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ABSTRACT

The potential of the parasitoid, *Gronotoma micromorpha* (Perkin) as a biological control agent for pea leafminer fly, *Liriomyza huidobrensis* (Blanchard) has been evaluated in a series of studies on the fly preference to host instars, biological characteristic and functional response to the host density. The studies were carried out at the Laboratory of Integrated Pest Management (IPMLab), Faculty of Agriculture Udayana University in Denpasar and Greenhouse Owned-Farmer in Pancasari Buleleng, Bali (1200 m asl; average temperature 23.67 ± 5.69 °C and average RH of 64 ± 17.69%). The results showed that *G. micromorpha* has a strong preference towards the 3rd instar larvae of *L. huidobrensis*. The pre-oviposition period was 0.4 ± 0.67^{d}, oviposition was 12.5 ± 2.0^{d} and post-oviposition period was 1 ± 0.0^{d} while the peak oviposition occurred on the 4th day. Meanwhile, all the offsprings produced were females which indicated that parthenogenesis thelytokous was present in *G. Micromorpha* and the fecundity was 29 ± 3.77 per female adult. Furthermore, the average life span for the adult was 13 ± 1.42 days with an oviposition period of 12.5 ± 2.01 days. The average post-oviposition period was 1 ± 0.0 days and average development period of egg to adult was 32.03 ± 0.98 days. Meanwhile, the breeding potential was high with an average daily percentage of 2.27 ± 0.27 fecundity and a maximum spawning occurred on day 4. The functional response was type-2 and it is the rate at which parasitism increase with increase in population of the host density. Hence, the biological characteristics and mode of functional response by the parasitoid showed its potential as a biological pesticide for pea leafminer *L. huidobrensis*.

1. Introduction

The pea leafminer fly, *Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae) is a global invasive pest that feeds on various vegetables including important fruit and flower crops [1]. It is assumed that these pests entered Indonesia around the 1990s and was first discovered in the vegetable crops of the Cisarua region, Bogor in 1994 [2]. Meanwhile, different types of plants affected include potatoes, celery, tomatoes, peas, kidney and long beans, etc. [3]. Among these host crops, potato crops suffered the most damage in the field [4].

Currently, the population of *L. huidobrensis* is still very large and this has caused a significant decrease in the production yields of vegetable crops, especially potatoes [5]. Meanwhile, farmers have taken control measures to overcome attacks from the pests by using synthetic insecticides which has a negative effect on the environment and its exposure poses direct or indirect risks to human health [6]. Furthermore, excessive use of chemicals causes pests to resist insecticides [7], reduce the abundance of natural enemies in the environment [8], and flies also have high mobility to escape insecticides [9]. However, biological control is an alternative method of controlling insect populations, weeds and disease management using natural enemies [10].

Biological control using parasitoids has been widely considered and is particularly important to farmers as an alternative in controlling leaf borer in Indonesia [11]. Meanwhile, the type of parasitoid associated

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with *L. huidobrensis* is *Gronotoma micromorpha* [5]. *The* parasitoid has the potential to control the population of flies on leaf of vegetable crops worldwide [12]. Moreover, previous study stated that *G. micromorpha* are the dominant parasitoid against *L. trifolii* in mainland Okinawa, Japan and Guam, USA [13]. The reproductive method in form of thelytoky, egg-pupal and larva-pupal parasitism shown by *G. Micromorpha* wasps is ideal for use as biocontrol agents [14,15]. In addition, the reproductive pattern of thelytoky in the Order Hymenoptera parasitoid is more profitable when compared to arrhenotoky in terms of mass breeding due to the asexual reproduction of offspring by female adults [13].

This study aims to evaluate the potential of *G. micromorpha* as a biocontrol agent for *L. huidobrensis* in vitro, by examining i) preference to host instar *L. huidobrensis*, ii) life history, iii) daily survival of adults and iv) the parasitiod functional response.

2. Materials and methods

2.1. Study area and host plant

The study was carried out in Pancasari Village, Sukasada District, Buleleng Regency at an altitude of 1200 m above the sea level, from January to September 2020. Meanwhile, the average room temperature for breeding was 23.67 ± 5.69 °C and the average humidity was 64 ± 17.69%.

Furthermore, the host plant used to breed leafminer flies was the red beans (*Vigna vulgaris*) which were planted in black plastic bags (20 cm high, 13 cm in diameter) using soil mixed with manure. Each of the black plastic bags was filled with ±0.35 kg of the medium and the plant seeds were watered daily. For insect breeding purposes, 10 days old seeds were used with the first fully developed leaves.

2.2. Insect

2.2.1. Host insect propagation

Identified *L. huidobrensis* adults were put in a maintenance cage with a wooden frame of 40 cm long, 40 cm wide and 45 cm high. The cage base was made of wooden planks to accommodate plants while the sides and front of the cage were made of batis cloth and both the back and top were made of clear plastic sheets. Furthermore, there was a door in front of the cage to enter plants and insects and 16 black plastic bags were filled with the host plant. The number of *L. huidobrensis* adults that was inventoried into confinement was 10–20 pairs per confinemenct and a 10% honey solution was given to maintain adult quality during the breeding process. Meanwhile, the administration of honey solution was carried out by wrapping a cotton swab on a stick and impregnating it with the solution which was later fixed on a black plastic bag according to the plant's height with a cotton. After 24 h, the host plant spawned by *L. huidobrensis* was transferred to another insect-free cage and was replaced with a new host plant. Meanwhile, plants infected with *L. huidobrensis* were grown until the eggs hatched into larvae. When the larvae approached to becoming buds, the petioles were cut and the leaves were put in a plastic hatching jar. The emerging adult was used for culture while some of the plants infested with the larvae were used for parasitoid breeding.

2.2.2. Parasitoid maintanence methods

The parasitoid, *G. Micromorpha* (Fig. 1) was obtained from the insect ecology laboratory of IPB University, Bogor - West Java, in form of a pupa in a plastic jar with a diameter of 18 cm and a height of 21 cm. Meanwhile, the jar was filled with wire to support the leaves from being rotten easily and *G. Micromorpha* was observed with a binocular stereo microscope (Ivymen System, Spain). A 7.5 cm × 5 cm ventilation hole was made on the side of the jar which was covered with batis cloth. Furthermore, a hole closed with an inverted plastic funnel was made at the top of the jar and a plastic tube was attached at the end of the funnel as fly trap to obtain the adult parasitiod that comes out as pupa while the plastic jar was inserted into a container filled with ice cubes to reduce temperature during the trip.

Meanwhile, the adult parasitoids that appeared were matched with a standard specimen that was available. Furthermore, the identified parasitoids were put in a rearing cage filled with red bean plants which its leaves had been infected with larvae of *L. huidobrensis* 3rd instar and 10% honey solution as supplementary. Inoculation was carried out every 24 h by moving the black plastic bag which contained the previous host plant and replacing it with the bag that already contain 3rd instar larvae as a new plant.

The parasite host larvae were transferred to hatching jars 3 days after inoculation by cutting the base of the infested host plant until the parasitoids hatched. After 30 to 34 days, the parasitoids that appear from the pupa were transferred to the culture cage and the reproduction result of adult *G. micromorpha* was used as material for further study.

2.2.3. Preference for host instars

The number of host selected was determined based on the number of eggs laid at the host instar while the number of eggs laid was calculated based on the number of adult that appeared. Moreover, the red bean plants infested with 1st larvae instar, 2nd larvae instar and 3rd larvae instar were used as treatment and the plants were later placed in a cage.

A 4 day old *G. micromorpha* adult was put in a cage containing 3rd larva of *L. huidobrensis* infested plants and parasitoids were released into the cage using a respirator. The released adult parasitoids were fed with 10% honey solution to maintain fitness. Meanwhile, after 24 h, the plants containing parasitoid were transferred to another empty cage

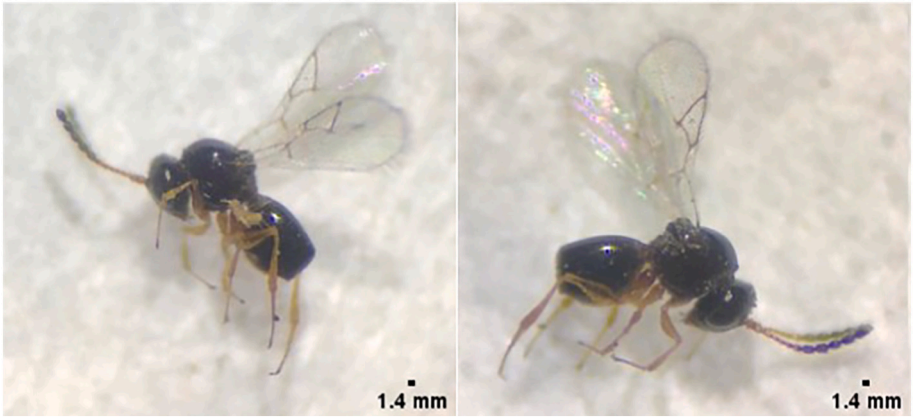

Fig. 1. The adult of *Gronotoma micromorpha.*

until complete pupation. When the larvae pupate, the petiole with the parasitoids was cut and placed in a plastic petri dish with a diameter of 10 cm and a height of 10 cm until it emerges and the number of emerged adult parasitoids was recorded daily.

This study used a randomized block design consisting of 3 treatments and 5 replications. The data were analyzed using analysis of variance and when there was a significant effect treatment effect and was continued with the LSD 5% test [16]. Furthermore, the index used to measure preference for instars was the Manly index [17] as follows:

$$bi = \ln(Ri/Ai) \Big/ \left(\sum_{i=1}^{k} \ln(Ri/Ai) \right)$$

Annotations:

bi = Index of preference for host instars.
Ai = initial host density, $I = 1,2, \ldots, k$.
Ri = Number of hosts that were not parasitized.
k = Number of host instars.

2.2.4. Life history of G. Micromorpha

The experiment was carried out in a cage which was 40 cm long and 45 cm high. Meanwhile, the sides and front were made of batis cloth while the back and top were made of transparent plastic sheets. Moreover, the red bean plants infested with larvae of *L. huidobrensis* 3rd instar with a total of 20 larvae were put in a cage and inoculated by a newly hatched *G. Micromorpha* adults. After 24 h, the plants were replaced with new ones and the *L. huidobrensis* larvae which had been parasitized were transferred to another cage free of pests and parasitoids.

When the larvae started becoming pupae, the plant leaves were cut and placed in a plastic cup of 10 cm in diameter and 10 cm high as a hatchery container. Daily observations were made on the life span, fecundity, oviposition rate, preoviposition as well as oviposition and post-oviposition periods, life cycle and number of adult parasitoid that appeared. Meanwhile, lifespan was estimated based on the life expectancy of parasitoids from emergence to death. Fecundity was the physiological ability to produce offspring and it is usually expressed as the number of offspring produced by a female adult throughout her lifetime.

The oviposition period was calculated based on the average number of offspring produced per day while the pre-oviposition period was calculated based on the time taken by the adult from the first appearance to the time it lays the first egg. Meanwhile, the oviposition period was calculated based on the time between the laying of first egg until the last egg while the post oviposition period was calculated based on the time between the laying of egg and the death of the adult. Furthermore, the life cycle was calculated based on the length of development from the egg phase until the female adult first lays the egg.

2.2.5. Demographic parameters

Demographic parameters were calculated based on the proportion of living females (Lx), laying (mx), net reproduction rate (Ro), average age of adult/individual (x), average period of one generation (T), intrinsic rate of increase (r), the rate of increase is limited (ƛ), limited birth rate (β), instantaneous birth rate (b) and population growth rate (Nt).

2.3. Functional response

To determine the functional response, a 4 day old *G. Micromorpha* adult was inoculated for 24 h on a larvae of *L. huidobrensis* 3rd instar on red bean leaves. The population densities of the larvae tested in each cage were 1, 3, 6, 9, 12 and 24 larvae respectively. Meanwhile, the experiment was repeated 5 times and the models used were as stated below:

$$y = \frac{Ttax}{1 + abx}$$

The values of a and b were calculated based on the above transformation as follows:

$$y/x = -aby + Tta$$

Annotation:

y = Number of parasitized hosts.
x = host population density.
Tt = Amount of time available.
a = Host search rate (coefficient).
b = Time required to parasitized one host [18,19].

3. Results

3.1. G. Micromorpha preference against L. huidobrensis host instars

The results showed that there was a strong preference for the 3rd instar larvae, namely 3.60 ± 0.49 adults compared to 2rd instar larvae, namely 3.2 ± 0.75 adults and 2.2 ± 1.17 adults or 1st instar larvae *($P < 0.05$)*. Moreover, the results of the analysis on *L. huidobrensis* instars are presented in Table 1.

The parasitoid firstly flew randomly on the upper side of the cage made of transparent plastic and later to the cage frame made of wood. This behavior was possible in some adults a moment, however, it was brief in others and settled on leaves after several minutes of inoculation into confinement.

Parasitoids that had settled on plant leaves moved on the upper or lower leaves surfaces by tapping the leaf surface with antennae and occasionally by touching abdomen until host is located. Meanwhile, the intensity of antenna clapping on leaf surface increased when the leafminer larvae has been found and thereafter inserted the ovipositor in a motionless mode. After laying the first egg, the parasitoid searched for another host to lay the next egg while some of the adult parasitoid that was inoculated into the cage spent a lot of time sitting on the walls and the cage.

3.2. Life history of G. Micromorpha

The life span of adult *G. Micromorpha* ranged from 9 to 17 days with an average of 13 days and the average number of adult obtained from the pupa was 29. Meanwhile, the oviposition rate in the host was 2.27 days, the pre-oviposition period was 0.4 days and oviposition period lasted for 12.5 days. Furthermore, the post-oviposition period of *G. Micromorpha* was 1 day and its life cycle lasted from 30 to 34 days with an average of 32.03 days. (Table 2). (See Table 3.)

The survival rate of adult inoculated against *L. huidobrensis* larvae is presented in Fig. 2, while the mortality rate increased rapidly with an increase in adult age. Meanwhile, the adult mortality began at the age of 9 days and increased dramatically from the age of 12 to 15 days which lasted until the age of 17 to 18 days.

Table 1
Average number of *Gronotoma micromorpha* adult emerging from pupa on the three host instars of *Liriomyza huidobrensis*.

Host larvae instar	Number of host larvae (adult)	Average number of paraitoid appear(adult)	Preference index
I	20	2.20 ± 1.17^{b}	0.23 ± 0.10^{b}
II	20	3.20 ± 0.75^{ab}	0.37 ± 0.11^{ab}
III	20	3.60 ± 0.49^{a}	0.41 ± 0.03^{a}
LSD 5%			0.1764

Annotation: Number followed by the same letter in the same column means that it is not significantly different at the 5% level of the LSD test.

Table 2
Life history parameters of *Gronotoma micromorpha.*

Parameter	N	Average
Life span (days)	10	13 ± 1.42
Keperidian (adult)	10	29 ± 3.77
Ovipotition rate (adult/female/days)	10	2.27 ± 0.27
The preoviposition period (days)	10	0.4 ± 0.67
The oviposition period (days)	10	12.5 ± 2.01
The post-oviposition period (days)	10	1 ± 0.0
Life cycle (days)	400	32.03 ± 0.98

Annotation: N is the number of samples observed (adults).

Table 3
Demographic parameters of *Gronotoma micromorpha.*

Proportion of living females Lx =	0.79
Laying (mx) =	20.12
Clean reproduction rate (Ro) =	15.97
Average age of adult/individual (x) =	9.60
Average period of one generation (T) =	9.60
Intrinsic rate of increase (r) =	0.29
The rate of increase is limited (λ) =	1.33
Limited birth rate (β) =	0.07
Instantaneous birth rate (b) =	0.06
Instantaneous mortality rate (d) =	−0.23
Population growth rate (Nt) =	41.83

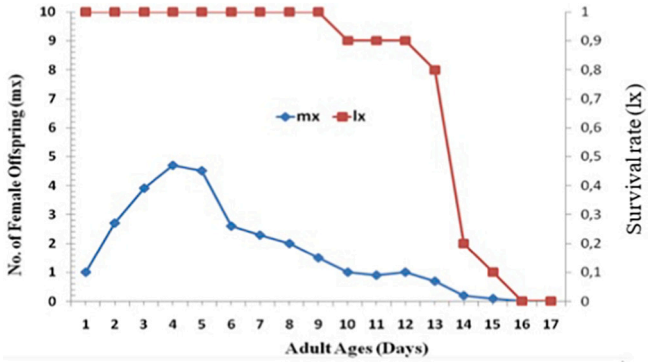

Fig. 2. Survival rate (lx) of *Gronotoma micromorpha* adult and daily fecundity of *Gronotoma micromorpha* adult.

The number of eggs laid daily was due to the number of offspring produced. Meanwhile, the results showed that *G. micromorpha* adult began laying eggs on the first day of emergence with the peak at 4 days old and hatched on day 30 to 34 after it laid the first egg on the host. Generally, the peak incidence of adult parasitoid on *L. huidobrensis* occurred on the 32nd day.

3.3. Functional response

The results showed that the number of parasite hosts obtained from the number of adult that appeared increased in accordance with the type-2 functional response. In addition, the curve also increased with increasing rate following a decreasing parabolic curve with respect to host density (Fig. 3).

4. Discussion

The difference in preference was caused by differences in the physical stimuli produced by each instar of the leafminer larvae in the process of searching for a host by parasitoids [20]. Meanwhile, this difference in stimulus occurred in form of vibrations produced by the host larvae [21]. For instance, the vibrations produced by small leafminer larvae of *Phytomyza ranunculi* schrank (Diptera: Agromyzidae)

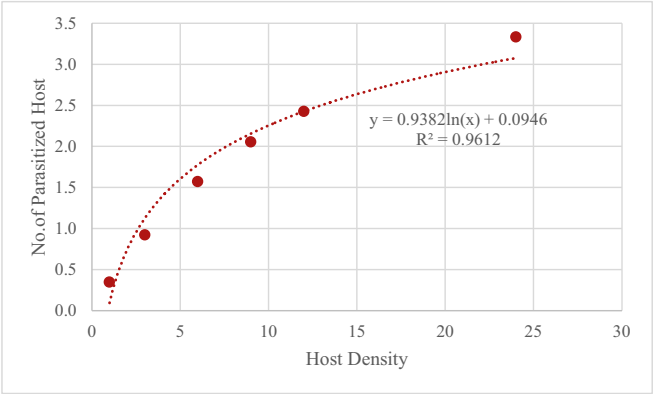

Fig. 3. The functional response of *Gronotoma micromorpha* to *Liriomyza huidobrensis.*

made searching of host harder for the parasitoid *Kratochoviliana* sp. (Hymenoptera: Eulophidae) [22]. Furthermore, the larger the host larvae, the stronger the vibrations, and the easierer it is for the parasitoids to detect [23]. Visually, the larger 3rd instar larval leafminer size was easier to find by the smaller litter parasitoid on the 1st instar [24].

The low average number of parasitoids that appear are presumed due to the rapid temperature changes at the study site which was between 19 and 30 °C with an average of 23.67 ± 5.69 °C while the age range of *G. micromorpha* adults decreased from 16.5 to 5.7 days with an increase in temperature between 15 and 25 °C. This showed that the age range for adults to be 15 °C or 20 °C is significantly longer than 25 °C [13].

Furthermore, the results showed that none of the adults were male. The sex of *G. micromorpha* was distinguished by the number of antennae which was 13 segments in female and 15 segments in male [25]. Meanwhile, one of the causes of thelytokous reproduction in Hymenoptera is the presence of endosymbiont bacteria of the genus Wolbachia [26].

Previous study showed that at high host population densities, parasitoids finds the host more easily than at low density [27]. Parasitoids have a type-2 functional response which showed that the rate of increase in parasitism decreases with the increase in host density [28]. Hence, the functional response is one of the essential elements in measuring the parasitoids effectiveness in regulating host populations in nature [29].

5. Conclusion

The results showed that *G. Micromorpha* has a strong prevalence against 3rd instar L.huidobrensis larvae. All the sexes that emerged were females which showed the occurrence of thelytokous parthenogenesis. Furthermore, the potential for breeding is quite high with the spawning peak on the 4th day and the functional response was type-2 which indicated that the rate of parasitism has increased with an increase in the number of host populations. Hence, the parasitoid has the ability as a biological control agent against *L. huidobensis.*

Declaration of Competing Interest

The authors reported no declarations of interest.

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