

Spatial distribution of parasitism on *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera: Gracillariidae) in citrus orchards

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(With 1 figure)

Abstract

Many species of microhymenopterous parasitoids have been registered on *Phyllocnistis citrella*, the citrus leafminer. The present study aimed to identify the spatial distribution pattern of the native and introduced parasitoids of *P. citrella* in two citrus orchards in Montenegro, RS. The new shoots from 24 randomly selected trees in each orchard were inspected at the bottom (0-1.5 m) and top (1.5-2.5 m) stratum and had their position relative to the quadrants (North, South, East and West) registered at every 15 days from July/2002 to June/2003. The leaves with pupae were collected and kept isolated until the emergence of parasitoids or of the leaf miner; so, the sampling was biased towards parasitoids that emerge in the host pupal phase. The horizontal spatial distribution was evaluated testing the fitness of data to the Poisson and negative binomial distributions. In Montenegrina, there was no significant difference in the number of parasitoids and in the mean number of pupae found in the top and bottom strata ($\chi^2 = 0.66$; $df = 1$; $P > 0.05$) ($\chi^2 = 0.27$; $df = 1$; $P > 0.05$), respectively. In relation to the quadrants, the highest average numbers of the leafminer pupae and of parasitoids were registered at the East quadrant ($\chi^2 = 11.81$; $df = 3$; $P < 0.05$), ($\chi^2 = 10.36$; $df = 3$; $P < 0.05$). In the Murcott orchard, a higher number of parasitoids was found at the top stratum (63.5%) ($\chi^2 = 7.24$; $df = 1$; $P < 0.05$), the same occurring with the average number of *P. citrella* pupae (62.9%) ($\chi^2 = 6.66$; $df = 1$; $P < 0.05$). The highest number of parasitoids and of miners was registered at the North quadrant ($\chi^2 = 19.29$; $df = 3$; $P < 0.05$), ($\chi^2 = 4.39$; $df = 3$; $P < 0.05$). In both orchards, there was no difference between the numbers of shoots either relative to the strata as well as to the quadrants. As the number of shoots did not varied much relative to the quadrants, it is possible that the higher number of miners and parasitoids in the East and West quadrants would be influenced by the higher solar exposure of these quadrants. The data of the horizontal spatial distribution of the parasitism fit to the negative binomial distribution in all sampling occasions, indicating an aggregated pattern.

Keywords: citrus leafminer, biological control, parasitoids, Hymenoptera.

Distribuição espacial do parasitismo *Phyllocnistis citrella* Stainton, 1856 (Lepidoptera: Gracillariidae) em pomares de citros

Resumo

Vários microimenópteros parasitóides têm sido registrados em populações de *Phyllocnistis citrella* (minador-dos-citros). Este estudo objetivou identificar o padrão de distribuição espacial de parasitóides nativos de *P. citrella* e do introduzido *Ageniaspis citricola* em dois pomares de citros em Montenegro, RS. Em amostragens quinzenais de julho de 2002 a junho de 2003, todos os brotos de 24 plantas sorteadas aleatoriamente em cada pomar foram inspecionados no estrato inferior (0-1,5 m) e superior (1,5-2,5 m) e registrada a localização destes em relação aos quadrantes (Norte, Sul, Leste e Oeste). As folhas com pupas foram coletadas e individualizadas até a emergência dos parasitóides ou do minador, assim, a amostragem foi focada em parasitóides que emergem na fase pupal do hospedeiro. A distribuição espacial horizontal foi avaliada testando-se os ajustes dos dados à distribuição Poisson e binomial negativa. Em Montenegrina não houve diferença significativa no número de parasitóides e no número médio de pupas encontrados no estrato superior e inferior, ($\chi^2 = 0,66$; $gl = 1$; $P > 0,05$) e ($\chi^2 = 0,27$; $gl = 1$; $P > 0,05$), respectivamente. Em re-

lação aos quadrantes, o maior número médio de pupas do minador e de parasitóides encontra-se no quadrante Leste ($\chi^2 = 11,81$; gl = 3; $P < 0,05$), ($\chi^2 = 10,36$; gl = 3; $P < 0,05$). No pomar de Murcott, um maior número de parasitóides foi constatado no estrato superior (63,5%) ($\chi^2 = 7,24$; gl = 1 $P < 0,05$), o mesmo ocorrendo com o número médio de pupas de *P. citrella* (62,9%) ($\chi^2 = 6,66$; gl = 1; $P < 0,05$). O maior número de parasitóides e de minadores foi registrado no quadrante Norte ($\chi^2 = 19,29$; gl = 3; $P < 0,05$), ($\chi^2 = 4,39$; gl = 3; $P < 0,05$). Em ambos os pomares, não houve diferença entre o número de brotos nos dois estratos ou entre os quadrantes. Como o número de brotos não variou muito em relação aos diferentes quadrantes, é possível que a presença maior de minadores e parasitóides no Leste e Oeste seja influenciada pela maior exposição solar destes. Os dados de distribuição espacial horizontal do parasitismo se ajustam à distribuição binomial negativa, em todas as ocasiões, evidenciando um padrão de distribuição agregado.

Palavras-chave: minador-dos-citros, controle biológico, parasitóides, Hymenoptera.

1. Introduction

Investigating patterns of spatial distribution of organisms in the environment is a central issue in population dynamics, considering that differences in the impact of several agents biotic and abiotic may occur in function of spatial variations in the proximity of individuals within a population (Heads and Lawton, 1983; Hassel, 1986).

The relationship between the spatial distribution of hosts and that of its natural enemies has a great influence on the dynamics of both populations. Many models describing this interaction assume that natural enemies have a random distribution relative to the host spatial distribution. This premise simplifies mathematical models, but is unrealistic because most natural enemies react to the spatial distribution of their prey (Pedigo, 1996).

The larvae of *P. citrella*, the citrus leafminer, dig into the subepithelial leaf tissue when feeding, affecting young leaves, shoots and, sometimes even small fruits. The miner causes reduction in the photosynthetic area and injuries that facilitate the infection by the bacteria *Xanthomonas citri* pv. *citri*, the citrus canker causal agent, considered one of the most important citrus pest (FUNDECITRUS, 2003).

The spatial distribution of the different stages of the leaf miner has been studied at different scales and in diverse species and cultivars of citrus in Brazil and abroad. In these studies was found a higher number of eggs in the terminal leaves of the shoots, a caterpillar preference by shoots in the north quadrant and an aggregated dispersion pattern of caterpillars (Peña and Schaffer, 1997; Paleari et al., 2001; Dantas, 2002).

According to Askew (1980), most miner insects are heavily attacked by eulophids. *P. citrella* has a number of natural enemies which belongs to this parasitoid family, and many are promising biological control agents of this pest. The effect of environmental spatial heterogeneity upon populations has been the target of several studies that seek to evaluate its role in the reduction of pest insect populations as well as in keeping their interaction with its natural enemies (Hassel, 1986). The aggregation of parasitoids in some areas of the environment seems to heavily influence the persistence of the interaction in non-cultivated areas as much as in agroecosystems (Hassel and May, 1974).

This kind of study is still scarce in the system citrus x leaf miner x parasitoid and is vital to the understand-

ing of the interactions between natives and introduced parasitoids, specially endeavouring a better management of the orchard.

Parasitoids will be detected and occur only in places where hosts are available and therefore their distribution will be linked to the host distribution. The present work aimed to identify the spatial distribution pattern of the citrus leaf miner parasitoids (those that emerge in the host pupal phase) in plants and in the host *P. citrella*, in two citrus orchards in Montenegro County, Rio Grande do Sul state, Brazil.

2. Material and Methods

Samplings were carried out in two adjacent organically grown orchards (approximately 2 ha each), one with *Citrus deliciosa* Tenore cv. Montenegrina and the other with a hybrid of *Citrus sinensis* L.Osbeck x *C. reticulata* Blanco Murcott, in Montenegro (29° 68' S and 51° 46' W), Rio Grande do Sul state. Each orchard, sampling was done in an area with approximately 0.6 ha, containing 315 plants.

Twenty-four randomly selected trees were inspected every 15 days from July/2002 to June/2003 and all new shoots of the bottom (0.5-1.5 m above the soil) and top (1.6-2.5 m) layers were examined and had their location relative to the quadrants (North, South, East and West), registered. All leaves containing pupae of *P. citrella* were collected and placed in identified plastic bags and transported to the laboratory, where they were individualized in sealed Petri dishes and kept until the emergence of either parasitoids or *P. citrella*.

The vertical distribution of the collected organisms in the plant was analyzed considering the bottom and top strata. The homogeneity of distribution among such strata was tested by the chi-square test of heterogeneity ($\chi^2 = \Sigma(O-E)^2/E$). The position of the collected leaves relative to the quadrants was also recorded and tested by Kruskal-Wallis.

The analysis of the horizontal distribution considered the total parasitism (without parasitoid species distinction). The sampling data was fitted to a Poisson and to a negative binomial series, respectively, by the dispersion index (I) and the dispersion parameter k, following Elliott (1983). Chi-square tests were utilized to test the goodness of fit of the observed distributions.

All calculations were done with Microsoft Excel, Bioestat 2.0 (Ayres et al., 2000) and Ecological Methodology (Krebs, 2000).

3. Results and Discussion

A total of 498 individuals of microhymenopterous parasitoids were collected in the Montenegrina orchard and 212 in the Murcott, all belonging to Eulophidae and Encyrtidae.

The Eulophidae species observed in this study foram *Cirrospilus neotropicus* (Diez and Fidalgo, 2003), *Cirrospilus floridensis* Evans, 1999, *Galeopsomyia fausta* LaSalle, 1997, *Sympiesis* sp., *Elasmus phyllocnistoides* Diez, Torrens and Fidalgo, 2006 e *Elasmus* sp. besides the exotic species, *Ageniaspis citricola* Logvinovskaya, 1983 (Hymenoptera: Encyrtidae) introduced in the region for the biological control of *P. citrella* (Jahnke et al., 2006).

The average percentage of parasitism was 30.2% in Murcott area and 37.6% in Montenegrina. In a simultaneous study carried out in these same areas it was found that the larval-pupal parasitism contributed 10.7% in average to the mortality of immature stages of the citrus leafminer (Jesus, 2005).

The observed parasitism rates among strata and quadrants of the tree canopy showed differences in the two cultivars (Tables 1). The high standard deviations registered on both cultivars could be explained considering flushing occurs only in certain periods of the year. In Montenegrina, there was no significant difference both in the number of parasitoids and of pupae found in the strata ($\chi^2 = 0.66$; $df = 1$; $P > 0.05$) and ($\chi^2 = 0.27$; $df = 1$; $P > 0.05$), respectively. Also, the number of new shoots recorded in the two layers did not show a significant difference ($H = 2.3472$; $P = 0.1255$). In the Murcott orchard, a higher parasitoid number (63.5%; $\chi^2 = 7.24$; $df = 1$ $P < 0.05$), and a higher number of *P. citrella* pu-

pae (62.9%; $\chi^2 = 6.66$; $gl = 1$; $P < 0.05$) was detected in the top layer. Nevertheless, the number of new shoots recorded did not differ among the strata ($H = 2.0411$; $P = 0.1531$).

In Montenegrina, the highest values of both parasitoid and of leafminer pupae numbers were registered in the East quadrant ($\chi^2 = 11.81$; $df = 3$; $P < 0.05$), ($\chi^2 = 10.36$; $df = 3$; $P < 0.05$). Nevertheless, in this cultivar the number of new shoots did not vary significantly among the quadrants ($H = 0.2571$ $P = 0.9679$). In Murcott, the largest number of parasitoids and of pupae occurred in the North quadrant ($\chi^2 = 19.29$; $df = 3$; $P < 0.05$), ($\chi^2 = 4.39$; $df = 3$; $P < 0.05$). The number of new shoots did not differ among the quadrants ($H = 0.5476$; $P = 0.9083$).

The differences found in the two orchards among quadrants, relative to the numbers of parasitoids and of pupae, may be explained by the plant arrangement in rows, and to the higher solar exposition of certain quadrants. In Montenegrina, rows were arranged in the north-south direction and, due to a larger distance between trees in different rows than to the distance between plants within a row, the east and west orientations probably receive more solar radiation. In the Murcott area, rows are oriented east-to-west and thus the north orientation is the one receiving more solar radiation.

The analysis of the horizontal distribution of the parasitism revealed aggregated patterns (Table 2). The data fit the Negative Binomial distribution in all tested occasions. This is reinforced by the high values of I obtained. The aggregated pattern of the citrus leaf miner distribution has been already registered in different cultivars (Vivas and Lopez, 1995; Peña and Schaeffer, 1997; Peña, 1998). In sweet orange orchards in São Paulo state, although Knapp et al. (1995) had considered the pest distribution as uniform, Paleari et al. (2001), when developing a sampling method to monitor *P. citrella* and its natu-

Table 1. Average number and standard deviation of new shoots in 0.1 m² of the plant canopy; average number and standard deviation of *Phyllocnistis citrella* pupae and parasitism rates recorded in strata and quadrants of the canopy of Montenegrina trees, from July/2002 to June/2003, Montenegro, RS.

	Layers							
	Top				Bottom			
	Mo		Mu		Mo		Mu	
New shoots 0.1 m ²	119.66 ± 237.89		69.5 ± 143.48		92.4 ± 198.51		47.3 ± 119.80	
Pupae	52.6 ± 89.56		62.9 ± 49.83		47.4 ± 93.06		37.1 ± 44.84	
Parasitism (%)	53.9		63.5		45.8		36.6	
	Quadrants							
	North		South		East		West	
	Mo	Mu	Mo	Mu	Mo	Mu	Mo	Mu
New shoots 0.1 m ²	14.9 ± 31.14	7.7 ± 24.83	16.0 ± 33.28	7.0 ± 23.86	16.0 ± 33.42	6.5 ± 2.25	16.1 ± 3.78	7.6 ± 32.38
Pupae	21.9 ± 38.10	32.8 ± 27.70	18.7 ± 35.95	29.3 ± 26.31	38.3 ± 67.96	19.9 ± 24.19	23.1 ± 45.70	22.9 ± 17.96
Parasitism (%)	13.2	41.4	25.8	13.1	37.4	28.3	23.6	17.2

ral enemies, verified that the number of damaged new shoots varied among the quadrants; in another words, it was found not homogeneous. Thus, the parasitism distribution will occur associated to the host, even though different patterns may arise.

The processes observed in the field that generate aggregated patterns of parasitism, besides the host presence, may be diverse: the continuous exploitation by parasitoids of previously colonized regions; the greater concentration of kairomones resulting either in a shorter searching time for new hosts (Waage, 1983) or increased residence time, which leads to higher parasitism rates; as well as the limited flight ability of these eulophids (Godfray, 1994).

The data for all sampling occasions analyzed fit to the Negative Binomial distribution; nevertheless, k values tended to be lower at smaller densities, indicating a higher aggregation in these periods (Figure 1). As the host and parasitoid populations increase, an increase in k

values occurs, indicating a lower aggregation of parasitism in these occasions.

The scarcity of foliar resources to the miner also leads to an increase in aggregation, because the parasitism becomes restricted to young shoots where larvae and host pupae occur.

Two peaks in parasitoid populations were registered for both orchards, although not simultaneously (Figure 1). In Montenegrina, the total number of parasitoids registered was larger probably due to the higher vegetative growth of this cultivar (Koller, 1994) and also to the larger leaf miner total population registered in this same area in a study carried out simultaneously to the present one (Cristiane Ramos de Jesus, personal communication), which may be related to the preference/suitability of this variety as a host.

In Montenegrina, a small populational peak was registered in February 17th and a larger one in March 17th, when 368 parasitoid individuals were collected; in

Table 2. Number of sampling units containing parasitized leafminers (N), average parasitism rate (%), I Index, and k values of the Negative Binomial, for sampling occasions when parasitoids were recorded, in Montenegrina and Murcott orchards, from July/2002 to June/2003, Montenegro, RS.

Sampling occasions	Montenegrina				Murcott			
	N	%	I	k	N	%	I	k
09/12/02	13	12.92	25	0.016	0	0	0	0
20/12/02	23	7.53	33.86	0.063	6	4.16	25	0.038
06/01/03	23	5.95	14.21	0.155	0	0	0	0
20/01/03	12	21.52	48.36	0.369	14	27.85	61.09	0.39
03/02/03	6	44.44	56.66	0.628	12	59.02	27.26	2.16
17/02/03	17	24.79	25.26	0.96	19	28.94	37.97	0.728
28/02/03	14	28.66	57.67	0.433	8	37.5	71.43	0.405
17/03/03	22	76.92	6.18	4.289	18	58.98	18	2.932
31/03/03	5	40	75	0.337	9	97.14	24.85	3.052

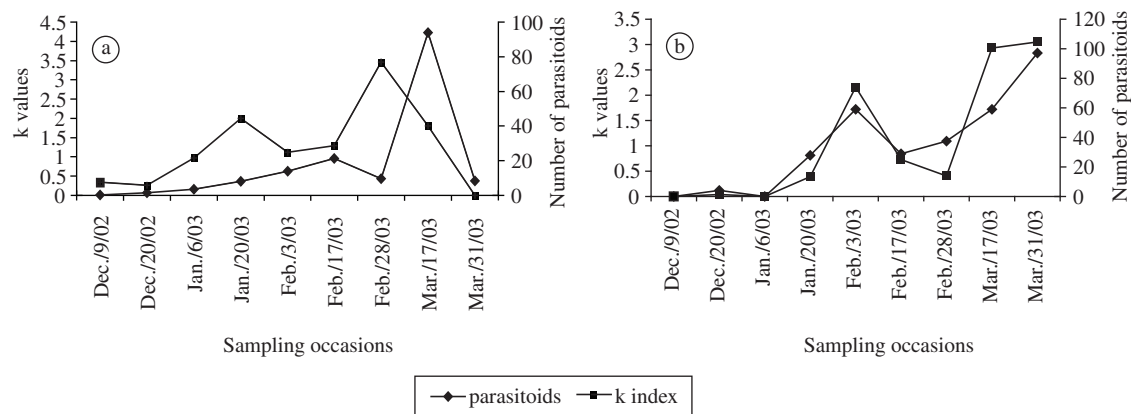


Figure 1. Number of parasitoids and k values of the negative binomial calculated per sampling occasion in a) Montenegrina and b) Murcott orchards, from July/2002 to June/2003, Montenegro, RS.

these occasions the largest k values were also obtained (Table 2). In Murcott, the first population peak occurred in February 3rd, concomitantly to those observed in Montenegrina. In these occasions, the calculated k , from the Negative Binomial, had larger values, also indicating in this orchard a weaker aggregation. It is important to emphasize that in spite of the continuing sampling until June/2003, after March 31 no pupae of the leaf miner were found in either orchard.

The observed aggregate pattern of parasitoids of *P. citrella* as well as the influence of this pattern in the dynamics of the system highlights the importance of this information to the development of a sequential sampling plan. Furthermore, it could provide elements to the elaboration of mathematical models to describe the interactions that occurs in the citrus system under organic management.

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