ENTOMON 39(2): 67-76 (2014)
Article No. ent. 39201

Effect of weather parameters on incidence of citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and its natural enemies in three commercially grown citrus cultivars

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ABSTRACT: Studies on the incidence of citrus leaf miner (CLM), *Phyllocnistis citrella* Stainton, species composition of its bioagents, percentage parasitism and the impact of weather parameters on their incidence on three major citrus cultivars, showed that higher levels of *P. citrella* infestation was found on acid lime and Nagpur mandarin cultivars than on mosambi and higher levels of infestation were observed during August-October and February-April. Different stages of *parasitoids and predators* were collected from leaf miner infested twigs. Parasitisation rates were high during *Hasta* season (October-early December) and least during *Mrig* season (June – July). Acid lime cultivar recorded up to 4.60 parasitized CLM larvae per 10 leaves, while on mosambi it was only 0.65. Maximum temperature was found to have significant negative correlation towards the CLM infestation as well as parasitisation rate on the three cultivars, while relative humidity was found to have significant positive role in favoring the incidence of CLM.

Keywords: Bioagent composition, parasitism levels, population fluctuation, *Phyllocnistis citrella*, weather parameters

INTRODUCTION

The citrus leaf miner (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was first described in Calcutta, India in 1856 (Stainton, 1856). Among 27 major species of insects and mites, *P. citrella* is the most serious pest of citrus, particularly on nursery and young plantations during hot and dry climatic conditions (Sharma *et al*., 2006). The larvae of *P.*

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citrella mine in leaf tissues of any citrus and related species (Heppner, 1993), and the larval feeding results in distorted and reduced young shoots of the citrus plants. Heavy infestation of *P. citrella* can severely damage young citrus trees in the field or in nurseries, while the damage is less in mature trees (Garcia-Marí *et al.,* 2002). Damage to host plants is incited by direct feeding and by providing an infection site for *Xanthomonas axonopodis* pv. *citri*, a bacterium that causes citrus canker (Jesus *et al*., 2006).

Various control methods have been reported for the management of citrus leaf miner including cultural practices, chemical control, use of petroleum spray oils and biological control (Shivankar *et al*., 2002; Rao and Shivankar, 2002; Jayanthi and Verghese, 2004). Over 80 hymenopteran species, mostly chalcidids, have been recorded to parasitize citrus leaf miner throughout the world (Schauff *et al*., 1998). The aim of this study was to monitor the seasonal abundance of citrus leaf miner, its bioagents and parasitism on major three citrus cultivars *viz*., Nagpur mandarin, acid lime and sweet orange (mosambi) as well as to correlate pest-parasitoid abundance with weather factors.

MATERIALS AND METHODS

In order to estimate the incidence levels of citrus leaf miner, species composition of bioagents and percent parasitism levels, fixed plot surveys were carried out at monthly intervals by collecting 4 flushes (each with 10-15 leaves from north, south, east and west direction in a tree) from ten randomly selected trees of *Citrus reticulata* Blanco (Nagpur mandarin), *Citrus aurantifolia* Swingle (acid lime) and *Citrus sinensis* (L.) (mosambi) for a total of 40 flushes (or 300-400 leaves) per cultivar at NRCC farm, Nagpur. No insecticide sprays were applied during the period of the study in the fixed blocks. Collected leaf samples were stored in properly labelled paper bags, in programmable environmental chamber (Remi scientific Limited) at 24±2^{0}C and RH of 65±5 per cent and were examined under the stereozoom microscope (Olympus SZX16) to determine the presence of mines (either occupied or abandoned), eggs, larvae and pupae of the citrus leaf miner, live and dead, as well as parasitoid immature stages. Leaves containing parasitized individuals of *P. citrella* were placed individually in plastic petridishes with water soaked cotton. Adults on emergence were collected and preserved in 70 per cent alcohol and got identified.

Predators including spiders observed on the surveyed trees were hand collected in glass vials containing ethyl alcohol (75 %) and brought to the laboratory for identification. The percentage parasitism of the samples was calculated as the ratio of the number of the parasitized host larvae and pupae to the total number of all host stages. Relative abundance of major bioagents including spiders was also expressed as percentage over the total bioagent collections obtained during the period of study.

The data of weather factors (maximum temperature, minimum temperature, relative humidity, rainfall) was obtained from www.*imdnagpur.gov.in.* The per cent infestation (CLM) and parasitization data were then correlated with temperature (maximum and minimum temperature), rainfall and humidity to find out any role of weather parameters on the incidence of CLM.

RESULTS

The citrus orchard at NRCC, Nagpur was sampled twelve times between October, 2013 and September, 2014 and out of 4305, 4133 and 3487 leaves of Nagpur mandarin(6 years old), acid lime (5 years old) and sweet orange (8 years old) examined, 1094,1131 and 804 leaves were found infested by *P. citrella*.

Per cent leaf miner infestation: In examination of leaf samples from three citrus cultivars, presence of any stages of *P. citrella* was not/the least observed during early May- early July in all the three cultivars. While there was abundant infestation during October - November, 2013 followed by Feb – April and late July-September, 2014. Results of this study showed that significantly more *P. citrella* infestation was recorded on acid lime and Nagpur mandarin cultivars than on mosambi (Fig.1). On Nagpur mandarin trees, two peaks of live immature stages were recorded *viz.*, August-September and February with 51.30, 60.90 and 41.99 per cent infestation, respectively. In acid lime cultivar, similar trend of infestation levels were observed with peaks from July-September and March with the highest infestation levels of 72.33% during the month of September. In comparison, the lowest levels of infestation were observed in Mosambi cultivar with the highest infestation levels of 37.22 per cent during the month of February and followed by September-October months during 2013-14.

The present results have revealed importance of temperature and relative humidity in bringing about change in incidence of CLM in the cultivars. Maximum temperature was found to have significant negative correlation towards the CLM infestation on the three cultivars. As the winter temperature increased during November to December, the per cent leaf miner infestation also decreased simultaneously. But with gradual increase in temperature during February to March, again a peak in per cent infestation was observed. It was also clearly evident that the relative humidity had a major role in favoring the incidence of citrus leaf miner (Table.1). Relation of incidence with rainfall was positive but not significant in all the three cultivars under study.

Table.1 Correlation of citrus leaf miner incidence and parasitism levels on three cultivars with weather parameters

Cultivars	Mean Max.Temp	Mean Min.Temp	Mean Humidity	Rainfall
Nagpur mandarin	-0.58*	-0.04	0.67*	0.48
Acid lime	-0.63*	-0.35	0.72*	0.04
Mosambi	-0.59*	-0.18	0.89*	0.22

*** Significant at 5%**

Bioagents and percent parasitism: The parasitoid complex of *P. citrella* consisted of *Citrostichus phyllocnistoides* Narayanan, *Cirrospilus quadristriatus* (SubbaRao and Ramamani), ***Elasmus brevicornis*** Gahan, ***Sympiesis striatipes*** (Ashmead) etc. Larva of *Mallada desjardinsi* (Navas) and adult stages of *Coccinella septumpunctata* (Lin.), *Serangium parcesetosum* Sicard were also found in the citrus ecosystem attacking larval stages of citrus leaf miner (Table.2). Three different species of spider's *viz.*, *Neoscona* cf. *theisi, Clubiona* sp. and *Thyene imperialis* were collected and constituted 7 per cent of the total bioagent collection. Similarly, grubs of coccinellid predators and *M. desjardinsi* larvae were found feeding on the larvae in mines which were exposed due to spider attack in the mines.

Relative abundance of the bioagents were also documented (Fig.2). Among them, *C. quadristriatus* was the predominant one apart from five unidentified *Cirrospilus* sp. in the collection. *C. phyllocnistoides* constituted 60 per cent of the total parasitoid complex and 30

Table.2 Natural enemies associated with citrus leaf miner, *P.citrella* during Oct, 2013-Sept, 2014

Bioagent	Order	Family
Parasitoids		
Citrostichus phyllocnistoides Narayanan	Hymenoptera	Eulophidae
Cirrospilus quadristriatus (SubbaRao and Ramamani)	Hymenoptera	Eulophidae
Cirrospilus sp. (Unidentified)	Hymenoptera	Eulophidae
Elasmus brevicornisGahan	Hymenoptera	Eulophidae
Sympiesis striatipes (Ashmead)	Hymenoptera	Eulophidae
Predators		
Mallada desjardinsi (Navas)	Neuroptera	Chrysopidae
Coccinella septumpunctata (Lin.)	Coleoptera	Coccinellidae
Serangium parcesetosum Sicard	Coleoptera	Coccinellidae
Spiders		
Neoscona cf. *theisi* (Walckenaer, 1842)	Araneae	Araneidae
Neoscona sp. 1	-do-	Araneidae
Clubiona sp.	-do-	Clubionidae
Thyene imperialis (Rossi, 1846)	-do-	Salticidae

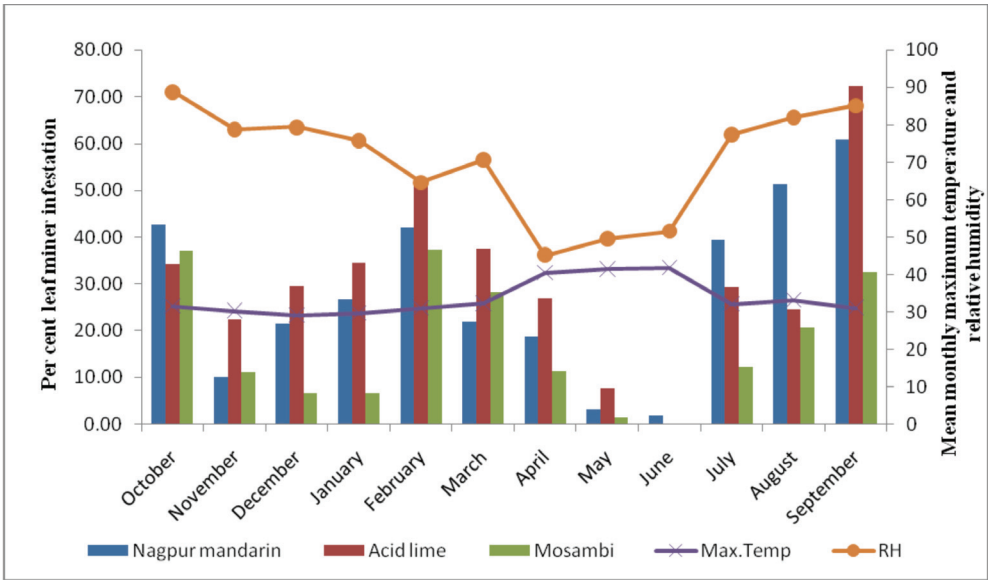

Fig.1. Percent infestation by citrus leaf miner, *P. citrella* and impact of major weather parameters on Nagpur mandarin, Acid lime and Mosambi cultivars during October, 2013-September, 2014

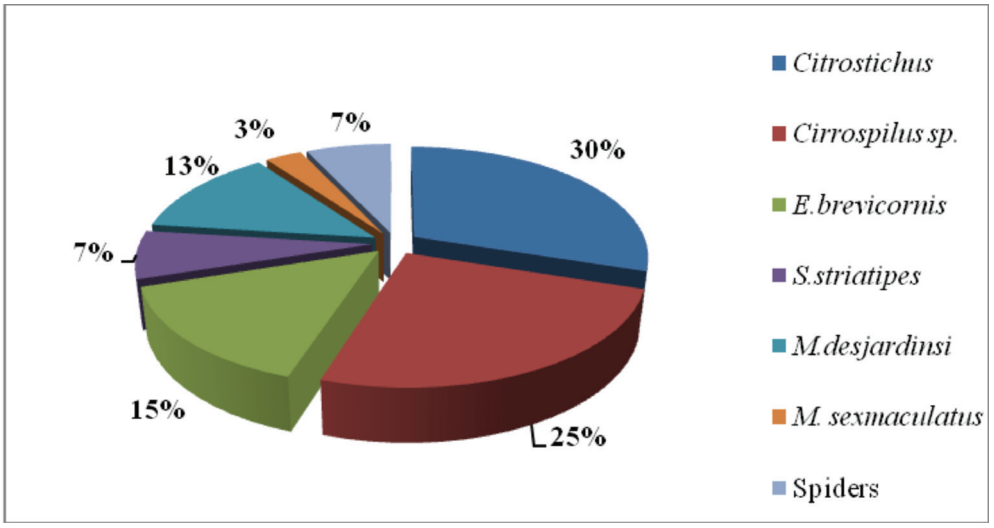

Fig.2 Relative abundance of bioagents recorded on citrus leaf miner from three citrus Cultivars

per cent of total bioagents collected. Diverse collection of Genus *Cirrospilus* including *C.quadristriatus* was obtained and comprised around 25% of total collection. *E. brevicornis* was found as an ecto-larval parasitoid of citrus leaf miner predominant during *Hasta* season. *S. striatipes* accounted for only 7% of the total bioagents collection and was found in low numbers.

The total number of parasitoids collected was maximum on acid lime followed by Nagpur mandarin compared with mosambi, probably due to the higher leaf miner population registered in the former cultivars. Maximum levels of parasitism were recorded during the months of October to November followed by February to March (Table. 3). Acid lime cultivar recorded up to 4.60 parasitized CLM larvae per 10 leaves while on mosambi, it was only 0.65 per 10 leaves. *C. phyllocnistoides* appeared for the first time in the samples during *Hasta* season *i.e* early October and November, having high rates of parasitism. Later as the winter started parasitism by this parasitoid decreased from December and again reaching its maximum during *Ambia* season *i.e* Feb to March. A complex of *Cirrospilus* sp. was also observed in the samples collected. This study also showed variability in *P. citrella* parasitism on the three commercial citrus cultivars. However, it was significantly higher with 9.45, 9.88 and 6.27 per cent, which were the mean percent parasitism found during the period of study in Nagpur mandarin, acid lime and mosambi cultivars, respectively. In general, a positive relationship

Table.3 Percent parasitism in Nagpur mandarin, Acid lime and Mosambi cultivars during October, 2013-September, 2014

Months	Nagpur mandarin	Acid lime	Mosambi
October	21.00	11.8	13.10
November	12.50	23.5	14.59
December	4.90	9.5	2.76
January	8.40	6.3	3.30
February	9.10	16.8	6.51
March	14.84	11.3	10.42
April	6.49	15.4	7.32
May	0.00	2.6	0.00
June	3.60	0.0	0.00
July	8.80	0.0	1.30
August	12.50	5.6	6.70
September	16.40	10.7	9.30

was evident between *P. citrella* and parasitoid abundance on the three citrus cultivars. An increase in the activity of parasitoids was observed when the average monthly temperature was between 29 to 33æ%C and the relative humidity was between 78 and 88 per cent. The role of spiders in the overall management of *P. citrella* populations was also studied. Maximum temperature was found to have significant impact on parasitisation rates but negative correlation (Table.4) while relative humidity was having positive correlation on parasitisation but not significant. Minimum temperature and rainfall was having negative and positive correlation, respectively *w.r.t* parasitisation on citrus leaf miner but not showing any significant influence.

Table.4 Correlation of parasitism with weather parameters

Cultivars	Mean Max.Temp	Mean Min.Temp	Relative Humidity	Rainfall
Nagpur mandarin	-0.57*	-0.16	0.74*	0.36
Acid lime	-0.63*	-0.35	0.52	0.04
Mosambi	-0.48	-0.18	0.49	0.23

*** Significant at 5%**

DISCUSSION

The citrus trees have generally three flush seasons; spring flush from Feb-April, summer flush from June to August and winter flush from October to November. The seasonal pattern of leaf miner activity was fairly higher with populations peaking during spring and late autumn flush and declining during the winter. Koli *et al.* (1981) observed maximum number of *P. citrella* larvae on citrus during August to October and February to March. The larval population of leaf-miner was higher during August-September than during the rest of the period (Ahmed *et al*. 2013). Knapp *et al*. (1995) and Jac-as *et al*. (1997) pointed out that the differences in susceptibility among different citrus species seem to be related to the flushing patterns of the trees. Acid lime cultivar was found to be highly susceptible to leaf miner attack with the maximum leaf miner infestation recorded in our study. The higher degree of susceptibility of acid lime among citrus cultivars may be induced due to certain anatomical modifications that can increase or decrease interaction between citrus cultivars and CLM (Gassmann and Hare 2005: Muller and Riederer 2005; Mathews *et al.* 2007). Lower preference of *P. citrella* was found on mosambi cultivar and could be due to the fact that mosambi do not develop intense vegetative flushes and consequently the microclimate in their canopy seems to be not ideal for the citrus leaf miner compared to acid lime and Nagpur mandarin.

The present results have revealed importance of temperature and relative humidity in bringing about change in incidence of CLM. High population densities of *P. citrella* are usually recorded

in spring and summer due to greater availability of leaf flushes and new shoots, as well as higher temperatures (Pena *et al*. 1996; Legaspi *et al*. 1999). Minimum temperature and relative humidity were the abiotic factors showing the strongest influence in the numbers of *P. citrella* mines and parasitisation.

Many parasitoids were recorded on the eggs and larvae of the leaf miner (Singh, 1993). Among them, *Tetrastichus phyllocnistoides* (Narayanan) and *Ageniaspis* sp. are important, the latter causing upto 80% parasitism (Atwal, 1964). Our collection mainly consisted of eulophid parasitoids, *viz*., *C. phyllocnistoides, Cirrospilus* sp., ***E. brevicornis*** **and** ***S. striatipes.*** *C. phyllocnistoides* is an ectoparasitoid of *P.citrella* and SubbaRao and Ramamani (1965) reported that parasitism by *C. phyllocnistoides* in India started in the fourth week of July, but did not exceed 2 to 4 per cent. Levels increased during the second week of August and during the third and fourth week of August ranging from 40 to 50 per cent. Parasitism levels decreased in the first and second week of September and disappeared by October. *Cirrospilus* Westwood is a large and widespread genus of Eulophinae, with over 130 species worldwide (Noyes, 1998) and has been reported as an ecto-parasitoid of late instar larvae of the CLM (Hoy and Nguyen, 1994) producing a single individual per host. *Sympiesis striatipes* was found in low number and it can be assumed that this parasitoid species have a very low impact on the reduction of pest population.

Parasitism in the orchards generally followed a trend similar to that of *P. citrella* density throughout the spring to winter during the study period. This is in agreement with previous studies, in which the spatial distribution of host and its natural enemies has a great influence on the dynamics of both populations (Jahnke *et al*. 2008) and most natural enemies react to the spatial distribution of their prey (Pedigo 1996). There is considerable difference in the flushing pattern of the three cultivars with regular and heavy flushing in acid lime cultivar and the least in mosambi and hence citrus leaf miner infestation also. Also measures should be taken to conserve the natural bioagents present in the citrus ecosystem especially during *Hasta* season during which per cent parasitization by parasitoids was highest. The present study helps in developing a spray programme based on active period of CLM coinciding with new flush and avoiding sprays during bioagent active periods.

ACKNOWLEDGEMENT

The author is grateful to Dr. Santhosh Shreevihar (Assistant Professor in Zoology, Kozhikode, Kerala) and Dr.Manju Siliwal (Wildlife Information Liaison Development Society, 9-A, Lal Bahadur Colony, Peelamedu, Coimbatore, Tamil Nadu 641004, India) for identification of insect specimens. The authors express their sincere gratitude to the staff of Entomology (Mrs. Sonali.P.Ghike, Mr. Mukesh, Mr.V.N.Dhengre and Mr.Sandep.Y) for their technical assistance and help rendered during the period. This work is a part of the Ph. D thesis of the first author AA.

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(Received 08 January 2015; accepted 13 February 2015)