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Research Article

Brown Planthopper Egg Parasitization in Rice Fields with Different Growth Ages

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ABSTRACT

The brown planthopper, *Nilaparvata lugens* Stål. (Hemiptera: Delphacidae), is one of the most important pests attacking rice plants. One of the strategies to control this pest is by using natural enemies, including egg parasitoids. This study aimed to investigate the species of *N. lugens* egg parasitoids and their parasitism levels in one rice ecosystem with different growth stages. The research was conducted by selecting three rice plots aged 3, 6, and 10 weeks after planting. Potted rice plants with *N. lugens* eggs were placed in those rice plots for two days and recollected for observations. The same release was repeated 3 weeks later at the same rice plots in the same site. Egg parasitoids of *N. lugens* found during this study were *Oligosita* sp. and *Anagrus* sp. The highest number of egg parasitoids were found at the rice plants aged 6 weeks after planting with 14.50 and 13.50 individuals collected during first and second trapping, respectively. In addition, the level of parasitism ranged from 6.89–22.26% with the highest parasitism was occurred in the 6-week old rice. These findings may suggest that different rice planting dates may benefit the egg parasitoids of *N. lugens* which could reduce the risk of outbreaks.

Keywords: brown planthopper; egg parasitoid; level of parasitism; plant age

INTRODUCTION

Brown planthoppers (BPH), *Nilaparvata lugens* Stål. (Hemiptera: Delphacidae), is the main rice pest, which is able to vastly reproduce and colonize rice plants. During the outbreak, the population may reach 200–500 of macropterous *N. lugens*/rice hill. Although a wide area of hopperburn does not happen yearly, spots of hopperburn could be found yearly (Trisyono *et al*., 2017; Y.A. Trisyono, 2020, field observations and unpublished), and these spots could spread and increase in the following years. Since the widespread outbreak in 2011 reaching ca 220,000 ha, the yearly data of damaged rice crops were continuously lower with 24,277 ha was infested and 571 ha of hopperburn in 2019 (Directorate General of Food Crops, 2019). The increase of damage and population density might be caused by several factors that could work simultaneously such as conducive climates, planting rice variety with no resistant gene(s), and high inputs of nitrogen and insecticides.

The population of *N. lugens* are dependent of biotic (natural enemies) and abiotic (temperature, rainfall) factors, which can suppress the population in the field. Natural enemies play an essential role in Integrated Pest Management (IPM) in rice and a significant disturbance of natural enemies would increase the probability of outbreaks. In alignment with IPM, Ecosystem Engineering (EE) techniques have been adopted to increase the role of natural enemies (Gurr *et al*., 2011; 2016; 2017). EE in rice fields involved the reduction of insecticide and increase the establishment of flowering plants on the bunds and field margins, with the ultimate goal to restore biodiversity and ecosystem services. The flora on the bunds provides shelter, nectar, alternate host, and pollen to conserve the natural enemy (Heong *et al.,* 2021a, 2021b).

One of the natural enemies that play an essential role in controlling the brown planthopper population is parasitoids. Egg parasitoids have a more effective level of parasitization than other parasites. Previous research showed that planting flowering plants in the bunds as a refuge increased the parasitism of *N. lugens* (Sinulingga *et al.*, 2019). The number of parasitoids that emerged from fields with refuge was 31.08 imagoes/trap compared to far from refuge (25.67 imagoes/trap) and no refuge (20.71 imagoes/trap). The number of parasitoids that did not emerge was higher from rice fields without refuge (14.59%) than with refuge plants (5.9%). This implies that refuge makes environments more beneficial for parasitoids by increasing the emergence rate which eventually produces more offspring that will help regulate *N. lugens* populations. Sugiharti *et al.* (2018) showed that when *Anagrus nilaparvatae* were reared with access to flowers or honey had higher hatching rates (37.4%) compared to without these food sources (8.19–15.6%). Offsprings of *A. nilaparvatae* that were fed with *Cosmos sulphureus, Turnera subulata,* or honey also had higher fecundity compared to without these food sources, which explain higher *N. lugens* parasitization in the rice field with refuge. Aldini *et al.* (2019) demonstrated that the diversity index of natural enemies associated with *Zinnia elegans*, *Cosmos sulphureus*, and *Tagetes erecta* were medium (1.328–1.581) with high dominances (0.314–0.453). The number of natural enemies associated with *C. sulphureus* and *Z. elegans* was higher compared to *T. erecta*.

Hymenopteran parasitoids is affected by landscape structure where the complex landscape structure could provide higher diversity compared to simple agricultural landscape (Ulina *et al.*, 2019). This is because the simple agricultural landscape cannot provide the needs of parasitoids, such as alternative host and prey, alternative sources of pollen and nectar, shelter, and favourable microclimates, or a combination of these resource. Although natural enemies moved through the whole landscape, there are positive and negative effects that include individual vigor even though at different levels of effects. Distribution of host changes over time and space, and flowering plants may add diversity of nectar/food source at similar time and space (Vollhardt *et al.*, 2010). In countries with four seasons, crop rotation can positively change habitat conditions through winter for natural enemies (Arrignon *et al.*, 2007).

Information regarding *N. lugens* egg parasitoids' diversity and their role in different rice ecosystems is still limited. Species diversity and richness of parasitoids are essential information to manage insect population balance. Research to investigate the species of *N. lugens* egg parasitoids has been done in Klaten and Bantul, reporting the three parasitoids found, *Anagrus nilaparvatae*, *A. optabilis* (Hymenoptera: Mymaridae), and *Oligosita* (Hymenoptera: Trichogrammatidae) (Torres-Moreno & Moya-Raygoza *et al.,* 2020). This study aimed to investigate egg parasitoids of *N. lugens* and their parasitism level in rice ecosystems with various growing stages and different crops surrounding the rice. These rice ecosystems are commonly found in relatively highland areas and lowland with a limited amount of water or labor.

MATERIALS AND METHODS

Study Location

This study was done at Paremono Village, Mungkid, Magelang, Central Java, (-7°34' N, 110°14' E). Rice was planted at different times resulting in a landscape with various ages of rice. In addition, other crops, such as long beans and chili pepper, were present in this landscape. The rice plots used for the research were selected far from these crops. This agricultural landscape also consisted of fields of various sizes; however, rice was still the most planted crop in this landscape.

Experimental Design

A completely randomized design (CRD) was employed consisting of three different ages of rice as treatments, and each treatment was replicated eight times. The first experiment (the first trapping) used rice plants aged 3, 6, and 10 weeks after transplanting. The second trapping was carried out three weeks later at the same rice plots. Therefore, the rice ages used in the second trap were 6-, 9-, and 13-week-old. The rice plots used for trapping were approximately 1000 m^2.

N. lugens Rearing

An initial population of *N. lugens* was obtained from the Laboratory of Control Technologies at the Faculty of Agriculture, Universitas Gadjah Mada, and the mass rearing followed the laboratory-established procedure (unpublished). This population has been reared in the laboratory since 1985 using rice seedlings and standard rearing protocols and now it has been reached the 706th generation. Rice seeds, Ciherang variety, were washed, immersed in water for 24 hours, and air-dried for 24 hours until seeds sprouted. Seedlings were then moved to plastic containers (diameter 20 cm, height 19 cm) and water was placed until seeds were not fully immersed and covered with cloth (30 mesh). Seven days after germination, rice seedlings were used as a food source and oviposition host for *N. lugens*. Imagoes were allowed to oviposit for 3–8 days and eggs hatched 6–8 days after. When the rice seedlings start yellowing, the rice seedlings with nymphs were moved to new seedlings.

Preparing the Rice Traps

Rice traps (Ciherang) were rice with *N. lugens* eggs. Five rice plants (1-month-old) were used as rice traps. The rice was planted in black plastic pots (top diameter 10 cm, bottom diameter 8 cm, height 8 cm) filled with wetted soil. Ten gravid females that were ready to oviposit, were moved to trap plants covered with a plastic cylinder (diameter 8 cm, height 50 cm) with tops covered with 30 mesh cloths. Female imagoes were left to oviposit and removed after 2 days to have a sufficient number of eggs laid while leaving sufficient time for field exposure before egg hatching. This procedure was adopted from Sinulingga *et al.* (2019). Eight rice traps were prepared for each treatment and each trapping time.

Field Exposure and Laboratory Handling

At each selected rice plot, eight rice traps were placed in the middle of the plot with a distance of approximately 3 m from each other (Sinulingga *et al.*, 2019) for 2 days to provide sufficient time for the parasitoids to find the eggs while avoiding the *N. lugens* eggs hatching in the field. Leaves of rice traps taken from study sites were cut and remaining stems were kept in previously described plastic cylinders. After 7 days, the remaining parts of rice traps were taken from the soil, washed, and wetted cotton was placed on their root ends. These stems were later placed in reaction tubes sealed with cloth mesh. Parasitoids that emerged were counted and then soaked into 70% alcohol. Parasitoids and *N. lugens* nymphs that emerged were counted daily. The number of *N. lugens* eggs that did not hatch (parasitized or not) were all counted by dissecting the plant tissue.

Identification of *N. lugens* Egg Parasitoids

Identification was conducted to the genus levels using morphological characteristics as described by Barrion and Litsinger (1994).

Data Analysis

Parasitization levels were calculated using the following formula previously used by Haryati *et al.* (2016):

$$\text{Parasitization level} = \left(\frac{A+T}{A+T+W}\right) \times 100\%$$

Where: A= total number of parasitoids that emerged; T= the total number of parasitized eggs, but no parasitoids emerged; and W= the total number of *N. lugens* nymphs and eggs that did not hatch.

An ANOVA using a CRD was used to determine differences between rice plant ages, and if a significant difference was found, a posthoc test was done. All analyses were done using SPSS 16.0 at α=5%.

RESULTS AND DISCUSSION

Parasitoids of *N. lugens* Eggs

Two species of *N. lugens* egg parasitoids were collected from this study, *Oligosita* sp. (Hymenoptera: Trichrogrammatidae) and *Anagrus* sp. (Hymenoptera: Mymaridae), and *Oligosita* sp. was more abundant than *Anagrus* sp. regardless of the rice age. During the first trapping, the highest number of parasitoids emerged were from the rice traps placed in the 6-week-old rice fields (*Oligosita* sp.: 9.75 individual/trap and *Anagrus* sp. 4.75 individual/trap, total parasitoid: 14.50 individual/trap). The same finding was found during the second trapping in which the highest total parasitoid emerged occurred from the traps placed in the rice plants aged 6-week-old (*Oligosita* sp. 9.75 individual/trap dan *Anagrus* sp. 3.75 individual/trap, total 13.50 individual/trap). These findings indicate that the 6 weeks-old rice plants were more

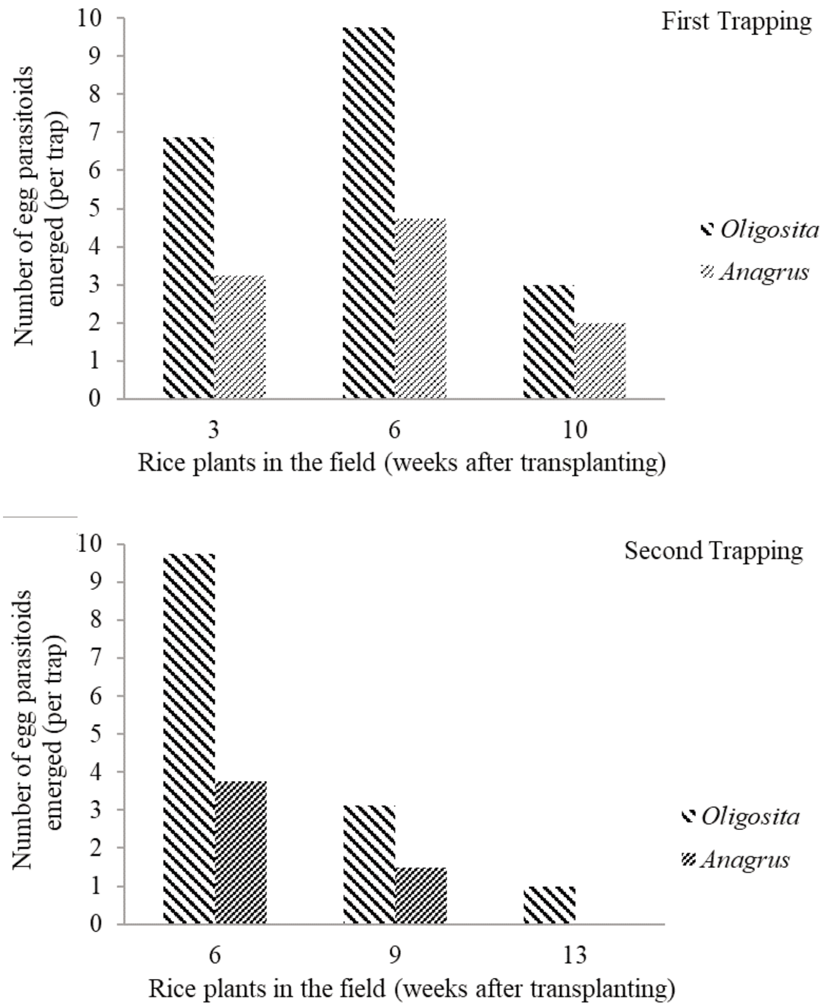

Figure 1. Parasitoids of *Nilaparvata lugens* eggs that emerged from *N. lugens* egg-infested trap plants on rice fields with different growing ages at Paremono Village, Mungkid, Magelang during March–April 2019

attractive for the egg parasitoids, and their attractiveness decreases when the plants get older (Figure 1). The differences in the abundance and parasitism by the egg parasitoids are mainly influenced by the population of hosts (the eggs of *N. lugens*), the microclimate, and the plant fitness which may work independently or in combinations. Previous researchers had showed the dependence of natural enemies with *N. lugens* in relation with the rice age (Ibrahim & Mugiasih, 2020; Hasan *et al.,* 2021) suggesting the phenomenon of density dependent factors. In this research, the population of *N. lugens* was very low (Y. Sulistyawati, unpublished). We do not have exact explanation supported by the empirical data on why the parasitism was the most at the 6-week rice age. However, we hypothesize that the difference in the rice ages might create different microclimate resulting in different level of attraction for the parasitoids to come and find *N. lugens* eggs.

The egg parasitoids from the genus of *Oligosita* and *Anagrus* were also commonly found in many Asian countries, such as north of India to China, north of Jepang and south of Malaysia, Singapore, and Vietnam (Gurr *et al.*, 2016), and Indonesia (Meilin, 2012; Sinulingga *et al.*, 2019). Different genera of egg parasitoids have been reported in Indonesia. For example, Minarni *et al.* (2018) reported *Gonatocerus* sp. dan *Oligosita* sp. in Banyumas Region. Abdillah (2015) reported that *Oligosita* sp. was the most abundant genus of egg parasitoids in Darmaga, Bogor compared to *Anagrus nilaparvata*, *Anagrus* sp., *Tetrastichus formosanus*, and *Gonatocerus* sp. Furthermore, Yaherwandi and Syam (2007) stated that *Anagrus* sp., *Gonatocerus* sp., and *Oligosita* sp. were also found

Table 1. Parasitization levels of *Nilaparvata lugens* eggs in rice fields at different growth stages in Paremono Village, Mungkid, Magelang from March–April 2019

Rice age (weeks)	Parasitoids that emerged	*N. lugens* *nymph*	*N. lugens* eggs that did not hatch	Parasitized *N. lugens* eggs but not hatching	Parasitization levels*
	(individual/trap)	(individuals/trap)	(eggs/trap)	(eggs/trap)	(%)
Trap 1					
3	10.13	50.00	29.13	4.13	15.16 ± 3.07b
6	14.50	45.25	30.00	7.13	22.26 ± 6.82a
10	5.00	49.75	31.13	8.25	14.24 ± 3.24b
Trap 2					
6	13.50	41.00	31.88	6.63	21.93 ± 4.61a
9	4.63	66.50	46.63	6.88	9.29 ± 1.73b
13	1.00	58.38	41.38	6.25	6.89 ± 1.82b

in the landscapes of Sungai Kapih and Kayu Tanduk, West Sumatera. These differences may indicate the diversity and the richness of *N. lugens* egg parasitoids which could be affected by the landscape and geographic differences as well as farmers' practices in rice production systems. Furthermore, it may lay the groundwork for maximizing the role of these parasitoids in regulating the population of *N. lugens*.

Parasitism Levels of *N. lugens* Eggs in Rice with Different Ages

The land structure of the study site consisted of rice plants of different ages and different crops within this landscape. Parasitization levels from 6-week-old rice plants were significantly higher (22.26%) compared to 3- (15.16%) and 10- (14.24%) week-old rice plants for the first trapping. The same pattern was found during the second trapping where parasitization levels from 6-week-old rice (21.93%) rice were higher compared to 9 (9.29%) and 13 weeks (6.89%) (Table 1). These results indicate that the parasitoids prefer living in the micro-ecosystem of 6-week-old rice than 3- or 9-week-old. Previous research by Claridge *et al*. (2002) reported the same pattern where *N. lugens* egg parasitization reached 29–91%, and 93% (Sann *et al*., 2018). Furthermore, Sinulingga *et al*. (2019) indicated that the parasitism in one rice season reached the highest when the rice plants were at 6-week-old and declined toward the harvesting time.

The decline of the parasitism at the older rice plants may increase the probability of *N. lugens* outbreak at the end of rice growing season. Planting flowering plants surrounding the rice field was able to maintain the parasitism remained high until the end of the rice season (Sinulingga *et al*. 2019). The flowering plants provide nectar, pollen, and shelter for natural enemies, and this phenomenon has been reported massively in publications. Therefore, managing the rice ecosystems to enhance the role of natural enemies may reduce the risk of *N. lugens* outbreaks and it could be one esential approach in ecological engineering (Heong *et al*., 2021a, 2021b). Despite the role of egg parasitoids has been well understood, the mechanism underlying the preference and high parasitism at the 6-week-old rice need to be further studied for practical implementation.

CONCLUSIONS

Two species of *N. lugens* egg parasitoids collected from the rice fields with different growing ages in Magelang were *Oligosita* sp. and *Anagrus* sp. The number of egg parasitoids emerged, and their parasitism level was the highest when the rice plants aged 6-week-old (13.5–14.5 individual/trap and 21.93–22.26%, respectively), and their number decreased when the plants get older.

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