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Relationships between the emergence and oviposition of ectoparasitoid *Spathius agrili* Yang and its host emerald ash borer, *Agrilus planipennis* Fairmaire

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Abstract Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), (= *A. marcopoli* Obenberger), is an important bark beetle attacking ash trees (*Fraxinus* spp.). It is very difficult to detect and control because of its highly concealed life history. This pest mainly distributed in partial Asian countries (China, Japan, Korea, Mongolia) and Far East Russia, while in China it presented in Heilongjiang, Jilin, Liaoning, Shandong, Inner Mongolia, Hebei, Taiwan Provinces and Tianjin City, etc. The important timber species *F. mandshurica* and gardening tree *F. velutina* were damaged severely in northern China. *Spathius agrili* Yang (Hymenoptera: Braconidae) is an important ectoparasitic wasp of the EAB larvae. This parasitoid has the potential to use as an excellent biological control agent for suppressing populations of EAB. The differences of emergence date between overwintered *S. agrili* and its host, parasitism rates at different periods, relations between parasitism rates and host densities, and relationships between ovipositions of braconid wasp and body sizes of host larvae were studied using methods of regular surveys in forests and observations in laboratory. Results revealed that the emergence of *S. agrili* was more than one month later than that of its host. It suggests good synchrony between parasitoid emergence and host availability. The overwintered *S. agrili* emerged from mid June to mid August with the peak in July in 2003, and it lasted from late May till late July with the peak during late June to early July in 2004. While the EAB emergence period ranged from mid May to late May in 2003, it ranged from mid

April to mid May in 2004 (in laboratory). The emergence date of parasitoid asynchronously inoculated with the optimum developmental instars of the earliest host larvae, which was the result of a long-term co-evolution between the two species. It was also suggested that the parasitoid *S. agrili* could be a specialized natural enemy for EAB. The natural parasitism rates on the whole gradually increased in field with time. The body sizes of host larvae, i.e. larval instar, affected the decision of parasitoid *S. agrili* to lay eggs or not. Under natural conditions, this parasitoid usually oviposited only on those host larvae with a pronotum and body width more than 1.5 mm, and a body length in excess of 12 mm, i.e. the third or fourth instar larvae. These findings would consequentially contribute to the further successfully biological control of the trunk borer.

Keywords emerald ash borer, *Spathius agrili*, emergence, oviposition, ecological relationship, biological control

1 Introduction

Emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), (= *A. marcopoli* Obenberger), is an important bark beetle attacking ash trees (*Fraxinus* spp.) (Yu, 1992). This pest is native in part of Asia (China, Japan, Korea, Mongolia) and Far East Russia, while in China it presented in Heilongjiang, Jilin, Liaoning, Shandong, Inner Mongolia, Hebei, Taiwan Provinces and Tianjin City, etc. The susceptible host plants include *Fraxinus americana*, *F. mandshurica*, *F. velutina*, *F. pennsylvanica*, *F. pennsylvanica* var. *lanceolata*, *F. nigra*, *F. chinensis*, and *F. rhynchophylla*. Currently, the EAB infestation becomes severe gradually and its distribution areas expand (Zhang et al., 1995; Liu et al., 1996). It was reported that EAB was introduced into North America in 2002 and severely damaged ash trees in Michigan, USA and Ontario, Canada. More than six million ash trees were infested or died of EAB attack (Haack et al.,

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2002). North America immediately listed this beetle as the important quarantine pest. For instance, Canada promulgated some new quarantine requests for the import of ash timber and seedlings, aiming at those native countries of EAB occurrence in part of Asia including China, Russia and United States.

The EAB larvae feed on the cambium. The newly hatched larvae penetrate bark and eat phloem first, then bore into cambium as the increase in age. An S-shaped gallery is produced between the xylem surface and phloem, packed with brown frass tightly. Seen from the outside bark, there is nearly no evident symptom in the early stage of infestation. The damaged ash trees never respond by producing sap at the site of the wound, and the only clear evidence of attack is a D-shaped exit hole produced by the emerging adult in the next year. Therefore, EAB is highly concealed and very difficult to be detected. Usually the infested trees died or were dying when EAB population was found. Through field investigation, a larval ectoparasitoid *Spathius agrili* Yang (Hymenoptera: Braconidae) was found and identified as a new species to science (Yang et al., 2005). The wasp has rather high parasitism rate in natural condition and is with restricted host specificity, which show better prospect for biological control of EAB. In order to utilize this natural enemy, the relationships among emergence, natural parasitism, oviposition of *S. agrili* and host pest were studied. Results were reported as follows.

2 Materials and methods

2.1 Field sites

Field surveys were conducted in the Guangang Forest Park in Dagang District, Tianjin City, China (38°56'N, 117°29'E). Around 130 hm² of velvet ash, *F. velutina* Torr., are planted in this park and approximately 5 hm² chosen as the regular stand for experimental investigation. This forest plantation is 9–10-year old ashes with row spacing of 1.5 m and plant spacing of 1.0 m. Both EAB larvae and *S. agrili* were collected at this site.

2.2 Adult emergence of overwintering generation

2.2.1 Emergence of EAB population in field

Thirty trees were selected at random in the research plot and marked on May 5, 2004 for the observation of the number of newly emerged adults. The conditions of the trees were recorded, including whether the bark was smooth or rough, live or dead and diameter at breast height (DBH). The numbers, direction and height of emergence holes in every tree were checked and recorded every day. The weather conditions in the field were also recorded. All new emergence holes were removed daily using a drawknife to prevent counting the same holes twice.

2.2.2 Emergence of EAB in laboratory

Forty-six EAB pupae were collected in the research field in Tianjin on April 19, 2003, and then cultured individually using glass test tubes of 1.5 cm in diameter and 10 cm in length in laboratory under room condition. A small piece of moistened folding filter paper was placed in each tube to maintain moisture. The insect developmental degree was checked daily, and recorded the emergence date and sex for each insect. In 2004, three batches of overwintered EAB pre-pupae or pupae were collected in field on March 7, March 20 and April 15, respectively, and cultured in laboratory with the same method and condition mentioned above.

2.2.3 Emergence of wasp *S. agrili* in laboratory

Fifteen coveys of wasp cocoons were collected from field on April 19, 2003, and then cultured using test tubes (diameter of 1.5 cm and length of 10 cm), one covey per tube. A small piece of moistened folding filter paper was placed in each tube to maintain moisture. The insect developmental degree was checked daily, and recorded the emergence date and sex for each wasp. In 2004, three batches of overwintered parasitic wasp cocoons were collected in field on March 7, April 15 and May 7, respectively, and cultured in laboratory with the same method and condition mentioned above. Every four days beginning from late May, forest areas were surveyed by a sweep net (mesh size of 0.02 mm) and field observation to establish the first appearance date of adults.

2.3 Survey of parasitism rates at different periods

From July to October 2003, field surveys were conducted every three days. During each survey, five to ten ash trees were sampled at random for emerald ash borer larvae and their parasites. The trunks of each tree were debarked at four to six sites (area: 10 cm × 30 cm) between 0.5–1.5 m in height above ground. Parasitism rates were calculated for each survey until no wasp appeared.

2.4 Determination on the relationship between host densities and parasitism rates

Twelve EAB infested ash trees with different DBHs were felled in the research plot on August 15, 2003, and transported to laboratory to peel bark carefully. The DBH of each tree, EAB number, and parasitized EAB number found in each tree were recorded. In addition, from late October to early November 2003, the percentages of the damaged ash trees and EAB densities in other seven plots in the park have been investigated. In each plot, 25 trees distributed in the east, west, south, north and center (five trees in every direction) were selected. Bark of the trunk in the height of 0–2.5 m was scraped off to examine insects. As the trees were not so big, all insects should be found at this height range. The EAB number and parasitized EAB number found in each tree were recorded.

2.5 Determination on the relationship between oviposition and host larval sizes

2.5.1 Field survey

During the investigations described in Sections 2.3 and 2.4 above, when the EAB larva was found to be parasitized by wasp *S. agrili*, the parasitoid eggs, larvae, and pupae associated with host larva were put into a single tube and transported to laboratory. Then the EAB larval length, larval width and larval pronotum width were examined by a binocular microscope with an eyepiece micrometer. The numbers of eggs, larvae or cocoons on each host larva were also recorded.

2.5.2 Determination in laboratory

The EAB larvae were fed fresh ash branch segments. Each ash branch of 1.0–1.5 cm in diameter was cut into 5–10 cm long segments. The segment was split in half. A 3 cm long groove approaching bark was excavated in one half. The field-collected emerald ash borer larvae were placed in the excavated groove, and the other half of the branch segment replaced and fastened with a rubber band. Two ends of the segment were sealed with wax to prevent the plant tissue from desiccation for the feeding of EAB larva. A pair of newly emerged *S. agrili* adults (female and male), with the segment, were all placed in a glass test tube (2.8 cm in diameter and 11.5 cm in length). Then the tube was sealed with a cotton plug. The segment was taken out to examine every three to four days until the female wasp died. If wasp eggs were observed on host larva, they were counted and the ash branch segment replaced. At the same time, host larval sizes, including lengths, widths and pronotum widths, were also measured. Treatments were divided into two groups: supply a 20%

honey solution in the tube or only distilled water to maintain humidity. Each group included 15 replicates.

2.6 Data statistical analysis

Data were analyzed with SAS software (version 8.2).

3 Results

3.1 Asynchronism in emergence between wasp *S. agrili* and host adults

From the 46 EAB pupae previously collected on April 19, a total of 42 adult beetles were successfully emerged from May 14 to May 24, with the peak about on May 20, 2003 (Fig. 1). In the collected 152 wasp cocoons from 15 coveys, 77 female and 20 male adults were emerged from June 18 to August 11, with the peak in July (Fig. 1). The emergence duration was short for EBA after overwintering, whereas parasitoid *S. agrili* came out more than one month later comparing with its host. And the wasp emergence lasted for a quite a long time, approximately three months.

In 2004, fifty-nine EAB adults emerged in laboratory, and the emergence period was from April 11 to May 16, with the peak from late April to early May. In field survey, a total of 534 emergency holes were recorded from May 10 to May 27, peak observation was in mid-May. Three hundred and forty-one *S. agrili* adults were collected in laboratory, including 229 females and 112 males. The emergence duration lasted from May 25 till to July 23, in which there were three evident emergence peaks on June 12–14, June 21–25 and June 28 to July 5 (Fig. 2). The earliest date for finding *S. agrili* adults in field was May 31, while the earliest observation of EAB adult beetles was May 5, 2004.

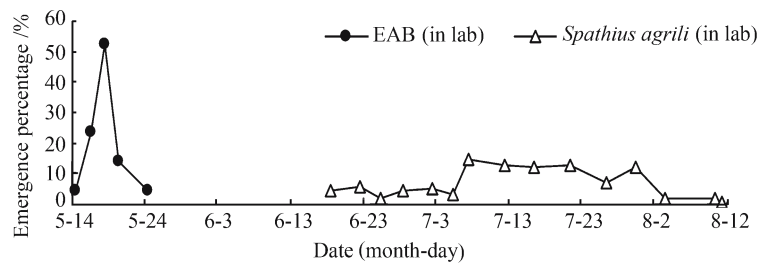


Fig. 1 Emergence of *Spathius agrili* and its host EAB in 2003

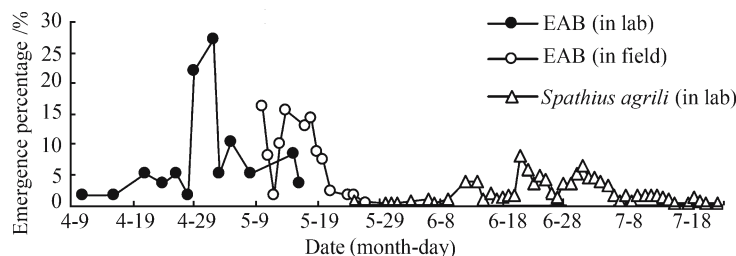


Fig. 2 Emergence of *Spathius agrili* and its host EAB in 2004

The emergence pattern of the two insects was similar in 2004 and 2003. The emergence date of *S. agrili* was later one and half months in laboratory, and was later more than 20 days in field comparing with its host. Based on the developmental duration of EAB, the pre-oviposition period is about seven days, the egg period 15 days, the first instar larval period 18 days, and the second instar larval period 11 days (Wang et al., 2007). Therefore, it occurred possibly in the end of June to early July when the earliest appropriate host larvae grew big enough for parasitization. In fact, the parasitized EAB larva was earliest found on June 28, 2004, while the wasp offspring entered the fourth larval instar. Thus the EAB larva might be parasitized on around June 22 actually, when calculated by the developmental rate of the *S. agrili* (Yang et al., 2007). Although the air temperature was higher than the threshold temperature for development of *S. agrili* in May, the wasp did not emerge largely until the host larvae developed big enough for oviposition. It was also suggested that the parasitoid *S. agrili* could be a specialized natural enemy of EAB. Moreover, according to life history of the two insects, *S. agrili* can absolutely complete its life cycles of all generations only on EAB in the whole year without any other alternative hosts. The emergence date of parasitoid asynchronously inoculated with the optimum developmental instars of the earliest host larvae, which was the results of a long-term co-evolution between the two species.

In addition, both the EAB and *S. agrili* emerged about three weeks earlier in laboratory in 2004 than those in 2003. It might be associated with different laboratories where the insects were cultured in the two years, besides the climatic differences between the two years. In 2003, the mean room temperature was relatively lower than that in 2004 in another room, which affected their emergence dates.

3.2 Parasitism rates of *S. agrili* at different periods

Although EAB larvae began to occur in field in June, *S. agrili* adults seldom come out during this period. Maybe the host larvae were still too young to meet the nutritional needs for wasp progeny. Until the end of July and early August, EAB larvae were found to be parasitized by *S. agrili* in field, and at this time the parasitism rates were rather low, only less than 10%. From mid-August the parasitism rates began to increase, and the mean parasitism rates were above 40% before overwintering, with the maximum even up to 70% (Fig. 3). As a result of sampling error and influence of insect

distribution pattern, the parasitism rates also showed some fluctuations at a certain stage, however, it escalated as a whole.

3.3 Relationships between host densities and parasitism rates

Analyzed from data of field survey, the densities of EAB population seemed no evident correlation with the parasitized number ($r = 0.361, 2, P = 0.000, 5$) (Fig. 4). However, through transforming parasitized number into the parasitism rates (y) and simulating the relation with EAB densities (x), a significantly negative correlation between the two variables ($F = 70.58, df = 1, 86, P < 0.000, 1$) was found (Fig. 5). In a certain habitat, the parasitism rates will decline as the increase in host population densities. Contrarily, if the host density is low, there should give a relatively high parasitism rate. This may be caused by the intraspecific competition on the host resources, or the mark pheromones remaining on the oviposition sites prevent other wasp individuals from searching hosts and producing eggs further.

3.4 Relationships between wasp oviposition and host larval sizes

The body sizes of host larvae, i.e. larval instar, affected the decision of parasitoid *S. agrili* to lay eggs or not. Under natural conditions, this parasitoid usually oviposited only on those host larvae with a pronotum and body width more than 1.5 mm, and a body length in excess of 12 mm, i.e. the third or fourth instar larvae (Fig. 6). *S. agrili* is an idiobiont parasitic wasp. The host larvae stop growing permanently as soon as they are parasitized. Therefore, the wasp females prefer older host larvae for laying eggs in order to provide adequate nutritional sources for more progeny. However, another reason why the wasp does not attack those small host larvae is that the host galleries are too small to cocoon subsequently by the wasp offspring after completing their development.

4 Discussion

When host quality varies, optimal foraging theory assumes that parasitic wasps select hosts in a manner that increases individual fitness of their progeny (Rivero, 2000). In idiobiont parasitoids, especially larval parasitoids, where the hosts

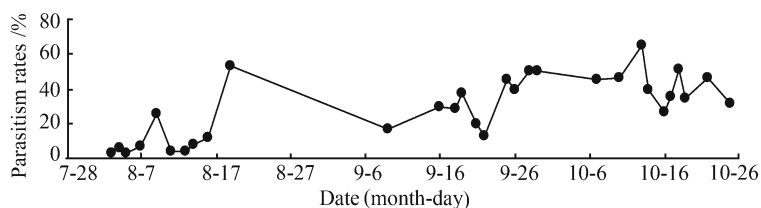


Fig. 3 Parasitism rates of EAB attacked by *Spathius agrili* Yang at different periods in field

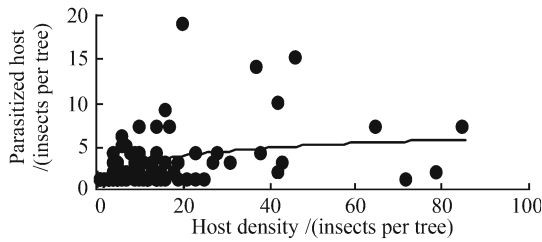


Fig. 4 Relationships between host larval densities and number of parasitized host larvae

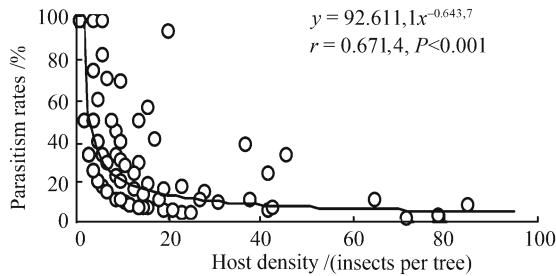


Fig. 5 Relationships between host larval densities and parasitism rates

are paralyzed, cease development and even die immediately after parasitization, host selection will prefer to large-sized hosts. The parasitic wasps need to provide sufficient food resource for their progeny to develop fully. For instance, in this study, *S. agrili* females only select the third and fourth instar host larvae for oviposition (EAB has four larval instars). Whereas in the koinobiont parasitoids, host selection may not reflect current host quality but may be based on an assessment of future growth rates and resources available for the developing larvae, because the hosts continue developing for a certain period after parasitization (Rivero, 2000). Furthermore, the reason why koinobiont parasitoids choose small hosts to attack may be little energy needed to overcome them. *Microplitis* sp. can parasitize one to four instar larvae of *Spodoptera litura*, but it prefer to attack those host larvae in the stages of the end of first instar and early second instar, but never attack larvae above five instars (Wang et al., 2001). Other parasitoids can allocate their egg number produced according to host sizes, although they lack of evident selection on host larvae instars. Lee (1992) reported that a

gregarious ectoparasitoid, *Goniozus legneri*, can parasitize any instar larvae of alternative host, *Anagasta kuehniella*, but the wasp oviposition was linearly positively associated with host weight.

It was reported that the probability of successful biological control against invasive pests significantly increases with the increase in maximum parasitism rate in the host's native range. No control is achieved for any exotic pest when the maximum parasitism rate is less than 32% in its native range (Hawkins and Cornell, 1994). In this case, field survey on parasitism rates of *S. agrili* for attacking hosts at different period revealed that the maximum parasitism rate was far more than 32%, even excess of 70% sometimes. This suggested a higher probability of successful control on EAB introduced from Asia in North America using the parasitoid *S. agrili*. The wasp *S. agrili* was a rather specialized parasitic natural enemy of buprestid beetle *A. planipennis*. Strong coupling between a host and a specialist parasite should lead to an increase in the host's dynamical dimension, which may greatly reduce the host population density and consequently help to the success of biological control (Bjørnstad et al., 2001). The EAB are mostly univoltine in Tianjin, China, and the larval stage lasts a very long time, about ten months (Wang et al., 2007). While the wasp *S. agrili* can reproduce three to four generations, seldom two generations, in one year (Yang et al., 2007). The population density of *S. agrili* increases naturally in field, thus the insect pest EAB would be effectively suppressed. The parasitoid shows a promising utilization prospect in practice as a biological agent when it is artificially reared by large scale.

Using principles of co-evolution among organisms for controlling pests is the theoretical fundament of biological control. It is important to understand the ecological relationships between insect natural enemies and their hosts when effectively utilizing natural enemies rationally against pests. Wood borers live in concealed environments, and are very difficult to be detected and controlled in time. Therefore, biological control would be superior to other measures such as chemical insecticides to manage these pests. Natural enemies can actively search, find, and kill host pests, self-reproduce repeatedly and increase their population naturally. Thereby sustainable pest control would be achieved to maintain the ecological balance and protect forest health ultimately.

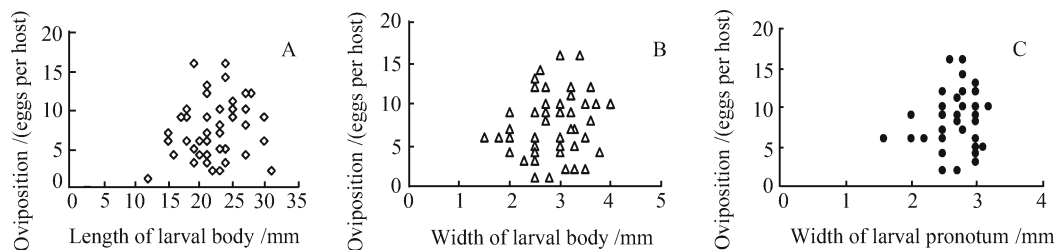


Fig. 6 Relationships among (A) oviposition and host larval length, (B) host larval width and (C) host larval pronotum width

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