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Research Article

Parasitization Levels of *Spodoptera frugiperda* Eggs (Smith) (Lepidoptera: Noctuidae) in Three Different Corn Ecosystems in East Java

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ABSTRACT

Spodoptera frugiperda (Lepidoptera: Noctuidae) has successfully invaded and spread to almost all provinces of Indonesia which may cause significant impacts on corn production. Local natural enemies could play an important role in managing this invasive insect, and more diverse ecosystems would benefit natural enemies. This study aimed to assess parasitization rates of *S. frugiperda* eggs by local egg parasitoids in three different corn ecosystems (agroforestry, rice field, and rainfed field) in East Java. Sentinel egg masses were used for this study by exposing eight-hour-old egg masses collected from the laboratory mass-rearing and left for 24 hours in corn plantations aged 7, 14, and 28 days after planting. *Telenomus* sp. was more abundant in the three ecosystems compared to *Trichogramma* sp. The egg mass parasitization varied from 15.6 to 52.5%. The number of egg masses parasitized was consistently higher in agroforestry, followed by rice fields and rainfed fields in all three different sampling times. Interestingly parasitization rates on egg masses were not different, and they ranged from 43.7 to 81.6%. These findings provide evidence on the importance of local egg parasitoids for managing *S. frugiperda* and some insights related to plant diversity to improving the services by these parasitoids.

Keywords: ecosystem; egg parasitoid; parasitism; *Spodoptera frugiperda*

INTRODUCTION

Spodoptera frugiperda (Smith) (Lepidoptera: Noctuidae) is a polyphagous pest that has been reported to damage more than 80 plant species from 23 families, including corn, sorghum, and other crops (Ashley *et al*., 1989). This species is widely spread in America and was first detected in Central and West Africa at the beginning of 2016 and quickly spread to Southeast Asia in 2018 (Food and Agriculture Organization [FAO], 2020). *Spodoptera frugiperda* was first detected in Indonesia at the beginning of 2019 and was first published from observation in Lampung by Trisyono *et al*. (2019). Later studies reported that this species has spread to other regions in Sumatra, Java, and Kalimantan (International Plant Protection Convention [IPPC], 2019). Currently, *S. frugiperda* has caused concern among farmers due to this species' ability to damage corn at all growth stages including vegetative to generative stages (Trisyono *et al*., 2019), and may occasionally cause complete yield loss (Sarmento *et al*., 2002). Centre for Agriculture and Bioscience International (CABI) has estimated that *S. frugiperda* cause corn yield loss to range between 4.1–17.7 million ton each year, which is equal to 1.088–4.611 million USD each year (Rwomushana *et al*., 2018).

The main strategies done in the United States of America to manage *S. frugiperda* are applying insecticides and planting genetically modified crops (Bt corn)(Abrahams *et al*., 2017). Massive insecticide spraying programs are also done in Africa (Abrahams *et al*., 2017; Prasanna *et al*., 2018). Application of synthetic insecticides is the main practice used to manage *S. frugiperda* in Indonesia. The Indonesian government have recommended insecticides containing four active ingredient namely emamectin benzoate, cyantranilirpole, spinetoram, and thiamethoxam (*Direktorat Jenderal Prasarana dan Sarana Pertanian*, 2019) under emergency permission. Synthetic insecticides should be used cautiously due to the adverse effects that are caused by their use, such as insecticide resistances among target pest species, reduce of natural enemy potency, and increase of production cost (Yu, 1991; Abrahams *et al.*, 2017; Prasanna *et al.*, 2018). Therefore, Integrated Pest Management (IPM) programs for *S. frugiperda* that utilize and increase ecosystem services are essential.

Indonesia is a country with rich biodiversity making exploration and use of local parasitoids of *S. frugiperda* a promising strategy to manage this invasive pest. Agroecosystem conditions that support the development of these parasitoids are essential. Several studies have revealed correlations between parasitoids and plant diversity within agroecosystems. Monoculture system used in intensified farming can reduce biodiversity and cause the loss of parasitoid functions (Buchori *et al.*, 2008). In contrary, habitat diversity can increase effectiveness of natural enemies by increasing alternative food sources, ensuring supply of host or prey, nectar source, and providing required microhabitats (Sheehan, 1998). In addition, planting orders also determine population dynamics of *S. frugiperda* due to its ability to provide food sources for this pest overtime (Midega *et al.*, 2018).

Utilization of natural enemies from introduction program or species that are locally available can reduce *S. frugiperda* damage, while also providing economically and environmentally friendly options. Many parasitoid have been identified and approximately 180 species of *S. frugiperda* parasitoid have been discovered in America (Molina-Ochoa *et al.*, 2003; Camargo *et al.*, 2015; Capinera, 2017; Shylesha *et al.*, 2018). Several species of egg and larvae parasitoids have been reported in Africa (Assefa & Ayalew, 2019; Kenis *et al.*, 2019; Sisay *et al.*, 2019; Agboyi *et al.*, 2020; Caniço *et al.*, 2020). The egg parasitoids, *Telenomus remus* (Hymenoptera: Scelionidae) and *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) are potential biocontrol agents that attack *S. frugiperda* before hatching and are easy to mass produced (Kenis *et al.*, 2019; Sari *et al.*, 2020; Jin *et al.,* 2021). Both parasitoids have been reported to reduce *S. frugiperda* infestation in the field with parasitization levels of 37.5–84.7% in Cameroon (Abang *et al.*, 2021) and even reach 64% di Nigeria, Africa (Laminou *et al.*, 2020). Besides that, corn is not only cultivated on ex-rice field land, but also dry and agroforestry conditions. Therefore, this study aimed to determine parasitization of local parasitoids on *S. frugiperda* egg masses at different ecosystem conditions.

MATERIALS AND METHODS

Research Location

This research was done using a sentinel technique to determine egg parasitization levels of *S. frugiperda* egg masses in Regency of Blitar, East Java. Three different locations were selected based on their ecosystems which were Village of Doko, District of Doko for the agroforestry ecosystems; Village Ngaringan, District of Wlingi for rice field ecosystems; and Village of Sumberasri, District of Nglegok for the rainfed field ecosystems. Agroforestry location was chosen based on characteristics stated by Wijayanto (2006) stating that it was an ecosystem with combination of perennial and annual crops, and secondary crops were not taller than the primary crop (forestry crop), and the diversity of plants within these ecosystems. Field samplings were done between March–July 2021. Agroforestry conditions were polyculture with perennial crops, corn, and other annual crops (Figure 1). Rice field were monoculture areas with irrigation, and other annual crops (Figure 2). Rainfed field were water-fed rice field that were planted with grown in monoculture systems (Figure 3).

Spodoptera frugiperda Rearing

Larvae of *S. frugiperda* (n=190) were collected from corn fields located in the Village of Doko, District of Doko, Regency of Blitar in January 2021. Population of *S. frugiperda* larvae were maintained using pieces of corn leaves. To prevent cannibalisms, each larva of *S. frugiperda* were reared in separated 50 mL plastic vials. As much as 200 *S. frugiperda* pupae were placed in a Petri dish inside a cage (50 cm×50 cm×60 cm) for mass rearing. Male and female *S. frugiperda* imagoes were allowed to mate randomly. Moths were fed with 10% of honey solutions using a cotton immersed in this solution, and replaced daily. Two corn plants grown in pots were placed into the cage as oviposition sites.

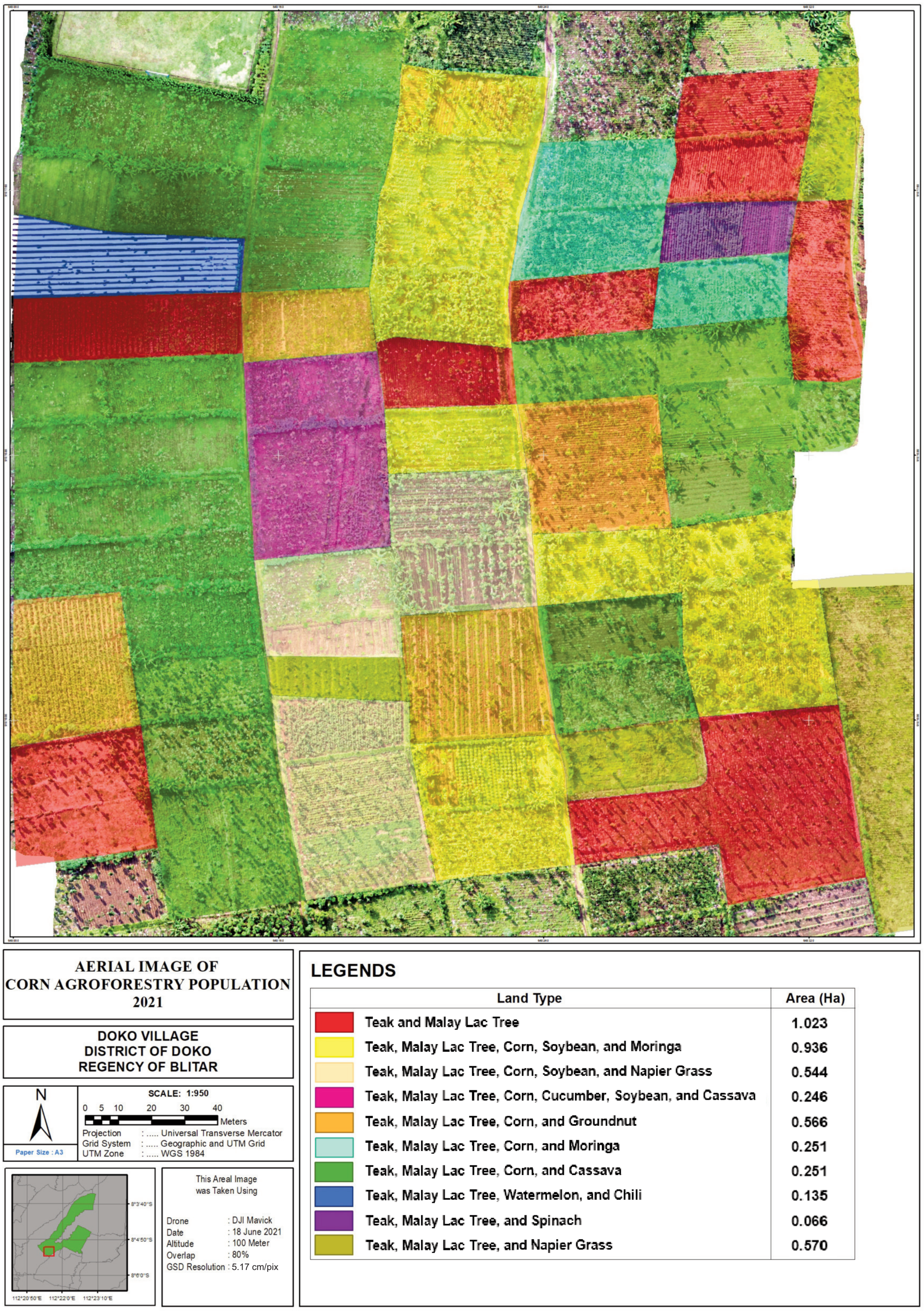

Figure 1. Vegetation types of agroforestry ecosystem in the District of Doko, Regency of Blitar, East Java in June 2021

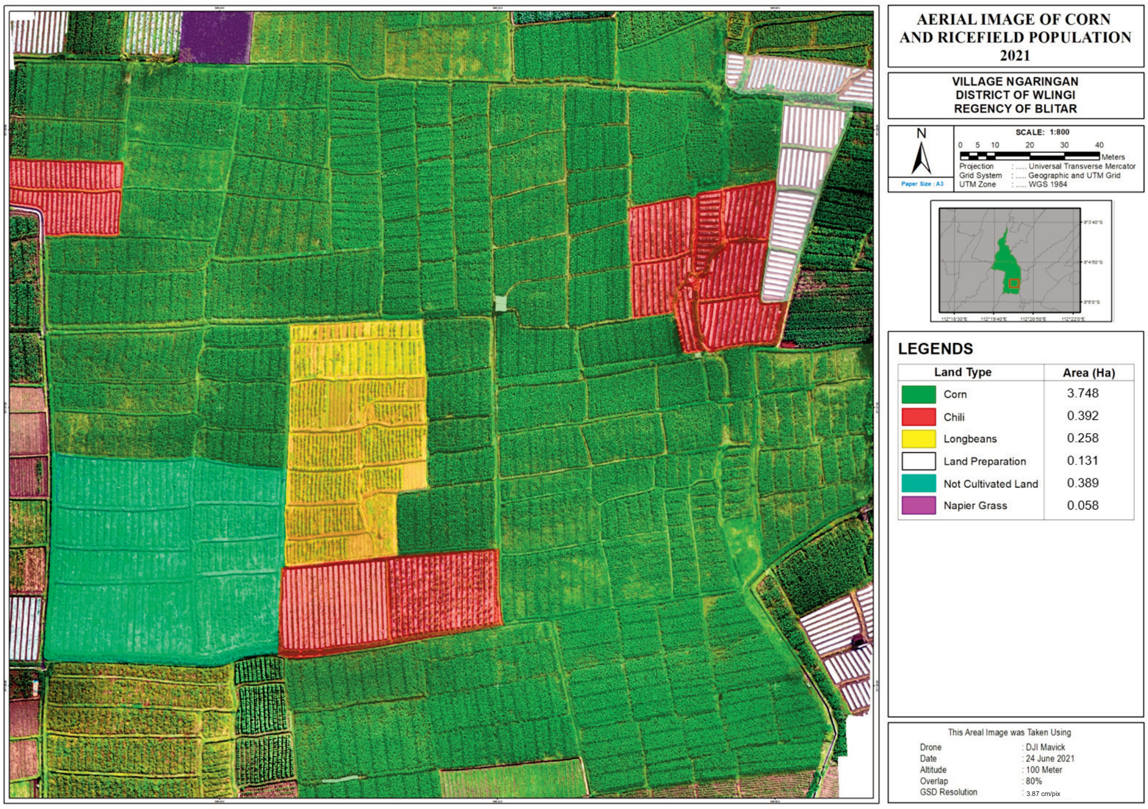

Figure 2. Vegetation types of rice field ecosystem in District of Wlingi, Regency of Blitar, East Java in June 2021

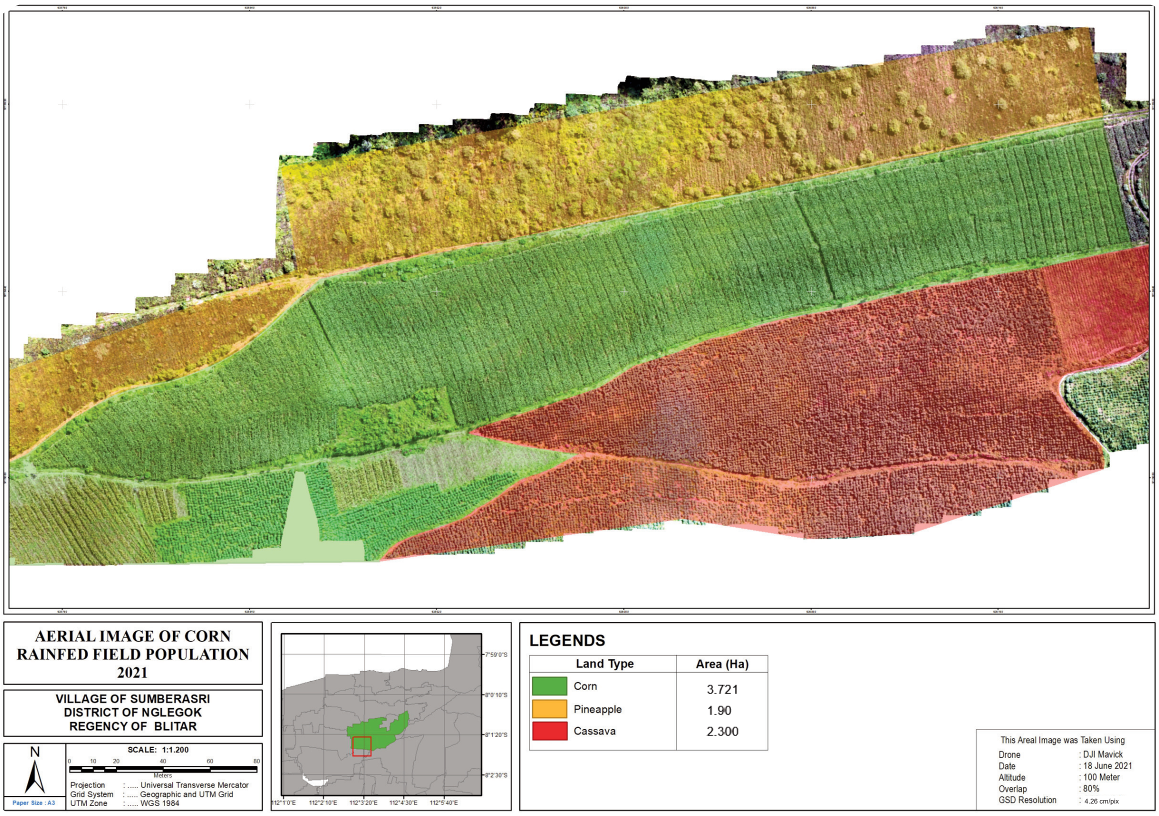

Figure 3. Vegetation types of rainfed land ecosystems in District of Nglegok, Regency of Blitar, East Java in June 2021

Every 8 hours, corn plants inside cages were changed with new ones. Egg masses of *S. frugiperda* (8 hours old) were cut with pieces of corn leaves and used for sentinel technique.

Egg Parasitoid Parasitization Levels

Egg masses of *S. frugiperda* used were 8-hours-old and collected from mass rearing. Exposure of egg masses using this sentinel technique and followed methods from Dasilva *et al.* (2015). Egg mass sentinels were placed in three agroecosystems (agroforestry, rice-field, and dryland). In each agroecosystem, egg masses were placed on corn plants aged 7, 14, and 28 days-after planting (DAP) (Beckingham, 2007; Mukkun *et al.*, 2021). As many as 90 egg masses were placed in agroforestry fields, 107 egg masses in rice fields, and 126 egg masses in rainfed fields. The difference in number of egg masses used in each agroecosystem was due to the risk of insecticide exposure on egg masses. Egg mass sentinels were placed 10 m from each other. Egg masses were left in the field for 24 hours and after that were collected and reared at the laboratory. Egg masses were kept in the laboratory at room temperature (24–26°C, RH 50–70%, and photoperiod 12:12 light:dark) until parasitoids emerged. Parasitoids were identified using the determination key from Johnson (1984), Borror *et al.* (2005), Querino and Zucchi (2005) to genus level at Invertebrate Plant Pest Laboratory, Department of Plant Protection, Universitas Gadjah Mada.

Data Analysis

Analysis were done quantitatively by calculating relative abundance of parasitoids (Canico *et al.*, 2020) and parasitization levels of *S. frugiperda* egg masses (Van Driesche, 1983). Percentage of parasiti-zation levels were analyzed using ANOVA. If significant differences were detected, an LSD post hoc test was using SPSS23. The t-test was done to compare proportion of parasitoids in each agroecosystem. ANOVA using a complete randomized design done for the parasitization level of *S. frugiperda* eggs and first transformed using arcsine before performing ANOVA.

Relative abundance (Ra) was calculated as the percentage of total number of parasitoid species (ni) divided by the total parasitoid collected (N).

$$Ra=(ni \div N)\times 100\%$$

Parasitization of egg masses (Pr) was calculated by dividing the number of parasitized egg masses (ne) by the total egg masses collected (N).

$$Pr=(ne \div N)\times 100\%$$

Parasitization levels (Para) was calculated by dividing the number or parasitoids that emerged (n) by the total number of eggs in each egg mass (number parasitoid that emerged + number larva that occurred + eggs that did not hatch) (N).

$$Para=(n \div N)\times 100\%$$

RESULTS AND DISCUSSION

Species of *Spodoptera frugiperda* Egg Parasitoids

As many as 323 *S. frugiperda* egg masses were placed in the field and two species of egg parasitoids collected evenly across three ecosystems were *Telenomus* sp. and *Trichogramma* sp. (Figure 4). Parasitoids collected were the most common species known to parasitize Lepidopteran pests. *Telenomus* sp. is an egg parasitoid of several Lepidopteran insect pest found in Asia and America (Cave, 2000). In addition to Lepidopteran insect, *Telenomus* sp. are known to emerge from eggs of insect from the Hemipteran, Dipteran, and Neuropteran order (Johnson, 1984). *Trichogramma* sp. is a main egg parasitoid of Lepidopteran insect together with eggs of some Coleopteran, Dipteran, Heteropteran, Hymenopteran, and Neuropteran insects (Smith, 1996). Most species of the Lepidoptera are parasitoid by approximately five species of Trichogrammatidae in Java (Buchori *et al.*, 2010).

The two parasitoids collected during this research were similar with reports of other researches from Indonesia. A survey done by Tawakkal *et al.* (2020) in Bogor, West Java, found seven genera of parasitoid related *S. frugiperda*, which also consisted of the two egg parasitoid genera, *Telenomus* sp. and *Trichogramma* sp. A survey in the Regency of Malang, Batu, and Kediri, East Java reported two species of *S. frugiperda* egg parasitoid, namely *Telenomus* sp. and one species from Mymaridae family (Rizali *et al.*, 2021). Meanwhile, another survey done in the Island of Lombok reported two parasitoids associated with *S. frugiperda* eggs, *Telenomus* sp. and *Trichogramma* sp.

(Supeno *et al.*, 2021). These findings were similar with research from Jaraleño-Teniente *et al.* (2020), that found *Tr. atopovirillia* and *Tr. pretiosum* in Middle Mexico. Shylesha *et al.* (2018) found five *S. frugiperda* parasitoid in India. Egg parasitoid documented in India, included *Telenomus* sp. and *Trichogramma* sp. Otim *et al.* (2021), reported 13 species of parasitoids that attack *S. frugiperda,* including *Telenomus* sp., *T. remus,* and Platygastridae.

Parasitoid Relative Abundances

Relative abundance of *Telenomus* sp. and *Trichogramma* sp. were different between plant age and ecosystems. Relative abundances of *Telenomus* sp. and *Trichogramma* sp. showed significantly differences across 7, 14, and 28 DAP in agroforestry, while 7 and 28 DAP from rice fields (Table 1). In general, percentage of *Telenomus* sp. abundance in the field was between 55.7 to 100%, while for *Trichogramma* sp. was between 0 to 44.2%. This shows that the occurrence of *Telenomus* sp. is more abundant compared to *Trichogramma* sp. in the field. High *Telenomus* population caused by behavior of female imagoes to search *S. frugiperda* egg masses to identify hosts' shape, texture, and chemical compounds to determine suitable hosts (Tawakkal *et al.*, 2020). Compared to *Trichogramma* sp., *Telenomus* sp. is a more aggressive parasitoid of *Spodoptera* spp. egg masses due to its larger size, being stronger, and increase ability to penetrate layer of egg masses (Cave, 2000). Female *Spodoptera* spp. moth covers their egg masses with scales of their body. Scales and egg layer can hinder *Trichogramma* sp. to parasite egg masses due to its smaller size compared to *Telenomus* sp. resulting in this species only being able to parasite eggs on the top layer (Salazar-Mendoza *et al.*, 2020).

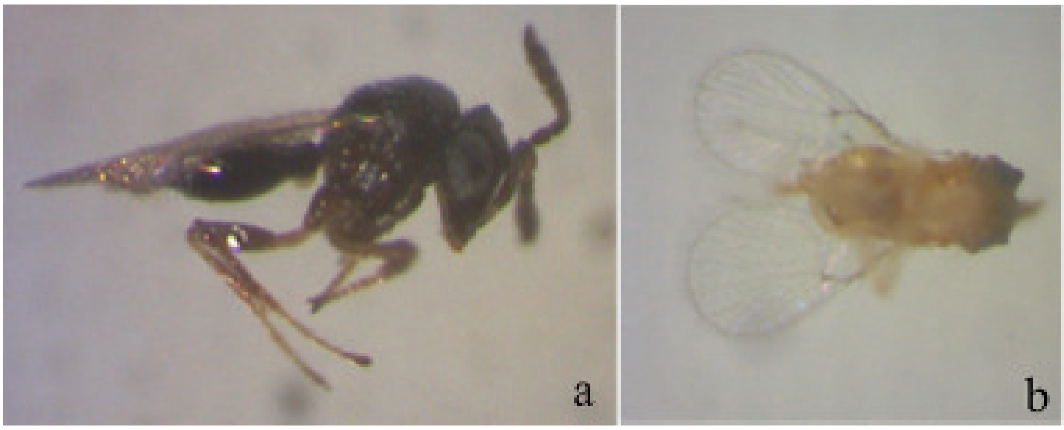

Figure 4. Parasitoids collected from the three ecosystems (agroforestry, rice field, and rainfed field) in Regency of Blitar, East Java during March–July2021: (a) *Telenomus* sp. and (b) *Trichogramma* sp.

Table 1. Relative abundance of each *Spodoptera frugiperda* egg mass parasitoids in corn fields from three ecosystems in Regency of Blitar, East Java

Ecosystem	Plant age (DAP)					
	7		14		28	
	Telenomus sp.	*Trichogramma* sp.	*Telenomus* sp.	*Trichogramma* sp.	*Telenomus* sp.	*Trichogramma* sp.
Agroforestry	67.5±40.1a	32.4±41.8b	65.6±42.1a	34.4±42.1b	64.8±42.2a	35.3±42.0b
Rice field	64.5±44.5a	35.5±44.5b	61.5±41.9a	38.4±41.5a	77.4±36.8a	22.6±36.8b
Rainfed field	55.7±47.8a	44.2±47.8a	100.0± 0.0a	0.0± 0.0a	100.0± 0.0a	0.0± 0.0a

Notes: Day after planting (DAP). Mean±SD followed by different lowercase alphabet showed significant differences between parasitoids in each ecosystem and plant age using a t-test ($P<0.05$) while the same alphabet indicates no significant different; *= significant different based on $LSD_{0.05}$ to compare mean parasitoid at three ecosystem and each plant age.

Table 2. Parasitization level of *Spodoptera frugiperda* egg masses in corn fields from three ecosystems from Regency of Blitar, East Java

Ecosystem	Plant age (DAP)	Number of egg masses	Number of parasitized egg masses	Parasitization (%)	$\bar{X}$±SE
Agroforestry	7	30	17	56.7	52.5± 7.7b
	14	30	17	56.7	
	28	30	13	43.3	
Rice field	7	35	13	37.1	30.0± 6.5a
	14	35	10	28.6	
	28	37	9	24.3	
Rainfed field	7	30	9	30.0	15.6±12.5a
	14	51	5	9.8	
	28	45	4	8.9	

Notes: Day after planting (DAP). Mean followed by the same alphabet are not significantly different based on $LSD_{0.05}$ post-hoc test.

Parasitization Level of *Spodoptera frugiperda* Egg Masses

Percentage of egg mass parasitization in the field were different among the ecosystems. Parasitization level reached 52.5% in agroforestry ecosystems and was higher than *S. frugiperda* egg masses parasitization in the rice field and rainfed field, 30 and 15.6% respectively (Table 2).

In agroforestry, corn was cultivated in polycultures with perennial and other annual crops (Figure 3) providing many host crops for polyphagous insects like *S. frugiperda*. High biodiversity conditions cause *S. frugiperda* to not only focus on corn, but also other plants, such as napier grass. This was consisted with the statement of Root (1973) that monophagous and oligophagous herbivores would easily find food in monoculture cropping systems, while polyphagous herbivores would migrate from these systems to other vegetation close by. Besides that, natural enemy populations were higher in polyculture systems due to existence of alternative prey, nectar source, and suitable microhabitats. Polyculture systems will increase plant diversity in agroecosystems and affect both pest and natural enemy insect diversity (Brotodjojo, 2009). Biocontrol using natural enemies are averagely 46% less effective in homogeneous landscape dominated by crops compared to more complexed landscapes (Rusch *et al.*, 2016). The complexity of agroecosystems in polycultures can decrease pest populations (Altieri & Letourneau, 1984). Research by Bianchi *et al.* (2006) demonstrated that natural enemies had stronger and consistent positive response in more complex landscapes.

Parasitization Levels of Parasitized *Spodoptera frugiperda* Egg Masses

Parasitization level of each parasitized *S. frugiperda* egg masses showed no significant differences across three ecosystems (Table 3). This may be caused by parasitoid behavior to parasite host and later whole egg masses. Percentage of parasitization of parasitized egg masses varied compared to plant age. Number of parasitized egg masses decrease as plant ages became older. However, agroforestry ecosystems showed that parasitization levels increased on parasitized egg masses as corn plant ages became older (Table 3). This is caused by the availability of hosts in the field. In general, *S. frugiperda* infest corn plants at all plant stages, but worst infestation happens during the vegetative stage. Survey from Rwomushana *et al.* (2018) in Ghana showed that 57% of *S. frugiperda* infest at early growing stages, while 84% attack during the vegetative stages in Zambia.

Parasitization level were about 43.7% in rainfed field ecosystem to 81.6% in agroforestry (Table 3). Highest parasitization levels were found in agroforestry at three different plant ages. Various factors affect parasitization levels. In rainfed field ecosystems, farmers may apply insecticide intensively to manage *S. frugiperda* and affect abundance of its natural enemies. Besides pesticide application (that cause low parasitization) differences of parasitization levels

Table 3. Number of *Spodoptera frugiperda* egg masses parasitized in corn field from three ecosystems at Regency of Blitar, East Java

Ecosystem	Day after planting (DAP)					
	7		14		28	
	n	Parasitization(%)*	n	Parasitization (%)*	n	Parasitization (%)*
Agroforestry	17	74.1±38.0a	17	76.1±35.2a	13	81.6±30.6a
Rice field	13	72.0±33.8a	10	65.8±36.9a	9	65.2±41.5a
Rainfed field	9	68.3±35.4a	5	51.7±45.3a	4	43.7±37.9a

Notes: Day after planting (DAP). Number of 8-hour-old egg masses parasitized after 24 hours in the field and brought to and kept in the laboratory (n). *Mean ± SD from each number of samples (n). Means followed by the same alphabet in each column are not significantly different based on $LSD_{0.05}$

were caused by parasitoid parasitization, ability of parasitoids to find and parasitize host, and different landscape structures (Buchori *et al*., 2010). Complex landscapes with various vegetation correlate with high parasitization incidences (Hunter, 2002; Buchori *et al*., 2008). In this study, agroforestry was example of complex landscapes causing higher parasitization compared to rice field and rainfed fields. Another research on the effect of landscapes on parasitization by Marino and Landis (1996) showed that *Pseudaletia unipuncta* as a model insect host and showed that *P. unipuncta* parasitization in simple agricultural systems were lower than more complex agricultural systems. Findings from this study support the importance of *S. frugiperda* management in area wide scales in addition to each farm management by the owners. Furthermore, the findings provide bases to design and utilize diverse landscapes to maximize the ecosystem services.

CONCLUSION

Two egg parasitoids of *S. frugiperda* collected from corn fields in agroforestry, rice fields, and rainfed fields were *Telenomus* sp. and *Trichogramma* sp. with the first species being more abundances in these three ecosystems. Highest parasitization of egg masses were in agroforestry ecosystems by 52.5% with parasitization level in each parasitized egg masses reaching 81.6%. Parasitization levels of egg masses decreased in rice fields and the lowest in rainfed fields. Attempts to increase the role of these parasitoids in managing *S. frugiperda* population can be done by increasing biodiversity of agricultural ecosystems to provide food sources and suitable habitats for both of these parasitoid species.

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