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# Some Biological Characteristics of Plum Sawfly *Monocellicampa pruni* (Hymenoptera: Tenthredinidae)

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# 자두수염잎벌(Monocellicampa pruni)의 생물학적 특성

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**ABSTRACT:** This study investigated the life history of the plum sawfly *Monocellicampa pruni* Wei, 1998 through field observations and laboratory experiments. *M. pruni* is a univoltine, complete metamorphosis species with five instars at the larval stage. Adults emerge from the soil around mid-March when Japanese plum trees are in their flowering period. The adults deposit one (rarely two) egg(s) under the epidermis layer of the calyx. After hatching, the larva soon burrows into the fruitlet, consumes the endocarp and remains inside the fruit within its four-time moulting transitions before reaching full growth. At maturity (in May), the larva leaves the infested fruitlet through an exit hole and burrows into the soil at a depth of 2 - 11 cm for spinning its cocoon and hibernates until the next spring as the larval form. Under laboratory conditions (T = 20°C; RH = 40 - 60%), the male lived for a slightly shorter period than the female, 6.03  $\pm$  0.40 and 7.55  $\pm$  0.45 days, respectively. A female could produce  $30.29 \pm 4.50$  eggs in her lifespan. In the field, the duration of the egg was approximately 10 - 11 days and that of the larva was approximately 31 - 34 days.

Key words: Plum sawfly, life history, developmental duration, longevity, fecundity

**조 록**: 본 연구는 포장 및 실험실 조사를 통해 자두수염잎벌(*Monocellicampa pruni* Wei, 1998)의 생활사를 확인하였다. 자두수염잎벌은 5령의 유충 단계를 가지는 일화성 곤충이다. 성충은 자두가 만개하는 3월 중순경에 흙에서 나와 꽃받침의 표피층 밑에 1개(드물게 2개)의 알을 낳는다. 부화 후, 유충은 곧 어린 과실 속으로 파고들어 성충이 되기 전 4번의 탈피 과정 동안 과실 안에서 과육을 섭식한다. 유충이 성숙하면(5월) 과실에서 나와 2 - 11 cm깊이의 땅속으로 파고 들어가 번데기방을 만들고 이듬해 봄까지 전용 형태로 월동한다. 실험실 조건(T = 20℃, RH = 40 - 60%)에서 수컷 성충의 생존기간은 암컷성충보다 약간 짧았고(수컷 6.03 ± 0.40일 및 암컷 7.55 ± 0.45일), 암컷은 평생 30.29 ± 4.50개의 알았다. 포장에서 알기 간은 약 10 - 11일이었고, 유충기간은 약 31 - 34일이었다.

검색어: 자두수염잎벌, 생활사, 발육기간, 수명, 생산력

Sawflies are amongst the most devastating pest of plum. Within 40 described species of genus *Hoplocampa*, Hartig 1837 (Liston et al., 2019; Taeger et al., 2010), the black plum sawflies *H. minuta* Christ, 1791 and yellow *H. flava* Linnaeus,

\*Corresponding author: curcul@scnu.ac.kr Received September 8 2021; Revised November 8 2021 Accepted November 29 2021 1761 have been the primary, host-specific pests of European plum (*Prunus domestica* L.) since the early XIX century (Almaši, 2010; Petherbridge et al., 1933; Tamosiunas et al., 2014). Those species can cause 36 - 96% loss of production and often have more severe effects in organic or non-treated orchards (Andreev and Kutinkova, 2010; Arnaoudov and Andreev, 2002; Caruso and Cera, 2004; Jaastad et al., 2007; Molinari et al., 1996; Oroian et al., 2009).

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The genus Monocellicampa, a small group of subfamily Nematinae, contains only two species of M. pruni Wei, 1998 and M. yangae Liu et al. (2017) that both damage Japanese plum (Prunus salicina Lindl.) in China (Liu et al., 2017; Wei, 1998). Their morphology is allied to the genus Hoplocampa. Characterisations, including the absence of the m-cu vein in the hind wing and epicnemial surface weakly outlined by a furrow, are the keys to distinguish the genus (Liu et al., 2017; Prous et al., 2014). When hosting in plum, larvae of M. pruni possess only six pairs of prolegs on abdominal segments II-VII after the first instar (Nguyen et al., 2020), which is a distinctive feature typical of Nematinea larvae species that have seven prolegs pairs (Stehr, 1987). M. pruni was discovered more than two decades ago. Somehow, this species has been ignored in many recent reports of the plum sawflies pest in the country of origin (Liu et al., 2017). There has been a lack of information about the infestation status until a report of *M. pruni* in South Korea. The damage resulted in slow development and early drop-down of the fruits and it was so severe that there was no harvest in the reported orchard (Park et al., 2019). M. pruni is rapidly spreading throughout the country, especially in organicbased farming orchards (Park et al., 2019), thereby raising urgent requirements for management and prevention of the pest. However, until now, knowledge about M. pruni has only been reported for its morphology.

In order to gain more insight about the pest, our present study aims to determine the main biological characteristics of *M. pruni* in discussion with some *Hoplocampa* species. These results will form the basis for management of this devastating species.

## Materials and Methods

#### Insect source

*M. pruni* at all developmental stages were collected in a 7 - 8 years old organic Japanese plum orchard in Okgok-myeon, Gwangyang-si, Jeollanam-do, South Korea at an altitude of 160 m above sea level.

#### Determining the biology of Monocellicampa pruni

We conducted a series of investigations both in the field and

laboratory conditions to determine the biology of *M. pruni*. From early April 2019, and from end of February 2020 and 2021, two insect tent traps (l = 2 m; w = 1 m; h = 1 m) were placed under the plum canopy covering  $2 m^2$  of soil to observe the adult's emergence from the ground. The traps were checked every two days until the first emergence of sawfly was found inside. Subsequently, the daily checking frequency was adjusted until no adults appeared for five days. Sawflies collected from the trap were counted, sexed and brought back to the laboratory in Sunchon National University for the pair-mating experiment.

Adults of the opposite sex on the same emergence day were coupled in a 450-ml rectangular transparent plastic container with a total of 29 pairs being crossed. Four plum flowers with water-soaked cotton covering the stem were placed in the container for oviposition. Pollens (Cheonji Herbs, Seoul, Korea) were supplied as food and a water-soaked cotton pad was placed on the container's bottom to retain moisture. Flowers were changed every day and the number of oviposited eggs was counted under a Leica EZ4HD microscope (Leica Microsystems, Wetzlar, Germany). The insects were reared until death in an incubator (Sanyo Incubator - MIR253, Sanyo Electric Co. Ltd, Osaka, Japan) set at  $T = 20^{\circ}C \pm 2^{\circ}C$ , RH = 40 - 60% to determine longevity and fecundity.

As it was difficult to rear the insect under cultured conditions, the immature stages of the plum sawfly M. pruni were investigated in the orchard. Five plum branches were selected and covered by net bags (l = 150 cm; d = 50 cm) before the flowering period. From 23 - 24 May 2020 when plum trees were in full blossom, forty to fifty adult pairs of M. pruni were released within 24 h into each net bag for oviposition. Twenty flowers (at the earlier stage) or fruits (later stage) were collected every day from each net bag until the first appearance of the exit hole on the fruitlet was confirmed. Eggs and larvae were observed by dissecting the fruits (or flowers) under the Leica EZ4HD microscope (Leica Microsystems, Wetzlar, Germany). Larval instar was determined by counting the number of head capsules inside the fruit. The first appearance date of each developmental stage in net bags was recorded. Time between each stage-first-appearance was considered the developmental duration. In addition, a data logger Hobo Pro v2 (Onset, Bourne, MA, USA) was placed at a height of 1.5 m above the ground in the orchard to gather data on temperature and humidity with the measurements being set at hour intervals.

The site where *M. pruni* sawflies pupate in the soil was investigated by a field experiment. Fifteen water draining PVC pipes (1 = 40; d = 10 cm) were vertically inserted into the soil to a depth of at least 30 cm. In early May 2019, thirty infested fruits with no identified exit holes were picked by hand and placed inside each pipe to allow the larva to escape and burrow into the soil for hibernation. All pipes were kept under field conditions until the spring of the following year 2020. From end February to early March 2020, the pipes were removed and taken back to the laboratory for further investigation. The pipes were opened using a cutting machine (Falcon - FD5016D, Hyunsung tools, Seoul, Korea) to access the soil column inside. Each soil column was then horizontally cut into slices of 1 cm. The soil slices of different depths were transferred to different zipper bags ( $20 \times 30$  cm) and labelled. The zipper bags were kept under room conditions (T =  $22^{\circ}C \pm 2^{\circ}C$ ). The number of adults that emerged from each zipper bag was counted to obtain data on the depth that sawfly pupated in the soil.

Data analysis

Daily temperature and humidity were calculated, based on the average hourly data gained from the logger equipment. The difference in longevity between male and female adults was determined by two samples t-test after checking the homogeneity of variances. Hypothesis tests were performed using Minitab software v.18.1 (Minitab LLC, State College, PA, USA). Charts were drawn using Microsoft Excel 2016 (Microsoft Corp., Redmond, DC, USA).

## Results

#### The adults

The sawfly adults start emerging in spring around mid- or end-March after a long hibernation period underground. Their emergence usually coincides with the flowering stage of plum in which they deposit eggs and occurs within about two weeks until the flower petals fall off (Fig. 1). Male and female longevity was 6.03 and 7.55 days, respectively, under laboratory rearing conditions. The longest lifespan was recorded at 12 days for females and 11 days for males. Females lived slightly longer than males (df = 56; t-statistic = 2.55; p-value = 0.014, Table 1).

#### Oviposition and the eggs

*M. pruni* started laying eggs about 1.5 days after emergence with an average of 30.29 eggs per female (Table 1). Notably,



**Fig. 1.** Cumulative spring emergence of *Monocellicampa pruni* in relationship with vegetation development of Japanese plum (Gwangyang-si, Korea, 2019 – 2021).

Table 1. Longevity and fecundity of adult plum sawfly Monocellicampa pruni reared in laboratory conditions (T = 20°C, RH: 40 - 60%)

Parameters	n	Mean ( $\pm$ SE)	Max	Min
Male longevity (days)	29	$6.03\pm0.40$	11	1
Female longevity (days)	29	$7.55\pm0.45*$	12	2
Pre-oviposition (days)	28	$1.54\pm0.16$	5	1
Fecundity (eggs/female)	28	$30.29\pm4.50$	86	2

(\*) indicates mean longevity between male and female are significantly different (df = 56, T-stat = 2.55, p-value = 0.014).



Fig. 2. Mean (± SE) number of eggs laid per day by a female and oviposition duration of plum sawfly Monocellicampa pruni.

Table 2. Appearance of immature stages of Monocellicampa pruniafter oviposition in field conditions (Gwangyang-si, Korea, 2020)

Stage	First appearance in net bag no.				Duration to next	Weather condition		
	1	2	3	4	5	first moulting	weather condition	
Egg (*)	23-Mar	23-Mar	23-Mar	24-Mar	24-Mar	10 - 11		
1st instar	02-Apr	02-Apr	02-Apr	03-Apr	04-Apr	8 - 9		RH (%) Mean: 60.08 Max: 97.90 Min: 32.07
2nd instar	10-Apr	11-Apr	11-Apr	11-Apr	13-Apr	5 - 6	T (°C) RH (%	
3rd instar	15-Apr	16-Apr	17-Apr	17-Apr	18-Apr	8 - 10	Mean: 11.89 Mean: 60 Max: 19.72 Max: 97	
4th instar	23-Apr	24-Apr	25-Apr	27-Apr	27-Apr	6 - 7	Min: 7.74 Min: 32.	
5th instar	30-Apr	01-May	02-May	04-May	03-May	3 - 4		
Exit hole	03-May	05-May	06-May	07-May	06-May	-		
Days from 1 <sup>st</sup> instar to exit hole					31 - 34			
Days from oviposition to exit hole						41 - 44		
		0 2	4 6 8	10 12	14 16 18	20 22 24 26		



Fig. 3. Percentage of plum sawfly Monocellicampa pruni pupated at different depths under the soil surface.

the highest number of eggs laid by a female reached 86 eggs. The oviposition duration of the female was over a period of up to 11 days. Eggs were mostly laid on the first five days after the emergence of adults with an average of 3.4 - 5.3 eggs/female/ day (Fig. 2).

The developmental duration of eggs was about 10-11 days in the field (Table 2).

#### The larvae

In the field condition, the first instar was recorded 10 - 11 days after oviposition. The second, third, fourth and fifth instars appear 8 - 9, 5 - 6, 8 - 10 and 6 - 7 days, respectively, after its relative previous stages. The larvae spend about 31 - 34 days developing mostly inside the fruitlet before escaping to spin their cocoons in the soil (Table 2).

#### Pupation site

The experiment involving the investigation of the pupation's depth indicated that the cocoons could be found at depths from 2 to 11 cm under the soil surface, with 89% of them pupated at 3 - 8 cm (Fig. 3).

#### Discussion

The life history of *M. pruni* here is similar to its relative Hoplocampa species. As a univoltine species, they also appear in a short period, damaging plum at the flowering and fruitsetting stages, then spend almost of their time hibernating under the ground. Due to the late installation of traps, the adult's first appearance was not known until the year 2019, but the last emergence of adults was still recorded at the petal-fall stage (Fig. 1, arrow). Additional observations in 2020 and 2021, comprising the whole emergence process of M. pruni, also revealed that adults waking in the spring coincided with the opening of blossoms, which occurred within a short period of 12 - 13 days (Fig. 1). Soon after crawling up from the soil, adults begin their flight activities and seeking mates. They are black, 4.5 - 6.5 mm in length. Females are usually larger than males and easy to distinguish by their roundish abdomen and an exposed saw-like ovipositor (Fig. 4). They are quite active on clear and sunny days, making it possible to observe their flight movement between flowers by shaking or tapping on the canopy. Adults were observed consuming flower stamens and pollens both in the field and laboratory.

Biological characteristics of adults M. pruni observed in the laboratory also reflected their features in the field. The relativelyshort lifespan observed under cultured rearing conditions supports the adult's brief introduction in the orchard. Our investigation indicated that M. pruni adults appeared for about 24 days in the field. This is quite similar to some species of the genus Hoplocampa, such as the apple sawfly H. testudinea presented within 2 - 3 weeks (Graf et al., 1996) or plum sawfly species H. minuta and H. flava needed 6 - 17 days to reach 75% cumulative flight (Tamosiunas et al., 2014) in the field. More works still need to be done to gain more insight into this species. The 20°C condition applied in our study here was significantly higher than the field conditions, which probably led to the adults' short lifespan. In addition, the limitation of flowers supplied for oviposition might also affect the egglaying activities of M. pruni. Further studies on temperaturedependent development or different host plants should be undertaken to address these issues.

While laying eggs, the female faces down to the stem of the flower, uses her hind legs to grasp the calyx and deposit into the cuticle layer (Fig. 5A). One (rarely two) egg(s) was found on each flower in field conditions (Fig. 5B). However, there were 13 eggs recorded in a single flower in the cultured rearing condition (Fig. 5C). Eggs are elongated (at oviposition), about 0.5 mm in length and transparent white (Fig. 5D). They shrink to an oval shape with evidence of two red spots of ocelli and a head capsule in preparation for hatching. It is possible to



Fig. 4. Adults of plum sawfly *Monocellicampa pruni*. A- female (left). B- male (right).



**Fig. 5.** Oviposition and eggs of plum sawfly *Monocellicampa pruni*. A - female depositing egg on calyx of the flower. B - egg laid under the cuticle layer (circle). C - a cluster of eggs laid under laboratory conditions. D - egg elongated at oviposition. E - egg shrinks to an oval shape in preparation for hatching with visible red ocelli.



Fig. 6. Larva of *Monocellicampa pruni*. A - First instar burrowing into the fruitlet; B - damaged (with entrance hole in circle, lower) and undamaged fruit (upper); C - five larval instars of *Monocellicampa pruni*; D - larva leave a hole on fruitlet after escape.

observe the movement of the larva inside the egg under a microscope (Fig. 5E);

On hatching, larvae are about 1 mm in length and soon start entering the developing fruitlet, which was still covered by the withering calyx at this time (Fig. 6A). After that, the entrance hole is filled up due to the fruit's growth and remains only as a small sting. The infestation leads to slow fruit development and subsequent early drop-down of fruit (Fig. 6B). There are five larval instars of *M. pruni*, which can be determined by counting the number of head capsules inside the fruit (Fig. 6C).

The larva consumes the endocarp part of the fruitlet and remains inside the invaded fruitlet until reaching mature. The condition of one exit hole indicates that one larva of M. pruni causes damage to one fruitlet only. At full growth, the last instar larva (which measures about 11 mm at this time), creates a hole (Fig. 6D) to escape from the fruit and enter the soil for hibernation, where they remain inside until the following spring as the larva form; The developmental duration of M. pruni larvae here was quite similar to H. flava which was about 33 days (Petherbridge et al., 1933). However, its biology associated with plum noticeably differs from other species of Hoplocampa. The larva of M. pruni infests only one plum fruitlet. In contrast, plum sawfly H. flava (Petherbridge et al., 1933), H. minuta (AgroAtlas, 2009) or apple sawfly H. testudinea (Miles, 1932) usually need several (3 - 6) fruits for their development. This might be the reason why the reduction of prolegs occurs in *M. pruni* since the insect does not migrate to other fruits.

Like other plum sawflies, *M. pruni* overwinter in a cocoon under the soil. Cocoons are oval, made of smooth brown solid filaments, about 6 - 6.2 mm in length and 3 - 3.2 mm in width. Due to the secretion from the spinning process, the cocoon's outer surface is formed by the particle texture of the soil, giving it a similar appearance to soil colour and making it extremely difficult to detect (Fig. 7). However, there are usually sparkling sand particles adhering to the outer surface, thereby making it like a small rough-coated stone. It is unclear whether different soil types or soil cover materials have any impact on migration of *M. pruni* into the ground or not. However, these findings still contribute significant academic information for further study management and control of *M. pruni*. Some sawfly species emergence in the spring is driven by soil temperature



Fig. 7. Cocoon of *Monocellicampa pruni*. A - cocoons appear like rough-coated stones; B - fifth instar larva living inside the cocoon (photo taken in October); C - a moulting pupa preparing for emergence (photo taken in March).

(Graf et al., 1996; Tamosiunas et al., 2014). Thus, the results here suggest the location of the site where we should place thermal measurement equipment to gain more reliable data or the application of bio-products and chemicals. Besides, concerns regarding the overwintering behaviour of *M. pruni* are still needed to be clarified, as some sawflies species can remain in the soil for several years (Hadzistevic, 1959). This information is not yet known for *M. pruni*.

Previous studies proved that environmentally-friendly approaches could be applied to control the plum sawflies species of *H. minuta* and *H. flava*. The timing of the control application is always the key factor for success. Until now, no programme has been proposed for control of M. pruni. However, according to observations in the field, control measures for the pest should be implemented prior to the larva's entrance into fruitlets. The duration from egg to larva hatch is probably the ideal time for action. Treatment, at around the time of petal falling, appears suitable in case of M. pruni here and this appears to have no negative impact on pollination. Applications of the entomopathogenic nematode are recommended for soil treatment, but it should be done before the sawfly settle inside their cocoons (Nježić and Ehlers, 2020). The developmental duration of larva, indicated in this study, could help optimising the effectiveness of this application.

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# Statements for Authorship position & contribution

- Nguyen, N.H.: Sunchon National University, researcher; carried out the survey, analysed data, and wrote the manuscript.
- Ki, W.: Sunchon National University, researcher; field survey, data collecting.
- Im, M.-H.: Bio-Plant Environment Research Center, researcher; field survey, data collecting.

Hong, K.J.: Sunchon National University, professor; designed the research and revised the manuscript.

All authors have read and approved the manuscript.

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