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Host stage preferences of three major parasitoids of the banana skipper (*Erionota thrax*) (Lepidoptera: Hesperiidae)

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ABSTRACT: **Oviposition preferences of three major identified parasitoids; *Ooencyrtus erionotae* Ferrière (Hymenoptera, Encyrtidae), *Cotesia erionotae* (Wilkinson) (Hymenoptera, Braconidae) and *Brachymeria albotibialis* (Ashmead) (Hymenoptera, Chalcididae) of *Erionota thrax* (L.) (Lepidoptera: Hersperiidae) eggs, larvae and pupae respectively were studied in the laboratory and field during a high-density period of the pest. Egg stage 1 (newly laid), stage 2 (intermediate), and stage 3 (advanced) were susceptible to *O. erionotae* oviposition. Stage 3 was the most preferred egg host with a mean of 3.4 adult parasitoids emerging per egg. For *C. erionotae*, second, third, and fourth larval instars were susceptible. The third instar larva was the most preferred stage for oviposition with a mean of 87 adult parasitoids emerging per larva. For *B. albotibialis*, only prepupae and pupae were susceptible. The pupa was the most preferred host stage with mean of 7.1 adult parasitoids emerging per pupa. In the case of *O. erionotae* and *C. erionotae*, no super-parasitism or multi-parasitism was observed.**

KEY WORDS: Banana, *Brachymeria albotibialis*, *Cotesia erionotae*, *Erionota thrax*, *Ooencyrtus erionotae*, oviposition, parasitoids

INTRODUCTION

Erionota thrax (L.,) (Lepidoptera: Hesperiidae) in its native or naturally occurring areas is most often found to exist at endemic levels. These persistent low-density levels have been attributed to natural enemies such as the hymenopteran parasitoids (Kalshoven, 1981; Ahmad and Balasubgramaniam, 1975; Khoo *et al.*, 1991; Hasyim *et al.*, 1994, Waterhouse *et al.*, 1998; Okolle *et al.*, 2006). These natural enemies attack *E. thrax* in all its life stages (except adults) and thus help regulate the population. However, these natural enemies have been surveyed and identified mainly from backyard and subsistence production systems, as well as research stations, and hence there is a need for studies on these natural enemies to be extended to large plantations that are intensively managed. In a plantation of Cavendish bananas in Penang, Malaysia, Okolle *et al.* (2006) recorded *Ooencyrtus erionotae* Ferrière (Hymenoptera: Encyrtidae), *Cotesia erionotae*

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(Wilkinson) (Hymenoptera, Braconidae) and *Brachymeria albotibialis* (Ashmead) (Hymenoptera, Chalcididae) as the major parasitoids of *E. thrax* eggs, larvae and pupae, respectively.

Furthermore, host stage preferences have been mentioned only for *C. erionotae* (Waterhouse and Norris, 1989; Sands *et al.*, 1993) with preference for second (L2) and third instar larvae (L3), although data to support this is not available. On the other hand, Hasyim *et al.* (1994) only specified the developmental stages of *E. thrax* larvae and pupae susceptible to oviposition by parasitoids. As far as the eggs of *E. thrax* are concerned, there is no study on host stage preferences. Such studies are very important, especially for mass production of parasitoids using their hosts. If the most susceptible or preferred host stage is not known, mass rearing of the parasitoids will be very difficult and could result in other effects on subsequent progenies of the parasitoids.

The objective of this research was to find the most preferred life stages of *E. thrax* for the major identified parasitoids, *O. erionotae, C. erionotae* and *B. albotibialis*.

MATERIALS AND METHODS

Study site

The research was carried out in a commercial plantation (176 acres) of Cavendish bananas, *Musa* sp. (Musaceae), located in Ara Kuda, Daerah Seberang Prai – Penang State, Malaysia, positioned at latitude 5°30' North and longitude 100°30' East with an altitude of 52 meters above sea level. The study duration was from October to November 2004 (i.e. months of high *E. thrax* density).

Host stage preference of the egg parasitoid, *Ooencyrtus erionotae*

From preliminary field and laboratory observations, the skipper eggs pass through four stages before hatching occurs: stage 1 – newly laid eggs, bright yellow in color, lasts 1-2 days; stage 2 - intermediate stages, orange in color and last 5-6 hours; and stage 3 - lasts 2-3 days, bright red in color; while stage 4 (most advanced stage with a pharate instar) lasts 2-3 days before hatching and is pale yellow.

In order to find out which of the egg stages were most susceptible or preferred, for *O. erionotae*, many egg batches of various stages in the field were then taken to the laboratory, kept separately and securely in enclosed vials until the emergence of first instar larvae of *E. thrax* or adult parasitoids. The total number of parasitized egg batches and individual eggs from each stage was then recorded. Oviposition behavior of female adults was also observed in the fields once a week throughout the study period. The adults were observed on eggs to find out whether two or more females would oviposit on the same egg or oviposit on eggs that are already parasitized.

Host stage preference of the larval parasitoid, *Cotesia erionotae*

Since it was very difficult to find *C. erionotae* in the field, its host stage preference was studied in the laboratory at a temperature of 29.9±2°C, relative humidity of 66.8±4%, and photoperiod of 14:10 (light: dark) hours. Eggs of *E. thrax* collected from the field were taken to the laboratory, placed in five cages, and reared in order to obtain pure (unparasitized) cultures of *E. thrax* instars. First and second instar larvae were provided with fresh banana leaves daily while third, fourth and fifth instar larvae were provided fresh leaves every three days. A culture of *C. erionotae* was also frequently maintained in the laboratory under the same conditions. To do this, *E. thrax* larvae were frequently collected randomly from the field and taken to the laboratory where those with signs of parasitism or with cocoons of *C. erionotae* were kept in three separate cages until adult parasitoids emerged. These parasitoids were provided with honey and sugar solution (one tablespoon of honey mixed with one tablespoon of tap water) and the various instar larvae of *E. thrax* were placed in the cages containing the parasitoids. The larvae were kept with these parasitoids for three days (24 hours for first instar larvae) and the various *E. thrax* larval instars were then removed from the cages and reared separately until adult skippers or parasitoids emerged or until they died. Dead larvae were dissected under a light microscope and those with parasitoid larvae were considered as parasitized. For each *E. thrax* larval stage or instar, the total number parasitized and the stage from which parasitoids emerged were recorded.

Host stage preference of the pupal parasitoid, *Brachymeria albotibialis*

For *B. albotibialis*, field studies were carried out to find out the most susceptible or preferred stages in which the parasitoids oviposit. In the field, female parasitoids were observed entering the leaf rolls to oviposit and the leaf rolls were collected as soon as the females came out of them. These leaf rolls were carefully opened in the laboratory and the *E. thrax* life stages found within were recorded. The stages obtained were separately kept in plastic vials (as with the eggs) until skipper adults or parasitoids emerged. The stages from which the adult parasitoids emerged were also recorded.

RESULTS AND DISCUSSION

Strand (1986) stated that since eggs lack a developed immune system, the most serious problem facing egg parasitoids are those associated with host development. With the exception of egg stage 4 (most advanced stage with a pharate instar), all the other stages were susceptible to *O. erionotae* oviposition, with stage 3 being the most preferred (Table 1). Waterhouse and Norris (1989) reported that *O. erionotae* oviposits in *E. thrax* eggs up to three days old, but seldom oviposit in older eggs. Egg parasitoids generally have been reported to prefer younger egg stages (Strand, 1986). This study showed that the younger *E. thrax* egg stages 1, 2 and 3 were preferred to the older and most advanced stage 4 (Table 1).

Although the focus of this study was not to find out the mechanisms that enable *O. erionotae* to select or prefer particular host stages, the females could have possibly learnt to associate the color changes of the eggs with susceptible or preferred stages. The use of visual cues by hymenopteran parasitoids to discriminate between rewarded and unrewarded microhabitats has been reported (Turlings *et al.*, 1993). In the field, although a maximum of three *O. erionotae* were found ovipositing on a single egg batch, they however avoided individual eggs that were already parasitized and this could possibly be an indication of the presence of a host marking chemical. Avoidance of parasitized eggs by *O. erionotae* could be a possible reason why this parasitoid causes higher parasitism of *E. thrax* eggs (Okolle *et al.*, 2006). This is because such avoidance will force the females to intensify their search for more unparasitized eggs. In all parasitized cases, the mean number of individual parasitoids emerged per host was 3.4±0.11 (range from 1 to 9, n = 156) and no superparasitism or multiparasitism was observed in the field. This is similar to the average of 3 reported by Hasyim *et al.* (1994).

For *C. erionotae*, second, third and fourth instar larvae were found to be the susceptible stages, with the third instar larvae being the most preferred stage for oviposition (Table 2) and no

Table 1. Stage preference of *O. erionotae* on *Erionota thrax* eggs

Egg stage	Number collected		Number parasitized		Percentage parasitized	
	Batch	Individuals	Batch	Individuals	Batch	Individuals
Stage 1	80	1567	34	325	42.5	20.7
Stage 2	52	1117	19	36	36.5	3.2
Stage 3	76	1248	62	780	81.6	62.5
Stage 4	64	1165	0	0	0	0

Table 2. Preference of *C. erionotae* to various larval stage of *E. thrax*

Host stage	Number collected	Number parasitized	Percentage parasitized	Stage emerged
First instars (L1)	190	0	0	-
Second instars (L2)	190	18	9.5	L4/L5
Third instars (L3)	120	49	40.8	L5
Fourth instars (L4)	120	2	1.7	L5
Fifth instars (L5)	120	0	0	-

parasitism of first and fifth instar larvae was recorded. These parasitized second and third instar larvae were capable of undergoing metamorphosis while the fourth instar larvae were not. All parasitized hosts did not feed much and at an advanced stage of parasitism, they became very sluggish. In all parasitized *E. thrax* instars, most late instars of *C. erionotae* emerged from fifth instar larvae with a few emerging from fourth instars, and the parasitoid instars formed cocoons at the sides of the hosts. When touched or pricked with a pin, most of the first and second instar larval stages did not show any signs of defense compared to fourth and fifth instar larvae, which suddenly bite back and regurgitate a green liquid. With the exception of first instar larvae, Hasyim *et al.* (1994) showed that all the other instar larvae were susceptible to oviposition and most parasitoids emerged from fourth and fifth instar larvae. Waterhouse and Norris (1989) reported second instar larvae as the most preferred host stage while Sands *et al.* (1993) mentioned second and third instar larvae as the most preferred for *C. erionotae* oviposition. Nealis (1990) also reported that late instar larvae of *Pieris rapae* (L.) were less susceptible to parasitism caused by *C. rubecula* than early instar larvae on the same plant. In this study, second and third instar larvae were the most preferred host stages probably because these two stages were found to have very little or no physical defensive characteristics or behavior compared to the fourth and fifth instar larvae that regurgitated a green liquid upon disturbance probably to deter natural enemies or intruders.

Furthermore, second and third instar larvae had smoother bodies due to the relatively little or no protective waxy powder compared to fourth and fifth instar larvae whose bodies are usually covered with large amounts of this powder. Such defensive behaviors and characteristics have been shown to be important predictors of parasitism avoidance for lepidopterous larvae (Gentry and Dyer, 2002; Dyer *et al.*, 2004). Also, the older fourth and fifth instar larvae could possibly have stronger defense or cellular immune systems and such systems have been found to decrease the survival of parasitoid eggs in older hosts (Van Alphen and Vet, 1986). The very small sizes of first instar larvae could also possibly be a reason why they are not susceptible to oviposition as they would not be able to contain the numerous first instars ensuing from the parasitoid eggs. In addition to the smaller size, trauma from ovipunctures caused by *C. erionotae* could possibly kill the first instars and hence immatures of the parasitoid would not survive. The mean number of *C. erionotae* emerging per host was 87±4.9 (range 14-192, n = 103) and after emergence of the parasitoid instars, most hosts stay for about 24 hours before dying. This mean value of *C. erionotae* emerging per host in this study is different from the average of 57 (Hasyim *et al.*, 1994) and 69 (Mau *et al.*, 1980).

In the case of *B. albotibialis*, only the prepupae and pupae were susceptible to oviposition while no parasitism was recorded from fifth instars. The most preferred stage for oviposition was the pupa (Table 3). This differed

Table 3. Preference of *B. albotibialis* for various stages *E. thrax*

Host stage emerged	Number collected	Percentage parasitized	Percentage of stage parasitoid
Pupae	196	188/196(100)=95.9	188/188(100)=100
Prepupae	154	17/154(100)=11.1	2/17(100)=11.8
Fifth instars (L5)	72	0	0

from studies by Hasyim *et al.* (1994) where fifth instars and pupae were the only susceptible stages. However, this difference could probably be due to the fact that fifth instars and prepupae in this study were considered as separate stages while Hasyim *et al.* (1994) considered them as one stage. Of the 188 pupae parasitized, parasitoids emerged from all but emerged only from two of the parasitized prepupae and all the parasitized prepupae were unable to transform to pupae. In the field, *B. albotibialis* females were never seen entering leaf rolls that contained parasitized hosts and avoided already parasitized pupae. Also, since the pupae were mostly found within tightly secured leaf rolls, females chewed the leaves and created holes in order to reach the pupae. These holes were created only in leaf rolls containing prepupae or pupae. Such a behavior indicates that they could be very effective in searching for susceptible hosts and hence be effective agents for biological control. In the course of oviposition, females inserted their ovipositor into different regions of the host (average of 8 insertions) and the mean number of *B. albotibialis* emerging per host was 7.1±0.46 (range 1-21, n = 106). Although the mean number of *B. albotibialis* emerged per host is slightly different from the average of 9 reported for *B. lasus* and *B. thracis* (Hasyim *et al.*, 1994), the range is within that (1 to 24) reported for *B. euploeae* (Waterhouse and Norris, 1989).

To conclude, younger eggs, second and third instar larvae, and pupae are therefore the most preferred life stages of *E. thrax*, respectively, for *O. erionotae*, *C. erionotae* and *B. albotibialis*.

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