New association between *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) and native natural enemies: Bioprospection of native natural enemies as biological control agents

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Abstract. Fall armyworm (FAW) *Spodoptera frugiperda* is an invasive pest that enters Indonesia which can pose a serious threat to the continued production of maize and other crops. This condition is exacerbated if there are no natural enemies who can control the pests. This study aims to explore potential native natural enemies to control the population of FAW in Indonesia and the influence of habitat conditions on the distribution and population of natural enemies. The research was conducted in seven sub-districts in Bogor and three plots were selected from each district. Observation of natural enemies was carried out directly by collecting samples of eggs and larvae of FAW which attacked maize with the transect method on 200 individual plants. The samples collected will be rearing until the parasitoids emerged. We collected seven genera of parasitoids that were associated with FAW: *Apanteles*, *Charops*, *Euplectrus*, *Microplitis*, *Telenomus*, *Trichogramma*, and two types of predators: ground beetles and Assassin bug. The highest parasitization rate of egg parasitoid was 93.4% while for larvae parasitoid was 12.44%. In conclusion, *Telenomus* and *Microplitis* are native parasitoids that can be used as potential natural enemies to control the *S. frugiperda* population in the field.

1. Introduction
Fall armyworm (FAW), *Spodoptera frugiperda* are invasive pests known to have entered Indonesian in early March 2019 [1]. Invasive pests are species, subspecies, or varieties (non-native, non-indigenous, foreign, exotic) that enter the territory of Indonesia a natural or semi-natural ecosystem that is not its natural habitat either directly or indirectly, settles and reproduces so that it can act as a modifying agent and threatens ecosystems, habitats, biodiversity, harming the economy and human health [2-3]. The increasing population of invasive alien species (IAS) has a rapid negative impact on species diversity, namely due to the homogenization of the world’s flora and fauna. Invasive species are now recognized as the main species causing the loss of biodiversity, second only to habitat destruction and this occurs in all taxonomic groups, and is at risk of occurring in all environments [4]. This could be due to the influence of invasive species on local species in competition to utilize the same resources [5].

Biological control of invasive pests is usually carried out by introducing natural enemies from outside of the country of origin of the pests to control pests in the newly entered areas [6] or known as classical
biological control. Since the introduction of the vedalia beetle *Rodolia cardinalis* to control the *Icerya purchasi* citrus pest purchased in California in 1886, nearly 6,000 introductions have been made and more than 2,000 insects are used as biological control agents for insect pest control worldwide [7]. The introduction of natural enemies in Indonesia has been carried out, to control the exotic pest of the *Heteropsylla cubana* by bringing in a predator *Curinus coerules* from Hawaii [8]. Besides, the introduction of the parasitoid *Anagyrus lopezi* from Thailand was carried out to control cassava mealybugs *Phenacoccus manihoti* in March 2014 [9].

*S. frugiperda* is one of the pests causing the highest economic losses in agriculture worldwide [10]. This insect is the main pest of corn and is polyphagous which can attack more than 80 types of plants. Yield losses caused by *S. frugiperda* in 2017 in Kenya, Africa were 924,000 tonnes (34%) and 883,000 tonnes (32%) in 2018 [11]. In July 2017, based on the results of the FAW attack survey resulted in an average national yield loss of 45% in Ghana (range 22-67%), and in Zambia 40% (range 25-50%) [12].

This pest originates from the tropical and subtropical regions of the American continent. Central and South America, the coastal parts of the United States, Argentina, and the Caribbean [10]. In West Africa, *S. frugiperda* was first reported to attack in late 2016 and has rapidly spread to various parts of the continent. At this time, it was officially reported that *S. frugiperda* was invading 44 African countries [13;14]. In 2018, *S. frugiperda* was first reported in India [15] and since then, this pest has reportedly continued to invade other Asian regions such as Bangladesh, Thailand, Myanmar, China, and Sri Lanka. The FAW case in Indonesia was first reported to attack corn plantations in West Pasaman Regency, West Sumatra in 2019 [1] and has spread to other provinces: Aceh, North Sumatra, Riau, South Sumatra, Lampung, Banten, West Java, Central Java, West Kalimantan, East Kalimantan and Gorontalo [16-17].

Parasitoids are natural enemies most commonly used to control insect pests [18]. More than 150 species of parasitoids are reported to attack *S. frugiperda* in America, one of which is *Telenomus remus* (Hymenoptera: Platygasteridae) which is also an egg parasitoid that attacks the eggs of various Lepidoptera species [19-21]. Sisay [22] reported that there are three species of egg parasitoids that attack *S. frugiperda* in Kenya, *T. remus, Trichogramma chilonis* (Hymenoptera: Trichogrammatidae), and *Chelonus curvimaculatus* (Hymenoptera: Braconidae). *T. remus* became the dominant egg parasitoid with a parasitization rate of 69.3% [22]. Research on natural enemies, especially the parasitoids that attack FAW in Indonesia, has not been widely carried out. Maharani [16] found the parasitoid *Archytas marmoratus* (Diptera: Tachinidae) and FAW larvae that were parasitized by the parasitoid family Braconidae. There are 7 genera of parasitoids found in parasites of *S. frugiperda* that attack maize crops in the Bogor area: *Trichogramma* sp., *Telenomus* sp., *Cotesia* sp., *Microplitis* sp., *Meteorus* sp. (Hymenoptera: Braconidae), *Euplectrus* sp. (Hymenoptera: Eulophidae), and *Anomaloninae* (Hymenoptera: Ichneumonidae) [23].

The discovery of several local parasitoids associated with UGJ then classical biological control did not apply in this case. For this reason, conservation biological control is necessary so that existing local natural enemies can thrive and survive in a habitat so that they can control pests. This study aims to obtain information on local natural enemies associated with *S. frugiperda* and to assess local natural enemies to be used as biocontrol agents in biological control.

2. Methods

2.1 Study site and observation unit

A total of seven sub-districts that are centers of maize plants in the Bogor Regency area will be selected for observation and sampling: Cijeruk, Ciomas, Dramaga, Kemang, Lewisadeng Pamijahan, and Tenjolaya Districts (figure 1) from July to September 2020. Observations were made on land 2-5 weeks after planting with a minimum area of 500 m$^2$. In each sub-district, three observation plots will be determined which are then used as replications. In each observation plot, additional information will be collected such as temperature, humidity, rainfall, altitude, land area, use of pesticides, plant varieties, and plant age.
2.2 Data collection on the abundance and diversity of *S. frugiperda* parasitoids

Data collection for the abundance and diversity of parasitoids in maize was carried out by two methods: direct observation of maize plants and rearing of sample insects to obtain parasitoids. Data collection was carried out once for each observation field. Samples were taken in the phase of eggs, larvae, and pupae of *S. frugiperda*. Besides, samples of other lepidopteran pests that attack maize were also taken. A sampling of eggs and larvae was carried out directly using a soft brush on corn plants that showed signs of damage. The samples obtained were put in a plastic container and labeled. Sampling was carried out on 20 transect lines. One transect line consists of 10 individual maize plants for each observation plot. The collected samples are then taken to the laboratory for rearing.

2.3 Rearing of insects and parasitization rate

Rearing of host insects is carried out by collecting Lepidopteran pests (eggs, larvae, and pupae) for rearing until the parasitoid imago insects appear. Samples from the field will be taken to the laboratory and put in a plastic container with a perforated lid so that air can enter and given a sheet of paper to keep moisture. The larvae sample will be given food in the form of young corn until the insects become imago or parasitoids have emerged. Egg samples are left until the insects or parasitoids come out. After the parasitoids died, they were put in a tube containing 70% alcohol and then labeled and stored. The level of parasitization was calculated based on the phase of the parasite-host. The level of parasitization is calculated using the formula:

\[
\text{Parasitization rate (\%)} = \frac{\text{(number of outgoing hosts)}}{\text{(number of hosts observed)}} \times 100\% 
\]
3. Results and discussion

At the time of observation, *S. frugiperda* was found in several phases. Starting from eggs to 5th instar larvae (Figure 2 c-e). Most of these phases are found in the leaves, especially the curling shoots. The difference in leaf position occurs during the larval phase. In the 1st instar larval phase, mostly found in leaves that have been opened or have passed the rolling phase. Whereas instars 2 to 5 are mostly found at the growing point. Symptoms of the attack caused by *S. frugiperda* also differ based on the larval stage. Larvae instar one to 2 will cause damage to the mesophyll of the leaf, causing a transparent layer on the damaged leaf (Figure 1a). This will cause a scattered form of damage such as white patches, which are leaves that have lost their mesophyll. 2 to 5 instar larvae will cause damage on the inside of the growing point or at the base of the curled leaves [1]. The damage is in the form of leaves that are cut into large holes, even in some observations the leaves have been damaged so that the curling leaves are cut to the point of growth (Figure 1b).

![Figure 2](image)

**Figure 2.** Symptoms of *S. frugiperda* in phase (a) larvae instar 1-2 and (b) larvae instar 3-5, phase found (c) eggs, (d) larvae instar 2, and (e) larvae instar 4.

A total of 993 individual parasitoids have been collected from the results of host rearing in the Bogor area. The parasitoids found were larvae and egg parasitoids. In this study, the most parasitic larvae hosts were *S. frugiperda* and *S. litura* with 2 and 3 larva phases. All of the parasitoids obtained belong to the order Hymenoptera, which consists of five families and six genera. Two genera are egg parasitoid *Telenomus* (Hymenoptera: Platygasteridae) and *Trichogramma* (Hymenoptera Trichogrammatidae), while the other four genera are larval parasitoids: *Apanteles* (Hymenoptera: Braconidae), *Charops* (Hymenoptera: Ichneumonidae), *Euplectrus* (Hymenoptera: Eulophidae), and *Microplitis* (Hymenoptera: Braconidae) (Table 1 and Figure 3). *Charops* and *Microplitis* were found to parasitize *S. frugiperda* and *S. litura* larvae, whereas *Apanteles* and *Euplectrus* were found to only parasitize and *S. frugiperda*. The parasitoid genus that has been obtained has also been found to parasitize *S. frugiperda* in the Americas and the Caribbean [19]. Other natural enemies found to belong to the predator group, namely ground beetle (Coleoptera: Carabidae) larvae, and assassin bug (Hemiptera: Reduviidae) (figure 3.g-i). Both predators are generalist predators that are commonly found preying on agricultural pests.
Figure 3. Several genera of parasitoids that parasitize larvae a) *Apanteles*, b) *Charops*, c) *Euplectrus*, d) *Microplitis* and egg parasitoid e) *Telenomus*, f) *Trichogramma*. Predators were found g) Assasin bug and h) larvae of ground beetle.

*Microplitis* and *Telenomus* are parasitoid genera that are almost always found in every observation location. The parasitoid wasp, *M. manilae* is known to parasitize larvae of many *Spodoptera* species in many countries [24]. *Telenomus* is an egg parasitoid that has the highest abundance, while *Trichogramma* is only found slightly, 16 individuals along with the egg group that has been parasitized by *Telenomus*.

Table 1. Parasitoids that were found associated with *S. frugiperda* and *S. litura*.

| Districts  | Genus      | Abundance | Types of parasitoids | Host          | Host phase | Parasitization |
|------------|------------|-----------|----------------------|---------------|------------|----------------|
| Kemang     | *Microplitis* | 19 Solitary | *S. frugiperda* and *S. litura* | Larvae 2-3   |            | 7.17           |
|            | *Telenomus*  | 284 Solitary | *S. frugiperda*   | Egg          |            | 76.54          |
| Cijeruk    | *Euplectrus* | 2 Gregarious | *S. frugiperda*   | Larvae 2     |            | 0.2            |
|            | *Microplitis* | 7 Solitary | *S. frugiperda*   | Larvae 2-3   |            | 1.4            |
|            | *Telenomus*  | 108 Solitary | *S. frugiperda*   | Egg          |            | 93.4           |
| Dramaga    | *Microplitis* | 27 Solitary | *S. frugiperda*   | Larvae 2-3   |            | 12.44          |
|            | *Telenomus*  | 135 Solitary | *S. frugiperda*   | Egg          |            | 91.84          |
|            | *Trichogramma* | 3 Solitary | *S. frugiperda*   | Egg          |            | 2.04           |
| Lewisadeng | *Microplitis* | 3 Solitary | *S. frugiperda*   | Larvae 2-3   |            | 1.23           |
|            | *Telenomus*  | 223 Solitary | *S. frugiperda*   | Egg          |            | 46.07          |
| Pamijahan  | *Euplectrus* | 1 Gregarious | *S. frugiperda*   | Larvae 2     |            | 0.26           |
|            | *Microplitis* | 14 Solitary | *S. frugiperda*   | Larvae 2-3   |            | 3.64           |
|            | *Trichogramma* | 9 Solitary | *S. frugiperda*   | Egg          |            | 1.46           |
| Taman Sari | *Apanteles* | 1 Gregarious | *S. frugiperda*   | Larvae 2     |            | 0.39           |
|            | *Charops*    | 2 Solitary | *S. frugiperda*   | Larvae 3     |            | 0.79           |
|            | *Microplitis* | 2 Solitary | *S. frugiperda*   | Larvae 2-3   |            | 0.79           |
|            | *Telenomus*  | 46 Solitary | *S. frugiperda*   | Larvae 2-3   |            | 12.23          |
|            | *Trichogramma* | 99 Solitary | *S. frugiperda*   | Egg          |            | 18.23          |
| Tenjolaya  | *Charops*    | 1 Solitary | *S. frugiperda*   | Larvae 2-3   |            | 0.27           |
|            | *Microplitis* | 46 Solitary | *S. frugiperda*   | Larvae 2-3   |            | 12.23          |
|            | *Telenomus*  | 99 Solitary | *S. frugiperda*   | Egg          |            | 18.23          |
|            | *Trichogramma* | 7 Solitary | *S. frugiperda*   | Egg          |            | 1.29           |

The parasitization rates of *Telenomus* ranged from 18.23% (Tenjolaya) to 93.4% (Cijeruk), while *Microplitis* ranged from 0.79% (Taman Sari) to 12.44% (Dramaga) (table 1). The high level of egg
parasitization by *Telenomus* was caused by the behavior of female Imago parasitoids from *Telenomus* who had the habit of placing their antennae (grumping) on the egg mass of *S. frugiperda* as a host recognition process to determine the shape, textures, and chemical substances of eggs to get a suitable host so that they could recognize the host properly.

Compared to other egg parasitoids such as *Tricogramma* spp. The ability to know a good host is owned by *Telenomus remus* making it more aggressive in *Spodoptera* spp memarasit eggs from the egg parasitoid more [25]. This can happen because when *Spodoptera* spp. layed the egg mass, then covered it with scales from its body. The scales and layers of eggs are the obstacles for *Trichogramma* spp. whose body size is smaller than *Telenomus* spp. thus it can only parasitize a few eggs in the upper layer [26].

Many factors influence the effectiveness of parasitoids in controlling pest populations such as reproductive capacity and searching capacity. Reproductive capacity is the parasitoid ability to reproduce or the ability to produce the next offspring, while the searching capacity is the ability to find a host. The abundance of larva population found did not correlate with the abundance of parasitoids (R² = 0.0211, P = 0.582), while the abundance of eggs and egg parasitoids had a positive correlation (0.00108) where the more number of eggs found the egg parasitoid population increased (figure 4).

![Figure 4. Correlation between host-parasitoid a) Larva and larvae parasitoid and b) egg and egg parasitoid.](image)

The absence of a correlation between larval parasitoids and the number of larvae found is thought to be due to the poor ability to find a host due to several factors such as the host phase that cannot be parasitized, plant phenology, distance, etc. 1-3 where there are also many older instars so that the parasitoids cannot parasitize them. Older instars are also on the unopened interior of the leaf shoots, causing the parasitoids to find them. The rate decreases with the increased distance from the point of release [27] are thought to be probably due to the increased distance from the point of release, which will expand the area to be searched for by parasitoids, thus requiring a large amount of energy and increasing the risk of death [28]. However, *Telenomus* egg parasitoids can detect semiochemical compounds to hitch a ride on adult insects or phoresy to save limited energy if a direct search for phoresy activities will make it easier for egg parasitoids to find hosts newly laid by adult insects [29].

4. Conclusion

Five families and six genera of parasitoids associated with *S. frugiperda*. Two genera of parasitoid eggs are *Telenomus* (Hymenoptera: Platygasteridae) and *Trichogramma* (Hymenoptera Trichogrammatidae),
while four other genera are the larval parasitoid: Apanteles (Hymenoptera: Braconidae), Charops (Hymenoptera: Ichneumonidae), Euplectrus (Hymenoptera: Eulophidae) (Hymenoptera: Braconidae). Telenomus and micropolitics are native parasitoids that can be used as potential natural enemies to control S. frugiperda populations in the field because they have high parasitization abilities and are found in all observation fields. So it is necessary to manipulate the habitat to support the survival of these parasitoids so that biological control can work properly.

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