doi: 10.12741/ebrasilis.v14.e926
e-ISSN 1983-0572
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EntomoBrasilis
e-ISSN 1983-0572

General Entomology

Sampling methods and metereological factors on pests and beneficial organisms in strawberries

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EntomoBrasilis 14: e926 (2021)

Edited by:
William Costa Rodrigues

Article History:
Received: 09.viii.2020
Accepted: 19.iv.2021
Published: 31.v.2021

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Funding agencies:
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Fundação Araucária

Abstract. We characterize the population dynamics of pests and beneficial organisms in strawberries to assess the impact of two meteorological factors (temperature and precipitation) and define the most appropriate method for monitoring insects in strawberries crop. Population dynamics were monitored weekly using the plant beat method and Möericke traps in Albion strawberries. Measurements were taken in organic strawberry farms over two growing seasons, from March 26, 2010 to August 23, 2010 (first season - 2009/2010) and from October 4, 2010 to March 21, 2011 (second season - 2009/2010) in the municipality of Pinhais, Paraná state, southern Brazil (25º25'S; 49º08'W). We collected a total of 7,971 pests and 1,987 beneficial organisms. The most abundant pests were *Chaetosiphon fragaefolii* (Cockerell) (Hemiptera: Aphididae) in the first growing season and *Tetranychus urticae* (Koch) (Acari: Tetranychidae) in the second. Beneficial organisms from Acari, Araneae, and Hymenoptera parasitoids predominated in both seasons. Temperature and precipitation were the the primary climatic factor associated with the population variation of aphids. Populations of *C. fragaefolii* grew when the temperature was between 16°C and 25°C. We found that plant beat was the fastest and most suitable method for strawberry pests monitoring since it captured insects that were effectively colonizing the plants. This study contributes to a better understanding of the entomofauna associated with cultivation of organic strawberry.

Keywords: Climate factors; entomofauna; monitoring method; organic strawberry.

Strawberry is the most widely consumed small fruit in the world, as well as having the great area under cultivation. Conditions in Brazil are suitable for the plant, whose cultivation is concentrated in the states of Minas Gerais (54.52%), Paraná (13.50%), Rio Grande do Sul (11.25%), and São Paulo (7.98%), while a smaller percentage (12.75%) is grown in other states (Madail 2016). Interest in the crop is driven by its high profitability, the fact that it is a well-known fruit to the consumer, and the variety of options for sale and processing (Fachinello *et al.* 2011; Madail 2016).

As the supply of strawberries on the Brazilian market has grown, consumers have become more demanding with respect to quality surrounding the fruit (Madail 2016). However, several factors can affect production, including the incidence of pests. Plant health management is especially laborious, with several pests reported in strawberry crops (Zawadneak *et al.* 2014). However, little is known about the dynamics between pest populations and beneficial organisms in Brazilian strawberry cultivation. Studies are therefore needed to identify the key pests and their natural enemies in strawberry producing fields and to assess their population dynamics to plan integrated pest management programs. In strawberry production systems, interactions between pests and natural enemies can promote natural reductions in pest populations (Kovanci *et al.* 2007; Zanuncio-Junior 2018; Canassa *et al.* 2019). In addition to the data on species, studies should also consider the impact of abiotic factors like climatic variables and the cropping system itself on the dynamics of pest populations (Castilho *et al.* 2015).

Arthropod sampling protocols are essential for basic studies in crops and the development of integrated pest management programs. Monitoring methods should be selected based on their applicability and the reproducibility of results (Degrande *et al.* 2003), including their ability to provide fast and accurate estimations of arthropod populations, which can aid in timely decision-making concerning pest control methods. Furthermore, there is currently no consolidated monitoring method to adequately assess arthropod diversity in strawberries (Bernardi *et al.* 2013).

This study characterizes the diversity of pests and beneficial organisms in strawberries, aiming to evaluate the impact of two meteorological factors (temperature and precipitation) and to identify the most appropriate method for monitoring insects in this crop.

MATERIALS AND METHODS

Plant material and experimental design. The study was conducted in an area of organic strawberry (*Fragaria* x *ananassa*) cultivation in Pinhais, Paraná state, Brazil (25º25' S, 49º08' W; altitude ~ 930 m). The climate of the region is humid subtropical and oceanic, without a dry season, and with temperate summer [Cfb], according to the Köppen's classification (Alvares *et al.* 2014).

The plants belonged to the Albion cultivar and were grown in a protected environment using a low tunnel structure (Figure 1). Sampling was carried out in two strawberry growing seasons: during the 2010-2011 growing season between March 26, 2010 to August 23, 2010, and between October 4, 2010 to March 21, 2011. All recommended agricultural practices for organic cultivation were followed, with no pesticide applications throughout the study period.

Figure 1. Experimental area of organic strawberry cultivation in the municipality of Pinhais, Paraná state, southern Brazil. Seasons (2009/2010; 2010/2011).

The experimental design was a randomized block, with two sampling methods (Möericke traps and plant beat) (Amaro & Baggiolini 1982) in six replicates. We demarcated 24 plots with 32 plants each during each growing season. The experimental plots were 5.6 m in length and 1.2 m in width, with 0.3 m spacing between plants.

Monitoring methods. Arthropod population dynamics were evaluated once per week using two monitoring methods, the Möericke modified trap and plant beat. The modified Möericke traps consisted of 2 L PET bottles longitudinally cut in half and painted yellow (Figure 2). The traps were arranged in the mulch at the center of each plot and filled with a water solution and neutral detergent (1:200). After one week, the trap contents were sieved (0.2 mm mesh), and the collected arthropods were transferred to a plastic container containing a 70% ethyl alcohol solution, when the trap solution was replaced. This procedure was followed throughout the experimental period.

Figure 2. Möericke modified trap in center of strawberry plot in the municipality of Pinhais, Paraná state, southern Brazil. Seasons (2009/2010; 2010/2011).

The plant beat method was performed by plant beating the aerial part of the plant three times and repeating on a total of four randomly chosen plants. Specimens were collected in trays (0.25 m x 0.30 m x 0.50 m) that were positioned beneath each plant. The tray bottom was lined with white ethylene-vinyl acetate (EVA), which was moistened with water solution and detergent at the time of sampling to keep insects from escaping. The specimens were transferred to identified plastic containers containing a 70% ethyl alcohol solution.

Collection and identification of pests and natural enemies. The identification and quantification of the collected material were carried out in the laboratory. The samples were separated into morphospecies using a Zeiss® stereoscopic microscope. They were subsequently stored in plastic containers containing a 70% ethyl alcohol solution. Samples were then identified using identification keys (Vockeroth & Thompson 1987; Booth *et al.* 1990; Burks 2003) and material from the Entomology Laboratory Prof. Angelo Moreira da Costa Lima reference collection at the Universidade Federal do Paraná (UFPR), Curitiba, Paraná, Brazil, or confirmed by specialists.

Agroclimatic conditions. Meteorological data including average temperature (°C) and precipitation (mm) were obtained from the Simepar (Sistema Meteorológico do Paraná) Technological Institute at the Meteorological Station in Pinhais.

Statistical analysis. The correlation between climatic factors and pest population was determined using Kendall correlation analysis considering weekly mean precipitation, temperature, and the number of pests collected during the two strawberry seasons. The level of statistical significance was set at 5% (Siegel 1969; Hollander *et al.* 2013). The Mann-Whitney test was used to compare the two sampling methods at a 5% significance level (Siegel 1969; Hollander *et al.* 2013). Statistical analysis was performed using the program R Development Core Team (2014).

RESULTS

Monitoring methods. We observed that Möericke traps were the most appropriate over both growing seasons to capture *Aphis gossypii* Glover (Hemiptera: Aphididae) ($p<0.001$) and *Aphis forbesi* Weed (Hemiptera: Aphididae) ($p<0.001$) (Figure 3). The same method was also efficient in capturing sweet ants of the genera *Camponotus* and *Solenopsis*, as well as dipterans of the family (Figure 3). Meanwhile, the plant beating method was effective at capturing the strawberry aphid *Chaetosiphon fragaefolii* (Cockerell) ($p<0.001$) (Figure 3).

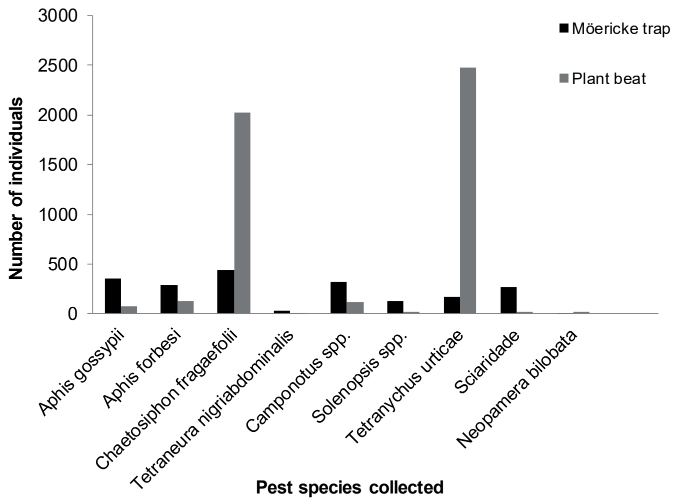

Figure 3. The number of pest individuals per species collected using Möericke trap and plant beat method, during two seasons (2009/2010; 2010/2011) of organic strawberry cultivation in the municipality of Pinhais, Paraná State, Southern Brazil.

Pests and natural enemies collected and identified. In total, 7,971 pest organisms and 1,987 natural pest enemies were collected over both strawberry seasons (Table 1). In the first season, 3,715 pests and 1,051 beneficial organisms associated with strawberries were collected. The most common pests found were from the order Hemiptera, especially *C. fragaefolii* (42.91%) and *A. gossypii* (11.33%). These were followed by representatives of Hymenoptera, being *Camponotus* sp. the dominant group of this order

Table 1. Total number and percentage of arthropods and mollusks collected during two seasons (S1= 2009/2010; S2 =2010/2011) in organic strawberry cultivation.

Phyllum	Class	Order	Family	Genus/Species	S 1	(%)	S 2	(%)	S1 + S2
	Arachnida	Acari	Tetranychidae	*Tetranychus urticae*	151	4.06	2507	58.91	
Mollusca	Gastropoda	Stylommatophora	Bradybaenidae	*Bradybaena similaris*	10	0.27	10	0.23	
		Eupulmonata	Agriolimacidae	*Deroceras leave*	3	0.08	2	0.05	
Arthropoda	Insecta	Coleoptera	Chrysomelidae	n.i.	37	1.0	114	2.68	
			Nitidulidae	n.i.	342	9.21	239	5.62	
		Diptera	Agromyzidae	*Liriomyza sativae*	24	0.65	1	0.02	
			Sciaridae	n.i.	242	6.51	46	1.08	
		Hemiptera	Aphididae	*Aphis forbesi*	320	8.61	103	2.42	
				Aphis gossypii	421	11.33	5	0.12	
				Chaetosiphon fragaefolii	1594	42.91	973	22.86	
				Capitophorus elaeagani	0	0	1	0.02	
				Myzus persicae	0	0	3	0.07	
				Therioaphis sp.	0	0	4	0.09	
				Urulecon sp.	0	0	1	0.02	
			Pemphigidae	*Tetraneura* sp.	33	0.89	2	0.05	
		Hemiptera	Rhyparochromidae	*Neopamera bilobata*	0	0	73	1.72	
		Hymenoptera	Formicidae	*Acromyrmex* sp.	1	0.03	28	0.66	
				Camponotus sp.	383	10.31	76	1.79	
				Solenopsis sp.	138	3.71	21	0.49	
		Thysanoptera	Thripidae	*Frankliniella occidentalis*	16	0.43	47	1.1	
		Total number of pests			**3715**		**4256**		**7971**
	Arachnida	Acari	Phytoseiidae	n.i.	332	31.59	160	17.09	
		Araneae	n.i.	n.i.	385	36.63	337	36	
	Insecta	Coleoptera	Coccinelidae	*Colleomegilla* sp.	0	0	14	1.5	
				Cycloneda pulchela	6	0.57	1	0.11	
				Eriopsis conexa	2	0.19	31	3.31	
				Harmonia axyridis	1	0.1	4	0.43	
				Hippodamia sp.	3	0,29	1	0.11	
				Psyllobora gratiosa	0	0	1	0.11	
				n.i.	5	0.48	1	0.11	
			Staphylinidae	n.i.	37	3.52	66	7.05	
		Diptera	Sirphidae	n.i.	2	0.19	1	0.11	
		Hemiptera	Ligaedae	*Geocoris* sp.	2	0.19	4	0.43	
		Hemiptera	Reduviidae	n.i.	9	0.86	16	1.71	
		Hymenoptera	n.i.	n.i.	235	22.36	284	30.34	
			Braconidae	n.i.	32	3.04	11	1.18	
		Neuroptera	Chrysopidae	n.i.	0	0	4	0.43	
		Total number of beneficial organisms			**1051**		**936**		**1987**

*Total number and percentage of arthropods and mollusks collected during two seasons (S1+S2)

(10.31%). The most abundant beneficial organisms were represented by species of the families Phytoseiidae (31.59%) and Araneae (36.63%) (Table 1).

In the second season, 4,256 pests and 936 beneficial organisms were collected (Table 1). In comparison to the first season, we observed a decrease in the populations of the aphids *C. fragaefolii* (22.86%) and *A. gossypii* (0.12%) (Table 1). At the same time, there was an increase (58.91%) in the population of the two-spotted spider mite *Tetranychus urticae* (Koch) (Trombidiformes: Tetranychidae) (Table 1). The main beneficial organisms from the orders Acari and Araneae decreased in number in the second season (36%), while those of Hymenoptera increased (31.52%) (Table 1).

Correlation between pest frequency and agroclimatic conditions. Mean weekly temperature and precipitation significantly affected aphid population dynamics. We observed an inversely proportional and significant correlation between the occurrence of *C. fragaefolii* and the mean weekly temperature ($p<0.001$; $\tau= -0.14$; Table 2). Similarly, in the first season, there was an inversely proportional correlation between the occurrence of *Aphis* spp. and mean weekly precipitation ($p= 0.03$; $\tau= -0.08$; Table 2). A negative

Table 2. Correlation between climatic factors and occurrence of insect pests during two seasons (2009/2010; 2010/2011) of organic strawberry cultivation. Acronym: NS, non-significant. Kendall's τ coefficient and p-value also shown.

Climatic factors	Species	Crop 1 (p-value)	Crop 1 (τ)	Crop 2 (p-value)	Crop 2 (τ)
Temperature	Sciaridae	NS	-	< 0.001	-0.18
	Formicidade *(Camponotus)*	< 0.001	-0.1	NS	-
	Lygaidae *(Neopamera bilobata)*	-	-	< 0.001	0.17
	(Aphididae) *Aphis gossypii*	< 0.001	0.09	NS	-
	(Aphididae) *Aphis forbesi*	0.003	-0.13	NS	-
	(Aphididae) *Chaetosiphon fragaefolii*	< 0.001	-0.14	< 0.001	-0.15
	(Acari) *Tetranychus urticae*	NS	-	NS	-
Precipitation	Sciaridae	NS	-	0.03	-0.08
	Formicidade *(Camponotus)*	NS	-	NS	-
	Lygaidae *(Neopamera bilobata)*	-	-	NS	-
	(Aphididae) *Aphis gossypii*	< 0.001	-0.08	NS	-
	(Aphididae) *Aphis forbesi*	0.03	-0.08	NS	-
	(Aphididae) *Chaetosiphon fragaefolii*	< 0.001	-0.62	< 0.001	-0.15
	(Acari) *Tetranychus urticae*	NS	-	NS	-

correlation between *C. fragaefolii* and precipitation was also observed, especially in the first season (p<0.001; τ= -0.62; Table 2).

The population fluctuations of *Camponotus* ant species, in the first season, had a negative correlation with the mean weekly temperature (p <0.001; τ= -0.10; Table 2). In the second season, we also observed a negative correlation between the mean weekly precipitation and the population number of Sciaridae dipterans (p= 0.03; τ= -0.08; Table 2). Specimens of the phytophagous hemipteran *Neopamera bilobata* (Say) (Hemiptera: Rhyparochromidae) occurred only in the second season, especially in December and January. We observed a positive correlation between the occurrence of *N. bilobata* and the mean weekly temperature (p<0.001; τ= 0.17; Table 2), but no correlation with mean weekly precipitation. The population of *C. fragaefolii* increased in mild temperatures (between 16 ºC and 25 ºC) (Table 3).

Table 3. Mean of temperature and precipitation during two seasons (2009/2010; 2010/2011) of organic strawberry cultivation in the municipality of Pinhais, Paraná state, southern Brazil.

Month/Year	Temperature mean (ºC)	Precipitation (mm)
mar/10	20.0	216.2
apr/10	17.2	306.2
may/10	14.6	103.2
jun/10	13.5	99.0
jul/10	14.7	86.6
aug/10	13.5	40.0
sep/10	15.8	99.6
oct/10	15.3	162.8
nov/10	18.0	143.0
dec/10	19.2	383.4
jan/11	21.1	389.8
feb/11	21.0	310.2
mar/11	18.4	104.2

DISCUSSION

Plant beating was the most effective sampling method for the two main pest species, the aphid *C. fragaefolii* and the mite *T. urticae,* as it provided a more effective quantification of the occurrence of these taxa. Arthropod sampling for integrated pest management in strawberry cultivation can be carried out using Möericke's trap (Amaro & Baggiolini 1982) and the plant beat method (Van Driesche *et al.* 1999; González-Zamora & Garcia-Marí 2003; Strand 2008). The plant beat method captures insects that are effectively colonizing the plants, meaning that it is faster and more suitable for monitoring strawberry crops. On the other hand, the Möericke's trap method was able to sample other groups of insects. Möericke's traps are mainly used to capture winged insects, while the plant beat method is better suited to wingless insects (Amaro & Baggiolini 1982). Sampling tests using these two traps have shown their effectiveness in capturing all strawberry pests, which may lead to a more realistic estimate of pest populations in the field (Zawadneak *et al.* 2014). Therefore, both methods tested should be used together to obtain more efficient monitoring of the area, given the diversity of arthropods associated with strawberry cultivation.

In this study, we showed the predominance of the strawberry aphid *C. fragaefolii* and the two-spotted spider mite *T. urticae* in organic strawberries and identified them as key pests of strawberries during the study period. *Chaetosiphon fragaefolii* occurred in higher numbers in both seasons. Our results are in line with a previous study (Bernardi *et al.* 2013), which showed the predominance of the aphids *C. fragaefolii, A. forbesi,* and *A. gossypii* in strawberry crops from southern Brazil (Paraná and Rio Grande do Sul) during spring. The prevalence of aphids in strawberries can be attributed to their high rates of reproduction and the plant's phenological stage, as the pest is most prevalent in developing plants, which offer high availability of amino acids and nitrogen in their growing parts (Pathania *et al.* 2019; Zhao *et al.* 2015; Wang *et al.* 2015). The carpenter ant *Camponotus* sp., associated with aphids, was the third most abundant pest. This result can be explained by their protocooperation with aphids, once the carpenter ants form nests in the vicinity of the plant (Rakhshan & Ahmad 2015).

Tetranychus urticae was the only pest mite collected during the two seasons and was found to be a key pest in the second season, when it occurred in greater intensity. The highest abundance of *T. urticae* can be attributed to the lower occurrence of its potential phytoseiid mite predators (Castilho *et al.* 2015; Abdallah *et al.* 2019; Urbaneja-Bernat *et al.* 2019).

The beneficial organisms that predominated during the two seasons were the species of Araneae, Acari, and Hymenoptera; which have been considered critical agents of biological pest control (Michalko & Pekar 2015; Nomano *et al.* 2015; Wäckers *et al.* 2008).

In addition to the presence of beneficial organisms, climatic factors can also be associated with the population dynamics of pests in strawberry crop (Pathania *et al.* 2019). As shown in this study, temperature was the primary climatic factor related to the population variation of aphids. This temperature range was also a determinant for a higher survival and fertility rate of lettuce aphids (Conti *et al.* 2010). Similarly, the population peaks of *C. fragaefolii* in the Festival, Aromas, and Camarosa strawberry cultivars occurred in the spring and autumn, seasons during which temperatures are lower, in La Plata, Argentina (Cédola & Greco 2010). In our study, the occurrence of *Camponotus* sp. also showed an inversely proportional correlation with temperature.

In our study, the occurrence of Sciaridae dipterans was inversely proportional to temperature. In contrast, the occurrence of *N. bilobata* had a positive correlation with mean weekly temperature. This result supports a previous study carried out in strawberry farms in Mexico, where a positive correlation also was observed between the presence of *N. bilobata* nymphs and high temperatures (Gallardo-Granados *et al.* 2016).

Precipitation was another critical climatic factor in pest population dynamics in this study. We found a negative correlation between precipitation and *Aphis* spp. and sciarids, since prolonged or heavy rains are harmful to these insects. The seasonality of winged species tends to decrease with rain, as precipitation reduces the number and duration of flights. Thereby, the drastic decrease in the number of flying insects affects their dispersion or colony establishment in other plants (Iversen *et al.* 2017). Moreover, prolonged periods of rain also provide favorable conditions for the propagation and growth of insect-associated entomopathogens. This situation can negatively affect the occurrence of the pest. All these characteristics explain, at least in part, the regulation of population dynamics by climatic factors.

The main groups of natural enemies present were predatory mites, spiders, and hymenopterans parasitoid. Precipitation influenced the population dynamics of *C. fragaefolii*. Information on the effect of climatic factors in the bioecology of the species enables a prediction of the pest population in different seasons can facilitate pest control and minimize the damage caused. The most effective way to prevent pest damage is to detect key pest populations early in their establishment by using one or more monitoring methods. Here, we conclude that the plant beating method was the most effective in sampling *T. urticae* and *C. fragaefolli*, while Möericke traps were better suited for the other taxa present in the cultivation area. These findings contribute to a better understanding of the entomofauna associated with strawberry cultivation and can support future management strategies.

ACKNOWLEDGMENTS

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Fundação Araucária for the Support of Scientific and Technological Development (FA) for the financial support. We are also grateful to BioAgro for providing the seedlings.

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