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A Larval Parasitoid Wasp Diachasmimorpha longicaudatus of the Oriental Fruit Fly in Taiwan
【Research report】

臺灣東方果實蠅幼蟲寄生蜂Diachasmimorpha longicaudatus之研究【研究報告】

Wen-Yung Lee*, Hsin-Che Chang and Li-Yun Chen
李文蓉*、張興哲、陳麗雲

*通訊作者E-mail :

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Abstract

The oriental fruit fly, Bactrocera (=Dacus) dorsalis Hendel, is an economic important pest in Taiwan. Government and agricultural organizations have used the annihilation technique with attractants of poisoned methyl eugenol as a control method. The Braconid wasp, Diachasmimorpha (=Opius, =Biosteres) longicaudatus Ashmead, an efficient larval parasitoid of the oriental fruit fly, was introduced from Hawaii and has been successfully raised in the laboratory. This wasp will be used as an agent of biological control, and together with the annihilation technique will form an integrated control method. The important factors influencing parasitoid yield such as the culturing apparatus, host larva age, parasitoid maternal age and the exposure period to parasitoid oviposition as well as the biology of the parasitoid were studied. The parasitoids have been released into orchards.

摘要

東方果實蠅Bactrocera (=Dacus) dorsalis臺灣重要之經濟害蟲，每年政府及農業機構均耗費巨資，利用含毒甲基丁香油誘殺之滅雄技術防治該蟲。果實蠅幼蟲寄生蜂，Diachasmimorpha (=Opius,=Biosteres) longicaudatus，為1896-1987年從夏威夷引進之寄生蜂，已在實驗室內飼育成功，並將應用於果實蠅生物防治與滅雄技術之綜合防治。室內飼育技術之逐步改進、寄主和寄生蜂之寄生年齡、接受寄生產卵時間之長短、以及寄生蜂之生物學等經詳細研究均在本文中報告，此外，田間釋放寄生蜂之研究亦在進行中。

Key words: Oriental fruit fly, larval parasitoid, parasitism, oviposition, mature larvae.

關鍵詞: 東方果實蠅，幼蟲寄生蜂，寄生，產卵，成熟期幼蟲。

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A Larval Parasitoid Wasp *Diachasmimorpha longicaudatus* of the Oriental Fruit Fly in Taiwan

Wen-Yung Lee*, Hsin-Che Chang and Li-Yun Chen Institute of Zoology, Academia Sinica, Nankang, Taipei, Taiwan, R.O.C.

ABSTRACT

The oriental fruit fly, *Bactrocera* (=*Dacus*) *dorsalis* Hendel, is an economic important pest in Taiwan. Government and agricultural organizations have used the annihilation technique with attractants of poisoned methyl eugenol as a control method. The Braconid wasp, *Diachasmimorpha* (=*Opius*, =*Biosteres*) *longicaudatus* Ashmead, an efficient larval parasitoid of the oriental fruit fly, was introduced from Hawaii and has been successfully raised in the laboratory. This wasp will be used as an agent of biological control, and together with the annihilation technique will form an integrated control method. The important factors influencing parasitoid yield such as the culturing apparatus, host larva age, parasitoid maternal age and the exposure period to parasitoid oviposition as well as the biology of the parasitoid were studied. The parasitoids have been released into orchards.

Key words: Oriental fruit fly, larval parasitoid, parasitism, oviposition, mature larvae.

Introduction

In Taiwan, *Bactrocera dorsalis* (=*Dacus dorsalis*) Hendel is an economical important insect pest of many fruits. This insect, commonly referred to as the Oriental fruit fly, is known as a polyphagous species which affects at least 32 genera and 89 species of fruit crops (Chu and Chen , 1985).

Given the polyphagous nature of *B. dorsalis,* effective biological control over a wide area supporting various host crops may depend on the introduction of different parasitoid species, especially in the field lack of management. Our laboratory imported parasitoids from Florida and Hawaii for laboratory studies during1986-1987; *Diachasmimorpha* (=*Opius*,=*Biosteres*) *longicaudatus* Ashmead (Hymenoptera: Braconidae) was successfully reared and a recovery survey was conducted from a small-scale field release in Baichi, Taipei (Yao, 1989).

D. longicaudatus is a solitary larval parasitoid and was recorded as 1 of the 5 native braconid parasitoid species of fruit flies in Taiwan (Clausen *et al.*, 1965). For over 10 years, a few *D. longicaudatus* var. *formosanus* have been collected from Nantou and Taipei, Taiwan (Yang, 1977; Yao and Lee, 1979). No further information on native parasitoid species on Taiwan was reported from the survey of *B. dorsalis* infestation (W.Y. Lee, unpublished data).

*Correspondence / reprint request address

We were able to rear *D. longicaudatus* in the laboratory and it appeared to be an efficient larval parasitoid (Greany *et al.*, 1976; Lawrence, 1981; Lawrence *et al.*, 1978; Yao, 1985). Three aspects of this parasitoid were studied: first, improving the technique for mass rearing; second, investigating the biology of the parasitoid and the mechanisms of the effects of the parasitoid on the host; and third, monitoring the progress of *D. longicaudats* establishment in the release field.

Our experiment used the mature late larvae for oviposition based on the behaviors of the host and the parasitoid. The young instar larvae of *B. dorsalis* live in the fruit and eat the pulp. Before the full-grown larvae fall to the ground for pupation, they move around underneath the skin of the fruit. At this time, the parasitoid lays its egg into the host larvae. Wong and Ramadan (1992) investigated the host age differences on progeny sex ratios of 3 parasitoid species, *D. longicaudatus, Psyttalia fletcheri and Diachasmimorpha tryoni*. In *D. longicaudatus,* and found that the progeny sex ratio increased significantly as their host larvae aged toward maturity: the highest percentage of emerged female offspring (66.7%±1.3% females) was obtained from the parasitoid ovipositing in the late 3rd instar host, while significantly more male offspring emerged from the 2nd instar larvae (9.5%±1.5% females).

Materials and Methods

Host larvae of *Bactrocera dorsalis* were mass cultured by methods currently used in the laboratory of the Institute of Zoology, Academia Sinica. A culture of *D. longicaudatus* reared in our laboratory was introduced from the Hawaiian Tropical Fruit and Vegetable Research Laboratory, USDA-ARS, Hawaii, USA during 1986-1987. The adult parasitoids were kept in $30\times30\times30cm^3$ plastic cages and provided with honey, water and sugar cubes as food. The laboratory conditions were 27±2℃, 75±1% RH, under a natural light period.

Improvement of Mass Rearing of Parasitoid Culture. Full-grown 3rd instar fruit flies (about 6 or 7 days old) were used for insect propagation of parasitoids. The oviposition unit was a modified plastic cover of a can (9cm in dia. and less than 0.5cm deep). Tight-filling lids with organdy covers were used to hold the host larvae inside the dish (Fig.1). Before packing the larvae in the oviposition unit, the larvae and medium were washed and the water was extracted. The following investigations were made.

(1) Influence of different exposure periods of the parasitoid on the parasitism of the mature late instar of the host larvae. These oviposition units were exposed to *D. longicaudatus* in cages for 2, 4, 6, and 8 h and overnight (about 16 h), respectively. After oviposition, the larval hosts were put into moist vermiculate to pupate, and, later, emerged as adults. The parasitism rates for each exposure time were calculated.

(2) Influence of female parasitoid ages to parasitism on the host larvae. In general, male parasitoids emerged 3 days before females, They mated immediately after the eclosion of females. Females began to lay eggs a day or so later. Ovipositions were obtained every day from 4- to 11-day old females. Comparisons of parasitism rates were determined each day.

The Biology of the Parasitoid and the Mechanisms of the Effects of the Parasitoid on the Host. The parasitoids laid their eggs into the mature late host larvae which would pupate the following day. Host pupae were dissected and examined with a light microscope every day from the time they were 1 day old until the parasitoid emerged.

Monitoring the Progress of *D.*

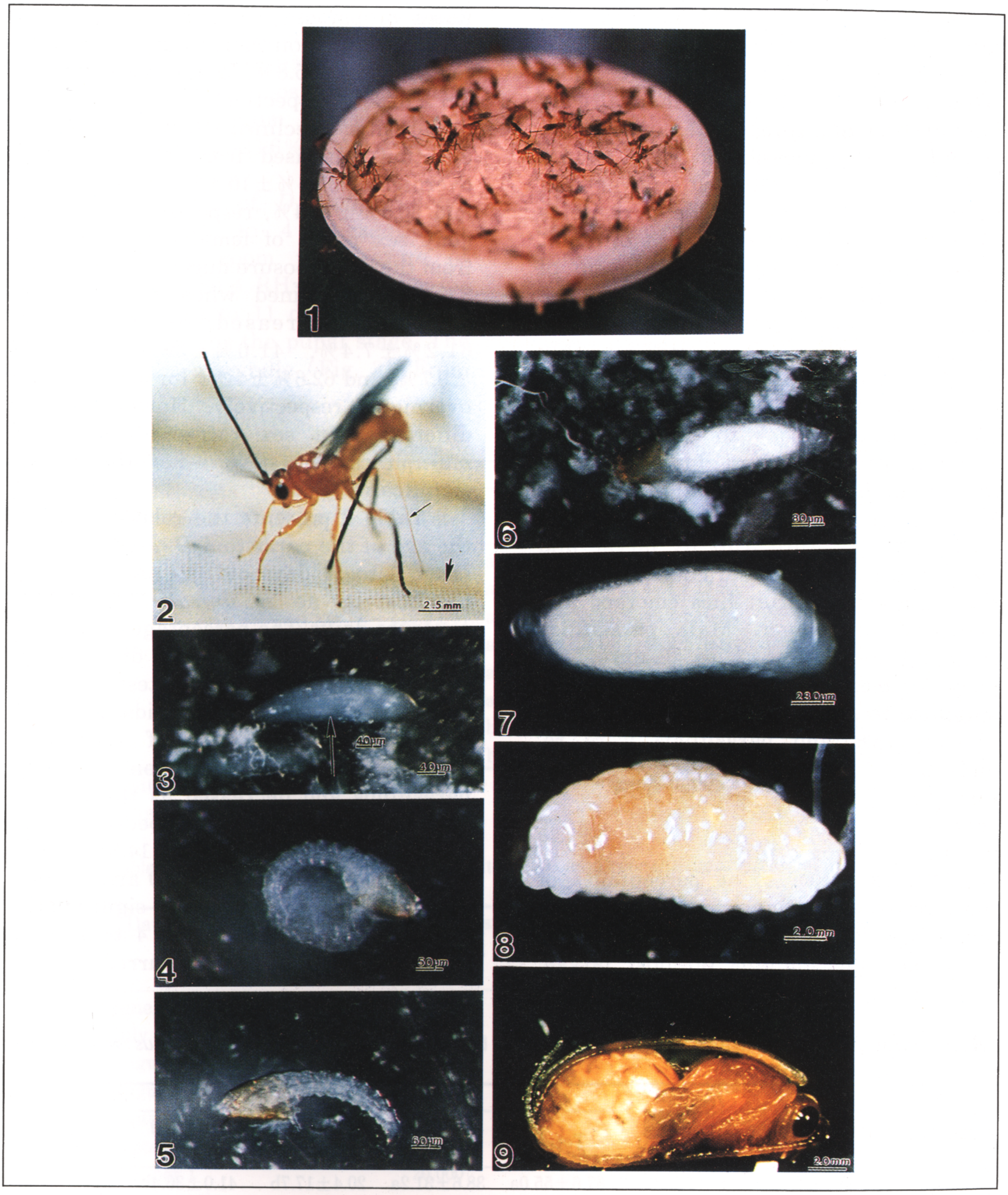

Fig. 1 Oviposition units and parasitoids.; Fig. 2 The female wasp ovipositing an egg into the host larva. Small arrow: ovipositor, big arrow: host larva.; Fig. 3 The egg of the parasitoid.; Fig. 4 The protopod phase I of the parasitoid, with a membrane wrapped around the body.; Fig. 5 The protopod phase II of the parasitoid; the membrane has disappeared.; Fig. 6 The parasitoid with transformed morphology between the protopod and apoid phases. Fig. 7 The apoid phase I of the parasitoid. Fig. 8 The apoid phase II of the parasitoid. Fig. 9 The pupal stage of the parasitoid.

***longicaudatus* Establishment in the Release Field.** Ten releases of parasitoids were made from June to December 1994. Most released females had already been mated or even oviposited once in the laboratory. The release field was a plantation with several hundred guava, wax apple and orange trees. The parasitoids were released on the ground at different spots. To determine the establishment of *D. longicaudatus,* fallen ripe fruit on the ground was sampled randomly at different distances from the release sites biweekly following the initial release on June 30, 1994. Procedures of handling the fruit samples brought back to the laboratory followed those of Yao (1989). Fallen fruits were placed into plastic containers with a layer of vermiculate on the bottom for 2 weeks. Mature fruit fly larvae and pupae were collected and counted. Several days later, the fruit flies emerged and died due to hunger. Male parasitoids emerged first. After 2 to 3 days, the females emerged. The parasitoids were identified to species and sex. The numbers of fruit flies and parasitoids were recorded.

Results

Table 1 presents the percentages of parasitism for different exposure durations (2, 4, 6, and 8 h and overnight (16 h.) of *D. longicaudatus* on host larvae. Rates of parasitism increased when the exposure duration increased from 2 and 4 to 6 h: 43.2%±5.8%, 52.3%±6.6% and 61.6%±10.4%, respectively. However, rates of parasitism declined when the exposure duration increased from 6 to 8 h and overnight: 61.6%±10.4%, 40.8%±6.1%, and 30.4%±13.4%, respectively. The progeny sex ratios of females were highest from the 6-h exposure duration (71.1%±8.8%), and declined when the exposure duration decreased or increased: 61.2%±7.4%, 41.0%±36.4%, 65.8%±9.7%, and 62.6%±4.3% for 4, 2, 8 h, and overnight, respectively. However, these differences in sex ratios by Duncan's multiple range test were not statistically significant.

Table 2 presents the relationships of the age of the parasitoid *D. longicaudatus* (from 4 to 11 days old) to parasitism on the host larvae, *B. dorsalis,* for 6 h of exposure daily. The percentages of parasitism were not significantly different by Duncan's multiple range test (<0.05) from 4- to 9-day old parasitoids, although the highest percentage of parasitism (58.2%±17.4%) occurred on 4-day-old parasitoids and decreased as the parasitoids got older. Parasitism became much lower (7.1%±12.0% and 14.8±28.5%) when the parasitoids were 10 and 11 days old. Progeny sex ratios were significantly high at the age interval of 4 to 9 days, while the low level occurred during

Table 1 Parasitism of the mature late instar host lavae by female parasitoids. *D. longicandatus* with different exposure periods.

Exposure Period(h)	Replicate (no.)	No. host (fly larvae)	Parasitoid production[1]				Parasitism[1] (%)
			Total	♀	♂	sex ratio (%♀)	
2	6	744	67.6±55.0a	38.6±31.2a	20.4±17.7b	41.0±36.4b	43.2± 5.9bc
4	6	1316	140.0±80.6a	83.3±42.1a	56.8±39.2a	61.2± 7.4ab	52.3± 6.6ab
6	6	1046	107.8±67.6a	75.0±45.3a	32.8±26.8ab	71.1± 8.8a	61.6±10.4a
8	6	1091	90.4±33.4a	60.0±26.2a	30.4±13.9ab	65.8± 9.7ab	40.8± 6.1bc
16(overnight)	6	1181	63.0±11.9a	40.4± 8.1a	24.4± 6.7ab	62.6± 4.3ab	30.4±13.4c

1. For means within columns, values followed by the same letter are not significantly different ($P<0.05$; Duncan's multiple range test).

parasitiod reproductive periods at 10 and 11 days old. The maximum percentage of female offspring was 56.0% ±5.3% at age 4 days. Progeny sex ratios declined every day and the minimum percentage of female offspring was 23.6% ±25.3% at age 11 days.

The life cycle of *D. longicaudatus* spanned about 3 to 4 weeks for 1 generation at a room temperature of 27±2℃ and 70%-80% RH: 1 to 2 days for the egg stage, 10 to 11 days for larvae, and 8 to 10 days for the pupal stage. The egg looked like a spindle (Fig. 3). From morphological characters, the larval stage could be divided into 2 forms. The 1- to 3-day-old parasitoid's appearance was like a carabiform larva with 3 pairs of thoracic legs, and a large head. It belonged to the protopod phase (Figs. 4 and 5), staying in the haemocoel of the host. After 3 days, the 4-day-old parasitoid became an apoid larva (Figs. 7 and 8) and attacked the inside of the host's body. At 5 or 6 days old, the larva grew and became very large, occupying the pupal case of the host. Between the protopod phase and the apoid larva there

Table 2 Parasitism of the host larvae by the female parasitoid, *D. longicaudatus* of different ages

Age of ♀ parasitoid (days)	Replicate (no.)	No. host (fly larvae)	Parasitoid production[1]				Parasitism[1] (%)
			Total	♀	♂	sex ratio (%♀)	
4	12	1757	114.0±35.1a	63.9±20.9a	49.8±15.5a	56.0± 5.3a	58.2±17.4a
5	12	1789	112.0±29.5a	67.9±25.1a	44.1±13.2a	57.7±12.1a	55.9±14.5a
6	12	1800	110.9±37.3a	63.8±25.5a	46.7±16.3a	56.4± 7.5a	54.3±18.5a
7	12	1759	107.2±36.8a	60.3±23.4a	46.9±18.8a	55.6± 8.1a	53.8±17.2a
8	12	1816	99.8±44.2a	51.9±24.7a	47.9±22.3a	51.6± 7.8a	49.2±20.8a
9	12	1400	97.6±50.8a	50.9±30.7a	46.7±20.2a	50.4± 3.8a	48.8±23.1a
10	8	1400	13.4±22.4b	8.0±14.1b	6.3± 8.2b	28.0±26.2b	7.1±12.0b
11	8	1000	23.4±47.7b	14.4±28.3b	11.3± 7.8b	23.6±25.3b	14.8±28.5b

1.For means within columns, values followed by the same letter are not significantly different ($P<0.05$); Duncan's multiple range test.

Table 3 Release results of the larval parasitoids *D. longicaudatus* at a guave field in Ilan, Taiwan (1994)

Date of parasitoid release	Approx. no. Parasitoids released(♀♂)	No host pupae collected	No. parasitoids obtained	
			♀	♂
June 30 (1994)	30,000	—	—	
July 15	20,000	—	—	
August 1	20,000	—	—	
August 15	20,000	145	6	2
September 1	20,000	96	1	0
September 16	20,000	77	0	0
October 1	20,000	271	7	3
October 15[1]	—	—	—	
November 1	20,000	160	4	5
November 15	20,000	25	5	6
December 1	20,000	325	23	20
December 15	60,000	84	12	8

1. Tyhoon Seth affected northeastern of Taiwan on the 9th of October with heavy rain afterward.

was a resting stage in which the larva had a head like the protopod phase but with the apoid larval body. It was termed the transformation (Fig. 6). The parasitoid pupated after the host body was completely digested at approximately 10 days old. The parasitoid pupae were the exerate type (Fig. 9).

The mechanism of the effect of parasitoids on the host was that *D. longicaudatus* was endoparasitic with females ovipositing their eggs into the host tissue (Fig. 2). Larvae grew and ate the tissues and organs of the host. The host body was completely digested before the parasitoid pupated. These parasitoids should not be considered a superparasite. Only 1 parasitoid grew in and pupated from a single host, although more than 1 protopod larvae might have occurred within that host.

Over 10,000 parasitoid wasps, with a male to female sex ratio of about 1:1.5, have been released to a guava field in the Yuanshen District of Ilan County since June 1994. This is a special plantation area with native varieties of guava, and also wax apple and orange. No parasitoid had been recorded here from a survey of *B. dorsalis* infestation during the past 10 years (Lee, unpublished data). It is usually cold and rainy in this area from January to May. Therefore, parasitoids were released beginning from June. The results of the releases and recovery of *D. longicaudatus* are given in Table 3. Six female and 2 male parasitoids were found from *B. dorsalis* larvae within the infested guava of the field survey of *B. dorsalis* in the middle of August 1994. Furthermore, different numbers of the same species of parasitoid were obtained: 1 female on the first of September, 7 females and 3 males in October, 9 females and 11 males in November, and 35 females and 28 males in December. All parasitoids were identified as *D. longicaudatus*.

Discussion

Diachasmimorpha longicaudatus is a larval parasitoid of *Bactrocera dorsalis*. It was one of many parasitoid species brought into Hawaii for controlling *B. dorsalis* (Clausen *et al.*, 1965) and has played a major role in the reduction of oriental fruit flies throughout the Hawaiian Islands (Vargas *et al.*, 1993).

Proper oviposition exposure period is an important factor in mass rearing of parasitoids. Ramadan *et al.* (1989) reported that 4- to 8-h exposure to parasitoids appeared optimum. Wong and Ramadan (1992) used a 6-h exposure to parasitoids in Hawaii. The present study also found that 4- to 6-h is a correct oviposition period for exposure to *D. longicaudatus*.

Information on reproductive activity in relation to cohort maternal age is useful to determine optimum conditions, and knowledge of specific cohort age groups will be helpful to determine when parasitoid brood colonies should be discarded (Carey *et al.*, 1988). Wong and Ramadan (1992) found that 5-day-old females of the parasitoid *D. longicaudatus* produced more progeny (parasitism 69.6%±3.3%) using middle 3rd instar larvae of *B. dorsalis* exposed to parasitoid oviposition for 6 h. Progeny production decreased when the age of female parasitoids increased. Thus, cohorts older than 15 days should be discarded, because parasitism was 2.6%±0.8% for 16-day-old cohorts. As for the cohorts in our experiment, 4-day-old parasitoids obtained the highest parasitism, 58.2%±17.4%. The parasitism rates decreased gradually for 5-to 9-day-old females, with a greater decline after 10 days old . These older cohorts, therefore, were discarded.

The parasitoid ovipositor injects its eggs into the host body by certain sensory structures which are important in host selection among endoparasitic Hymenoptera (Gutierrez, 1970; Vinson, 1976). Supernumerary 1st instars of parasitoids compete with each other when the host is superparasitized (Pemberton and Willard, 1918), until the 1st instar parasitoid molts to the subsequent instar, leaving only 1 larva within the host. To minimize the rates of superparasitization and use the high rates of each parasitoid, proper oviposition exposure period is an important factor in the mass-rearing of parasitoids.

Between 1947 and 1952, 21 different species of parasitoids were brought into Hawaii to control *B. dorsalis*. Most were species of *Opius* (Hymenoptera: Braconidae: Opiinae) (Clausen *et al.*, 1965). Some of these parasitoids became established and reduced the population of *B. dorsalis* significantly (Bess, 1953). The field experiments of suppression of the medfly, *Ceratitis capitata* (Wiedmann), population on Kauai Island suggests that the concurrent release of parasitoids and sterile flies represents a valuable approach to eradicate these flies (Wong *et al.*, 1992). In Taiwan, several *Opius oophilus* were brought in from Hawaii by Bess and 38 parasitoids were captured 2 years later (Yang, 1977). Yao (1989) recovered of *Biosteres (Diachasmimorpha) longicaudatus* Ashmead from a small-scale field release in the Baichi area, Taipei. The present study documents releases of *D. longicaudatus* into a guava tree plantation area located in Ilan, during 1994 and also recoveries since August of the same year.

The biological control method, i.e., the release of the larval parasitoid *D. longicaudatus,* should be used to suppress the population of the oriental fruit flies, *B. dorsalis,* and, together with annihilation methods, should constitute an integrated control program in Taiwan. These parasitoids can migrate to and become established in areas where *B. dorsalis* is abundant, and where the effects of methyl eugenol can not reach. The mass rearing of parasitoids is important for initiation of this biological control method. Host larva age, parasitoid maternal age and the exposure peroid of parasitoid oviposition are important factors influencing parasitoid yield. Thus, successful propagation of these valuable parasitoids also depends on careful control of host quality.

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臺灣東方果實蠅幼蟲寄生蜂*Diachasmimorpha longicaudatus*之研究

李文蓉* 張與哲 陳麗雲 中央研究院動物研究所 臺北市南港區研究院路二段128號

摘　　要

東方果實蠅*Bactrocera*（=*Dacus*）*dorsalis*為臺灣重要之經濟害蟲，每年政府及農業機構均耗費巨資，利用含毒甲基丁香油誘殺之滅雄技術防治該蟲。果實蠅幼蟲寄生蜂，*Diachasmimorpha*（=*Opius*,=*Biosteres*）*longicaudatus*，為1986－1987年從夏威夷引進之寄生蜂，已在實驗室內飼育成功，並將應用於果實蠅生物防治與滅雄技術之綜合防治。室內飼育技術之逐步改進、寄主和寄生蜂之寄生年齡、接受寄生產卵時間之長短、以及寄生蜂之生物學等經詳細研究均在本文中報告，此外，田間釋放寄生蜂之研究亦在進行中。

關鍵詞：東方果實蠅，幼蟲寄生蜂，寄生，產卵，成熟期幼蟲。

*抽印本索取及論文聯繫之負責人