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Daily Rhythm, Factors Controlling Oviposition Behavior and Dispersal of Reproducing Adults of the Leaf Beetle *Gastrophysa atrocyanea* (Coleoptera: Chrysomelidae)

Seiji Tanaka

Matsushiro, Tsukuba, Ibaraki 305-0035, Japan
* Correspondence author: stanaka117@yahoo.co.jp

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ABSTRACT

Leaf beetles (*Gastrophysa atrocyanea*) lay eggs on the leaves of dock plants. The study aimed to investigate the environmental factors influencing their oviposition behavior. The beetles were observed laying eggs continuously during both the light and dark periods at 25°C, indicating their around-the-clock oviposition activity. When leaves were glued to the ceiling of Petri dishes, adults deposited egg masses on the front and undersides at similar rates, suggesting that the adults did not discriminate between the two sides of the leaf for oviposition. However, when the beetles and dock plants were placed in a box covered with a blackout curtain and illuminated from the floor, most of the egg masses were found on the undersides of the leaves. This pattern resembled the observations made under semi-outdoor conditions, indicating a strong preference for laying eggs on the undersides of leaves. Nevertheless, the oviposition behavior was not influenced by the direction of light or differences in light intensity between the two sides of the leaves. In late March, when numerous larvae were growing on dock plants, it was noted that most adults were found on plants without larvae. Conversely, most dock plants infested by larvae were without adults. These findings suggest the possibility that adults dispersed in response to cues associated with larvae or severely defoliated plants.

Key words: daily rhythm, egg mass, oviposition behavior, dispersal

Introduction

Overwintered leaf beetles (*Gastrophysa atrocyanea* Motschulsky) deposit eggs in masses on the leaves of dock plants belonging to the Polygonaceae family (Miyazaki, 1979; Naito *et al.,* 1979a; Lee, 2022). In central Japan, numerous egg masses are observed on the leaves of dock plants, such as the Japanese dock plant (*Rumex japonicus*) and the bitter dock plant (*R. obtusifolius*), in spring. In a previous study (Tanaka, 2023), it was documented that *G. atrocyanea* females deposited 1 to more than 10 egg masses per dock plant leaf and that more egg masses were observed on larger host plants in the field. However, female adults did not have a preference for a particular size of host plant or leaf as the oviposition site, although they showed a preference for the underside of leaves.

To further understand the oviposition behavior of this beetle, this study aimed to observe the oviposition behavior directly through several experiments. The objectives were to determine the time of day when adults lay eggs, how and why they deposit eggs on the undersides of leaves, and whether the dispersal of reproducing adults is related to the appearance of larvae on their current host plant.

Some insects lay eggs at a particular time of day, although the time of oviposition may vary depending on the species (Saunders *et al.*, 2002). For example, mosquitoes exhibit diverse temporal patterns of oviposition, some being diurnal and others nocturnal (Brrozo *et al.,* 2004). Some crickets predominantly lay eggs during the light phase (Loher, 1979). In hemipteran insects, oviposition is controlled by circadian rhythms that persist even in constant dark conditions after a transfer from light/dark cycles (Numata and Matsui, 1988; Ampleford and Davey, 1989). Although the mechanisms underlying these circadian rhythms have been analyzed in some insects, the adaptive significance of these rhythms is not well understood. Gathering information from such observations is important for understanding the oviposition behavior and patterns of egg deposition on host plants. In the present study, the daily oviposition activity of *G. atrocyanea* was examined at room temperature and in an incubator with constant temperature and light/dark cycles.

In leaf beetles, including *G. atrocyanea*, eggs are often deposited on the undersides of host plant leaves (Oliveria and Solomon, 2004; Eaton, 2016; Gibb and Sadof, 2017; Tanaka, 2023). Understanding the mechanism that governs oviposition behavior on the host plant is a crucial aspect of research in this field. Insects employ visual, chemical, and tactile cues to orient themselves, locate suitable host plants, and recognize them as potential oviposition substrates (Finch and Collier, 2000; Schowalter, 2011; Day, 2016; Cury *et al.,* 2019). For example, in the Colorado potato beetle (*Leptinotarsa decemlineata* Say), a pest of domesticated potato (*Solanum tuberosum* L.) plants, adults use visual and olfactory cues from a distance (De Wilde, 1958; Dickens, 2001; Sablon *et al.,* 2013). Once they are in close proximity, they are attracted to volatile compounds from the host plants, locate the host plant, and accept it as a food and oviposition substrate if they perceive appropriate chemical stimuli with their mouthpart sensillae (Visser and Nielsen, 1977; Mitchell and McCashin, 1994). However, corresponding information for *G. atrocyanea* is currently lacking. In this study, experiments were conducted to determine how adult beetles discriminate between the front and undersides of host plant leaves and identify the important stimuli for selecting the oviposition site.

G. atrocyanea adults reach their host plants by flying or walking for development and reproduction (Naito *et al.,* 1979b) and sometimes move from one dock plant to another during oviposition (Suzuki, 1985). This movement appears to be adaptive, allowing them to avoid overcrowding and efficiently utilize unexploited resources. Once female adults start reproducing, their abdomen swells, and flight activity is strongly inhibited (Naito *et al.*, 1979b; Miyazaki and Naito, 1981; Suzuki, 1985). However, they are capable of walking relatively fast (Naito *et al.,* 1979b). Nevertheless, dispersal by walking in the field is likely less efficient due to obstacles such as the leaves of other plants that the beetles must traverse (Miyazaki and Naito, 1981). Despite these challenges, some adults appear to disperse to new dock plants and successfully lay eggs. The final subject examined in this study is related to the dispersal of reproducing adults. Although adults are abundant and actively reproducing in March, their numbers on each plant ranged from 9 to 19 in mid-March (Tanaka, 2023), suggesting that overcrowding of adults on plants may not cause their dispersal. Multiple depositions of egg masses on leaves and plants are common, with as many as 157 egg masses observed on a single dock plant (Tanaka, 2023). Therefore, it seems unlikely that adults leave the current oviposition site in response to the presence of too many egg masses. During the collection of adults in late March for other experiments, it was noticed that adults were more commonly found on dock plants without larvae than on those with larvae. Based on this observation, it was speculated that adults might leave for a new host plant in response to some cue from the larvae. A preliminary census was conducted to verify this speculation by checking the presence of adults and larvae on dock plants. This paper describes the results of these observations and discusses the control mechanism of oviposition behavior in *G. atrocyanea*.

Materials and Methods

Insects

G. atrocyanea beetles were collected from leaves of *R. japonicus* and *R. obtusifolius* plants along two unpaved roads (each measuring 4 x 80 m) and grasslands adjacent to the Hasunuma River in Tsukuba, Ibaraki, Japan (36.1°N, 140.1°E) during March and April 2023. These areas covered an approximate total area of 500 m^2. The mean daily air temperature in Tsukuba during March and April 2023 was 13.2°C, ranging from 5.5 to 20.8°C. The daily minimum and maximum temperatures recorded were -2.4°C and 27.3°C, respectively, according to the Japan Meteorological Agency (2023).

Daily oviposition activity

To investigate the daily rhythms of oviposition activity in *G. atrocyanea*, five groups of 10 female and 10 male adults collected in the field on March 31 were held in plastic containers (14 cm in diameter, 7 cm in height) with two pieces of dock plant leaves. The containers were covered with a lid with a screen window and kept in an unheated indoor room (semi-natural conditions), which was slightly warmer than the outdoor temperature during the night due to its connection to an adjacent heated room. During the day, the room was heated by direct sunlight entering through a large window. The containers were positioned 1 m away from the window, ensuring that they did not receive direct sunlight but still experienced semi-natural light/dark cycles. Sunrise and sunset on April 1 were at 05:26 and 18:01, respectively. The temperature and light intensity (lux) near one of the containers were recorded hourly using a TR-74Ui light meter (T&D, Nagano, Japan). The average air temperature during the experimental period (April 1-8) was 19.9°C, with a maximum mean of 21.5°C and a minimum of 16.2°C. The corresponding temperature figures for Tsukuba, as reported by the Japan Meteorological Agency (2023), were 14.2°C (maximum mean = 19.9°C; minimum mean = 8.8°C). Egg masses on the leaves were collected at 07:30 and 18:30 each day, and fresh leaves were provided. The number of egg masses and eggs was recorded. When adult beetles died, they were replaced with live individuals reared in an extra container in the same room. Since the egg masses were collected at 07:30 and 18:30 each day, the number of egg masses and eggs deposited during the 13-hour period, including the 'nighttime' (nighttime period), and the 11-hour period, including the 'daytime' (daytime period), were compared on an hourly basis. In this study, an egg mass was defined as a cluster of 5 or more eggs deposited in contact with one another.

A similar experiment was carried out at 25 ± 1°C and LD 12:12 h (light on at 08:00 and light off at 20:00) in an incubator (CN-40A; Mitsubishi Electric Engineering Co, Tokyo, Japan) from April 1 to April 7. In this experiment, 3 groups of 10 female adults collected in the field on March 31 were prepared. Leaves with eggs were removed from the containers shortly before and after the dark period each day, and fresh leaves were supplied. The numbers of both egg masses and eggs per egg mass during the light and dark periods were recorded for each container. Dead adults were replaced by live ones reared in an extra container in the same incubator.

Factors causing adults to lay eggs on the undersides of leaves

Three experiments were conducted to investigate the factors influencing the preference of *G. atrocyanea* adults for laying eggs on the undersides of leaves. In Experiment 1, leaf discs (6 cm in diameter) were cut in half, and two halves of a leaf disc were glued to the center of the bottom of a 9-cm plastic Petri dish using double-sided tape. One half was glued with the underside facing up and the other half with the front side facing up. A female and a male beetle were introduced into the dish, covered with a lid and then placed upside down in a plastic tray (35 × 28 × 11 cm). The tray, containing 10 dishes prepared similarly, was placed in the unheated room mentioned earlier. The dishes were inspected daily, and the locations where egg masses were deposited were recorded. Fresh leaves were provided daily, and the mean temperature in the room during the observation period (April 4-7) was 19.9°C.

Experiment 2 was similar to Experiment 1, except that all dishes containing insects were placed in a light-proof cardboard box (38 × 30 × 21 cm) to assess the role of visual stimuli in selecting the oviposition site. The experiment was conducted at room temperature, and the mean temperature during the 7-day period (March 24-30) was 17.8°C. In both Experiments 1 and 2, all egg masses were deposited on the ceiling of the dishes.

In Experiment 3, to investigate the influence of the placement of leaf discs, two halves of a leaf disc were glued to both the ceiling and floor of the plastic dishes. Each dish contained two adult females, and 12 dishes were incubated under LD 12:12 h at 25°C. The experiment lasted 6 days (March 24-29), following the same procedure described above.

Overall, these experiments aimed to investigate the influence of the leaf side, visual stimuli, and the placement of leaf discs on the oviposition behavior of *G. atrocyanea* adults.

Effect of direction of light on oviposition behavior

A previous study by Tanaka (2023) confirmed that the majority of egg masses of *G. atrocyanea* were found on the undersides of leaves in the field. This observation led to the hypothesis that the beetles might use sunlight direction or differences in light intensity between the two sides of leaves to select their oviposition site. To test this hypothesis, 50 adult females and a potted bitter dock plant with 25 leaves were placed inside a transparent plastic box (31 × 29 × 40 cm). The box was covered with a blackout curtain, except for the floor, and illuminated from underneath the floor continuously with a 15-W fluorescent lamp in an unheated room. Light intensity measurements using a light meter revealed that the front side of the leaves had an average intensity of 15.5 lux (range: 7-23 lux), while the underside had an average intensity of 391.0 lux (range: 97-970 lux). This significant difference (t = -4.49; df = 9; p < 0.01) indicated that the underside of the leaves was much brighter than the front side. As a control, a transparent plastic box containing a dock plant with 21 leaves and 30 female adults was prepared and placed in the same unheated room, 1 m away from the window. This box was exposed to light coming through the window during the daytime but received no direct sunlight. Light intensity measurements on the front side and underside of 10 leaves at 13:00 on March 23 showed that the front side had an average intensity of 436.6 lux (range: 113-919 lux), while the underside had an average intensity of 229.1 lux (range: 12-533 lux). This difference (t = 2.33; df = 14; p < 0.05) indicated that the front side of the leaves was brighter than the underside. The number of egg masses deposited on the front side and undersides of the leaves over 2 days was recorded. The experimental period lasted from March 22 to March 24; the mean temperature was 20.9°C. These experiments aimed to investigate the potential influence of sunlight direction and differences in light intensity on the oviposition behavior of *G. atrocyanea* adults.

Dispersal of ovipositing adults in relation to the presence of larvae

A field census was conducted to examine the possibility that adults left for new host plants in response to the presence of larvae. In mid-March, numerous egg masses were observed (Tanaka 2023), and larvae at the second or third (last) instar were commonly found on dock plants by late March. On March 30, 2023, 566 dock plants of the species mentioned above were examined at the study sites and adjacent areas. The presence of adults and larvae on each plant was recorded, while the observed egg masses were disregarded. The plants were categorized into four types: plants with both adults and larvae, plants with neither adults nor larvae, plants with only adult(s), and plants with only larvae. In this census, the sex and number of insects were not recorded, so no distinction was made between plants with a single adult and those with more than 10 adults.

Statistical analyses

A t-test was employed to compare the light intensities and the numbers of eggs and egg

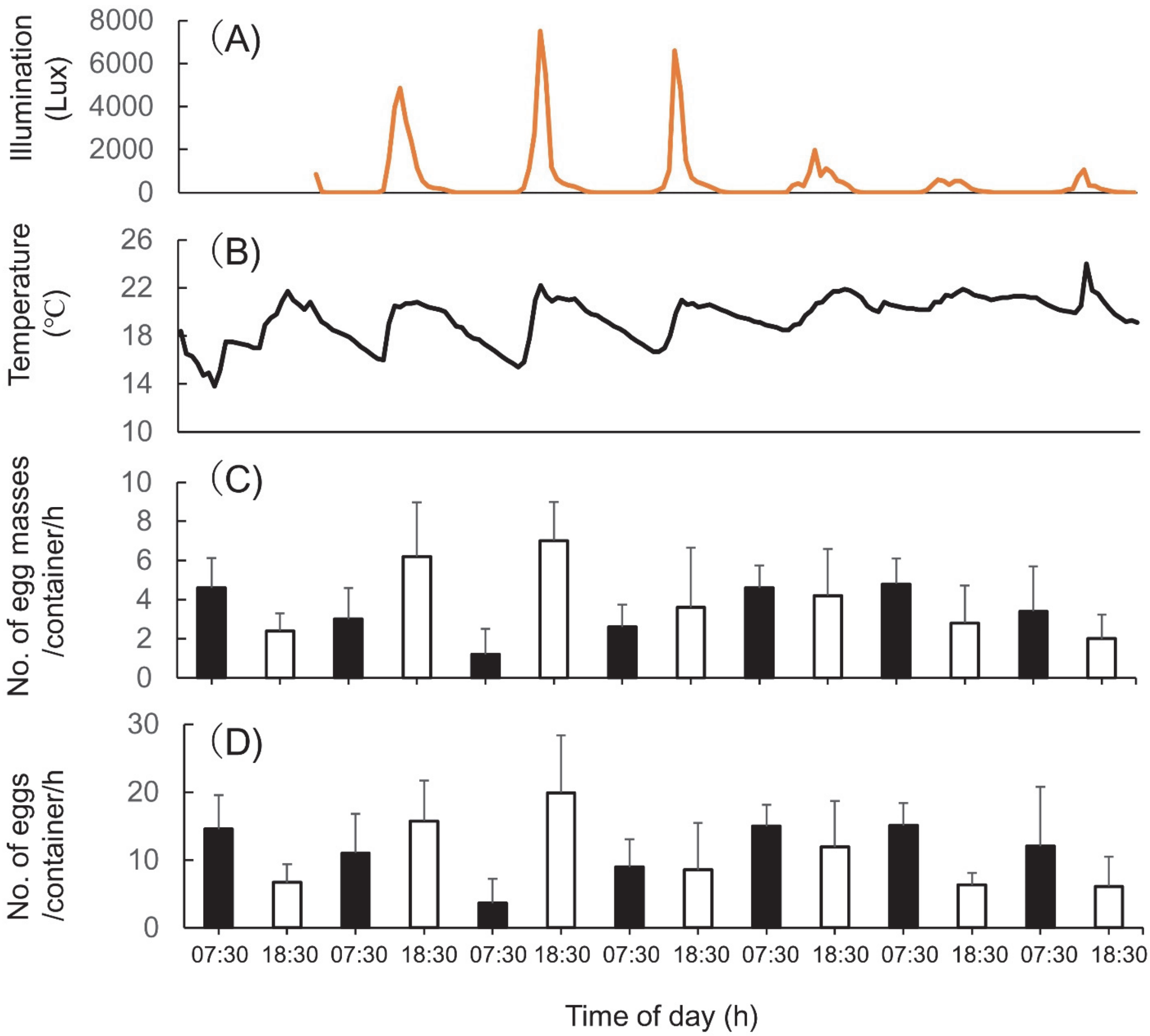

Fig. 1. Daily oviposition activity of *G. atrocyanea* under semi-natural light conditions in an unheated room. Light intensity (A). Temperature (B). Numbers of egg masses (C) and eggs (D) deposited per container per hour during the daytime (07:30-18:30) and the nighttime periods (18:30-07:30). Ten female adults were housed in each of 5 containers. Bars indicate standard deviation (SD). Closed and open histograms indicate the mean values during the nighttime period and those during the daytime periods, respectively.

masses. In certain experiments, the numbers of egg masses in different treatments were assessed for unity using a $\chi 2$-test. These statistical analyses were conducted using Descriptive Statistics (Excel, Microsoft Office 365) or StatView (SAS Institute Inc., NC, USA). Significance was determined at $P < 0.05$.

Results

Daily oviposition activity

The light intensity and temperature in an unheated room exhibited daily fluctuations (Fig. 1A, B). A total of 262 egg masses and 9,296 eggs were collected from five containers, each containing 10 female and 10 male adults of *G. atrocyanea*, during a 7-day observation period. Egg masses and eggs were continuously deposited during both daytime (07:30-18:30) and nighttime (18:30-07:30) periods (Fig. 1C, D). There was no significant difference in the mean number of egg masses (number of egg masses per container per hour; $t = 0.12$; $df = 12$; $P = 0.91$; Fig. 2A) or eggs (Fig. 2B; $t = 0.05$; $df = 9$; $P = 0.96$) between the daytime and nighttime periods. A similar conclusion was reached under LD 12:12 h and 25°C conditions, where 162 egg masses and 6,087 eggs were obtained from three

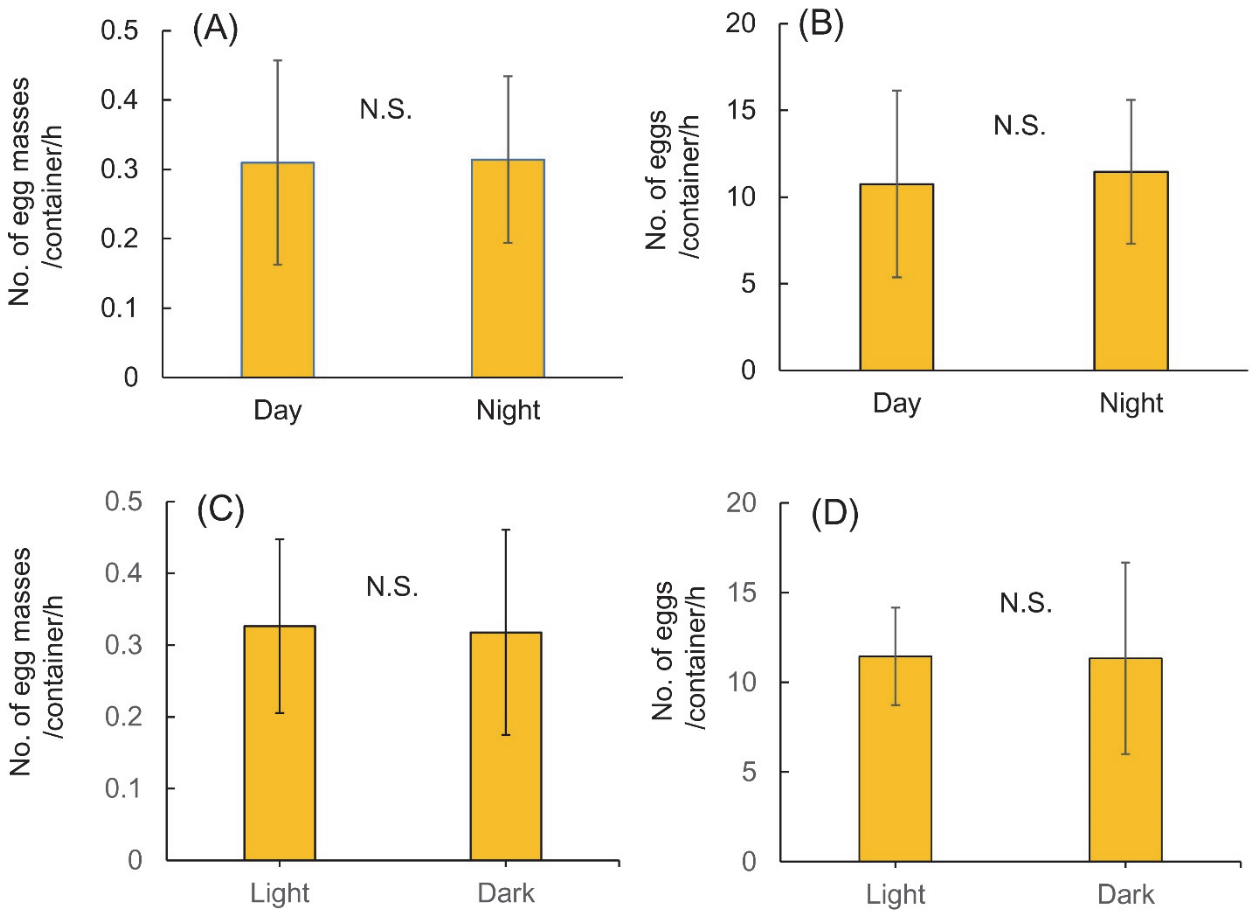

Fig. 2. Comparison of mean numbers of egg masses (A, C) and eggs (B, D) laid by *G. atrocyanea* under semi-natural light conditions (A, B) and LD 12:12 h (C, D). The ordinate axis indicates the number of egg masses or eggs per container per hour during the day and night periods in an unheated room and during the light and dark periods at 25°C. Five and three containers, each housing 10 female adults, were used in experiments under semi-natural light conditions and LD 12:12 h, respectively.

containers. Egg masses and eggs were continuously deposited during the light (08:00-20:00) and dark (20:00-08:00) periods throughout the observation period (Fig. 3). There was no significant difference between the light and dark periods in the mean number of egg masses (Fig. 2C) or eggs (Fig. 2D). These findings do not provide any evidence for the presence of daily rhythms in the oviposition activity of *G. atrocyanea*.

What causes adult insects to lay eggs on the undersides of leaves?

Experiment 1 determined which side of leaves the adults of *G. atrocyanea* deposited their egg masses by gluing two halves of a leaf to the ceiling of a Petri dish, as depicted in Fig. 4A. The results showed that egg masses were present on both the front (FL) and undersides (UL) of leaves, and there was no significant difference in the total number of egg masses between the two sides ($\chi2$ = 0.01; df = 1; P > 0.05; Fig. 4B), indicating that the beetles did not discriminate the two sides of leaves. However, the number of egg masses directly deposited on the plastic surface (DS) of the ceiling was significantly smaller compared to FL ($\chi2$ = 4.31; df = 1; P < 0.05; Fig. 4B), UL ($\chi2$ = 3.91; df = 1; P < 0.05), or the combined number of egg masses on both sides ($\chi2$ = 14.0; df = 1; P < 0.05).

In Experiment 2, the same procedure was followed, but the adults were kept in a light-proof box within the same room. The results were essentially similar (Fig. 4C): no significant difference was observed in the number of egg masses between the two sides of the leaf ($\chi2$ = 0.02; df = 1; P > 0.05). However, the number of egg masses deposited on the plastic surface (DS) was significantly smaller than that on FL ($\chi2$ = 5.21; df = 1; P < 0.05), UL ($\chi2$ = 5.90; df = 1; P <

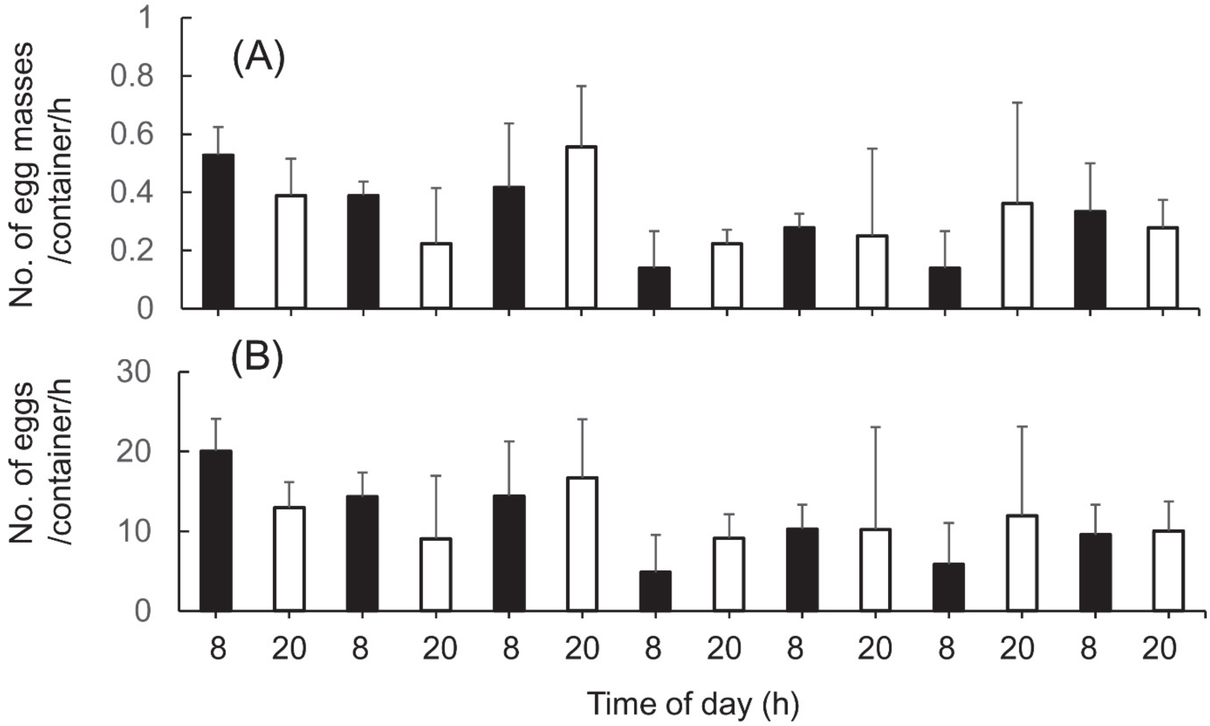

Fig. 3. Daily oviposition activity of *G. atrocyanea* at LD 12:12 h and 25 °C. Number of egg masses (A) and eggs (B) deposited per container per hour during the light period (08:00-20:00) and dark period (20:00-08:00). Three containers, each housing ten female adults, were used. Bars indicate standard deviation (SD). Closed and open histograms indicate the mean values during the dark period and those during the light periods, respectively.

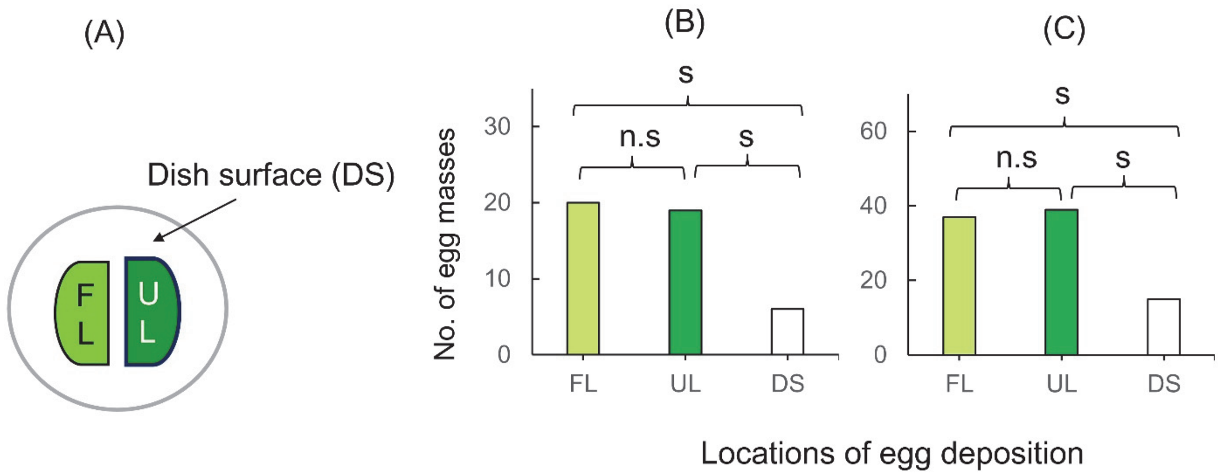

Fig. 4. Number of egg masses laid by *G. atrocyanea* on leaves glued to the ceiling of dishes (A). Numbers of egg masses laid on the front sides of leaves (FL), undersides of leaves (UL), and dish surfaces (DS) in light/dark cycles (B) and in darkness (C) in an unheated room. 's' indicates the proportions significantly deviating from unity with a $\chi2$-test at P< 0.05; 'n.s.' indicates not significant.

0.05), or the combined number of egg masses on both sides ($\chi2$ = 23.0; df = 1; P < 0.05). These results suggest that the adults preferred leaves over the plastic surface, but they did not exhibit a preference for the undersides of leaves, and this oviposition behavior was unaffected by the presence or absence of light.

In Experiment 3, two halves of leaf discs were glued to both the ceiling and floor of dishes, and the adults were introduced into the dishes at 25°C and a light-dark cycle of 12:12h. The objective was to determine if the adults would deposit eggs only on the ceiling. The results showed that 24 egg masses were deposited on the ceiling and 10 on the floor. However, this difference was not statistically significant ($\chi2$ =

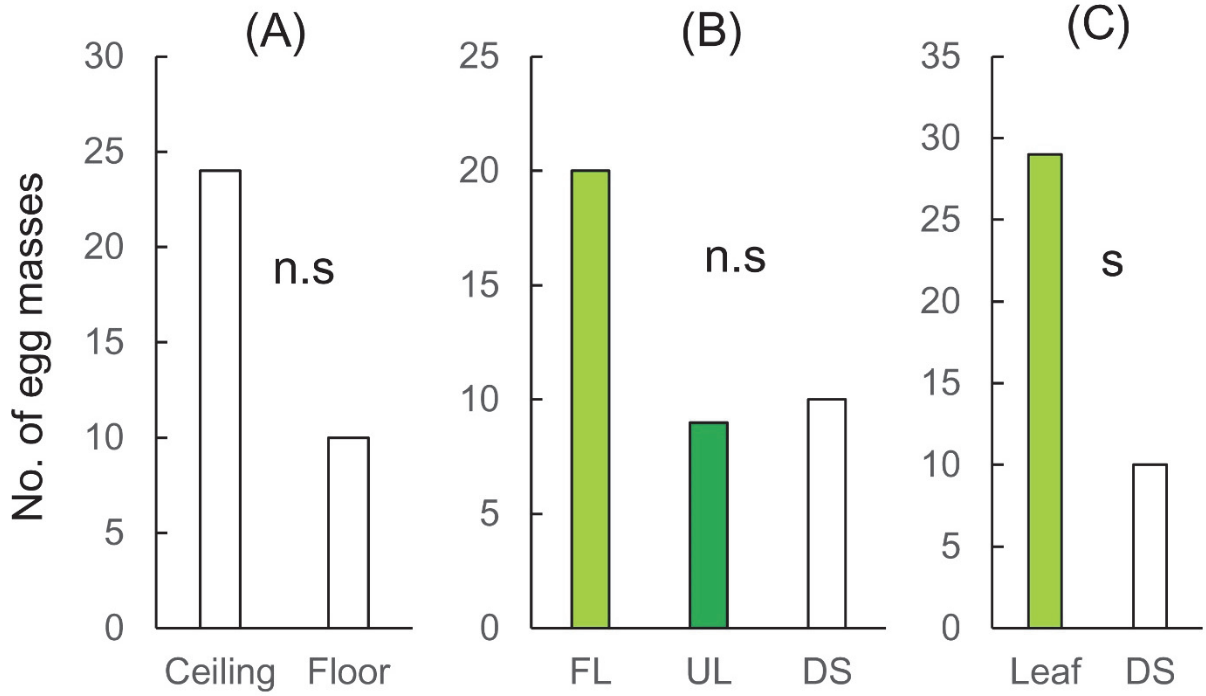

Fig. 5. Oviposition activity of *G. atrocyanea* when dock leaves were glued to the ceiling and floor of dishes. (A) Numbers of egg masses laid on the ceiling and floor. (B) Numbers of egg masses laid on the front sides of leaves (FL), undersides of leaves (UL), and dish surfaces (DS). (C) Numbers of egg masses laid on leaves and DS. 's' indicates the proportions significantly deviating from unity with a $\chi 2$-test at P < 0.05; 'n.s.' indicates not significant.

3.00; df = 1; P > 0.05; Fig. 5A), likely due to the small sample size. There was no significant difference in the total number of egg masses between FL and UL or between DS and FL or UL ($\chi 2$-test; P > 0.05 each; Fig. 5B). Conversely, the total number of egg masses laid on the combined FL and UL was significantly larger than that on the DS of the combined ceiling, floor, and side areas of the dishes. This difference became more pronounced when considering the difference in area ($\chi 2$ = 4.92; df = 2; P < 0.05; Fig. 5C) between the leaf discs (56.5 cm^2) and the DS (127.2 cm^2), suggesting that female adults exhibited a strong preference for leaves as the substrate for oviposition.

Effect of direction of light on oviposition behavior

In an experiment where female adults and a potted dock plant were placed in a box covered with a blackout curtain, and the light was directed onto the plant through the floor (Fig. 6A), the majority of egg masses (93.3%) were deposited on the undersides of leaves, while only a few egg masses (6.7%) were found on the front sides ($\chi 2$ = 27.7; df = 1; P < 0.05; Fig. 6B). These egg masses were observed on 11 green leaves, while no egg mass was found on 5 green leaves and 9 wilted leaves that had turned yellow or brown. As mentioned earlier, the light intensity on the undersides of leaves was significantly higher than that on the front sides, indicating that the beetles did not choose the darker surface as their preferred oviposition site. In the control group, female adults were kept with a potted dock plant in a transparent box without a blackout curtain cover, receiving daylight through the large window of the room. As previously described, the light intensity measured during the daytime was higher on the front sides than on the undersides of leaves. The control beetles exclusively laid their egg masses on the undersides of 10 green leaves (Fig. 6C). No egg mass was found on 5 green leaves and 6 wilted leaves. These results suggest that the beetles did not rely on the differences in light intensity between the two sides of leaves to determine their oviposition site and that they appeared to avoid wilted leaves.

Dispersal of ovipositing adults in relation to the presence of larvae

Out of the 566 dock plants examined, 504 (89.0%) were found to be infested with *G.*

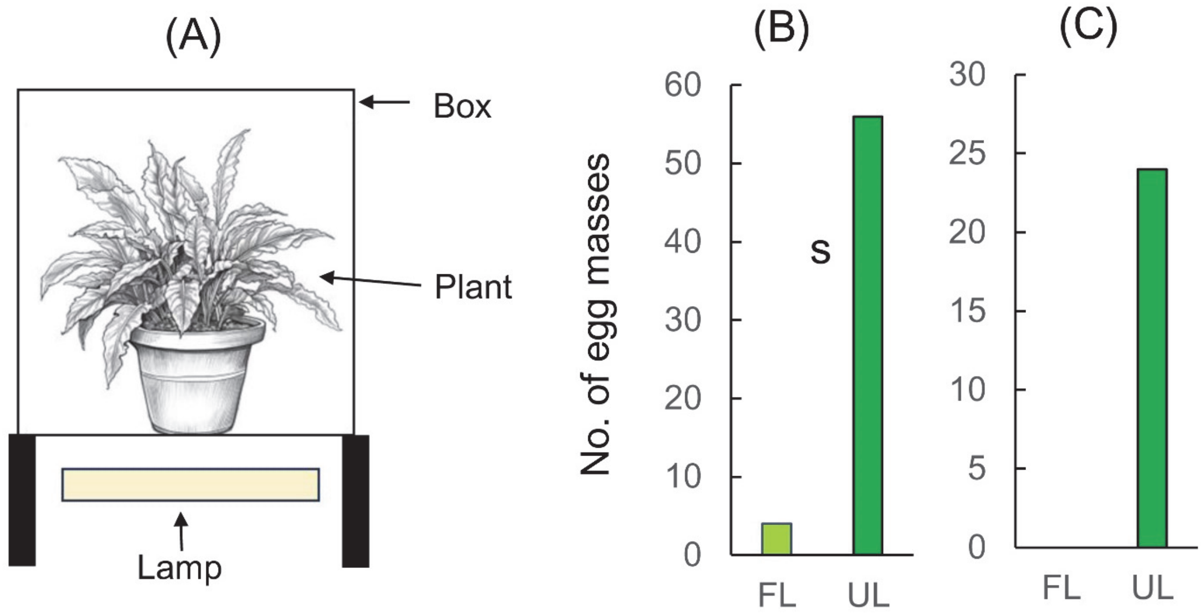

Fig. 6. Oviposition activity of *G. atrocyanea* when the adults and a dock plant were illuminated from the floor. Diagram showing the treatment setup after removing a blackout curtain (A). Numbers of egg masses laid on the front sides of leaves (FL) and undersides of leaves (UL) in the treatment (B) and control that was exposed to semi-natural light/dark cycles in an unheated room (C). 's' indicates the proportions significantly deviating from unity with a $\chi 2$-test at P < 0.05.

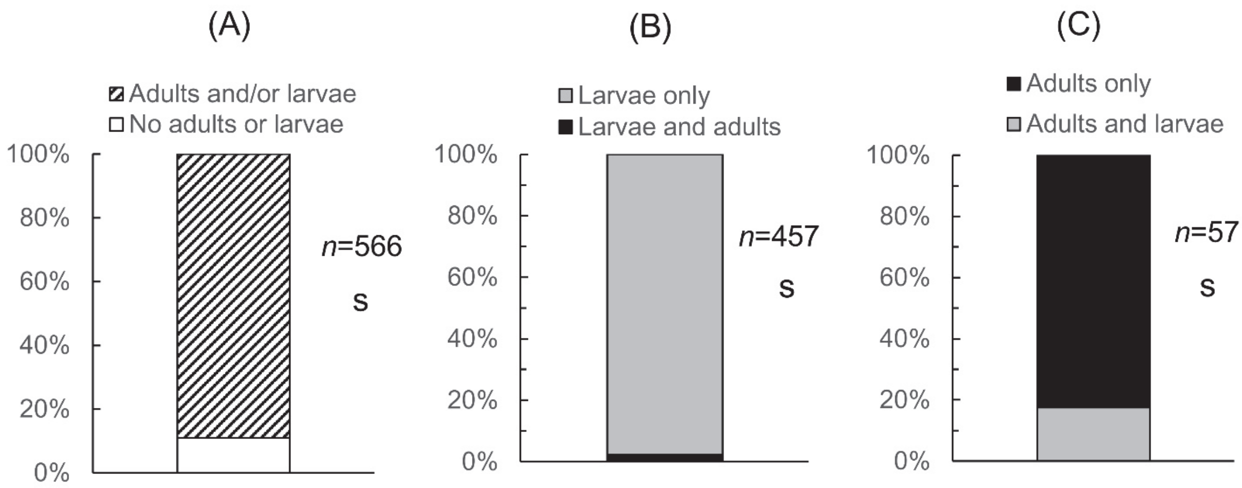

Fig. 7. Proportions of dock plants with and without *G. atrocyanea* individuals (A), those with adults and larvae or adults only (B), and those with larvae and adults or larvae only (C) observed on March 30, 2023. 's' indicates the proportions significantly deviating from unity with a $\chi 2$-test at P < 0.05.

atrocyanea on March 31 (Fig. 7A), representing the majority compared to the intact plants (11.0%; $\chi 2$ = 203.6; df = 1; P < 0.05). Larvae were observed on 457 dock plants, of which 97.8% were devoid of adults (Fig. 7B), suggesting that the majority of parents of these larvae had either abandoned these plants or perished. Adults were observed on 57 dock plants (Fig. 7C), of which 82.5% exclusively hosted adults, while the remaining 17.5% hosted both adults and larvae ($\chi 2$ = 13.4; *df* = 1; *p* < 0.05). This result suggests that the former group likely moved to these plants shortly before this census.

Discussion

The present study focused on three subjects related to the oviposition behavior of overwintered *G. atrocyanea*. The first subject examined the daily rhythm of oviposition activity; the second subject explored why the beetles laid eggs on the undersides of leaves; and the final subject investigated adult dispersal concerning the presence of larvae. These subjects will be discussed below.

As mentioned previously, some insect species exhibit daily rhythms in their oviposition activity. However, in *G. atrocyanea*, no evidence was found to suggest the occurrence of daily periodicity in oviposition activity, either under semi-natural or constant temperature conditions. Instead, this beetle displayed continuous oviposition activity throughout the day and night. The absence of overt rhythms is observed in various animals, including polar animals, long-distance migratory birds, cave-dwelling fishes, and eusocial mammals and insects (Bloch *et al.,* 2013). Honeybee and ant queens, for example, lay eggs continuously without regard to the time of day (McCluskey, 1967; Sharma *et al.,* 2004; Harano *et al.,* 2007; Johnson *et al.,* 2010), allowing for rapid colony growth in a short period (Bloch *et al.,* 2013). In *G. atrocyanea*, it is unclear whether the around-the-clock oviposition activity is due to the absence of a circadian rhythm or a mixture of individuals displaying circadian rhythms in different phases. While this issue should be addressed by observing oviposition activity on an individual basis in future studies, it is undeniable that oviposition occurs continuously within populations, resulting in the rapid deposition of egg masses on a host plant within a short period, which might be favorable for better survival of eggs and larvae. Additional information on hatching behavior and mortality rates during the egg and larval stages may be necessary to fully understand the significance of this phenomenon.

G. atrocyanea adults have been observed to deposit egg masses on the underside of host plant leaves in field conditions (Tanaka, 2023). This behavior is likely adaptive due to the susceptibility of eggs to desiccation and leaf friction caused by strong winds (Miyazaki and Naito, 1981). Additionally, the detection and predation rates of eggs by other insects and animals are probably lower on the undersides of the leaves than on the front sides. However, egg masses were found on both the front and undersides of leaves at similar frequencies in laboratory experiments when leaf discs were glued to the ceiling. Similarly, egg masses were observed on both surfaces when leaf discs were glued to both the ceiling and floor of dishes. These results clearly indicate that *G. atrocyanea* adults do not exhibit a preference for either side of the leaf when it comes to oviposition.

When leaf discs were glued to the ceiling of the dishes, *G. atrocyanea* adults deposited egg masses on the ceiling. However, adults laid egg masses on both surfaces when leaf discs were glued to the ceiling and floor. Because adults feed on leaves during the oviposition period, they were likely attracted to the leaf discs for feeding, which led to the deposition of eggs on the discs and the adjacent surfaces of the dish. In preliminary tests (Tanaka S, unpublished), I observed instances where adults laid eggs on the underside of a partially detached leaf disc from the floor, suggesting that this beetle may prefer to lay eggs by bending its abdomen underneath a substrate. This behavior may explain why the beetles frequently lay eggs on the undersides of leaves, although they do not show a preference for laying eggs on either side of the leaf when both sides are presented.

Possible factors that influence the selection of oviposition sites in insects include visual stimuli, odors, and contact chemicals (Cury *et al.*, 2019). However, in the case of *G. atrocyanea*, eggs were found not only on leaves but also on plastic surfaces of dishes. In my unpublished observations, egg masses were even deposited on wet tissue in a plastic dish without any host plants present, suggesting that oviposition can occur without specific stimuli from the host plant. In the field, I observed eggs deposited on plants (*Stellaria* sp.) adjacent to dock plants. Although more egg masses were found on leaves than on the dish surface, both under light-dark cycles and in darkness, this may suggest that certain plant-derived compounds and/or moisture directly or indirectly facilitate oviposition, making visual stimuli less important in this beetle. The direction of light or differences in light intensity between the front and underside of leaves also proved unimportant, as most egg masses were laid on the undersides of leaves when the plants and adults were illuminated from the floor (Fig. 6).

In the strawberry leaf beetle (*Galerucella* *grisescens* Joannis), which also uses dock plants as an oviposition substrate, Nakamura (2008) demonstrated that oviposition is greatly facilitated under high humidity or wet conditions. Hori *et al.* (2006) reported that adults of this species are attracted to *cis*-3-hexenyl acetate, commonly known as leaf alcohol, which insects often use for host recognition. Nakamura (2008) also tested the oviposition activity of this beetle on 18 different plant species, including both host and non-host plants, and observed oviposition on all plant species tested, although fewer beetles oviposited on the non-host species. In the strawberry leaf beetle, tarsal sensilla have been found to recognize two wax components from dock plants and are believed to play an important role in host plant recognition (Yasano *et al.*, 2020). However, Nakamura (2008) did not find evidence of the involvement of water and hexane extracts from dock plants in stimulating oviposition in this beetle.

Regarding *G. atrocyanea*, studies have examined the role of organic acids in feeding behavior (Matsuda, 1981), and a correlation has been observed between beetle densities and the levels of phenolic compounds and tannins present in host plants (Ohsaki *et al.*, 2021). However, no information is currently available about the mechanism that controls oviposition behavior in this species. It is possible that these leaf beetles locate a host plant using chemical cues from the plant, feed on the leaves and then move to the undersides of leaves for oviposition without relying on additional environmental cues except for moisture. Nakamura (2008) suggested that the low oviposition rates on non-host plants in the strawberry leaf beetle could be attributed to inhibitory compounds. A similar explanation may be possible for the lack of egg deposition on wilted leaves by *G. atrocyanea* adults.

Miyazaki and Naito (1981) investigated the survival rates and mortality factors in *G. atrocyanea* in detail, and the results show that the mortality rate from egg deposition to adult emergence was high, ranging from 65% to 99%. Both physical and biological factors may contribute to this high mortality, but a common factor is the occurrence of food shortages, often caused by high larval densities, which is particularly crucial for survival during the egg and larval stages. During the present study, dock plants seriously defoliated by crowded larvae were frequently observed in April. Given the high lifetime fecundity of this beetle (725-1156 eggs; Wei, 1991), it is not surprising that reproducing adults have evolved mechanisms to avoid overcrowding in their offspring.

Naito *et al.* (1979b) demonstrated that *G. atrocyanea* adults dispersed up to a radius of 50 m from the release point and mentioned that females dispersed over greater distances than males. However, these observations were limited to the early stage of the reproductive season. Suzuki (1985) conducted a longer-term study on the movement of reproducing *G. atrocyanea* adults in the field and reported that they tended to concentrate in relatively large patches of dock plants. He found that only 17% of adults moved among patches, including those that disappeared from their original patches, from March to May. However, it is important to note that his observations focused on dispersal among patches rather than movement between plants. The present study observed the dispersal behavior of this beetle between plants.

During the present study, an interesting observation was made regarding the distribution of adults on dock plants in late March. It was noticed that adults were primarily found on dock plants without larvae. These plants appeared nearly intact or had only a few leaf holes. This anecdotal observation aligns with the census conducted on March 30, where more than 80% of adults were observed on dock plants without larvae, suggesting that the adults migrated to these plants shortly before the census. In contrast, larvae were observed on 457 dock plants, of which 98% were without adults. While it is possible that many adults had died, it is likely that at least some of them successfully moved to new host plants. This behavior may reduce intraspecific competition and allow the beetles to exploit unused resources. The specific stimulus triggering adults to leave the original plant remains unknown, but it could be valuable to explore the role of chemical compounds associated with *G. atrocyanea* larvae under crowded conditions or on severely defoliated host plants. Unfortunately, this study did not measure the distance between the infested plants and the newly occupied plants. However, unpublished observations noted that some plants were separated by at least 8 steps (approximately 4.8 m) from the nearest infested plants. Additionally, it should be noted that the proportions of eggs, larvae, and reproducing adults on dock plants would likely vary significantly over time. Therefore, conducting a thorough census on different occasions throughout the entire reproduction season while collecting such information would be valuable for a comprehensive understanding of the population dynamics of *G. atrocyanea*.

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References

Ampleford EJ, Davey KG. 1989. Egg laying in the insect *Rhodnius prolixus* is timed in a circadian fashion. J Insect Physiol 35: 183-187. DOI: 10.1016/0022-1910(89)90003-6

Barrozo RB, Schilman PE, Minoli SA, Lazzari CR. 2004. Daily rhythms in disease-vector insects. Biol Rhythm Res 35: 79-92.

Bloch G, Barnes BM, Gerjena MP, Helm B. 2013. Animal activity around the clock with no overt circadian rhythms: patterns, mechanisms and adaptive value. Proc Biol Sci 280: 20130019. DOI: 10.1098/rspb.2013.0019

Cury K, Prud'homme B, Gompel N. 2019. A short guide to insect oviposition: when, where and how to lay an egg. J Neurogen 33: 75-89. ff10.1080/01677063.2019.1586898ff. ffhal-02368283

Day JF. 2016. Mosquito oviposition behavior and vector control. *Insects* 7: 65. DOI: 10.3390/insects7040065

De Wilde J. 1958. Host plant selection in the Colorado beetle larva (*Leptinotarsa decemlineata* say) - An ethological approach to food finding in insects. Entomol Exp Appl 1: 14-22.

Dickens JC. 2001. Orientation of Colorado potato beetle to natural and synthetic blends of volatiles emitted by potato plants. Agri Forest Entomol 2: 167-172. DOI: 10.1046/j.1461-9563.2000.00065.x

Eaton AT. 2016. Colorado potato beetle. UNH Coop Ext, Fact sheet 16. https://extension.unh.edu/sites/default/files/migrated_unmanaged_files/Resource002811_Rep4166.pdf

Finch S, Collier RH. 2000. Mini review host-plant selection by insects – a theory based on 'appropriate/inappropriate landings' by pest insects of cruciferous plants. Entomol Exp Appl 96: 9-102.

Gibb TJ, Sadof CS. 2017. Elm leaf beetle. Household and Structural E-25-W. https://extension.entm.purdue.edu/publications/E-25/E-25.pdf

Harano K, Sasaki M, Sasaki K. 2007. Effects of reproductive state on rhythmicity, locomotor activity and body weight in the European honeybee, *Apis mellifera* (Hymenoptera, Apini) queens. Sociobiology 50: 189-200.

Hori M, Ohuchi K, Matsuda K. 2006. Role of host plant volatile in the host-finding behavior of the strawberry leaf beetle, *Galerucella vittaticollis* Baly (Coleoptera: Chrysomelidae). Appl Entomol Zool 41: 357-363. DOI: 10.1303/aez.2006.357

Japan Meteorological Agency. 2023. Retrieved from https://www.jma.go.jp/jma/index.html

Johnson JN, Hardgrave E, Gill C, Moore D. 2010. Absence of consistent daily rhythmicity in mated honey bee queen behavior. J Insect Physiol 56: 761-773. DOI: 10.1016/j.jinsphys.2010.01.004

Lee C-F. 2022. Redescription of *Gastrophysa atrocyanea* (Motschulsky) and *G. polygoni* (Linnaeus) (Insecta: Coleoptera: Chrysomelidae: Chrysomelinae), with Notes on Their Biology. J Taiwan Agric Res 71: 281-292. DOI: 10.6156/JTAR.202212_71(4).0001

Loher W. 1979. Circadian rhythmicity in locomotor behavior and oviposition in female *Teleogrylly commodus*. Behav Ecol Sociobiol 5: 253-262.

Matsuda K. 1981. Feeding stimulation of nutrient chemicals in *Gastrophysa atrocyanea* Motschulsky (Coleoptera: Chrysomelidae).

Jap J Appl Ent Zool 25: 84-88.

McCluskey ES. 1967. Circadian rhythms in female ants, and loss after mating flight. Comp Biochem Physiol 23: 665-677. DOI: 10.1016/0010-406X(67)90418-5

Mitchell BK, McCashin BG. 1994. Tasting green leaf volatiles by larvae and adults of Colorado potato beetle, *Leptinotarsa decemlineata*. J Chem Ecol 20: 753-69.

Miyazaki M. 1979. An annotated list of insects feeding on *Rumex obtusifolius* L. (Polygonaceae) in Japan. Bull Natl Grassl Res Inst 14: 124-139.

Miyazaki M, Naito A. 1981. Studies on the biological control of *Rumex obtusifolius* L., a grassland weed, by *Gastrophysa atrocyanea* Mots. (Coleoptera: Chrysomelidae). III. Survival rate and mortality factors of *G. atrocyanea*. Bull Natl Grassl Res Inst 20: 103-111. (in Japanese with English abstract)

Naito A, Miyazaki M, Emura K. 1979a. Studies on the biological control of *Rumex obtusifolius* L., a grassland weed, by *Gastrophysa atrocyanea* Mots. (Chrysomelidae, Coleoptera) 1. On the host specificity of the insect. Bull Natl Grassl Res Inst 14: 117-123. (in Japanese with English abstract)

Naito A, Miyazaki M, Kanda K. 1979b. Studies on the biological control of *Rumex obtusifolius* L., a grassland weed, by *Gastrophysa atrocyanea* Mots. (Coleoptera; Chrysomelidae). II. Dispersal of overwintered adult insects. Appl Entomol Zool 14: 51-55.

Nakamura Y. 2008. Ichigohamushi no koubikoudou oyobi sanrankoudou (Mating and oviposition behaviors in the strawberry leaf beetle). [Master's thesis]. Morioka (Japan): Tohoku University. 65 pp. (in Japanese). http://hdl.handle.net/10097/17507

Numata H, Matsui N. 1988. Circadian rhythm of oviposition in the bean bug, *Riptortus clavatus* Thunberg (Heteroptera: Alydidae). Appl Entomol Zool 23: 493-495.

Ohsaki H, Miyagi A, Kawai-Yamada M, Yamao A. 2021. Intraspecific interaction of host plants leads to concentrated distribution of a specialist herbivore through metabolic alterations in the leaves. Func Ecol 36: 779-793. DOI: 10.1111/1365-2435.13988

Oliveria FL, Solomon JD. 2004. Forest pests of North America: Cottonwood leaf beetle. http://www.forestpests.org/nursery/cwleafbeetle.html.

Sablon L, Dickens JC, Haubruge E, Verheggen FJ. 2013. Chemical ecology of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), and potential for alternative control methods. Insects 4: 31-54. DOI: 10.3390/insects4010031

Saunders DS, Steel CGH, Vafopoulou X, Lewis RD. 2002. Insect Clocks, third ed. Amsterdam: Elsevier. 559 pp.

Sharma VK, Lone SR, Goel A. 2004. Clocks for sex: loss of circadian rhythms in ants after mating? Sci Nat 91: 334-337. DOI: 10.1007/s00114-004-0526-8

Schowalter TD. 2011. Insect Ecology, An Ecosystem Approach, 3rd ed. Tokyo: Academic Press. 633 pp.

Suzuki N. 1985. Resource utilization of three chrysomelid beetles feeding on *Rumex* plants with diverse vegetational background. Jap J Ecol 35: 225-235.

Tanaka S. 2023. Characteristics of eggs and egg masses of the leaf beetle, *Gastrophysa atrocyanea* (Motschulsky) (Coleoptera, Chrysomelidae). Formosan Entomol 43: 24-33.

Visser JH, Nielsen JK. 1977. Specificity in the olfactory orientation of the Colorado beetle, *Leptinotarsa Decemlineata*. Entomol Exp Appl 21: 14-22.

Wei X. 1991. *Gastrophysa atrocyaneae* (Col: Chrysomelidae), an agent for biological control of the dock, *Rumex japonicus* (Polygonaceae) in China. Trop Pest Manag 37: 383-386. DOI: 10.1080/09670879109371619

Yasano S, Kutsuwada Y, Akatsu M, Masuta S, Kakazu R, Masuoka N, Matsuda K. Hori M. 2020. Taste recognition through tarsal gustatory sensilla potentially important for host selection in leaf beetles (Coleoptera: Chrysomelidae). Sci Rep 10: 4931. DOI: 10.1038/s41598-020-61935-x.

日常節律，控制臺灣斑馬葉甲蟲（*Gastrophysa atrocyanea*，鞘翅目：金花蟲科）成年產卵行為和散佈的因素

田中誠二

日本茨城縣筑波市松代市
* 通訊作者 email: stanaka117@yahoo.co.jp

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摘　　要

蓼藍齒脛金花蟲（*Gastrophysa atrocyanea*）會在羊蹄的葉子上產卵。本研究旨在調查影響其產卵行為的環境因素。觀察發現，在 25°C 的光照和黑暗期間，蓼藍齒脛金花蟲持續地產卵，顯示牠們具有全天候的產卵活動。當將葉子黏貼在皮氏培養皿的上層時，成蟲以相似的速率在葉子的正面和底面產卵，這表明成蟲在產卵時並不區分葉子的兩面。然而，當將金花蟲和羊蹄放置在一個被遮蔽簾子覆蓋並由地面照明的盒子裡時，大部分的卵塊被發現在葉子的背面，類似於在半戶外條件下的觀察結果。這表明產卵行為並不受光線方向或葉子兩面之間光線強度的影響。在三月底，當許多幼蟲在蒲公英植物上生長時，觀察到大多數成蟲出現在沒有幼蟲的植物上。相反地，大多數被幼蟲侵害的羊蹄上沒有成蟲。這些發現暗示著成蟲的擴散現象可能與對有幼蟲出現或被蟲蛀的植物有關。

關鍵詞：日夜節律、卵塊、產卵行為、擴散