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Citrus Leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Phyllocnistidae) and Its Natural Enemies

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ABSTRACT

Biological study of the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Phyllocnistidae) revealed that egg eclosion was 3.10±0.46 days. The larva consisted of four instars before pupation. The mean durations of larval, pupal, male and female adults were 4.20±0.53, 8.04±0.92, 2.59±0.93 and 4.04±1.26 days, respectively. The analysis of biological life table resulted in the following biological attributes: the net reproductive rate of increase (R_o) was 23.3946; the capacity for increase (r_c) was 0.1944; the finite rate of increase (λ) was 1.2146 and the cohort generation time (T_c) was 16.2158 days. The investigation on natural enemy complex of *P. citrella* was conducted in many citrus areas in Thailand. Survey carried out from July 1998 to June 2000 found 11 species of hymenopterous parasites namely *Quadrastichus* sp., *Citrostichus phyllocnistoides* (Narayanan), *Teleopterus* sp. and *Microbracon* sp. as larval parasites, and *Ageniaspis citricola* Logvinovskaya, *Cirrospilus ingenuus* Gahan, *Sympiesis striatipes* (Ashmead), *Closterocerus trifasciatus* Westwood, *Eurytoma* sp., *Zaommomentedon brevipetiolatus* Kamijo and *Tetrastichus* sp. as pupal parasites. The other natural enemies as predators were found in the larvae of *Chrysoperla* sp. and some spiders feeding on larvae of *P. citrella*. The evaluation revealed that *Quadrastichus* sp. was the most important larval parasite and *A. citricola* was the most important pupal parasite. These parasites would be used as biological control agents of *P. citrella* in Thailand.

Key words: natural enemy complex, *Phyllocnistis citrella* Stainton, biology, biological life table, hymenopterous parasites

INTRODUCTION

The citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Phyllocnistidae), is an important pest of citrus plantations in recent years. *P. citrella* is widely distributed. It is found in many citrus growing areas of the world, including Southeast Asia, Japan, Taiwan, Australia, Africa, Florida, Mediterranean countries, and Israel (Hill, 1983; Heppner, 1995 and Argov and Rössler, 1998). *P. citrella* may attack any *Citrus reticulata* Blanco; sweet orange, *Citrus sinensis* Osbeck; acid lime, *Citrus aurantifolia* Swingle and grapefruit, *Citrus paradise* Macf. Damage is caused by the larvae mining the surfaces of young citrus leaves. It makes the leaf fold over and

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twisted, with a high degree of distortion, then damaged leaves dry out and resulting in reduced photosynthetically (Hill, 1983). It retards the growth of nursery citrus stock and newly planted citrus trees. It reduces yields in bearing trees. Beside these, the damage from *P. citrella* could be infected by citrus canker, *Pseudomonas citri* Hasses (Smith and Hoy, 1995).

To control *P. citrella*, insecticides are usually employed to prevent serious losses in citrus yields. However, the residue of insecticides and environmental pollution are the important problems that must be considered. The use of natural enemies has now been considered as a potential way to control this insect. Thus, the objective of this study was to conduct an investigation on the biological attributes, including the construction and analysis of the life tables of *P. citrella*; to conduct a survey and evaluation of the parasites of *P. citrella*, including the biology of the important parasites; to study the population of *P. citrella* and its parasites and to evaluate the effective parasites of *P. citrella* as potential biological control agents. It was expected that the results obtained from this study would be utilized as a basis for biological control of *P. citrella* in Thailand.

MATERIALS AND METHODS

Stock culture of *P. citrella*

Pupae of *P. citrella* were collected from citrus orchards by picking infected citrus leaves with pupal chambers kept in plastic boxes (11.5 cm high and 13 cm in diameter) and supplied with soaked filter paper for moisture. Then the boxes were put in the growth chamber with conditions of 25 °C 80 %RH and 12:12 hrs. Dark:Light until the adult of *P. citrella* emerged, after which they were transferred to the oviposition cages (80×101×91 cm in dimensions) of 1 cm long with newly shoots of small citrus trees. Honey was streaked in small drops on wax paper taped on the citrus trees as food for adult moths. The small citrus trees were replaced every day until the moths died. Using this method, it was possible to maintain a stock culture of *P. citrella* for all experiments.

Biological study of *P. citrella*

The citrus trees with eggs of *P. citrella* were collected from the stock culture and kept in the oviposition cage. Daily observation was made and history data were recorded throughout the developmental period. The length of larvae were measured by an ocular micrometer to study growth of the larval stages. When the adult stage was reached, a male and female were paired in another cage for the observation on the reproductivity of the female in terms of preovipositional period, fecundity, and longevity. The small citrus trees with oviposition were separated every day for counting the eggs laid. The life span of adults and other biological data were also recorded.

Biological life table study of *P. citrella*

Study on biological life table were carried out by using 230 newly laid eggs of *P. citrella*. Egg counting were made under the microscope. The newly hatched larvae were maintained in the citrus trees. When the pupal stage occurred, leaf tissues were cut in small pieces and kept in plastic box (11.5 cm high and 13 cm in diameter) with soaked filter paper for moisture. Boxes were kept in the growth chamber with the conditions of 25°C 80 %RH and 12:12 hrs Dark:Light until the adults emerged. The moths were then removed to the oviposition cage of 1 cm long with newly shoot of small citrus trees for egg laying. Data on the number of adults survived and the number of eggs laid on leaves were recorded daily until the adult died. The recorded data were used for the construction of the biological life table using technique given by Napompeth (1973).

Survey of the natural enemies of *P. citrella*

Field survey of the natural enemies of *P. citrella* were carried out by collecting the parasitized larvae and pupae of *P. citrella* from the pummelo and lime orchards in the central region of Thailand, specifically Nakhon Pathom, Chai Nat, Ratcha Buri, Samut Sakhon, Prachin Buri and Chantha Buri provinces. The citrus leaves with larvae and pupae of *P. citrella* were cut in small pieces and kept in the plastic boxes (11.5 cm high and 13 cm in diameter) with soaked filter paper for moisture. Boxes were placed in the growth chamber with the conditions of 25°C 80%RH and 12:12 hrs Dark:Light until the adult parasites emerged for identification and other laboratory studies.

RESULTS AND DISCUSSION

Biology of *P. citrella*

Description of developmental stages of *P. citrella*

Egg: *P. citrella* usually laid a single egg on the young citrus leaves, mainly near the midrib and also on the leaf blade. It could laid eggs both on the upper and under sides of citrus leaf. Mostly high population of eggs were found on the under side of the leaf. The egg was translucent oval shape. The size of individual egg was 0.30±0.03 mm in length, ranging from 0.20 mm to 0.33 mm and 0.23±0.02 mm in width, ranging from 0.18 mm to 0.26 mm.

Larva: When the larva emerged, it immediately bored through the lower wall of the egg directly into the epidermis of the leaf. The newly hatched larva was translucent and changed to green only after feeding. The larva sucked liquid from epidermis cells and made a serpentine mine along the leaf and causing the leaf curled. The larva of *P. citrella* exhibited hypermetamorphosis with two distinct forms that the first to third instars were sap-sucking stage possessing a flattened, apodal body. Legs were reduced and prolegs were absent, and had scissor like mouthpart. The fourth instar became a prepupa that was a spinning stage by changing to a cylindrical body with spinnerate mouthpart. The larva had three molts and the head capsule was triangular. The exuvies and head capsules were found in the tunnel.

Pupa: The pupal stage occurred when the mine of third instar larva reached the leaf edge and molting to the fourth instar occurred resulting in a pupal chamber made of a cocoon of silk and folding over the leaf edge. The pupa was an obtected form. Sex differentiation could be detected by using morphological differentiation at the tip of abdomen, in female the abdomen was stunt but in male it was slender. The distance between the female genital and anal pores was longer than male. It was pale-yellow and became brown and wings and antennae became visible before adult emerged.

Adult: Adults of the a microlepidopteran *P. citrella* usually emerged from evening to dawn but sometimes occurred at noon. Male and female were similar in color that had silvery forewings, four black stripes across each other with dark spot at the terminal edge and long fringes. The hind wings were white pale and feathery. Antennae were filiform, 31-segments in male and 33-segments in female. They were active at night and found mating on citrus leaves in morning hours. The body measurement of various stages are shown in Table 1.

Duration of developmental stages of *P. citrella*

The incubation period was 3.10±0.46 days, ranging from 2 days to 4 days under laboratory conditions (32.14±1.4°C and 61.18±1.2 %RH). *P. citrella* larva consisted of four instars. The duration from first to third instars were 1 day; 1 day and 1.18±0.39 day, ranging from 1 day to 2 days, respectively. And the fourth instar was the prepupal stage that was 1 day development. The total larval period was 4.20±0.53 days, ranging from 3 days to 6 days. The pupal stage was

8.04±0.92 days, ranging from 6 days to 13 days. The longevity of male and female adults were 2.59±0.93 days, ranging from 1 day to 4 days and 4.04±1.26 days, ranging from 2 days to 7 days respectively. The total life cycle of *P. citrella* from egg to male and female adults were 17.15±1.85 days, ranging from 13 days to 20 days and 20.04±1.64 days, ranging from 17 days to 24 days respectively. The data on the duration of developmental stages of *P. citrella* is presented in Table 2. The citrus leafminer, *P. citrella* was an important pest of citrus in Thailand. This study indicated that the life cycle of *P. citrella* was completed in about 13 to 20 days in male and 17 to 24 days in female. This was similar to Smith and Hoy (1995) who reported that a generation of *P. citrella* was completed in 14 to 17 days during the warm summer months. The female adults could lay eggs both on the upper and under sides of the citrus leaf and twig but preferred the under side of leaves.

These results agreed with the experiments of Knapp *et al*. (1993) which reported that the incubation period was 2 to 10 days. The larval development was 5 to 20 days and the prepupa was one day before forming a pupal

Table 1 Body measurement of various stages of *Phyllocnistis citrella* Stainton (n = 50).

Stage of development		Length (mm)		Width (mm)	
		Mean±S.D.	range	Mean±S.D.	range
Egg:		0.30±0.03	0.20-0.33	0.23±0.02	0.18-0.26
Larva:	Instar I	0.66±0.07	0.51-0.82	0.15±0.04	0.08-0.23
	Instar II	1.10±0.18	0.82-1.64	0.34±0.04	0.26-0.41
	Instar III	1.85±0.18	1.46-2.10	0.55±0.07	0.43-0.67
Prepupa:	Instar IV	3.59±0.13	3.40-3.90	0.69±0.08	0.59-0.84
Pupa:	Male	2.46±0.19	2.03-2.83	0.54±0.04	0.46-0.61
	Female	2.49±0.18	2.13-2.93	0.56±0.04	0.49-0.64
Adult:	Male	1.78±0.10	1.56-2.00	4.39±0.28	3.53-4.80
	Female	2.08±0.11	1.87-2.30	5.13±0.30	4.47-5.54

Table 2 Duration of various development stages of *Phyllocnistis citrella* Stainton under laboratory conditions (32.14±1.4°C and 61.18±1.2 % RH).

Stage of development		N	Mean±S.D. (days)	Range (days)
Egg:		50	3.10±1.46	2-5
Larva:	Instar I	50	1	1
	Instar II	50	1	1
	Instar III	50	1.18±0.39	1-2
Prepupa:	Instar IV	50	1	1
Pupa:		50	8.04±0.92	6-13
Total:	first to last Instar	50	4.20±0.53	3-6
Adult:	Male	27	2.59±0.93	1-4
	Female	23	4.04±1.26	2-7
Total life cycle:				
	Male	27	17.15±1.85	13-20
	Female	23	20.04±1.64	17-24

chamber. The pupal stage was 6 to 22 days and the total development of *P. citrella* fluctuated between 13 to 52 days. Smith and Hoy (1995) also reported that life cycle of this insect pest was completed in 14 to 17 days during warm summer months but could be as long as 52 days in the winter.

Life table of *P. citrella*

The biological life table of *P. citrella* was investigated and constructed by using the techniques given by Napompeth (1973) (Table 4), The population statistics were calculated, various population parameters calculated from this table were as follows: the net reproductive rate (R_o) was 23.3946, the capacity for increase (r_c) was 0.1944, the finite rate of increase (λ) was 1.2146 and the cohort generation time (T_c) was 16.2458 days (Table 3). These calculated parameters indicated that the population of *P. citrella* might multiply 23.3946 times in each generation or 1.2146 times per day.

The life table of *P. citrella* was not all completed and still required additional investigation. The partial ecological life table could provide information on the survival in each stage of development under favorable conditions. The mortality obtained in each stage was apparently lower than that observed under the field condition, because of various regulatory factors in the environmental factors. The parasites, especially *Quadrastichus* sp. and *A. citricola* were highly efficient and considered as the important mortality factors on the general population of *P. citrella* in the field. The role of predators was not fully investigated in this study but some predators such as the larvae of *Chrysoperla* sp. and some spiders could extent high mortality of *P. citrella* in the fields as well.

Survey of natural enemies of *P. citrella*

The field survey and evaluation of natural enemies of *P. citrella* was carried out in pummelo and lime orchards in many locations in central Thailand. Suan Mai Det, Amphoe Muang, Prachin Buri was the main site for this investigation. Other sites included in this survey were Nakhon Pathom, Chai Nat, Ratcha Buri, Samut Sakhon and Chantha Buri. The survey of parasites and predators of *P. citrella* revealed 11 species of hymenopterous parasites. Four of these parasites were larval parasites, identified as *Quadrastichus* sp. (Hymenoptera: Eulophidae), *Citrostichus phyllocnistoides* (Narayanan) (Hymenoptera: Eulophidae), *Teleopterus* sp. (Hymenoptera: Eulophidae), and *Microbracon* sp. (Hymenoptera: Braconidae) and 7 species were pupal parasites, identified as *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae), *Cirrosilus ingenuus* Gahan (Hymenoptera: Eulophidae), *Sympiesis striatipes* (Ashmead) (Hymenoptera: Eulophidae), *Eurytoma* sp. (Hymenoptera: Eurytomidae), *Tetrastichus* sp. (Hymenoptera: Eulophidae) and *Zaommomentedon brevipetiolatus* Kamijo (Hymenoptera: Eulophidae). In this study, the egg parasite was not found. As for the predator, larvae of *Chrysoperla* sp. fed on *P. citrella* larvae as well as some species of spiders. Morakote *et al.* surveyed natural enemies of this insect pest in 1992 and reported that only 9 species of hymenopterous

Table 3 Parameters calculated for biological attributes of *Phyllocnistis citrella* Stainton under laboratory conditions (32.14±1.4°C and 61.18±1.2 % RH).

Biological attribute	Notation	Calculated value
Net reproductive rate of increase	R_o	23.3946
Capacity for increase	r_c	0.1944
Finite rate of increase	l	1.2146
Cohort generation time	T_c	16.2158

parasites attacked *P. citrella*. Pena *et al*. (1996) suggested that *P. citrella* populations could be reduced by biological control methods by specifically hymenopterous parasites. Thirty-nine species of Hymenoptera have been recorded from its area of origin in Southeast Asia.

Beside the parasites, the larvae of *Chrysoperla* sp. and some spiders were found as predators attacking the larvae and adults of *P. citrella*. Browning and Pena (1995) reported that in Florida and Hondurus, lacewing larvae, *Chrysoperla rufilabris* were observed feeding on the larvae. Two species of this predator, *Anklyopteryx octopunctata* and *A. boninensis* were found effective against *P. citrella* in south China. The former could ate eggs, larvae and pupae and the latter fed on all stages of *P. citrella*.

Biology of important parasites

***Quadrastichus* sp. (Hymenoptera: Eulophidae)**

Quadrastichus sp. was a solitary ectoparasite which laid 1 egg to 3 eggs on the first or second larval instars of *P. citrella*. The egg was elongate. The larvae of *Quadrastichus* sp. was vermiform, white in color. When the larvae hatched, it fed on the *P. citrella* larva and killed the third and fourth larval instars and pupated in *P. citrella* tunnel. The only one adult emerged from one host.

Male pupa 0.98±0.14 mm in length, ranging from 0.79 mm to 1.20 mm and 0.35±0.04 mm, ranging from 0.28 mm to 0.38 mm in width. Female pupa 1.36±0.14 mm in length, ranging from 1.07 mm to 1.59 mm and 0.45±0.07 mm, ranging from 0.38 mm to 0.56 mm in width. Adult yellow. Compound eyes and occelli red. Antenna, funicle and club elongated each part 3-segments with long setae both male and female. All tarsi 4-segmend. Male third thorax and abdomen tip dark brown and female with one brown stripe on the abdomen. Male smaller than female. Average length from head to the tip of abdomen of male and female 0.89±0.11 mm, ranging from 0.74 mm to 1.15 mm and 1.20±0.15 mm, ranging from 0.95 mm to 1.54 mm. Wing expanses of male and female 1.68±0.19 mm, ranging from 1.23 mm to 2.00 mm and 2.04±0.17 mm, ranging from 1.56 mm to 2.30 mm. Male longevity 3.34±1.77 days, ranging from 1 day to 8 days and the female 4.50±1.83 days, ranging from 1 day to 11 days.

***Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae)**

A. citricola was endoparasite. Female adult often laid eggs into the third larval instar of *P. citrella*. *A. citricola* larvae were fed and developed inside *P. citrella* larva until reached the last instar. The host died after form the pupal chamber then *A. citricola* pupated instead. The pupae of *A. citricola* were inside the cocoons. The cocoon was chained, looked like sausage, stoutly constructed and dark brown in color. The individual cocoon was 1.07±0.16 mm, ranging from 0.90 mm to 1.28 mm in length and 0.53±0.04 mm, ranging from 0.43 mm to 0.56 mm in width. From the survey, it was found that *A. citricola* produced 1 to 7 pupae per single host.

Adult black. All legs dark brown, tasi and distal one-third of tibiae yellow. Compound eyes red brown. Fore and hind wing hyaline. Radicle, scape, and pedicel dark brown, first to third segments of funicle light brown, fourth to sixth segments and club yellow. Funicle with 6-segments and club 1-segment. Fore- and hind-tarsi 5-segments, mid tarsi 6-segments with long spur both male and female.

Male and female were similar in shape and color and differed in the length of the antennal segments. In female, first and second segments of funicle were very short which were broader than long whereas in male, these segments were quadrate and similar to the other segments. Average lengths from head to tip of abdomen of the male and female were 0.64±0.06 mm, ranging

from 0.54 mm to 0.74 mm and 0.73±0.05 mm, ranging from 0.67 mm to 0.84 mm, respectively. The wing expanses were 1.78±0.06 mm, ranging from 1.66 mm to 1.92 mm and 1.78±0.09 mm, ranging from 1.54 mm to 1.95 mm for male and female, respectively. The longevity of male and female adults were 1.18±0.39 days, ranging from 1 day to 2 days.

***Cirrospilus ingenuus* Gahan (Hymenoptera: Eulophidae)**

C. ingenuus was a solitary ectoparasite. Female deposited several eggs on the second or third larval instars of *P. citrella*. When the larva hatched, it fed on *P. citrella* larva. *P. citrella* died in the pupal stage. Then *C. ingenuus* produced a single shiny pupa in *P. citrella* pupal chamber. The pupa was translucent and black in color. The average size of pupa was 1.58±0.07 mm, ranging from 1.46 mm to 1.74 mm in length and 0.59±0.08 mm, ranging from 0.46 mm to 0.69 mm in width.

Adult yellowish. Male smaller than female. Female with five brown stripes on the abdomen. Compound eyes and ocelli red. Antenna stoutly dark brown with 2-segments funicle and 3-segments club. Fore- and mid-tarsi 4-segments and hind tarsi 6-segments. All legs yellowish. Length measured from head to abdomen of male and female 1.21±0.13 mm, ranging from 1.03 mm to 1.56 mm and 1.52±0.10 mm, ranging from 1.36 mm to 1.66 mm, respectively. Wing expanse 2.36±0.21 mm, ranging from 2.03 mm to 2.90 mm and 2.71±0.14 mm, ranging from 2.26 mm to 2.96 mm for male and female, respectively. Males longevity 4.20±1.85 days, ranging from 1 day to 9 days and females 4.54±2.40 days, ranging from 1 day to 11 days.

***Citrostichus phyllocnistoides* (Narayanan) (Hymenoptera: Eulophidae)**

C. phyllocnistoides was a solitary ectoparasite. The female adult laid one or more eggs on the first or second larval instars of *P. citrella*. *C. phyllocnistoides* larvae attached and fed on the larva of *P. citrella* larva. Then *P. citrella* larva died in the third or fourth instars. Only one pupa could survive from one host and pupated in the tunnel.

Adult black. Both male and female with one translucent patch on the abdomen. Compound eyes and ocelli red. Antenna yellow, funicle elongate 3-segments, club elongate 3-segments with setae. All legs yellow, tarsi 4-segments. Male smaller than female. The length from head to abdomen of male and female 0.82±0.12 mm, ranging from 0.51 mm to 0.95 and 1.12±0.05mm, ranging from 099 mm to 1.23 mm, respectively. Wing expanses 1.43±0.15 mm, ranging from 1.10 mm to 1.64 mm. and 1.90±0.11 mm, ranging from 1.66 mm to 2.12 mm for male and female, respectively. Male longevity 3.17±2.56 days, ranging from 1 day to 10 days and female longevity 3.80±2.26 days, ranging from 1 day to 11 days.

In Israel, Argov and Rössler (1998) who evaluated the biology of these parasites reported that life cycles of *Quadrastichus* sp., *A. citricola*, *C. ingenuus* and *C. phyllocnistoides* were 10, 15, 8 and 12 days at 25°C.

The biological study of *P. citrella* and these parasites still requires a more refined technique for further investigation of the biological control and the integrated pest management system. Thus, these basic investigations were essential for the operation of integrated pest management program for controlling *P. citrella* in the citrus orchards and economically acceptable basis and conservation of the environment from pesticides.

CONCLUSION

Biological study of the citrus leafminer, *P. citrella* under laboratory found the larva to burrow through the cuticle of the leaf and fed the epidermis cell only. The larval stages from the first to fourth instars were 4.20±0.53 days and the

pupa stage was 8.04±0.92 days. The longevity of male adult was 2.59±0.93 days and the female was 4.04±1.26 days. The life cycles from egg to adult were 17.15±1.85 days in male and 20.04±1.64 days in female. The analysis of biological life table and partial ecological life table revealed important population attributes: net reproductive rate of increase (R_o) was 23.3946; the capacity for increase (r_c) was 0.1944; the finite rate of increase (l) was 1.2146 and the cohort generation time (T_c) was 16.2158.

The natural enemies survey of *P. citrella* found 11 species of parasites, among these *Quadrastichus* sp., *A. citricola*, *C. ingenuus* and *C. phyllocnistoides* dominated all locations investigated. In Thailand, *Quadrastichus* sp. was the most important larval parasite and *A. citricola* was the most important pupal parasite of *P. citrella.* These two parasites played an important role for biological control of *P. citrella* population. The biological and population statistics obtained from this investigation could be useful in the development of *P. citrella* management program in Thailand and could be improved for an effective biological control method for use instead of costly and environmentally damaging insecticides.

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