brassicae* and *D. rapae* assumed activity in the fourth week of January and remained active till May end, with peak during the fourth week of March. In the present study, the maximum parasitism (47.05 %) was observed when the aphid population was minimum (2.72 mummified aphids/plant).

The present findings align with the research conducted by Jankowska and Wiech (2003), who also observed that the highest parasitization occurred during periods when the population of aphids on cruciferous vegetables was at its lowest. Similarly, Zhang and Hassan (2003) reported that a wasp-to-aphid ratio of 1.2:1 for the parasitoid *D. rapae* resulted in effective control of aphids by the second generation of the parasitoid. This suggests that the peak

parasitization of aphids may be attributed to the activity of the second generation of *D. rapae* when the aphid population is at its lowest. Furthermore, Zhang and Hassan (2003) found that parasitization by *D. rapae* led to a decrease in the number of offspring produced by the cabbage aphid and a shortened reproduction period, highlighting the significant role of *D. rapae* in regulating the aphid population.

Mutual interference of *D. rapae* parasitizing third and fourth nymphal instars at constant density of 30 nymphs of *B. brassicae*

The observations on mutual interference of parasitoids at different density variables (2, 4, 6 and 8) showed that there was a gradual increase in the number of parasitized nymphs with increasing parasitoid density whereas, a gradual decline was observed in number of nymphs parasitizedper female as evident from Table 2. When a constant density of 30 *B. brassicae* nymphs was used for observation, Table 2 shows the mutual interference of parasitoids at different densities variables (2, 4, 6 and 8). The parasitization of nymphs was observed to be 13.6, 14.8, 18.8, and 20.8 respectively, at third nymphal instars, whereas 9.8, 11.8, 15.2, and 18.2 parasitization was observed for fourth nymphal instar. At densities of 2, 4, 6, and 8, the parasitoid's single female was able to parasitize 6.80, 3.70, 3.13, and 2.60 third-instar nymphs, respectively, and 4.90, 2.95, 2.53, and 2.28 fourth-instar nymphs, respectively. For the third nymphal instar, the area of discovery for parasitoids was 0.30, 0.17, 0.16, and 0.15, respectively, at densities of 2, 4, 6, and 8 parasitoids per 30 host nymphs. In contrast, the area of discovery for fourth instar nymphs was 0.20, 0.12, 0.12, and 0.11,

Table 1. Seasonal abundance of *D. rapae* parasitizing *B. brassicae* in cauliflower during 2020-2021

Month	Standard Week	Cabbage aphid (nymphs/plant)	Mummified aphids (Mummies/plant)	Parasitization (%)
December	52	2.10	0	0
January	1	6.28	0	0
	2	17.32	0	0
	3	32.04	0	0
	4	69.82	2.58	3.68
February	5	89.16	4.12	4.62
	6	134.02	6.04	4.49
	7	208.34	11.48	5.51
	8	263.04	17.96	6.83
March	9	319.48	22.48	7.03
	10	221.36	27.28	12.32
	11	98.08	20.08	20.47
	12	34.34	12.28	35.76
April	13	11.04	5.16	46.73
	14	2.72	1.28	47.05

Table 2. Mutual interference at different densities of *D. rapae* parasitizing third and fourth nymphal instars at constant density (30 nymphs) of *B. brassicae*

Parasitoid density (females) (P)	Number of nymphs parasitized (Mean±SE)		Number of nymphs parasitized per female (Mean±SE)		Area of discovery/ searching efficiency (E)	
	Third instar nymphs	Fourth instar nymphs	Third instar nymphs	Fourth instar nymphs	Third instar nymphs	Fourth instar nymphs
2	13.60±0.51	9.80±0.37	6.80±0.26	4.90±0.19	0.30	0.20
4	14.80±1.02	11.80±0.66	3.70±0.26	2.95±0.17	0.17	0.12
6	18.80±0.73	15.20±0.92	3.13±0.12	2.53±0.15	0.16	0.12
8	20.80±0.73	18.20±0.86	2.60±0.09	2.28±0.10	0.15	0.11
Mutual interference coefficient			0.507, 0.391			
R^2			0.90, 0.86			
Hassell and Varley equation			log E = -0.399– 0.507 log P, log E = -0.615– 0.391 log P			
Quest constant			0.29, 0.41			

respectively. For the third and fourth nymphal instars, the mutual interference coefficient was noted to be 0.507 and 0.391, respectively. The Hassell and Varley equation on a log scale for third instar nymphs was log E = -0.399-0.507 log P, whereas for fourth instar nymph the equation was log E = -0.615- 0.391 log P, where E is the parasitoid searching efficiency/area of discovery and P is the parasitoid density. The regression slope's negative value observed for third and fourth instar nymphs was -0.507 and -0.391, respectively.

Mutual interference of *D. rapae* parasitizing third and fourth nymphal instars at constant density of 50 nymphs of *B. brassicae*

When the observations were made for mutual interference at a constant density of 50 nymphs, the results indicated the similar trend of increasing number of nymphs parasitized and a decline number of nymphs parasitized per female, with an increasing parasitoid density. The perusal of data in Table 3 showed that the parasitization rates of nymphs were 19.8, 28.8, 35.9, and 38.2 within 24 hours when the parasitoids densities were kept as variable, i.e. 2, 4, 6, and 8 parasitoids, and the host density was kept as 50 third instar nymphs of *B. brassicae* (constant). However, the parasitization rates of fourth instar nymphs were 15.4, 25.4, 29.6, and 34 within 24 hours. For third nymphal instars, the single female of the parasitoid was able to parasitize 9.90, 7.20, 5.98, and 4.78 nymphs, respectively; for fourth nymphal instars, the parasitization rate was 7.70, 6.35, 4.93, and 4.25 nymphs, respectively. When compared to the fourth region of discovery, which had concentrations of 2, 4, 6, and 8 parasitoids per 50 host nymphs, the parasitoids' area of discovery was 0.25, 0.21, 0.21, and 0.18. For the third and fourth nymphal instars, the mutual interference coefficient was 0.217 and 0.196, respectively. For fourth instar nymphs, the equation was log E = -0.664- 0.196 log P, where E is the parasitoid seeking efficiency/area of discovery and P is

the parasitoid density. The Hassell and Varley equation on a log scale was log E = -0.531- 0.217 log P for fourth instar nymphs. The third and fourth instar nymphs' regression slopes' negative values, (-0.217) and (-0.196), respectively.

In the present study, mutual interference was observed in *D. rapae* when more than two female parasitoids were searching for *B. brassicae* in the same arena. As the density of searching parasitoids increased, the efficiency of their search declined. The results indicated that third instar (m = 0.217, 0.507) exhibited higher interference compared to fourth instar (m = -0.196, -0.391) at both host densities. The quest constant, which represents the area of discovery in the absence of mutual interference or when only one parasitoid is searching in a given arena, was calculated as 0.66 and 0.29 at 50 host nymphs and 0.71 and 0.41 at 30 host nymphs.

The phenomenon of inverse density dependence in searching efficiency, referred to as mutual interference, was initially described by Hassell and Varley (1969), who observed an inverse relationship between the area of discovery by the parasitoid and the density of the parasite. Subsequently, Beddington (1975) further developed this mutual interference model as an inverse density dependence in searching efficiency.

In the present findings, an inverse relationship between parasitoid density and per capita searching efficiency was observed. These results are in agreement with the results of Abidi *et al.,* (1987), Shukla *et al.* (1997), Fathipour *et al.* (2006) and Soni and Kumar (2020), who reported a negative relationship between parasitoid density of *D. rapae* parasitizing *B. brassicae* and *L. erysimi,* respectively.

Similar to the present findings, previous studies by Hassell (1978) and Visser and Driessen (1991) also reported

Table 3. Mutual interference at different densities of *D. rapae* parasitizing third and fourth nymphal instars at constant density (50 nymphs) of *B. brassicae*

Parasitoid density (females) (P)	Number of nymphs parasitized (Mean±SE)		Number of nymphs parasitized per female (Mean±SE)		Area of discovery/ searching efficiency (E)	
	Third instar nymphs	Fourth instar nymphs	Third instar nymphs	Fourth instar nymphs	Third instar nymphs	Fourth instar nymphs
2	19.80±0.37	15.40±0.75	9.90±0.19	7.70±0.38	0.25	0.18
4	28.80±1.02	25.40±0.75	7.20 ±**0.26**	6.35 ±0.19	0.21	0.18
6	35.90±1.16	29.60±0.93	5.98 ±**0.19**	4.93 ±0.16	0.21	0.15
8	38.20±1.46	34.00±1.05	4.78±**0.18**	4.25±0.13	0.18	0.14
Mutual interference coefficient	0.217, 0.196					
R^2	0.91, 0.87					
Hassell and Varley equation	log E = -0.531– 0.217 log P, log E = -0.664– 0.196 log P					
Quest constant	0.66, 0.71					

a decline in per capita searching efficiency, which may be attributed to various factors. One possible explanation is that females may remain on otherwise favorable host patches due to agonistic interactions with conspecifics, leading to reduced efficiency in locating new hosts. Another factor could be the deposition of eggs in already parasitized hosts, as well as the distribution of eggs during the egg-laying process. Mutual interference among parasitoids can arise from competition for a common resource, resulting in a decrease in the searching efficiency of individual parasitoids. Hassell and Varley (1969) and van Alphen and Vet (1986) have previously observed that mutual interference occurs when competition for a shared resource leads to a decrease in searching efficiency.

In the present study, the specific behavioral mechanisms underlying the decline in attack efficiency were not directly observed. The assessment of mutual interference was based solely on the net outcome, determined by counting the number of parasitized nymphs of the cabbage aphid after a 24-hour exposure to the parasitoids. The low parasitization of nymphs at high densities of parasitoids may also be attributed to the rapid host location and subsequent parasitization by the parasitoid females at higher densities, resulting in the saturation of available hosts. In related studies, Free *et al*. (1977) coined the term "pseudo-interference" to describe phenomena that do not directly stem from agonistic interactions between parasitoids but still contribute to the observed decline in attack efficiency.

In all the scenarios examined, the presence of mutual interference among the searching female parasitoids was evident, as indicated by a decrease in the area of discovery. The area of discovery serves as a measure of the effectiveness of the parasitoid's search activity. Moreover, the negative regression slopes observed indicate an inverse relationship between the density of parasitoids and their individual searching efficiency, further supporting the occurrence of mutual interference.

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