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Effects of climate, seasonality, and parasitoid abundance on Liriomyza Mik (Diptera: Agromyzidae) populations on important crops in Northeastern Brazil

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ABSTRACT. Agromyzidae (Insecta: Diptera) is a cosmopolitan family of acalyptrate flies, with almost 3,000 species worldwide distributed. Most species are leaf miners on a large number of plants. Among them, Liriomyza Mik, 1894 is a large genus of leaf-miner species that can cause significant damage to economically important crops and is considered agricultural pests, such as Liriomyza sativae Blanchard, 1938 and Liriomyza huidobrensis (Blanchard, 1926), which are herein investigated. The present study deals with the impact of climatic factors (temperature, humidity, and precipitation), seasonality, and parasitoid abundance on leaf-miner infestation during one year in five cultivated crops in Northeastern Brazil. Climatic factors for instance had different effects on L. sativae populations in melon and watermelon crops. Larval abundances were greater during the dry season for both species, L. sativae and L. huidobrensis, and abundance of adult parasitoids followed the increase of mining larvae.

KEYWORDS. Leaf-miners, climatic factors, plant-insect interaction.

Agromyzidae (Diptera) is a large family of phytophagous acalyptrate flies, with almost 3,000 known species around the world (ITIS, 2016), more than 75% of them have larvae feeding on plant leafs. Some species may attack other parts of the plant, such as root and stem of herbaceous plants and seed-head and cambium of trees (SPENCER, 1973, 1990). According to BENAVENT-CORAI et al. (2005), approximately 900 species in the world have their host plants known, distributed on more than 140 botanical families. More sporadically, some species can be gall-inducing (SPENCER, 1973), as Agromyza Fallén, 1810, Japanagromyza Sasakawa, 1958, Hexomyza Enderlein, 1936 (exclusively galler), Melanagromyza Hendel, 1920, Ophiomyia Braschnikov, 1897, Phytoliriomyza Hendel, 1931, and *Phytomyza* Fallén, 1810 species (DEMPEWOLF, 2005).

Most species have a specific host plant, however, some occur in more than one species of a single genus (monophagous) or botanical family (oligophagous). Some species are polyphagous, attacking a wide variety of species from different botanical families, and are considered as important agricultural pests (BOUCHER, 2010). Liriomyza Mik, 1894 is a large genus with 456 leaf-miner species (ITIS, 2016), four of them considered truly polyphagous: Liriomyza sativae Blanchard, 1938, Liriomyza langei Frick,

1951, Liriomyza huidobrensis (Blanchard, 1926), and Liriomyza trifolii (Burgess, 1880) attacking a wide range of plant species, and Liriomyza brassicae (Riley, 1885), more restricted to the Brassicaceae family (LONSDALE, 2011).

In Brazil, Liriomyza species occurs on several crops as bean, potato, and tomato being able to attack in the early days of cultivation (GALLO et al., 2002) and also may cause problems on ornamental plants (PARRELLA, 1987). The biological control of pest species is hampered due to the protection that the leaf provides to the mine and the scarcity of biological information for their species is also a further difficulty (SPENCER, 1973). Synthetic insecticides and parasitoid hymenopterans have been used for population control of pest species (BOUCHER, 2010).

Currently, the wide variety of insecticides used to control Liriomyza pest species does not affect resistant populations. It is still unknown whether parasitoids are effectively able to control fly populations due to high levels of insecticides in some regions (MURPHY & LASALLE, 1999).

Liriomyza huidobrensis and L. sativae are currently considered the most serious pests among the Agromyzidae (WEINTRAUB et al., 2017). Liriomyza huidobrensis occurs in a large number of hosts and its damage is mainly caused by larval activity and also by the feeding punctures made by adult females. *Liriomyza sativae*, many times mistakenly identified under different species names, has been recorded as a serious agricultural pest, with a large number of crops attacked all over the world. Their pest status may vary over the years and location, but damage may increase due to extensive use of DDT and other pesticides (SPENCER, 1973).

Several environmental factors can affect the metabolism and the development of insects. In general, higher temperatures favor a faster metabolism and relative humidity directly affects development, which can be retarded when the humidity is low and can cause drowning eggs and facilitate pathogen contamination when it is too high (GULLAN & CRANSTON, 2014). Climate changes have notable impacts on agriculture, by influencing, directly or indirectly, crop plants and their associated pests (PRAKASH *et al.*, 2014). In general, climatic factors such as temperature, precipitation, humidity, and wind speed and direction directly influence pest distribution and growth by affecting their rate of development, reproduction, distribution, migration, and adaptation. For leaf-miners, temperature certainly affects growth and development (TSHIALA *et al.*, 2012).

Among the many commercially produced plants in Brazil, melon and watermelon crops are two of the main products of large national and international interest, with more than 25 and 31 million tons, respectively, produced in the year 2018 (IBGE, 2018). The climate of Northeastern Brazil favors these plantations, as it has low humidity and little rainfall, favoring production of sweeter fruits (SEBRAE, 2016). Tomatoes are another important crop produced in Northeastern Brazil, and due to the dry climate and low rainfall rates, it can grow almost all year round (COSTA et al., 2019). In 2017, tomato production exceeded 134 million tons in Ceará state, 32 million of them only from Guaraciaba do Norte (IBGE, 2018). Additionally, Northeastern Brazil represents the third most important Brazilian macroregion for ornamental horticulture, with 11.8% of national producers. The state of Ceará also stands out in the production of ornamental flowers, with roses as the main product. São Benedito has important companies focused on the international market, which stand out in the production of chrysanthemum, gypsophila, among others (SEBRAE, 2015).

The main goal of this paper is to assess the seasonal abundance of *L. sativae* and *L. huidobrensis* larvae and

their parasitoids on five crops: melon, watermelon, tomato, gypsophila, and chrysanthemum. We also analyzed the influence of climate variables, such as, temperature, humidity, and precipitation on *L. sativae* leaf-miner populations on melon and watermelon crops in Northeastern Brazil. For the tomato, gypsophila and chrysanthemum crops no investigation on the climatic effects were carried out due to the absence of annual climatic data for these localities.

MATERIAL AND METHODS

In order to analyze the seasonal abundance of *L. sativae* and *L. huidobrensis* larvae and their parasitoids, specimen collections were carried out in melon, watermelon, tomato, gypsophila, and chrysanthemum crops in Northeastern Brazil (Tab. I). Melon (*Cucumis melo* L.) (Fig. 1) and watermelon [*Citrullus lanatus* (Thunb.) Matsum. &Nakai] (Fig. 2) crops were located in the municipality of Mossoró (state of Rio Grande do Norte), tomato (*Solanum lycopersicum* L.) in Guaraciaba do Norte (state of Ceará) (Fig. 3), and gypsophila (*Gypsophila paniculata* L.) (Fig. 4) and chrysanthemum (*Chrysanthemum morifolium* Ramat.) (Fig. 5) in São Benedito (state of Ceará). All crops are in the open field, except chrysanthemum in greenhouses.

Collections were carried out bimonthly from November 2016 to September 2017 in the studied sites. For each crop, individual plants were investigated in their intermediate age relative to the total time of development until harvest, except for tomato crops that were investigated independent from age as they are perennials. A total number of ten plants of each crop were randomly selected, and from them, 25 leaves, also randomly selected, were investigated for the absence or presence of Mines. For *L. huidobrensis*, only information on larval numbers and their parasitoids was used. Population density was based on the number of larvae.

Leaves with mines were detached, stored under styrofoam with refrigerated plates and taken to the laboratory for analysis and count of the number of insect mines, larvae, and pupae, and parasitoid in each leaf. Some infested branches were removed with pruning shears and placed for rearing in the laboratory of Entomology of *Empresa Brasileira de Pesquisa Agropecuária, Embrapa Agroindústria Tropical* (EMBRAPA) under temperature of 27°C, until adult emergence for identification of the species.

Tab. I. Information about locality, crops, variety and geographical coordinates in Northeastern Brazil where the collections were carried out.

Locality	Crop	Variety	Geographical coordinates, Elevation
Mossoró, RN	Melon	Goldex	S04°03'53.7" W40°53'34.0" - 32 m
	Watermelon	Quetzali	S04°53'43.2" W37°21'36.7" - 36 m
Guaraciaba do Norte, CE	Tomato	Paron, Janaina	S04°03'53.7" W40°53'43.4" - 865 m
São Benedito, CE	Chrysanthemum	Omega time golden, Sunny Reagan	S04°03'53.6" W40°53'43.4" - 889 m
	Gypsophila	Dynamic love	S04°03'50.5" W40°53'14.5" - 855 m



Figs 1-5. Overview of crops. Figs 1, 2, Mossoró, Rio Grande do Norte, Brazil: 1, melon (*Cucumis melo* L.); 2, watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. Fig. 3, tomato (*Solanum lycopersicum* L.) in Guaraciaba do Norte, Ceará, Brazil. Figs 4, 5, São Benedito, Ceará, Brazil: 4, *Gypsophila paniculata* L.; 5, chrysanthemum (*Chrysanthemum morifolium* Ramat.).

The identifications of the specimens were made with use some keys and morphological comparisons. *Liriomyza sativae* was identified on melon, watermelon and tomato crops, while *L. huidobrensis* was identified on gypsophila and chrysanthemum crops. The material were deposited on Diptera collection at Museu Nacional, Universidade Federal do Rio de Janeiro.

Meteorological data [minimum and maximum temperature (°C), relative humidity (%), and precipitation (mm)] were collected from *Centro de Previsão de Tempo*

e Estudos Climáticos (CPTEC) of *Instituto Nacional de Pesquisas Espaciais* (INPE) located at Mossoró (Tab. IV).

The role of climatic variables (Tab. VI) on leaf-miner density (number of leaves with mines, number of leaf-miner larvae and number of parasitoids) was assessed through a Generalized Linear Model (GLM) method with Poisson distribution for each of the dependent variables. All data were analyzed using program R (v.3.5.3.) (R CORE TEAM, 2019). Information on the number of mines on leaves, number of affected leaves, number of *L. sativae* larvae and number of parasitoids (larvae or pupae) have been accounted in melon (Tab. II) and watermelon (Tab. III) crops.

Tab. II. Biotic data of *Liriomyza sativae* Blanchard, 1938 on melon (*Cucumis melo* L.) crop from November 2016 to September 2017 in Mossoró, Rio Grande do Norte, Northeastern Brazil.

Date	N° leaves attacked	N° of larvae	N° of parasitoids
November/2016	78	146	8
January/2017	110	448	7
March/2017	153	745	390
May/2017	117	584	7
July/2017	110	317	3
September/2017	134	1763	321

Tab. III. Biotic data of *Liriomyza sativae* Blanchard, 1938 on watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) crop from November 2016 to September 2017 in Mossoró, Rio Grande do Norte, Northeastern Brazil.

Date	N° leaves attacked	N° of larvae	N° of parasitoids	
November/2016	95	160	6	
January/2017	83	446	69	
March/2017	2	0	1	
May/2017	34	23	3	
July/2017	56	104	3	
September/2017	105	1767	512	

Tab. IV. Meteorological data from November 2016 to September 2017 in Mossoró, Rio Grande do Norte, Northeastern Brazil. The values refer to the annual mean of each month.

Date	Tempera	ture (°C)	\mathbf{D} -1-time term: \mathbf{J} ter (0/)	Den sinitation (mm)	
Date	Min.	Max.	— Relative humidity (%)	Precipitation (mm)	
November/2016	22.4	35.3	31.0	1.0	
January/2017	22.6	36.4	29.0	6.4	
March/2017	21.7	33.7	44	74.6	
May/2017	21.1	35.1	31.0	42.8	
July/2017	19.0	34.6	25.0	63.6	
September/2017	18.7	35.6	24.0	0.6	

RESULTS

As regards the role of climatic variables on *L. sativae* and *L. huidobrensis* larvae and their parasitoids for melon and watermelon, results of the GLM analysis (Tab. V) on melon (Figs 6-17) showed that the best model included all variables. Maximum temperatures had significant positive relation with all dependent variables (*i.e.*, number of leaves with mines, number of leaf-miner larvae, and number of parasitoids). *Liriomyza sativae* density, number of leaves affected, and parasitoid population showed an association with larger thermal amplitudes, since their density was higher

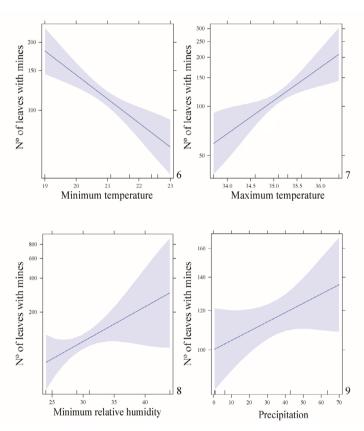
when minimum temperatures were lower and the maximum temperatures were higher. On the other hand, leaf-miner density was lower when the minimum temperature was larger and the maximum was shorter (Figs 6, 7, 10, 11, 14, 15). All dependent variables also increased with the increase of the minimum relative humidity (Figs 8, 12, 16). Precipitation (Figs 9, 13, 17) caused different effects among variables. While higher precipitations caused a positive relation with the number of leaves and larvae, which continued to increase even in rainy seasons; it caused a decrease in the number of parasitoids.

Tab. V. Generalized Linear Model (GLM) result used to evaluate effects of climate variables on *Liriomyza sativae* Blanchard, 1938 population in melon crop from November 2016 to September 2017. Values in columns correspond to the coefficient of each explanatory variable in the best model with respective degrees of freedom. Log likelihood and AICc of each model (Tmin, minimum temperature; Tmax, maximum temperature; Rhmin, minimum relative humidity).

Melon crop	Tmin	Tmax	Rhmin	Rain	df	logLik	AICc
Nº of leaves	-0.24490	0.466700	0.071270	0.0043140	6	-19.742	-32.5
Nº of larvae	-1.1350	1.81400	0.14880	0.005703	6	-24.168	-23.7
Nº of parasitoids	-1.9810	1.5330	0.87450	-0.056110	6	-14.980	-42.0

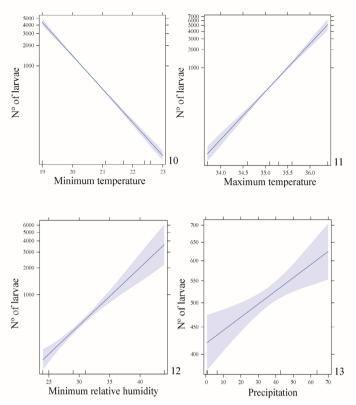
Tab. VI. Generalized Linear Model (GLM) result used to evaluate effects of climate variables on *Liriomyza sativae* Blanchard, 1938 population in watermelon crop from November 2016 to September 2017. Values in columns correspond to the coefficient of each explanatory variable in the best model with respective degrees of freedom. Log likelihood and AICc of each model (Tmin, minimum temperature; Tmax, maximum temperature; Rhmin, minimum relative humidity).

Watermelon crop	Tmin	Tmax	Rhmin	Rain	df	logLik	AICc
N° of leaves	0.37990	-0.53860	-0.28260	-0.01556	6	-16.497	-39.0
Nº of larvae	1.76700	-1.40100	-3.41900	-0.008127	6	-17.816	-36.4
N° of parasitoids	-1.3500	2.9630	0.6880	-0.04209	6	-12.896	-46.2

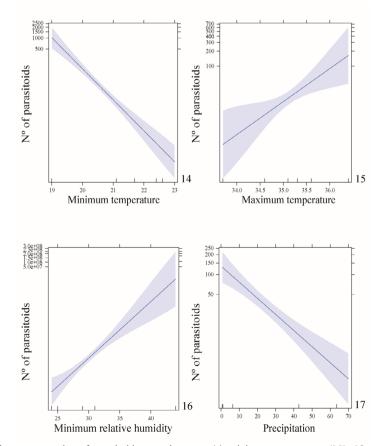


Figs 6-9. Effect of climate factors on number of mined leaves on melon crop: 6, minimum temperature (°C); 7, maximum temperature (°C); 8, minimum relative humidity (%); 9, precipitation (mm).

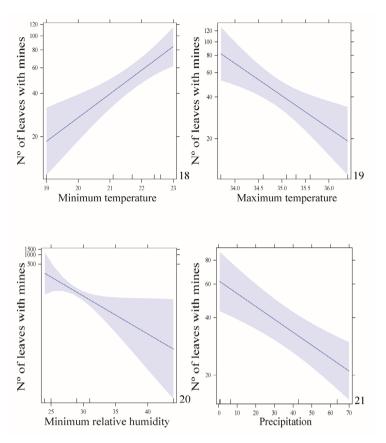
In watermelon crop (Figs 18-29), GLM best model also included all variables, and results showed that the number of attacked leaves was more associated with the shorter thermal amplitudes, so it was higher when minimum temperatures were higher and maximum temperatures were lower. On the other hand, density of mined leaves was lower when the minimum temperature was lower and the maximum was higher (Figs 18, 19). Density of leaf-miner larvae and parasitoids showed a different pattern with higher densities when minimum temperatures were lower and maximum temperatures were higher (Figs 22, 23, 26, 27). Concerning the relative humidity, the number of leaves with mines was higher when minimum relative humidity was lower (Fig. 20), while density of larvae and parasitoids were higher when minimum relative humidity was also higher (Figs 24, 28). Precipitation negatively affected all biotic variables that decreased during rainy periods (Figs 21, 25, 29).



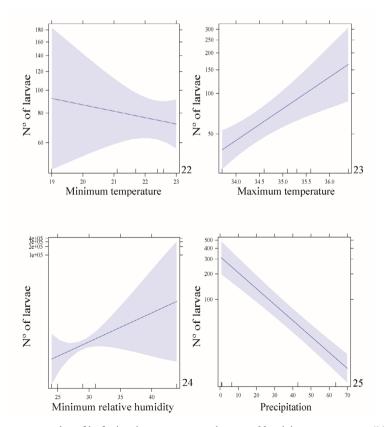
Figs 10-13. Effect of climate factors on number of leaf-miner larvae on melon crop: 10, minimum temperature (°C); 11, maximum temperature (°C); 12, minimum relative humidity (%); 13, precipitation (mm).



Figs 14-17. Effect of climate factors on number of parasitoids on melon crop: 14, minimum temperature (°C); 15, maximum temperature (°C); 16, minimum relative humidity (%); 17, precipitation (mm).



Figs 18-21. Effect of climate factors on number of mined leaves on watermelon crop: 18, minimum temperature (°C); 19, maximum temperature (°C); 20, minimum relative humidity (%); 21, precipitation (mm).



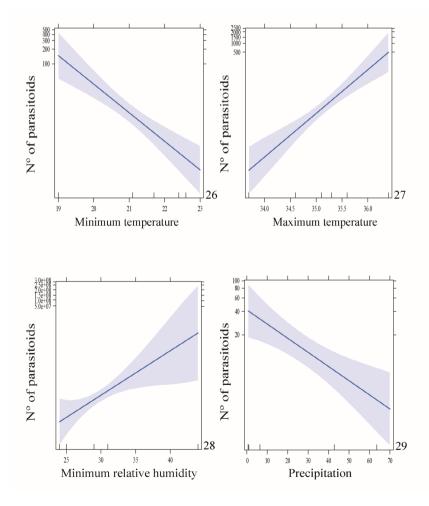
Figs 22-25. Effect of climate factors on number of leaf-miner larvae on watermelon crop: 22, minimum temperature (°C); 23, maximum temperature (°C); 24, minimum relative humidity (%); 25, precipitation (mm).

Regarding the seasonal abundance of *L. sativae* and *L. huidobrensis* larvae and their parasitoids on five crops, the GLM analysis (P<0.05) indicated that *Liriomyza* species were present throughout the year in all crops. In melon and watermelon crops, abundances of *L. sativae* were higher during September in the dry season (Figs 30, 31). In the tomato crop, the highest abundance of *L. sativae* occurred in January (Fig. 32). The abundance of *L. huidobrensis* on gypsophila was quite similar throughout the year, with a slight increase in the months of July and September (Fig. 33). In chrysanthemum, the population had a small increase in July, May and September (Fig. 34). In general, both *L. sativae* and *L. huidobrensis* infestations had a population increase in the dry season, when the temperature was higher.

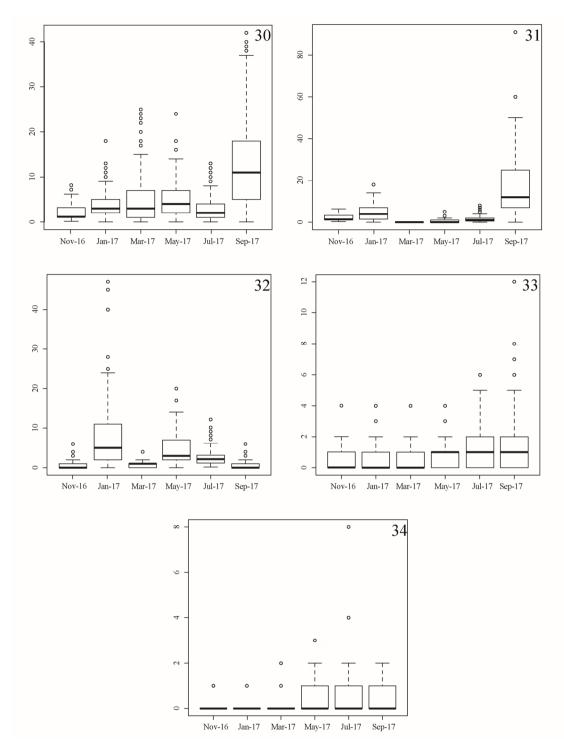
The abundance of parasitoids increased in the same proportion as the increase of mining larvae, with P values (P<0.05) (Tab.VII) significant for all crops (Figs 35-38). For the gypsophyla crop no parasitism was observed during the collection period.

DISCUSSION

Regarding L. sativae on melon and watermelon crops the larval density showed to be more related to higher thermal amplitudes. According to RODRIGUES (2004), the optimal threshold considered for rapid development, with a higher number of offspring for most insects is close to 25°C. To COSTA-LIMA et al. (2009), larvae of L. sativae can survive under different temperatures from 15-32°C, 30°C being the best for their development. Observed temperatures in the present experiment were more or less constant throughout the year, with the maximum varying only from 34.6°C to 36.4°C. Similar to our analyses, results for L. sativae on tomato, demonstrated that the rate of infestation exhibited an increasing trend with increasing temperature (MAZUMDAR & BHUIYA, 2015). On the other hand, L. huidobrensis populations can remain viable at maximum temperatures of 28°C (MACVEAN, 1999) and the increasing solar intensity and aridity approaching summer months may trigger estivation, thus allowing them to survive for a long time in subtropical climates (WEINTRAUB et al., 2017).



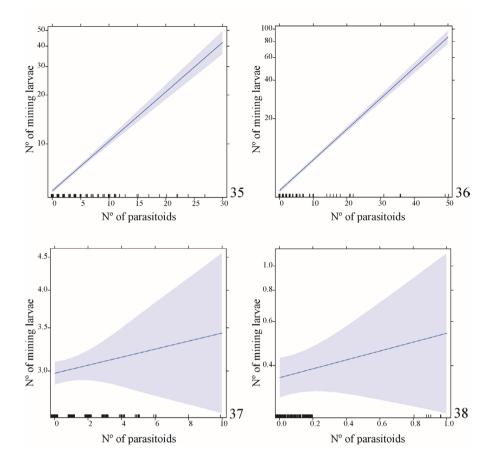
Figs 26-29. Effect of climate factors on number of parasitoids on watermelon crop: 26, minimum temperature (°C); 27, maximum temperature (°C); 28, minimum relative humidity (%); 29, precipitation (mm).



Figs 30-34. Seasonal abundance from November 2016 to September 2017. *Liriomyza sativae* Blanchard, 1938: 30, on melon (*Cucumis melo* L.); 31, on watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]; 32, on tomato (*Solanum lycopersicum* L.). *Liriomyza huidobrensis* (Blanchard, 1926): 33, on *Gypsophila paniculata* L.; 34, on chrysanthemum (*Chrysanthemum morifolium* Ramat.).

Tab. VII. General Linear Model (GLM) result used to evaluate the level of parasitism on fly larvae on all crops from November 2016 to September 2017.

Crops	P value
Melon	<2e-16 ***
Watermelon	<2e-16 ***
Tomato	<2e-16 ***
Chrysanthemum	<2e-16 ***



Figs 35-38. Abundance of leaf-miner larvae compared to the abundance of their parasitoids from November 2016 to September 2017: 35, *Liriomyza sativae* on melon; 36, *L. sativae* on watermelon; 37, *L. sativae* on tomato; 38, *Liriomyza huidobrensis* on chrysanthemum.

For the leaf-miner *L. trifolii*, studies demonstrated that the temperature, relative humidity, and rainfall had a negative influence on number of mines and larvae, while bright sunshine hours positively correlated with *L. trifolii* incidence (VARIYA & BHUT, 2014).

Mining insects have an added protection that the leaf provides, a microenvironment protected from the most extreme temperatures and with reduced evaporation and possibly a lower chance of larval desiccation (CONNOR & TAVERNER, 1997). Ideal conditions on temperature, dampness, and protection from wind are more favorable conditions for Agromyzid infestations (MAZUMDAR & BHUIYA, 2015). Our observations showed few differences in how *L. sativae* density responds to temperature changes even under the same environmental conditions. This may be due to the fact that host insect populations and their natural enemies may respond differently to changes in temperature (PRAKASH *et al.*, 2014), besides that, larval development of leaf-miners also may vary by host plant (PARRELLA, 1987).

For both crops, the minimum relative humidity around 25%, started the beginning of the increase of larval density, which continued to increase according to higher relative humidity, different from what was observed by MAZUMDAR & BHUIYA (2015), where no significant relation with humidity

was observed. According to COSTA-LIMA *et al.* (2009), the relative humidity has a more direct effect on larval survival, with an ideal around 50%, while below 30%, the leaves are less turgid, compromising larval development. Persistent high relative humidity, between 83-91% has a significantly negative impact on the abundance of leaf-miner, especially at the late growth stage (CHAKRABORTY, 2011).

In our results, the abundance of larvae of L. sativae on melon crop continued to increase even in periods with higher precipitation, while the abundance decreased in watermelon crop. Drizzling rainfall had no significant effect on the pest structure. But heavy rainfall within a short period had imparted significant negative effect on leafminer incidence. Number of rainy days however showed insignificant positive effect in all the years (CHAKRABORTY, 2011). A similar pattern, as in our results, in the abundance of L. sativae in watermelon, was found in L. trifolii population on tomato, where the rainfall had a positive and significant effect (SHARMA et al., 2013). The population peaks of L. trifolii in coffee plantations occurred after a period of low rainfall (SILVA et al., 2015). In other cases, the climatic factors did not affect the population of L. huidobrensis as well as the population of related parasitoids (AHYA & LIYANA, 2018). Generally, most of the climatic factors act independently,

any change of a single climatic factor can lead multiple effects on pest population (CHAKRABORTY, 2011). Moreover, these differences in climate effects related to leaf-miners populations, especially what we observed in watermelon population, could be attributed by other factors such as types of pesticides used to control the leaf-miner (AHYA & LIYANA, 2018).

For all crops the largest infestation of both *Liriomyza* species was observed during the dry season, as well as previously documented with the most serious infestations during the same season (TRAN *et al.*, 2007). The presence of parasitoids during all the period of cultivation and the increase of this number in proportion to the increase in larvae. Similar to that observed for *L. trifolii* on bean that the abundance of the parasitoids was positively correlated with leaf-miner host density (LI *et al.*, 2012).

In general, the population of insect pests can be larger in open field comparing to greenhouse (SRI *et al.*, 2017), as we observed here. In addition, both biotic and abiotic factors may together contribute to the establishment of populations in a host plant and the importance of each factor may vary, taking into account the location, season and spatial pattern determined in each host plant (ABOU-FAKHR *et al.*, 2000).

We conclude that all the climatic factors investigated caused rather different effects on *L. sativae* populations in melon and watermelon crops. These effects varied according to the crop, though temperature, both minimum and maximum, seems to affect most variations in population density among crops.

For all crops the seasonal abundance demonstrated higher population density during the dry season. The population of the parasitoids on each crop (except gypsophyla) had grown with the increase of the mining larvae, possibly due to the greater availability of host larvae. Population seasonality in both *L. sativae* and *L. huidobrensis* was also firstly demonstrated in the crops located in Northeastern Brazil. In addition, it was reported that the abundance of parasitoids corresponded to the abundance of mining fly larvae. We also point out the importance to know the factors that can interfere in these pest populations, in order to establish a pattern that could be used for future studies.

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