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Biology and Control of the Citrus Leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in Japan

Takeshi UJIYE*
Department of Plant Protection, National Institute of Fruit Tree Science
(Tsukuba, Ibaraki, 305-8605 Japan)

Abstract
Recent advances in research on the life history, natural enemies, sex attractant, and practical control of the citrus lceafminer (CLM) in Japan are described, Adult CLM overwinters within canopies of the citrus trees in the warmer districts of the citrus belt where the overwintering females begin to oviposit in mid-March. The moth passes 9 to 10 and 5 generations per year in the southwestern and northeastern parts of the citrus belt, respectively. Developmental zero and effective heat units for development from egg to adult emergence are 12.1°C and 206 degree-days, respectively. Eighty per cent or more larvae were killed by parasitoids and unknown factors before pupation during the summer-autumn seasons. Dominant *parasitoids* are *Sympiesis striatipes* in mainland-Japan and *Cirrospilus ingenuus* and *Citrostichus phyllocnistoides* in the Southwestern Islands. Biological control of CLM by native parasitoids is not sufficiently effective in Japan because *Ageniaspis citricola*, a most effective biological control agent in tropical Asia, has not been detected in Japan. A sex attractant, (Z, Z)-7, 11-hexadecadienal showed a high activity for male moths in Japan, but was ineffective for those in foreign countries. The sex attrac tant appeared to be useful for the forecasting of seasonal occurrence, and control measures by using this substance should be initiated. Insecticides are commonly used for the control of CLM on young trees. Nicotine sulfate, IGRs, imidacloprid, alanycarb and pyrethroids are sprayed 4 to 5 times at 5- to 10-day intervals during the flush developing period. Recently, CLM has become resistant to pyrethroids in some districts of Kyushu. To avoid the spread of insecticide resistance of CLM, an attempt was made to use the effective parasitoid, *A. citricola*, through introduction from Taiwan and Thailand to Japan.

Discipline: Insect pest
Additional key words: parasitoid, sex attractant

Introduction

The citrus leafminer, *Phyllocnistis citrella* Stainton (CLM), is a serious pest in nurseries and on top-grafting trees of citrus, and also on citrus growing in plastic greenhouses in Japan.

CLM originated in tropical Asia from Afghanistan to China, and was introduced to Japan in the 1600s[6]. CLM had been spreading slowly over adjacent areas, and became known also in the temperate zone of Asia, Australia, Pacific Islands, South Africa (miss-identification?) and eastern part of Africa by the beginning of the 1980s. Then its distribution spread rapidly: it was confirmed in southern Florida in late May of 1993[6,7], in Florida, most of the Caribbean region, the southern part of the USA[2,8] and the northern part of South America by September in 1994, and lastly in Uruguay in January, 1997. It also first occurred simultaneously on the Mediterranean coast of Europe[11], in the Middle East[19], North Africa[11] and Reunion Island[13] in 1993 to 1995.

This paper describes recent contributions to studies on CLM biology and current status of control of CLM in Japan.

Outline of citriculture in Japan

The citrus cultivation belt of Japan stretches along

Present address:
Ushiku, Ibaraki, 300–1216 Japan.
* Corresponding author: tel +81–298–71–1265.
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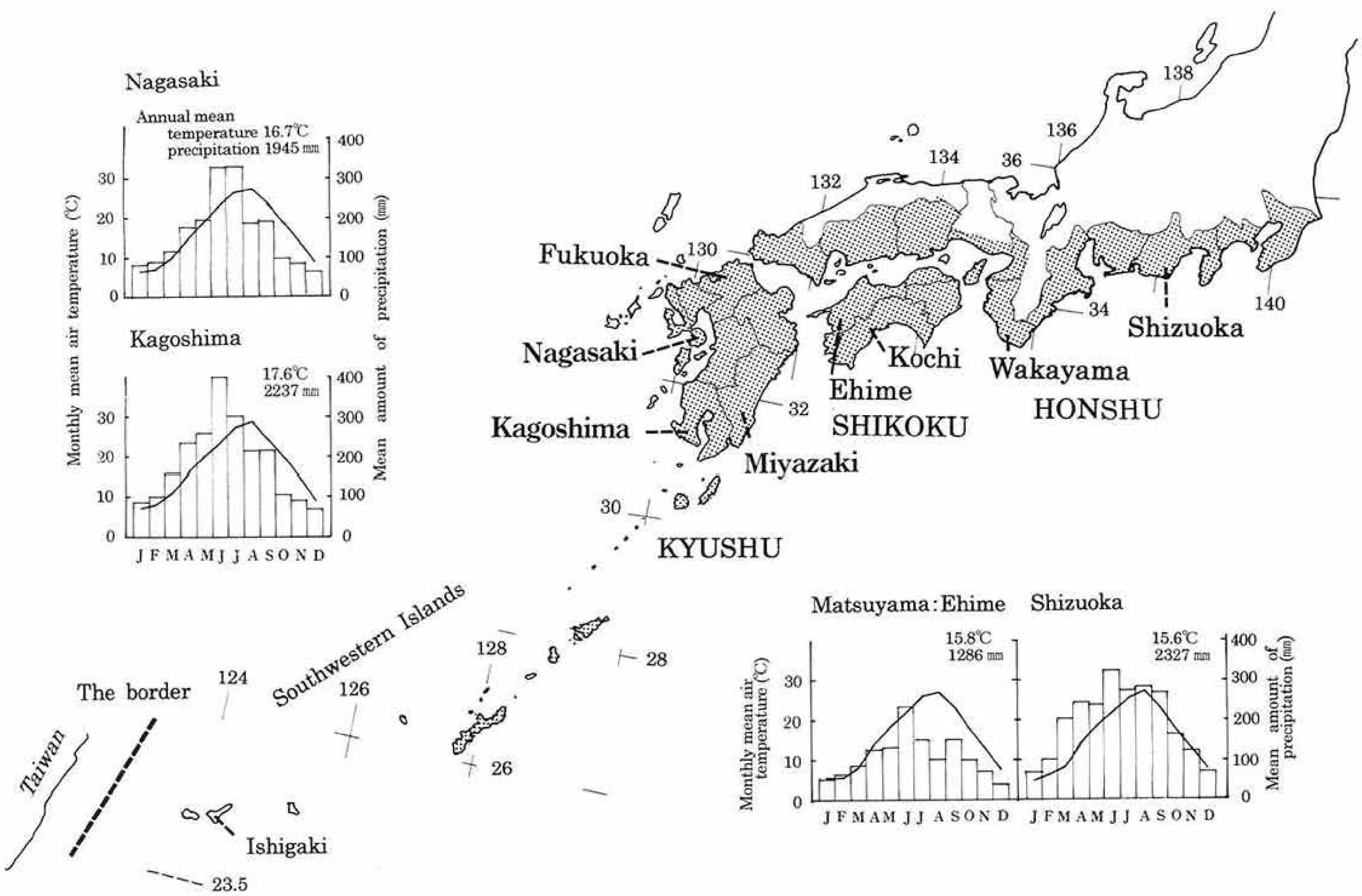

Fig. 1. The citrus belt in Japan

the southwestern coast of Honshu, Shikoku and Kyushu, extending from 35°N to 31°N (Fig. 1). It belongs to an area with relatively cool winter temperatures among the citrus cultivation zones of the world. Mean air temperature in a year and in January ranges from 14 to 17°C and 4 to 7°C, respectively, and the daily minimum air temperature sometimes falls below 0°C in mid-winter. Small-scale citrus orchards are also dispersed about all over the Southwestern Islands.

Among the citrus cultivars planted in Japan, about 65% consist of Satsuma mandarin, followed by Iyo (10%), Natsudaidai (5%) and Hassaku (4%).

About 77% of the citrus orchards are located on slopes with an inclination above 5° for the following reasons: plains are used for rice paddy fields, citrus trees on plains are often damaged owing to freezing night temperatures in winter, and high quality fruits are obtained usually from sloping orchards with good drainage.

The citrus trees have generally 3 flush seasons; the spring flush from early April to early May, the summer flush from June to August and the autumn flush from early to late September. Because of the low winter temperature, there are no young leaves during more than 5 months from October to early March. The spring shoots are very important as fruit-bearing branches of the next year, while the summer and autumn shoots are less important or often useless in mature trees. Therefore, the optimum method of tree management is to suppress the growth of the new shoots from summer to autumn; or, secondarily, to cut off the new shoots emerging during these seasons.

On the other hand, in young or top-grafting trees, the summer shoots are necessary to build up the tree shape. In addition, in plastic greenhouses, the summer shoots are also useful as fruit-bearing twigs of the next year. The summer and autumn shoots must be healthy for cultivation, and not damaged by pests such as CLM.

Life history of CLM

Yamamoto[20,21] first observed that the adult of CLM overwintered within canopies of citrus trees in Miyazaki Prefecture in the southern part of Kyushu. Thereafter, overwintering adults were observed in several warmer districts of the citrus belt such as Kagoshima, Nagasaki (Kyushu), Kochi (Shikoku) and Wakayama (Honshu), though their presence has not been confirmed in some orchards located in rather cool districts of the belt. For example, based on observations made in 1992–1995 in Shimizu (Shizuoka Prefecture: near the northeastern end of the belt), mines formed by the 1st-generation larvae were found in 1992, 1994 and 1995, but not in 1993. These results suggest that the density of the 1st generation varies among years and that CLM probably does not always overwinter every year in Shimizu.

Key factors for the fluctuations in the density of overwintering CLM adults have not been clarified, but it is assumed that the population density before winter and temperatures during winter are important. A small amount of flushes and/or too early flushing in autumn tend to reduce the number of overwintering adults. Low temperatures during winter also appear to be harmful to CLM. Winter diapause of CLM has not been confirmed. Because the mature eggs inside of the ovaries of female moths emerging in autumn are absorbed while exposed to low temperatures before winter[5], CLM females seem to be in diapause. On the contrary, because male moths are sexually active during winter as described later, CLM males do not appear to have a winter diapause.

Most adults emerging in autumn seem to die during the winter, because their density in spring is very low even in the warmer districts of southern Kyushu where the moths constantly overwinter every year. In rather cool regions such as Fukuoka and Ehime, adults of CLM often occur from June corresponding to the 2nd-generation flight season in the overwintering districts. It is suggested that short distance migration or movement of adult moths occurs from the overwintering sites to northern and non-overwintering parts of the belt during the 1st flight season every year. It was confirmed that the overwintering female moths started to oviposit from mid-March on the sprouts of cultivars with early germination such as oranges[21] and Ponkan.

Based on observations using traps baited with sex attractant in Kuchinotsu (Nagasaki Prefecture), the emergence of the 1st generation seemed to start from early to mid-May[15]. Developmental zero and effective heat units for the development of CLM were estimated at 12.1°C and 238 degree-days respectively, by Yamamoto[23]. However, the latter value was rectified to 206 degree-days by Ujiye[15] based on the results of further tests. Using these values, CLM was estimated to have 7 to 8 complete generations per year in Kuchinotsu[15]. It was also estimated based on field population trends that CLM passed 9 to 10 generations per year in Miyazaki[21], 7 to 8 in Kochi[9] and 7 in Wakayama[10]. In these districts, the presence of overwintering adults of CLM was confirmed. On the other hand, Yoshida and Takei[25] observed 5 generations in Shizuoka, where the winter conditions are sometimes too severe for adults to overwinter as mentioned above.

Sex ratio of CLM was about 1:1. A female moth mated on the early morning of the day after emergence[22], and laid 55 to 133 eggs during her life span[21].

Fig. 2. Third instar larva feeding on sap from ruptured epidermal cells

Fig. 3. Infestation of summer flushes of Satsuma mandarin tree with the citrus leafminer

Eggs of CLM are laid singly on both the upper and lower surfaces of a citrus leaf, sometimes also on green shoots and seldom on young fruits. Oviposition occurs on leaves more than 5 mm in length. Eggs are seldom laid on hardened leaves; if eggs were deposited on such leaves, hatching larvae would die without mining within the leaves.

The larvae have 4 instars; the 1st to the 3rd instar larvae are sap-feeders, and the 4th is a spinning larva which can not feed on the leaf epidermis due to the degeneration of the mouth parts. The sap-feeder makes a serpentine mine in which it feeds within the epidermis leaving only the cuticular layer. It finally reaches the leaf margin where it molts to a spinning larva after enlarging the terminal part of the mine (Figs. 2, 3). The spinning larva makes a pupal cell by bending the leaf edge and pupates inside[14].

Table 1. List of parasitoids reared from the citrus leaf-miner in Japan

Pteromalidae
Trichomalopsis oryzae Kamijo et Grissell
Eupelmidae
Eupelmus sp.
Elasmidae
Elasmus sp. (A)
Eulophidae
Cirrospilus phyllocnistis (Ishii)
Cirrospilus ingenuus Gahan: (SWI[z])
Cirrospilus sp. (near lyncus Walker)
Elachertus sp. (A)
Elachertus sp. (B)
Pnigalio sp. (A)
Pnigalio sp. (B)
Pnigalio sp. (C)
Stenomesius japonicus (Ashmead)
Sympiesis striatipes (Ashmead)
Citrostichus phyllocnistoides (Narayanan)
Quadrastichus sp.
Achrysocharoides sp. (A)
Chrysocharis pentheus (Walker)
Closterocerus trifasciatus Westwood
Kratoysma sp. (B)
Neochrysocharis formosa (Westwood)
Pleurotroppopsis japonica (Kamijo)
Teleopterus delucchii Bouček:(SWI[z])
Teleopterus erxias (Walker)
Zaommomentedon brevipetiolatus Kamijo

z: Southwestern Islands.
A, B and C refer to the difference in the leg color (see also ref. 17).

Natural enemies

As shown in Table 1, 24 species of parasitoids emerged from the mines of CLM collected from about 50 locations all over Japan[17]. Among them, *Sympiesis striatipes* and *Quadrastichus* sp. were the dominant species in many citrus orchards. *Cirrospilus phyllocnistis*, *Chrysocharis pentheus* and *Zaommomentedon brevipetiolatus* were also major parasitoids in some orchards of the mainland. *Citrostichus phyllocnistoides* and *Cirrospilus ingenuus* were the dominant or major species in the Southwestern Islands in the subtropical region. During the larval stages of the summer generations, 80% or more miners were killed by parasitoids and unknown factors. Most of the parasitoids attacked mainly the late 3rd to 4th instar larvae when the host mines were fully developed with a certain amount of injury[16]. Heavily infested leaves curl and fall off the tree. Citrus canker, *Xanthomonas campestris* pv. *citri* (Hasse) Dye, can easily invade from the cuticle-removed mine traces to internal tissues of the leaf. For these reasons, in spite of the high parasitism rates, biological control of CLM by the native parasitoid complex seems to be difficult in Japan.

In Taiwan and Thailand, the polyembryonic encyrtid, *Ageniaspis citricola* was recognized as the most effective biological control agent of CLM[17,18]. Because *A. citricola* does not occur in Japan, attempts were made to introduce it from Thailand and Taiwan in the summer of 1995. Although the attempt failed because of the death of the wasps during transport, further efforts should be made in future.

Although larvae and pupae were found to be killed by unknown predators, the predator species and their effects have not been identified.

Sex attractant

Ando et al.[1] confirmed that (Z,Z)-7,11-hexadecadienal strongly attracted male CLM moths in the field. In a trap baited with a rubber septum containing 1 mg of the substance, the number of male moths caught per day was 30 to 90 times higher than that in a trap with a virgin female, and this level of attracting ability was maintained for more than 2 months. Male moths were caught not only in the traps placed in the citrus orchard, but also in those placed in a wood located at a distance of 200 m or more from the nearest citrus groves[15]. Males were attracted to the sex attractant mainly from May to November, and a small number of males were also caught on warm and windless days even in the winter season. Based on these results, it was confirmed that the sex attractant was useful for forecasting the CLM seasonal occurrence.

The attracting ability of the substance to male moths was evaluated throughout Japan and in some areas in foreign countries in which CLM occurred. The male moths were caught in traps placed in the citrus-growing regions from Shizuoka to Ishigaki Island in Japan. A small number of CLM moths were also caught in traps set in Nepal. However, in other countries such as Thailand, Taiwan, Turkey, China, Egypt, Spain, Reunion Island and Uruguay, males were not attracted by this substance.

Control

As stated above, since the spring population density of CLM is very low, it is not necessary to control CLM before late June in most parts of the citrus belt in Japan.

On the other hand, it is so important to protect the new shoots of the young or top-grafting citrus trees from the infestation of the CLM summer generations, that insecticides such as nicotine sulfate, IGRs, imidacloplid, alanycarb and pyrethroids need to be sprayed. These were applied 4 to 6 times at several-day intervals from the beginning of germination to the end of shoot growth. As a rule, nicotine sulfate, IGRs such as diflubenzuron and tiflubenzuron, and pyrethroids and imidacloplrid are sprayed at intervals of 5 days, 7 days, 7 to 10 days, respectively, in Japan.

Susceptibility of CLM to many pyrethroids has decreased recently in the western part of Kyushu[3,4,12] and populations resistant to pyrethroids are expanding gradually to the east, so that this group of insecticides is becoming ineffective in these areas. To control CLM, organo-phosphorous insecticides such as penthoate, phosmet, and isoxathion had been used until about 20 years ago, but they also can not be used presently because of resistance[24]. Petroleum oils are commonly sprayed in summer to control CLM in Australia, Israel, etc., but oil sprays after late July should be avoided owing to the decrease of the Brix content of fruits in Japan.

In general, special insecticide sprays for CLM are not applied on the mature Satsuma mandarin trees. However, some insecticides are customarily applied to control other main pests such as yellow tea thrips (*Sciltothrips dorsalis*), white-spotted longicorn beetle (*Anoplophora malasiaca*), and arrowhead scale (*Unaspis yanonensis*) in the citrus orchards. Although these insecticides seemed to influence the incidence of mortality of CLM, under these conditions, about 100% of new leaves are likely to be heavily infested with CLM during the summer to autumn seasons every year. In order to suppress the population density of CLM, it is recommended to cut off all of the infested shoots as a cultural control method for CLM.

Conclusion (some problems for the future)

For nurseries, top-grafting trees, trees planted in plastic greenhouses, and susceptible cultivars to citrus canker, many insecticides are applied to control CLM at short-term intervals during the seasons of shoot development in Japan. These continuous sprays of insecticides can not only induce CLM resistance to insecticides but also eliminate natural enemies of many citrus pests from citrus orchards. An additional problem is the increased cost of operating pesticide sprays. Consequently, attempts should be made to use only natural enemies and sex attractants for the control of CLM in Japanese citrus orchards.

Natural enemies: As stated above, the native natural enemies were not sufficiently effective as biological control agents for CLM in Japan. An encyrtid parasitoid, *Ageniaspis citricola* appeared to be the most effective parasitoid for the natural control of CLM in Thailand and Taiwan. The wasp which has been recently introduced from these countries to Australia, United States and Israel, established successfully and was able to reduce the CLM population markedly under the new circumstances. An attempt to introduce *A. citricola* from Thailand and Taiwan to Japan was made in the summer of 1995, resulting in failure because of the death of the wasps during transport. Additional efforts should be made to introduce *A. citricola* from tropical Asia or secondarily colonized areas such as Florida and Australia to Japan. Though it remains to be determined whether *A. citricola* is able to overwinter under the long, cold Japanese winter conditions, attempts should at least be made to introduce the encyrtid from Taiwan to Ishigaki Island which is located at the same latitude as the northern part of Taiwan.

Sex attractant: It was suggested that (Z,Z)-7,11-hexadecadienal could be utilized for forecasting the occurrence of CLM moth, but suppressive effects on CLM were obtained based on small-scale field tests using the sex attractant as a mating disruptant. It appears that many mated females flew into the sex-attractant-treated field from the surrounding untreated ones. Large-scale testing using large amounts of the sex attractant should be performed in large fields. In Japan, however, as most of the citrus orchards are located on slopes where a gassy sex attractant can not drift in the air for a long period of time, it may be difficult for the synthesized sex attractant to disturb the sensibility of the male moths occurring throughout the citrus orchards.

Kuroko and Kumata, Japanese experts in the taxonomy of Gracillariidae, identified the specimens collected from Thailand and Morocco as *Phyllocnistis citrella*, which is the same species as the citrus leafminer occurring in Japan. However, the synthesized substance strongly attracted Japanese CLM males, while it did not attract the moths occurring in other countries. This difference of sensibility to the synthesized sex attractant may indicate that *P. citrella* has differentiated into some biotypes. Consequently, (Z,Z)-7,11-hexadecadienal used in these studies was synthesized as an analogue from one component of the pink bollworm sex pheromone and confirmed to be a sex attractant of CLM based on random screening tests in the field[1]. The substance was assumed to be at least one component of the sex pheromone of CLM based on its strong attractiveness for male moths of CLM. It is necessary, however, to extract the true pheromone from the virgin female, to determine whether it is the same material as the synthetic one, and whether it contains other minor components, in order to utilize this substance for effective control or monitoring of the populations in various parts of the world.

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