**Translation of**

**Seo, Meeja, Jeong Hwan Kim, Bo Yoon Seo, Changgyu Park, Byeong Ryeol Choi, Kwang Ho Kim, Chang Woo Ji and Jum Rae Cho. 2018.** Mass-rearing techniques of Anastatus orientalis (Hymenoptera: Eupelmidae), as the egg-parasitoid of Lycorma delicatula (Hemiptera: Fulgoridae): An using method [sic] of Antheraea pernyi (Lepidoptera: Saturniidae) and L. delicatula eggs in laboratory. Korean Journal of Applied Entomology 57(4): 243–251. https://doi.org/10.5656/KSAE.2018.08.0.035.

**INTRODUCTION**

Spotted lanternfly (*Lycorma delicatula*)is a major domestic pest belonging to the planthoppers called lantern fly (Han et al. 2008). Spotted lanternfly was discovered in Cheonan, Chungcheong Province in 2004 and it has been increasing all over the country including Seoul, Gyeonggi, Chungbuk, Jeonbuk, and Gyeongbuk (KFRI, 2007; Han et al., 2008; Choi et al., 2012). Spotted lanternfly feeds on the sap of grape, apple, pear, tree of heaven, and Chinese mahogany, resulting in weakening of the tree by sooty mold formed by the excrements after feeding on the sap (Jung et al. 2017). Spotted lanternfly can be controlled using pesticides, trap trees using tree of heaven, physical control using sticky traps, and also through biological control (Choi et al., 2012; Park et al., 2009; et al., 2010). In recent years, chemical control of forests and farmland has been carried out at the same time, but problems of residual pesticides remain in fruits or trees. Therefore, to solve pesticide residue problems and preserve ecosystem it is necessary to try classical biological control system to suppress the density of spotted lanternfly perennially by fixing natural enemies in ecosystem. Historically, S. Korea had success in control of woolly apple aphid and red wax scale using biological control (Jeon et al., 2003; Kim et al., 1979). Choi et al. (2012) and Choi et al. (2014) surveyed South Korea for native natural enemies and recorded birds (sparrows, magpies, etc.), amphibians (tree frogs, etc.), insects (praying mantis, robber flies, Reduviidae *Sphedanolestes impressicollis* and *Velinus nodipes*, etc.), and spiders feeding on spotted lanternfly. However, *Sphedanolestes impressicollis* was found not to be a suitable natural enemy since predation rate on immature sootted lanternfly was low (Choi et al., 2012). On the other hand, Choi et al. (2014) investigated the natural enemies of spotted lanternfly in China for the introduction of natural enemies and found *A. orientalis* parasitized 33.3% - 69.0% and *Dryinus ircoroae* parasitized 43% depending on the area. It has also been reported that in China *A. orientalis* parasitized 80% of spotted lanternfly eggs (Xiao, 1992; Zhang, 1993).

There were seven Eupelmidae found in South Korea; *Anastatus albitarsis*, *A. bifascuatys*, *A. dendrolini*, *A. gastropachea*, *A. japonicas*, *Eupelmus formosae*, *E. urozonus* (Paek et al., 2011). However, there are no records demonstrating the Eupelmidae parasitizing spotted lanternfly eggs. Therefore, *A. orientalis* was introduced from China Forest Science Research Institute in 2011, and it is confirmed that it can be utilized as a natural enemy for controlling the spotted lanternfly in Korea. We would like to report on the technique of mass rearing of parasitoid.

**CONCLUSION**

There are seven species belonging to Eupelmidae that are found in South Korea (Paek et al. 2011), but their parasitism of spotted lanternfly in the field has not been confirmed (Kim et al., 2011). *Anastatus orientalis* are well known as parasitoids of spotted lanternfly in China and have a high parasitism rate (Xiao, 1992; Xhang, 1993). In this study, we examined the feasibility of using *A. orientalis* from China in South Korea. In this study, it was confirmed that *A. pernyi* immature eggs was the only suitable alternative host among the seven tested for parasitism by *A. orientalis*. It has been confirmed that *Anastatus orientalis* does not lay eggs on immature Hemiptera, but do lay eggs on Lepidoptera eggs (Fay and Huwer, 1993; Žikić et al., 2017). Therefore, immature *A. pernyi* eggs were selected as an alternative host for mass rearing once parasitism was confirmed. Eggs of *Lymantria dispar* and *Antheraea yamamai* were not suitable for *A. orientalis* due to the small size of the egg resulting in not enough nutrients being available for development of the parasitoid. However, *A. pernyi* eggs are 3.0-3.7 mm long and 2.3-2.6 mm wide which is larger than spotted lanternfly eggs (3.0-3.1 mm long and 1.6-2.0 mm wide). Although the size of larval development in spotted lanternfly egg host versus *A. pernyi* was not compared, we expected development in *A. pernyi* to be superior.

*Anastatus orientalis* has been artificially raised for the production of cocoons and silk from the 17th century, and currently China produces more than 90% over 60,000 tons annually of worldwide production (Li et al., 2017). Also, *A. pernyi* eggs have been used to mass rear *Trichogramma* wasps for biological control of *Astrinia furnacalis* (Guenée) (Wang et al., 2001). *Antheraea pernyi* has also been used as host for *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae), *Propylea japonica* (Thunberg), and *Harmonia axyridis* (Pallas) (Nguyen et al., 2013; Ali et al., 2016). Since the breeding technique, production and use of the moths were confirmed, it was considered that breeding as an alternative host was relatively easy. In this study, we attempted mass rearing of *A. orientalis* using immature eggs of *A. pernyi*.

In this study it was possible to rear *A. pernyi* as an alternative host on oak leaves indoors and was able to obtain cocoons in early July and early October. The process of obtaining cocoons is an important step in the production and storage of host insects. The amount of immature eggs that can be obtained at this time can be estimated, and the timing of securing the host can be controlled by adjusting the timing of emergence when necessary. It was not difficult to obtain immature eggs by cutting the abdomen of female adult. The abdomen was cut carefully to avoid crushing the eggs to obtain about 150 to 200 eggs per female. When used as a host for *Trichogramma*, eggs obtained from dissecting adult females had a higher parasitism rate than eggs that were laid naturally (Wang et al. 2014). Although *A. pernyi* eggs can be stored up to 6 months, the parasitism rate greatly decreases when parasitoids are given long term stored eggs or eggs dissected from long term stored females (Wang et al. 2014). Results of a studying using *Trichogramma evanescens* parasitism on *A. pernyi* demonstrated that parasitized eggs stored at 2 ° C for up to 89 days were still viable (Ding et al., 1993). Storing the host in an egg or pupa state at low temperatures is a very effective method for raising, transporting, and inoculating natural enemies for commercial purposes to control pests (Lu et al., 2005). However, conditions such as storage time and storage temperature in the egg state of the host can have a negative impact on the parasitic capacity, release rate and longevity of the parasitoid from the host (Wang et al., 2014). Therefore, further study is needed on the optimal conditions for effective parasitism of long-term storage of *A. pernyi* immature eggs and spotted lanternfly eggs used in this study. The developmental stages from egg to pupa stage were 37.3 days and 36.1 days, respectively. In addition, there was no significant difference in the parasitism rate between the two hosts. Therefore it was concluded that the use of *A. pernyi* eggs as a host for *A. orientalis* was possible. In order to maximize the life span of *A. orientalis* we tested the life span by comparing wasps feed honey and those feed with nothing. The supply of honey seems to have played a role in longevity as a food source for newly emerging parasitoids. In this study, an immature oocyte of *A. pernyi*, which can be continuously supplied as a substitute host of the spotted lanternfly, was selected to establish a large-scale breeding system for *A. orientalis*. In order to successfully grow and sustain growth in the future, it is necessary to study the spawning season and habitat environment of the larvae.

**MATERIALS AND METHODS**

**Experimental Insects**

Spotted lanternfly parasitoids were introduced in 2011 from the Forest Science Research Institute of China for classical biological control of spotted lanternfly and has been used for experiments in a breeding program by the Department of Crop Protection in the Rural Development Administration, National Institute of Agricultural Science. Spotted lanterfly eggs oviposited onto grapevines were collected from a vineyard (37.0’N, 126.8’E) at 25 ± 1℃, 50-65% relative humidity, 14L: 10D photoperiod for use in parasitoid breeding. Spotted lanternfly eggs were stored at 3~4℃ until used for host parasitism.

**Alternate host selection for mass breeding of spotted lanternfly parasitoid**

In order to select an alternative host which can be used for mass breeding of spotted lanternfly parasitoids, eggs and immature eggs of different sizes were reviewed (Table 1), Brown marmorated stink bug (*Halyomopha halys* Halyha), the bean bug (*Riptortus pedestris* Fabricius), Gypsy moth (*Lymantria dispar* L.), Japanese oak silk moth (*Antheraea yamamai* Guérin-Méneville), cricket (*Verlarifictorus* spp.), house fly (*Musca domestica* L.) pupa, Chinese oak silk moth (*Antheraea pernyi* Guérin-Méneville). A total of 8 hosts including field collected spotted lanternfly eggs (30~60 eggs per week) and each was placed in a 90mm petri dish with five females and three males 3 day old adult parasitoids. Petri dishes were stored at 25 ± 1℃, 60~70% relative humidity, 16L: 8D photoperiod and presence of parasitism was monitored after seven days. After seven days development of parasitoids were observed daily. Among the seven alternative hosts, immature eggs of the *A. pernyi* confirmed to be parasitized and a substitute host, the development period, life span, and number of eggs were compared.

**Obtaining immature eggs of *A. pernyi***

Larvae that emerged from *Antheraea pernyi* eggs received from the National Institute of Agricultural Science and Technology were reared on leaves of five year old Chinese cork oak (*Quercus variabilis*). Pupae were collected twice per year, the first collection occurred mid-May to mid-July and the second collection end of July to early October. Females that emerged from pupae were stored at 1~5℃. Immature eggs from cold stored females were removed by excising the abdomen, rinsed with water, dried, and then stored at 1~5℃ until use. When needed, *A. pernyi* eggs were removed from cold storage and placed on either a plastic Petri dish (90mm) or cardboard to allow for parasitization.

**Developmental characteristics of *Anastatus orientalis* on immature *Antheraea pernyi* eggs**

*Anastatus orientalis* were reared in transparent acrylic cages (50 x 40 30 cm) with immature *A. pernyi* eggs (200-300 eggs) placed on either egg cards or plastic Petri dish (90 mm) at the bottom of the cage and kept at 25 ± 1℃, 60~70% relative humidity, 16L: 8D photoperiod. About 50~100 *A. orientalis* adults (20~25 females) were placed into the transparent cages for 48 h to allow for parasitization. After the parasitization period, the egg cards or petri dish was removed and incubated under the same conditions mentioned above. From the parasitized eggs, 100 eggs were placed into a plastic Petri dish (90 mm) and the development of *A. orientalis* was visually observed from egg until adult emergence during the 25 day incubation period. Emerged adults were given a honeyed water, water only, or nothing as a food source through a wetted into a cotton wool placed on the lid of a 50 mm Petri dish lid. Cotton wool was replaced every 2~3 days and *A. pernyi* immature eggs were replaced every 2 days.

**Developmental characteristics of *Anastatus orientalis* on spotted lanternfly eggs**

The development and longevity of *A. orientalis* on spotted lanternfly eggs was observed under similar conditions as mentioned above. Spotted lanternfly eggs were collected from a vineyard in songseon-myeon, Hwaseong-si, Gyeonggi-do and stored at 3~4℃. Before *A, orientalis* were exposed to spotted lanternfly eggs, the wax covering the eggs were removed with a brush. One newly emerged *A. orientalis* female and two males were placed into the transparent acrylic cage (50 x 40 x 30 cm) and exposed to spotted lanternfly eggs for 48 hours. After exposure, parasitized eggs were observed from egg to adult emergence under similar conditions as mentioned in previous experiments to determine life span. Newly emerged adults were placed in the transparent acrylic cages with honeyed water, water only, or nothing as a food source in cotton wool along with spotted lanternfly eggs to observe life span. The food source was replaced 2~3 days while spotted lanternfly eggs were every 2 days.

**Comparison of *A. orientalis* development between spotted lanternfly eggs and *A. pernyi* eggs**

To compare development between immature *A. pernyi* eggs and spotted lanternfly eggs, 15 eggs of each were placed into the same plastic container (100 mm diameter, 40 mm height) with a 40 mm (diameter) ventilation hole. One pair of seven day old *A. orientalis* male and female was added to the container and was allowed to parasitize the eggs for 24 h. A total of 20 females were used for each host type (20 replicates each) and was incubated under similar conditions as the developmental experiments.

**Data Analysis**

The difference in the developmental periods between *A. orientalis* parasitized spotted lanternfly eggs and immature *A. pernyi* eggs were compared using the independent sample T-test. Life span comparison of different food sources was compared using Tukey’s HSD (Honest significant difference) test at 5% significance level. Parasitization comparison was analyzed using a paired T-test. All statistical analysis was performed using IBM SPSS Statistics 22.

**RESULTS**

**Alternate host selection for mass breeding of spotted lanternfly parasitoid**

The alternative hosts (egg, immature eggs, or pupae) used in the selection are listed in Table 1. From the eggs and pupa shown in Fig. 1, no *A. orientalis* was seen to emerge except from immature *A. pernyi* eggs and spotted lanternfly eggs.

**Breeding of *A. pernyi* (immature eggs) for use as alternative host**

The method of breeding and securing immature eggs from *A. pernyi* is documented in Fig 2. Five year old Chinese cork oak (*Quercus variabilis*) trees were planted in a house shaped steel structure, which was covered with mesh to prevent birds from entering. The main stem was cut 30 cm from the ground to induce more growth of the oak leaves like how a white mulberry tree is pruned. Adult emergence began in late May to mid-July and egg laying started 4~5 days later, with eggs starting to hatch in 10 days. Second instar larva were placed onto leaves of the Chinese cork oak to feed and develop, pupae were removed from rolled leaves in early July and early October (Fig 2A~D).

Chinese oak silk moth cocoons harvested in early July had adult emergence in August which allowed for another breeding cycle. However, cocoons collected in early October needed to overwinter and began emerging in mid-May. Cocoons collected in July were stored in the breeding room (20~25℃, 30~60% relative humidity) for 1-~15 days to allow for eclosion. Four to five days after eclosion female moths began producing eggs, which increased the size of the abdomen. These females were stored at 1~5℃ until ready for use. Females were not stored at temperatures below 0℃ because lower temperatures deformed/crushed the eggs making them not suitable for use. When eggs were needed, females placed in cold storage were removed, abdomens were removed, soaked in water, and then using dissecting scissors and blade the abdomen was cut vertically to remove the eggs. The eggs were placed in a 35 mesh sieve, washed with running water, and then air dried on fine mesh. The dried eggs, around 150~200 eggs were stored at 1~5℃ until use (Fig 2E to L).

**Breeding of *A. orientalis* on immature *A. pernyi* eggs**

The breeding of *A. orientalis* on *A. pernyi* eggs is showing in Fig 3. *A. pernyi* eggs collected in the procedures mentioned above were placed onto the lid of a plastic Petri dish (90mm) or egg card (eggs glued to cardstock), placed in the center of the acrylic cage (50 x 40 x 30 cm), and then *A. orientalis* adults were released into the cage. Observation of adult *A. orientalis* behavior demonstrated that adults would make holes in the *A. pernyi* eggs to feed and then lay eggs inside *A. pernyi* eggs (Fig 3D). Parasitized spotted lanternfly eggs were placed into Petri dishes (90mm) and in the cage and were replaced every 2~3 days. It took *A. orientalis* around one month to develop from egg to adult emergence. After emergence, adults were given honey as food source along with host eggs (*A. pernyi*) (Fig. 3A~F) and stored at 25~28℃, 60~70% relative humidity, 14L: 10D photoperiod.

**Breeding of *A. orientalis* on spotted lanternfly eggs**

Spotted lanternfly eggs were collected December to March from a vineyard in Songsan-myeon, Hwaseong-si, Gyeonggi-do and stored for up to a year at 3-4℃ until use. Before use of spotted lanternfly eggs, the wax covering on the eggs were removed using a brush. These eggs were then placed onto a Petri dish (90 mm) and into the cages (50 x 40 x 30 cm) containing *A. orientalis* adults for 48 hours. The eggs were kept in the Petri dish and kept at 25~28℃, 60~70% relative humidity, 14L: 10D photoperiod until adult emergence.

**Comparison of *A. orientalis* development between spotted lanternfly eggs and *A. pernyi* eggs**

In order to determine possibility of using *A. pernyi* eggs as an alternative host for mass rearing of *A. orentalis*, the development period between the two hosts were compared. The development period from egg to adult was 36.1 days for *A. pernyi* as the host and 36.8 days for spotted lanternfly as the host (Fig 4), there was no statistical difference between the two hosts (T = 1.790, P = 0.074). Results from host preference demonstrated similar results in that there was no significant differences (t = 2.093, P = 0.563) between the numbers of parasitized eggs of spotted lanternfly (4.2) versus *A. pernyi* (3.3) after a 24 h period (Fig 5).

**Effect of food source on life span of *A. orientalis***

In order to maintain the longevity of female adults for an extended period of time, different food sources were tested and results from the test are shown in Table 2. Honey water as the food source had a significant difference in longevity at 64.3 days, while water only was 3.9 days, and nothing at all was 4.0 days showing no significant difference between water only and nothing (F 2, 55 = 278.24, P = 0.000).