

Spatial distribution of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) larvae in three scales

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Received June 19, 2006 – Accepted September 18, 2006 – Distributed February 29, 2008

(With 1 figure)

Abstract

The spatial distribution of larvae of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), the citrus leaf miner, in leaves, shoots, and tree crowns of Montenegrina tangerine (*Citrus deliciosa* Tenore) and Murcott tangor (*Citrus sinensis* L. Osbeck x *Citrus reticulata* Blanco) was determined. Fortnightly samplings from July/2001 to June/2003, in orchards located in Montenegro (29° 68' S and 51° 46' W), RS, Brazil, were carried out. At each sampling occasion eighth shoots randomly selected were collected. The spatial distribution pattern of *P. citrella* larvae between tree crowns and in the shoots was aggregated in most sampling occasions in both citrus species. Nevertheless, on the leaves, this pattern followed a random distribution, as indicated by the indices I , I_g and k parameter.

Keywords: citrus leafminer, spatial distribution, 'Montenegrina', 'Murcott'.

Distribuição espacial das larvas de *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) em três escalas

Resumo

Com o objetivo de determinar a distribuição espacial das larvas de *Phyllocnistis citrella* Stainton, (Lepidoptera: Gracillariidae), o minador-dos-citros, nas folhas, nos brotos e nas copas das plantas de tangerineira 'Montenegrina' (*Citrus deliciosa* Tenore) e de tangor 'Murcott' (*Citrus sinensis* L. Osbeck x *Citrus reticulata* Blanco), foram realizadas amostragens quinzenais de julho de 2001 a junho de 2003 em pomares localizados em Montenegro (29° 68' S e 51° 46' W), RS, Brasil. Em cada ocasião de amostragem foram coletados aleatoriamente oito brotos. Através da análise, pelos índices de dispersão I e I_g , o padrão de distribuição espacial das larvas de *P. citrella* entre as plantas e nos brotos de 'Montenegrina' e 'Murcott' foi agregado na maioria das ocasiões de amostragem. O padrão de distribuição espacial das larvas do minador-dos-citros nas folhas mostrou-se diferente do registrado em copas das plantas e brotos, os índices I , I_g e o parâmetro k indicaram distribuição aleatória.

Palavras-chave: minador-dos-citros, distribuição espacial, 'Montenegrina', 'Murcott'.

1. Introduction

The spatial distribution of a population, that is, the position that individuals occupy in environment ones relative to the others, at a given time, is a central issue in population dynamics studies. A population may show three basic patterns of distribution: random, uniform (regular) and aggregated (clustered). The pattern may change in time, among developmental stages of a same species or even occur superposed in a given population (Southwood, 1978; Elliott, 1983). The identification of the distribution pattern is essential to the development of sampling programs, especially those involving species considered pests (Davis, 1993).

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), has been considered one

of the most important pests of the citrus culture, occurring practically worldwide (Heppner, 1993; Amalin et al., 2002). The eggs are placed in new leaves (shoots) and the larvae feed under the leaf epidermis making galleries or mines. The epidermis detaching may cause a reduction of the photosynthetic area and leaf curling causing losses mainly in nurseries and young orchards (Schaffer et al., 1997; Volpe et al., 1998). Indirectly, the chief damage caused by *P. citrella* facilitates the entrance of the bacteria *Xanthomonas axonopodis* pv. citri (Hasse) Vauterin et al., the causal agent of the citric canker (Chagas et al., 2001; Amaral, 2003). This disease is responsible for serious economic losses because fruit spoilage depreciates its commercial value. Besides this, the control of this

disease is difficult, the eradication of the affected plants being the main measure (Amaral, 2003).

The spatial distribution of the immature stages of *P. citrella* have been studied in several citrus cultivars and in different spatial scales, mainly in the USA (Knapp, 1995), in India (Rao et al., 2002) and in Brazil (Dantas, 2002).

This study aimed to determinate the spatial distribution of *P. citrella* larvae on leaves, on shoots and on plants of 'Montenegrina' tangerine and 'Murcott' tangor, in orchards under organic management, in Montenegro, RS.

2. Material and Methods

The samplings were carried out in an orchard of 'Montenegrina' tangerine trees and in other of the hybrid 'Murcott' (*Citrus sinensis* L. Osbeck x *Citrus reticulata* Blanco), located at Montenegro (29° 68' S and 51° 46' W), RS, Brazil.

Each orchard, with an area of 0.6 ha, comprehends 315 plants, 12 years old, spaced 3 m between plants and 6 m between rows. The orchards are maintained under organic management since their implantation.

The orchards were subdivided in three subareas approximately 0.2 ha each with a similar number of plants to ensure that all the area be represented in the samplings. Fortnightly samplings were carried out from July 2001 to June 2003, and in each occasion 12 plants were randomly selected, being four in each subarea. At each selected plant, if present, eight shoots were collected.

The collected shoots were individually packed in labeled plastic bags, stored in thermal box and transported to the laboratory.

At the laboratory, with the aid of a stereomicroscope Leica MZ 12, each leaf was examined and the number of larvae per leaf registered.

The spatial distribution of *P. citrella* larvae was analyzed in the leaves, shoots and tree crowns, only on those sampling occasions when the number of individuals (n) was higher than eight. It was calculated the ratio between the variance and the sample average (I), the Morisita Index (I_δ) and the k parameter of the Negative Binomial, using the software Ecological Methodology (Krebs, 2000). Values of I and I_δ equal to unity indicates a random distribution, lesser than one a uniform distribution and significantly higher than one an aggregated distribution. Otherwise, values of 0 < k ≤ 8 indicate a random distribution (Poole, 1974). Significance was tested by the χ-square (α = 0.05).

The dispersion index of Iwao was calculated by the ratio of the accumulated average (x' = x̄ + [(s² / x̄) - 1]) and the average (x̄), resulting in the linear equation: x' = β x̄ + α, where β gives the aggregation coefficient (Southwood, 1978; Elliott, 1983).

3. Results and Discussion

Along the two study years 53 sampling occasions occurred. In 'Montenegrina' orchard, 1,487 shoots were collected totaling 7,313 examined leaves and 767 larvae of *P. citrella* registered. In 'Murcott' orchard, were collected 1,045 shoots, 4, 536 leaves and 655 larvae.

The greatest number of larvae was registered in summer and in autumn either in 'Montenegrina' as in 'Murcott' (Table 1). In both orchards, along the study there was a marked decrease of the citrus leaf miner

Table 1. Number of *Phyllocnistis citrella* larvae (n), I index (I), Morisita (I_δ) and k parameter of the Negative Binomial distribution (k), used to determine the spatial distribution of larvae in tree crowns of Montenegrina (*Citrus deliciosa* Tenore) and of the hybrid Murcott (*Citrus sinensis* x *Citrus reticulata*), chi-square test (χ²) to test the goodness of fit of the observed distributions and probability (P), from July 2001 to June 2003, Montenegro (29° 68' S and 51° 46' W), RS.

	'Montenegrina'						'Murcott'					
	n	I	I _δ	χ²	P	k	n	I	I _δ	χ²	P	k
15/10/2001	-	-	-	-	-	-	9	0.95	0.90	7.6	0.52	1.89
12/11/2001	11	3.31	2.61	23.1	0	0.73	29	6.50	1.78	26	0	1.81
26/11/2001	27	8.83	4.01	88.2	0	0.35	11	2.61	2.29	20.9	0	0.92
10/12/2001	-	-	-	-	-	-	11	4.23	2.61	21.1	0	0.74
26/12/2001	45	6.08	3.88	152	0	0.35	-	-	-	-	-	-
07/01/2002	19	5.86	2.35	29.3	0	0.88	26	5.52	2.91	60.7	0	0.54
21/01/2002	-	-	-	-	-	-	29	2.55	1.66	30.6	0	1.76
04/02/2002	60	4.59	1.54	41.3	0	2.29	-	-	-	-	-	-
04/03/2002	-	-	-	-	-	-	28	3.91	1.64	23.5	0	2.09
18/03/2002	-	-	-	-	-	-	17	2.11	1.69	21.1	0.01	1.75
01/04/2002	97	5.55	1.53	66.3	0	2.06	78	4.12	1.44	45.3	0	2.82
15/04/2002	31	4.12	2.66	66.0	0	0.64	69	9.23	2.81	138.5	0	0.57
29/04/2002	12	1.51	1.69	22.6	0.09	1.72	29	3.59	2.20	46.7	0	0.91
13/05/2002	9	1.80	1.50	9	0.10	3.6	8	2.75	2.50	16.5	0.01	0.83
25/11/2002	16	2	1.47	14	0.05	3.22	-	-	-	-	-	-
09/12/2002	44	2.14	1.31	25.7	0.01	4.30	-	-	-	-	-	-
20/12/2002	44	3.18	1.72	44.6	0	1.58	-	-	-	-	-	-
06/01/2003	191	5.51	1.57	132	0	1.89	83	4.15	1.26	29.0	0	7.83
21/01/2003	118	12.10	1.66	84.4	0	190	66	5.76	1.73	57.6	0	1.58
03/02/2003	67	3.66	1.44	40.2	0	2.85	67	3.50	1.60	56.0	0	1.85
17/02/2003	16	2.06	1.38	18.6	0.02	3.75	-	-	-	-	-	-
17/03/2003	-	-	-	-	-	-	15	1.80	2.09	12	0.21	1.02
28/04/2003	15	1.40	1.14	7	0.21	-18	20	1.33	1.15	34.3	0.01	22.2

population in the winter ($n < 8$ in every sampling occasion).

The analysis through the dispersion indexes I and I_8 and the spatial distribution pattern of *P. citrella* larvae in plants of 'Montenegrina' cultivar, revealed an aggregated distribution in 100% of the sampling occasions and in only three occasions was not detected significance (Table 1).

In plants of the tanger 'Murcott' in 17 occasions the distribution pattern was aggregated and in two it was random. The k parameter also indicated the aggregated distribution as the most frequent pattern, in just one occasion a random distribution was registered (Table 1). Dantas (2002) obtained a similar result, which registered an aggregated distribution pattern of the *P. citrella* larvae in orchards of 'Pera-Rio' orange in São Paulo state. The aggregation observed in both studies may be resultant of an intrinsic behavior of the individuals and/or a response to the food and habitat resources distribution. Concentration of a suitable resource in some areas has been considered as the prevailing cause of aggregation of most organisms (Ricklefs and Miller, 2000). According to Knapp (1995), the citrus leaf miner distribution among plants seems to be related to the availability of shootings in the plant, which may differ among them.

In 'Montenegrina' shootings, it was observed an aggregated distribution pattern through the use of index I in 94.7% of the sampling occasions (Table 2). Otherwise, the Morisita index rendered an aggregated distribution in 84.2%, random in 10.5% and regular in 5.3% of the sam-

pling occasions. The k parameter indicated aggregated distribution in 89.5% and random in 5.9%.

In the 'Murcott' orchard, indexes I and I_8 revealed an aggregated distribution in 88.2% and regular in 11.8% of the sampling occasions. Considering the k parameter, 94.1% and 5.9% of the occasions showed respectively an aggregated and a regular distribution. These results indicate that besides the preference for some plants in the orchard there is also a selection of most suitable areas to the offspring in the host plant. Dantas (2002) also registered an aggregated distribution pattern of larvae in shoots. Otherwise Knapp (1995) in a study realized in southern Florida in lime orchards, found a regular distribution pattern in shoots.

The spatial distribution pattern of the citrus leaf miner larvae, in leaves, was different from those found in plants and shoots. In 'Montenegrina', the indexes I and I_8 and the k parameter indicated a random distribution in 77.7% and aggregated in 22.2% of the sampling occasions (Table 3). In 'Murcott', it was registered a random distribution in 64.7% and aggregated in 35.3%. The k parameter evidenced that in 52.9% of the sampling occasions the distribution was random and in 47% aggregated. This pattern observed in leaves reflects one of the strategies showed by miner insects to maintain the population, considering that competition by interference in leaves with numerous mines represents one of the main mortality factors. Aggregation could increase the occurrence of intraspecific interference (Auerbach et al., 1995). Rao et al. (2002), in a study carried out in

Table 2. Number of *Phyllocnistis citrella* larvae (n), I index (I), Morisita (I_8) and k parameter of the Negative Binomial distribution (k), used to determine the spatial distribution of larvae in shoots of Montenegrina trees (*Citrus deliciosa*) and of the hybrid Murcott (*Citrus sinensis* X *Citrus reticulata*), chi-square test (χ^2) to test the goodness of fit of the observed distributions and probability (P), from July 2001 to June 2003, Montenegro (29° 68' S and 51° 46' W), RS.

	'Montenegrina'						'Murcott'					
	n	I	I_8	χ^2	P	k	n	I	I_8	χ^2	P	k
15/10/2001	-	-	-	-	-	-	9	1.48	1.36	8.8	0.17	5.62
12/11/2001	11	2.6	2.60	26	0	0.72	29	4.86	2.24	43.7	0	0.90
26/11/2001	27	4.9	2.85	49	0	0.59	11	2.86	3.05	31.5	0	0.55
10/12/2001	-	-	-	-	-	-	11	2.3	1.78	13.8	0.03	1.73
26/12/2001	45	1.15	1.18	13.8	0.31	11.09	-	-	-	-	-	-
07/01/2002	19	3.74	2.39	93.5	0	0.75	26	3.61	2.78	54.2	0	0.60
21/01/2002	25	18.66	4.68	93.3	0	0.28	29	1.81	1.72	43.4	0	1.52
04/02/2002	60	18.66	1.47	47.4	0	2.39	-	-	-	-	-	-
04/03/2002	-	-	-	-	-	-	28	1.76	1.34	21.2	0.04	3.99
18/03/2002	-	-	-	-	-	-	17	2.34	2.00	28.1	0.005	1.14
01/04/2002	97	2.37	1.91	119	0	1.13	78	5.66	2.42	101.9	0	1.00
15/04/2002	31	3.72	3.68	96.1	0	0.39	69	4.16	2.52	95.6	0	0.73
29/04/2002	12	4.58	1.68	24.8	0.07	1.73	29	3.22	2.85	61.3	0	0.70
13/05/2002	9	1.55	1.77	14	0.05	1.71	8	2.85	1.14	20	0	0.65
25/11/2002	16	2	1.66	19	0.02	1.89	-	-	-	-	-	-
09/12/2002	44	2.11	1.56	40.7	0	2.03	-	-	-	-	-	-
20/12/2002	44	2.54	1.55	54.4	0	1.96	-	-	-	-	-	-
06/01/2003	191	2.17	1.40	137	0	2.57	83	1.14	1.55	8	0.33	5.88
21/01/2003	118	8.68	2.39	199.7	0	0.74	66	5.06	1.71	55.7	0	2.14
03/02/2003	67	3.05	1.65	61.3	0	1.67	67	5.20	1.38	57.2	0	1.62
17/02/2003	16	2.34	1.77	37.5	0	1.40	-	-	-	-	-	-
17/03/2003	-	-	-	-	-	-	15	2.00	1.16	54.0	0	2.90
28/04/2003	15	1.46	1.20	8.8	0.18	31.25	20	1.16	1.35	50.1	0.2	7

Table 3. Number of *Phyllocnistis citrella* larvae (n), I index (I), Morisita (I_g) and k parameter of the Negative Binomial distribution (k), used to determine the spatial distribution of larvae in leaves of Montenegrina trees (*Citrus deliciosa*) and of the hybrid Murcott (*Citrus sinensis* X *Citrus reticulata*), chi-square test (χ^2) to test the goodness of fit of the observed distributions and probability (P), from July 2001 to June 2003, Montenegro (29° 68' S and 51° 46' W), RS.

	'Montenegrina'						'Murcott'					
	n	I	I_g	χ^2	P	k	n	I	I_g	χ^2	P	k
15/10/2001	-	-	-	-	-	-	9	1.17	1.22	11.7	0.29	9.11
12/11/2001	11	1.57	2.23	44	0.02	0.9	29	1.84	1.57	35.1	0.01	1.97
26/11/2001	27	2.77	1.12	58.1	0	2.87	11	1.67	1.41	29.1	0.25	2.90
10/12/2001	-	-	-	-	-	-	11	0.95	0.92	15.2	0.50	-8.10
26/12/2001	45	0.77	0.41	23.9	0.81	-1.7	-	-	-	-	-	-
07/01/2002	19	1.10	1.12	75.2	0.25	9.05	26	0.84	0.82	21.0	0.68	-4.79
21/01/2002	-	-	-	-	-	-	29	0.96	0.91	65.6	0.55	10.16
04/02/2002	60	7.53	1.25	444	0	-1.8	-	-	-	-	-	-
04/03/2002	-	-	-	-	-	-	28	0.66	0.73	14.7	0.87	-3.28
18/03/2002	-	-	-	-	-	-	17	0.71	0.29	22.7	0.911	-1.45
01/04/2002	97	0.88	0.91	62.6	0.74	-10	78	1.35	1.21	66.2	0.05	5.11
15/04/2002	31	1.27	1.72	100.6	0.05	1.4	69	1.33	1.46	94.7	0.03	2.25
29/04/2002	12	0.92	0.69	41.6	0.61	-3.3	29	1.40	1.52	60.5	0.04	1.71
13/05/2002	9	0.89	0.8	15.2	0.58	-4.2	8	1.5	1.85	18	0.11	1.46
25/11/2002	16	0.67	0.55	14.2	0.85	-2.1	-	-	-	-	-	-
09/12/2002	44	0.66	0.64	31.2	0.96	-2.7	-	-	-	-	-	-
20/12/2002	44	0.46	0.20	30.9	0.99	-1.2	-	-	-	-	-	-
06/01/2003	191	0.94	0.91	250	0.75	-10	83	0.95	0.97	44.8	0.56	-21.00
21/01/2003	118	15.37	1.38	191	0	3.3	66	2.21	1.71	86.4	0.00	-1.46
03/02/2003	67	0.56	0.50	35.8	0.99	-1.9	67	0.77	0.75	59.7	0.92	-3.90
17/02/2003	16	0.54	0	30	0.99	-1.0	-	-	-	-	-	-
17/03/2003	-	-	-	-	-	-	15	0.52	0.33	18.3	0.99	-1.49
28/04/2003	15	0.55	0.61	3.6	0.87	-2.2	20	1.25	1.94	101.7	0.05	1.12

India, verified that in low densities *P. citrella* present an aggregated distribution in leaves, and at high densities it is random.

The β values and the determination coefficients obtained through Iwao regression in tree crowns ($\beta = 1.40$ and $\beta = 1.17$) and shoots ($\beta = 3.25$ and $\beta = 1.54$) of 'Montenegrina' (Figure 1a,c) and 'Murcott' (Figure 1b,d), respectively, indicated that larval distribution was aggregated in these levels along all the sampling period.

The aggregation coefficient of the larval distribution on the leaves of 'Montenegrina' trees (Figure 1e) was near one ($\beta = 0.09$) but not significant, nevertheless indicating a trend to a regular distribution. Otherwise, in 'Murcott' tree crowns, this coefficient denoted an aggregated distribution of *P. citrella* larvae in the leaves ($\beta = 1.31$) (Figure 1f).

The results obtained in the present study corroborate the findings of Hespeneide (1991), which emphasize that the spatial distribution of miners insects should be approached at different spatial scales, considering that in a given habitat the distribution is not homogeneous. The mine distribution is determined by the oviposition preference of the adults (Mopper et al., 1984). According to

Faeth (1990), aggregation in miner insects is widespread, because the immature stages are usually sedentary due to their feeding habit. Meanwhile, the formation of aggregates in individual leaves could result in an increase of the intraspecific competition or cannibalism (Auerbach and Simberloff, 1989).

The aggregated distribution of *P. citrella* larvae originates from the females' preference to oviposit only in young leaves of the shoots (Garijo and Garcia, 1994; Peña and Schaffer, 1997). Nevertheless, the distribution pattern in shoots was also aggregated, suggesting that even among young leaves, some are preferred. This female oviposition preference could be due to factors as leaf position in the tree crowns and/or to an asynchrony between flushings and the adults' presence in the orchard (Faeth, 1990).

Based upon the obtained results it was verified that the spatial distribution of the citrus leafminer was not homogeneous in the orchards of 'Montenegrina' and 'Murcott', mainly due to plant heterogeneity as a consequence of the organic management. These results indicate that for the monitoring and sampling of *P. citrella*, the spatial distribution pattern should be considered mainly in the tree crown and shoot scales.

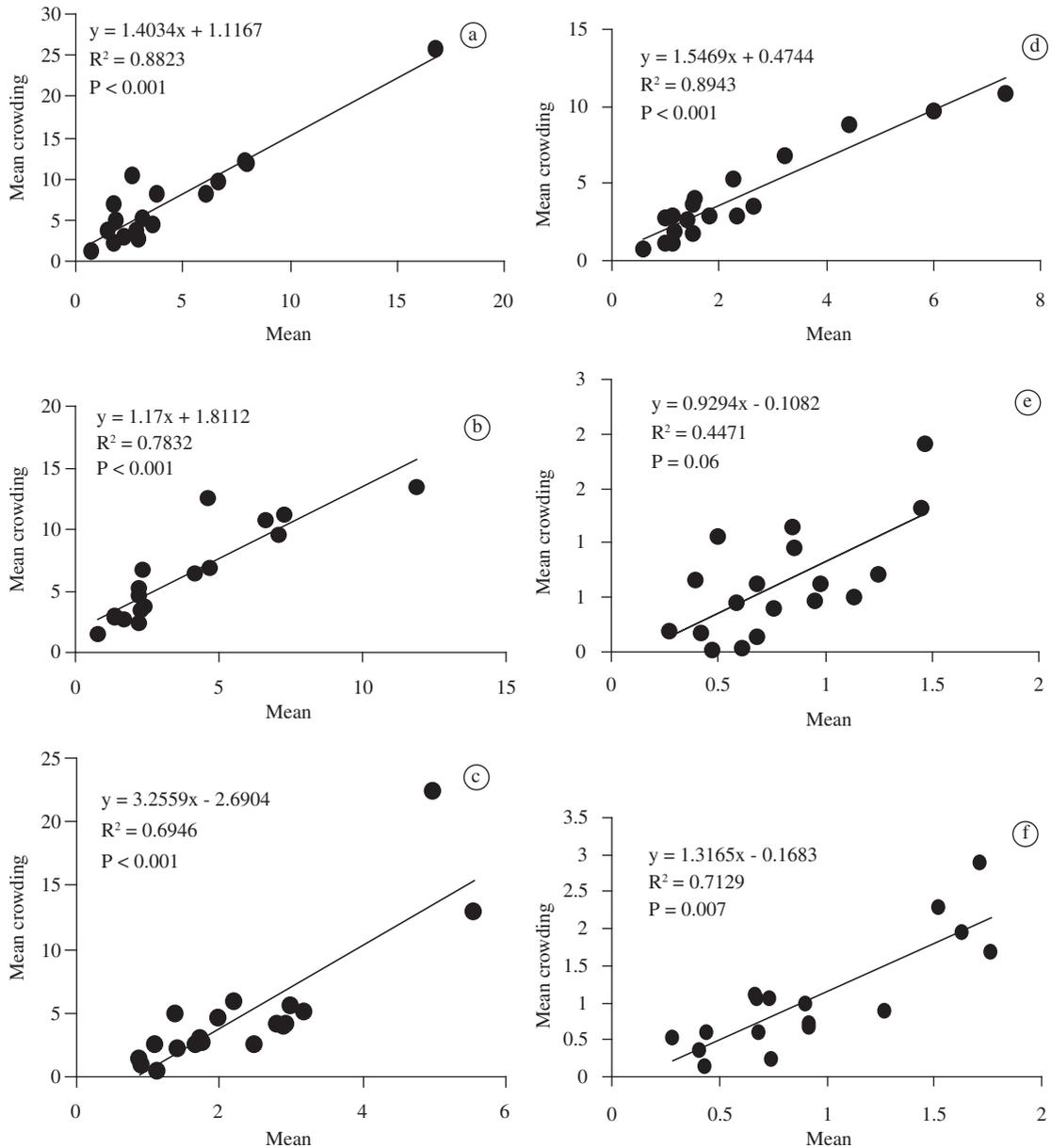


Figure 1. Iwao regression for *Phyllocnistis citrella* larvae in tree crowns (a,b), shoots (c,d) and leaves (e,f) of Montenegrina tangerine trees (*Citrus deliciosa*) (a,c,e) and Murcott tangor trees (*Citrus sinensis* x *Citrus reticulata*), (b,d,f), Montenegro (29° 68' S e 51° 46' W), RS.

Acknowledgments — The authors gratefully, acknowledge Programa RS-Rural for funding and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for fellowships conceded to the authors.

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